

Safety of long-term isoniazid preventive therapy in children with HIV

by

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PREAMBLE

DECLARATION

I, Stanzi Maria le Roux, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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For Fred and Irene

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Abstract

BACKGROUND: Tuberculosis (TB) and HIV co-infection is associated with significant morbidity and mortality, especially in young children. Prevention of tuberculosis in children with HIV is a global health priority and is best achieved through a combination of antiretroviral therapy (ART) and isoniazid preventive therapy (IPT). Current WHO guidelines recommend 6 months of IPT for all HIV-infected children older than 1 year without TB disease; up to 3 years is recommended in high TB prevalence areas. Although both ART and IPT can cause liver injury, data on the safety of IPT in HIV-infected children accessing ART is limited, and no published data exist on the hepatotoxicity risk of prolonged IPT. This thesis aims to address these knowledge gaps.

METHODS: The main aim of this thesis was to investigate the incidence of severe liver injury in HIV-infected children receiving IPT. The specific objectives were to: (1) Present a structured research proposal and plan; (2) Conduct a structured review of published literature on severe liver injury among children receiving IPT, with a specific focus on those with HIV; and (3) Present the findings of a prospective study evaluating risk of liver injury among HIV-infected children receiving prolonged IPT. A randomized, placebo-controlled trial of IPT (the “INH-study”) commenced in December 2002, in Cape Town, South Africa. Participants were HIV-infected children. Placebo was discontinued in May 2004, due to a marked survival benefit among children receiving IPT. Thereafter, all children receiving placebo were switched to IPT and followed until December 2007, to assess safety and adherence. Alanine transaminase (ALT) was measured at baseline, six-monthly and during illness: an increase ≥ 10 -fold the upper limit of normal defined severe liver injury.

RESULTS: A structured literature search strategy was developed and implemented by one author. Apart from the published findings of this thesis project, only two other published papers presented risk estimates of liver injury in HIV-infected children receiving IPT (range of 0.4-5% of study population). No other published, prospective data on IPT-related liver injury in HIV-infected children older than one year or in symptomatic HIV-infected children were found. In the INH study, 297 of 324 children (91.6%) received IPT, and 207(63.9%) received ART. Although 16 children developed severe liver injury while receiving IPT, isoniazid was considered causative in only five cases (1.7%). No child developed hepatic failure; one died of an unrelated cause. All surviving children subsequently tolerated IPT.

CONCLUSIONS: Taken together with other published studies of IPT in children, these findings suggest that long-term IPT has a low toxicity risk in pediatric populations irrespective of HIV or ART status.

Acknowledgements

Thank you to my two supervisors, Professors Landon Myer and Heather Zar, for mentoring and guiding me through this process with great patience, support and encouragement; for reading multiple drafts of the manuscript and providing thoughtful and detailed feedback.

Thank you to the principle investigators of the parent study, Professors Heather Zar, Mark Cotton and Simon Schaaf, for allowing me access to the data of the INH study, and for inspiring me to work in the field of pediatric infectious diseases; Dr Carl Lombard and Professor Landon Myer for overseeing my statistical analysis of this project; and Dr Dave le Roux, my husband, colleague and friend, for everything.

This thesis addresses a secondary aim of the INH parent study. Professors Heather Zar, Mark Cotton, and Simon Schaaf conceived the parent study, wrote the protocol and grant applications, obtained funding and supervised the study. Dr Carl Lombard was the trial statistician, and provided support for the statistical analysis of this thesis, along with Dr Dave le Roux and Professor Landon Myer.

To the two long-suffering and dedicated INH study teams at Red Cross War Memorial Children's Hospital, and Tygerberg Children's Hospital – thank you for your hard work, your assistance with data abstraction and your advice. An especial thanks to Drs Liz Walters and Teresa Edwards.

Thank you to Professor Peter Donald for helpful discussions, encouragement and access to various journal articles from the previous century.

Lastly, thank you to the mothers who brought their children for countless study visits in a time when antiretroviral therapy and hope for South African children with HIV seemed so far away.

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List of Abbreviations

aHR	Adjusted hazard ratio
ALT	Alanine transaminase
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
ATS	American Thoracic Society
CDC	Centers for Disease Control and Prevention
CMV	Cytomegalovirus
DAIDS	Division of AIDS
DILI	Drug-induced liver injury
EBV	Ebstein-Barr Virus
ELISA	Enzyme-linked immune-sorbent assay
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hazard ratio
IgG	Immunoglobulin G
IgM	Immunoglobulin M

INH	Isoniazid
IPT	Isoniazid preventive therapy
IQR	Inter-quartile range
IRR	incidence rate ratio
Kg	Kilogram
LTBI	Latent tuberculosis infection
Mg	Milligram
NAT2	N-acetyl transferase 2
PCR	Polymerase chain reaction
PY	Person-years
RCT	Randomized controlled trial
TB	Tuberculosis
TMP-SMX	Trimethoprim-sulphamethoxazole
TTE	Transient transaminase elevation
ULN	Upper limit of normal
USA	United States of America

A. RESEARCH PROTOCOL

1. BACKGROUND

Tuberculosis (TB) and HIV co-infection is associated with significant mortality and morbidity; young, HIV-infected children are particularly vulnerable to the development of TB disease, with poor outcomes in the absence of antiretroviral therapy¹⁻³. Although antiretroviral therapy (ART) or isoniazid preventive therapy (IPT) given individually significantly reduce TB risk, there is evidence that IPT given with ART has even greater efficacy in both adults and children^{1, 2}. The World Health Organization (WHO) has identified the “three I’s” (IPT, intensified case finding and infection control) as key to reducing the incidence of TB among people living with HIV³. Current WHO guidelines recommend 6 months of IPT for all HIV-infected children older than 1 year without TB disease, even in the absence of a known TB contact; up to 3 years is recommended in high TB prevalence areas^{4, 5}. Despite these recommendations, uptake of IPT for HIV infected individuals remains disappointingly low, even in high TB burden areas⁹. Drug side effects, including liver injury, are known barriers to successful isoniazid preventive therapy among adults^{10, 11}. Isoniazid can cause idiosyncratic liver injury¹². Its predominant metabolizing pathway is acetylation by N-acetyltransferase 2 (NAT2); liver toxicity is mostly attributed to metabolites such as hydrazine¹². In general, idiosyncratic drug induced liver injury (DILI) is thought to result from a complex “multi-hit” process, where drug-specific upstream injury is exacerbated or ameliorated by less specific downstream factors^{13, 14}. Known predisposing factors for isoniazid induced liver injury can be categorized accordingly: drug-specific risk factors include higher

dosages and polymorphisms of NAT2¹². During TB treatment, daily dosing appears to confer higher risk than intermittent dosing¹². Previously described “downstream” factors include malnutrition, other hepatotoxic drugs (such as nevirapine and pyrazinamide), viral hepatitis, and in adults, age and alcoholism^{13, 14}. Children generally tolerate isoniazid better than adults. Although death and hepatic failure can occur¹⁵, less than 0.8% of HIV-uninfected children experience moderate to severe liver injury⁶⁻¹⁰, compared to 0.1- 6.4% of HIV-uninfected adults^{8, 11, 12}. Although the independent effect of HIV on DILI is not yet fully understood, it is biologically plausible that the chronic state of immune activation associated with HIV can predispose to idiosyncratic DILI¹³. In Botswana, HIV-infected adults receiving 6 months of IPT appeared to have a risk of liver injury similar to that of HIV-uninfected adults^{13, 14}; another study described severe liver injury in a higher proportion (5.5%) of adults on a similar IPT regimen²⁴. Data for HIV-infected children are limited. A retrospective cohort study reported severe liver injury in 5% of 112 HIV-infected children receiving IPT, most of which was ascribed to other causes such as viral hepatitis¹⁵. In a recent study of pre-exposure IPT used early in infancy, HIV-infected infants received isoniazid for up to 2 years; 0.4% developed significantly raised alanine transaminase (ALT), and 4.0% developed significantly raised aspartate aminotransferase (AST) ¹⁶. However, there are no published, prospective long-term data on liver injury in HIV-infected children older than 1 year, or in symptomatic HIV-infected children, who commence IPT. As risk factors for idiosyncratic DILI are dynamic^{13, 14}, it is possible that the cumulative risk for

isoniazid induced liver injury is greater during long-term exposure, as was observed among HIV-infected adults receiving 3 to 6 years of IPT²⁴. There are no published studies reporting adverse effects of long-term exposure to isoniazid in children. This study aims to investigate the incidence, predictors and outcomes of severe liver injury in a cohort of HIV-infected infants and children receiving IPT for up to five years.

2. STUDY OBJECTIVE, AIMS & HYPOTHESES

Study objective:

To investigate the incidence, predictors and outcomes of severe liver injury in a cohort of HIV-infected children enrolled in a 5-year prospective trial of isoniazid preventive therapy (IPT).

Specific aims:

- 1) To describe the incidence, clinical presentation and outcomes of severe liver injury among study participants, including liver injury probably/possibly related to isoniazid preventive therapy
- 2) To identify predictors of severe liver injury

Hypotheses:

1. Isoniazid-induced severe liver injury is rare among children with HIV.
2. The incidence of severe liver injury is higher among children receiving isoniazid daily, compared to thrice weekly; it is also higher among those receiving anti-retroviral therapy and anti-tuberculosis therapy containing rifampicin/pyrazinamide, and in children with more advanced HIV disease.

3. METHODS

3.1 Study design

Secondary analysis of data obtained, with permission, from a closed clinical trial database (“Long Term Study of 2 Isoniazid (INH) Prophylactic Regimens With Concomitant Trimethoprim-Sulphamethoxazole (TMP-SMX) in HIV-infected Children - Impact on Morbidity, Mortality, Bacterial Resistance and Incidence of Tuberculosis”); registered as Clinical Trials NCT00330304, with Principle Investigators Prof Heather J Zar, University of Cape Town and Professor Mark F Cotton, Stellenbosch University²⁷.

The original study was conducted prospectively at two sites in Cape Town, South Africa, between December 2002 and December 2007. The study had a factorial design: participants were randomized to receive either isoniazid or placebo (first level of randomization, double-blind with allocation concealment), with trimethoprim- sulphamethoxazole (TMP-SMX). The second level of randomization (allocation not concealed) was to either a daily or thrice weekly dosing schedule for all study drugs (isoniazid with TMP-SMX, or placebo with TMP-SMX).

Incidence of severe liver injury was an a priori secondary outcome specified in the original trial protocol, as approved by the Ethics Committees of University of Cape Town (HREC REF 057/2002) and Stellenbosch University. The primary outcome for the IPT component of the study was mortality; the sample size achieved (324 participants) was sufficiently powered to detect a significantly lower mortality risk

in the IPT group as compared to the placebo group. This result has been published²⁸.

Sample size and statistical power

Although the primary focus of this secondary outcome analysis is descriptive, some comparisons will be made between different subgroups. With a sample size of only 324, it is anticipated that only marked differences will be statistically detectable. However, a secondary power analysis will not be conducted, as the sample size was calculated for a different outcome, and the study is closed. As currently recommended, 95% confidence intervals will be used to demonstrate the precision achieved for all estimates^{29,30}.

3.2 Study population

HIV-infected children older than 8 weeks of age and weighing more than 2.5kg, resident within the Cape Metropole area, and whose parents/legal guardian provided informed consent, were eligible for enrolment if the caregivers had access to transport. Exclusion criteria included: chronic diarrhea, current use of (or need for) IPT according to national TB control guidelines, history of hypersensitivity to isoniazid or allergy to sulphur drugs, severe anemia, neutropenia, and/or thrombocytopenia, irreversible kidney failure and/or clinical hepatitis. Asymptomatic children with significant baseline ALT elevation were thoroughly investigated; after full biochemical recovery, study drugs were systematically introduced under close monitoring. Children younger than 18 years with HIV infection (hence, vulnerable population) were the subjects of this study, as the aim was to identify efficacious strategies to lower mortality and morbidity risk for this particular population. Of the 339

children enrolled, 324 will be included in this analysis: 15 will be excluded, as 10 children ultimately tested HIV-negative, and 5 were lost to follow-up within a month of randomization. These are the same 324 study participants who were included in other published analyses from the same database^{28, 31, 32}.

3.3 Recruitment and enrolment

For the original study, children were recruited from the two tertiary hospitals (RCWMCH and TCH), as well as New Somerset and Paarl hospitals; there were also study eligibility assessment referrals from various district hospitals and clinics. Details of this process have been published²⁸.

3.4 Research procedures and data collection methods

Extensive clinical history, clinical examination and laboratory testing were conducted on all enrolled participants at baseline, and repeated according to the study protocol at specified follow-up times. Extensive clinical data were collected onto standard forms every three months; at every six month interval, chest X-rays and laboratory investigations were also conducted, and results captured.

Relevant to this study, alanine transaminase (ALT) was measured at baseline and six-monthly; children on ART also had ALT measured 1 and 3 months after enrolment. Other baseline and six-monthly investigations have been described elsewhere^{17, 18}. Tests were repeated more frequently if clinically indicated. All deaths were investigated by accessing clinical records, or if unavailable, by verbal autopsy where feasible.

Toxicity

Toxicity events were graded according to the Division of AIDS (DAIDS) toxicity criteria¹⁹. For liver injury, an ALT 10 times the upper limit of normal was defined as a grade 3 event; above 15-fold increase was considered grade 4. Gender and age-specific reference ranges were used to define the upper limit of normal, according to local laboratory practice. Each toxic event was assessed as definitely, probably, possibly or unrelated to study drugs.

Data capture

All information was entered by hand onto standardized paper forms designed by the study team leaders. Electronic data capture was done in intervals by two experienced data capturers, at both sites, onto a central database. Before final analyses commenced, all data entries were double checked and discrepancies resolved by an external data management expert in consultation with the project manager. The database is now closed.

3.5 Data safety and monitoring

A data safety and monitoring board (DSMB) comprising international and South African experts reviewed safety and progress of the study based on three- to six-monthly interim data analyses^{28, 31, 32}. The placebo arm of the study was terminated by the DSMB after 19 months of enrolment due to the early survival benefit observed on IPT²⁸; the DSMB continued to monitor the study until isoniazid was discontinued for all participants at the earliest study visit following December 2007.

This secondary outcome analysis will be supervised by two experienced researchers, as indicated previously.

3.6 Data analysis

Data will be extracted from the closed database, without any personal identifiers, by the study data manager. The data will be analyzed and saved on the personal computer of Dr Stanzi le Roux; no other person will have access to the data. The data, and results from analyses, will also be saved onto an external hard drive for back-up purposes.

The main outcome of interest will be severe liver injury, defined as a grade 3 or 4 elevation in ALT at any time during follow-up. To address all the study aims, three levels of analysis will be conducted, using Stata version 10.0 (Stata Corporation, College Station, Texas, USA):

(1) Proportions

Risk will be summarized as the proportion of children who ever developed severe liver injury, and also as the proportion of children who ever developed severe liver injury possibly/probably related to isoniazid.

(2) Survival analysis: Kaplan-Meier method with incidence rates (IR) and incidence rate ratios (IRR)

The Kaplan-Meier method³⁴ will be used to analyze time to first episode of severe liver injury. To account for potential changes in clinical management of a patient following severe liver injury, follow-up time will be censored at the first episode; for those who were event-free, last known time alive will be used. The main predictor

will be dosing schedule (thrice weekly compared to daily). Using crude IRR, risk for severe liver injury will be compared between dosing schedules (intent-to-treat analysis) and between different categories of drug exposure (as-treated analysis).

(3) Survival analysis: Cox proportional hazards regression

Predictors of severe liver injury will be analyzed with Cox proportional hazards regression analysis³⁴. The following potential confounders will be assessed: baseline age, Centers for Disease Control and Prevention (CDC) clinical and immune categories, CD4 cell percentage (CD4%), weight-for-age Z-score, gender and study site. Current drug exposure will be modeled as a time-varying covariate. Study site will be adjusted for in all analyses, as randomization was stratified by site. After conducting an exploratory data analysis, likelihood ratio tests and Akaike's Information Criterion will be used for model building³⁴. Effect modification by dosing schedule will be tested for. Model checking will be done using residuals analysis; the proportional hazards assumption will be tested with the Grambsch-Therneau test³⁴. Statistical tests will be two-sided at $\alpha=0.05$.

3.7 Other methodological aspects

A description of risks and benefits, the informed consent processes and reimbursement details of the original study have been provided and approved previously (HREC REF 057/2002).

3.8 Privacy and confidentiality

No personal identifiers will be present in the data used for analysis.

3.9 Contributions, authorship and acknowledgements

The principle investigator of this secondary study, Dr Stanzi le Roux, originally participated in data collection as medical officer for the UCT site. She will be conducting the data cleaning, analysis and writing the mini-thesis protocol, literature review and journal-ready manuscript under the supervision of Professors Zar and Myer during her time as a registered MPH student (2009-2013). Her two supervisors are Prof Heather J Zar (principle investigator of the overall study) and Prof Landon Myer (convener of the MPH Clinical Research track). Along with Professors Mark F Cotton and H Simon Schaaf, Professor Zar conceived the original study, wrote the protocol and grants applications, obtained funding and supervised the study. Dr Carl Lombard was the trial statistician.

In the event of publication of this secondary outcome analysis, authorship will be attributed according to standard authorship guidelines.

4. REFERENCES

1. Walters E, Cotton MF, Rabie H, Schaaf HS, Walters LO, Marais BJ. Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on anti-retroviral therapy. *BMC Pediatr* 2008;**8**:1.
2. Moore D, Liechty C, Ekwaru P, Were W, Mwima G, Solberg P, et al. Prevalence, incidence and mortality associated with tuberculosis in HIV-infected patients initiating antiretroviral therapy in rural Uganda. *AIDS* 2007;**21**:713-9.
3. Hesseling AC, Cotton MF, Jennings T, Whitelaw A, Johnson LF, Eley B, et al. High incidence of tuberculosis among HIV-infected infants: evidence from a South African

- population-based study highlights the need for improved tuberculosis control strategies. *Clin Infect Dis* 2009;**48**:108-14.
4. Frigati LJ, Kranzer K, Cotton MF, Schaaf HS, Lombard CJ, Zar HJ. The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011;**66**:496-501.
 5. Golub JE, Pronyk P, Mohapi L, Thsabangu N, Moshabela M, Struthers H, et al. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: a prospective cohort. *AIDS* 2009;**23**:631-6.
 6. WHO Three I's Meeting: Intensified Case Finding (ICF), Isoniazid Preventive Therapy (IPT) and TB Infection Control (IC) for people living with HIV. Report of a Joint World Health Organization HIV/AIDS and TB Department Meeting. Geneva , Switzerland., 2008. Available from:
http://www.who.int/hiv/pub/meetingreports/WHO_3Is_meeting_report.pdf.
(accessed 1 June 2011)
 7. World Health Organization. Antiretroviral therapy for HIV infection in infants and children: Towards universal access. Recommendations for a public health approach: 2010 revision. Geneva World Health Organization, 2010. Available from:
<http://www.who.int/hiv/pub/paediatric/infants2010/en/index.html>. (accessed 1 June 2011)
 8. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource constrained settings. Geneva, Switzerland, 2011. Available from:
http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf. (accessed 1 June 2011)
 9. Getahun H, Granich R, Sculier D, Gunneberg C, Blanc L, Nunn P, Raviglione M. Implementation of isoniazid preventive therapy for people living with HIV worldwide: barriers and solutions. *AIDS*. 2010 Nov;**24** Suppl 5:S57-65.
 10. Ziakas PD, Mylonakis E. [4 months of rifampin compared with 9 months of isoniazid for the management of latent tuberculosis infection: a meta-analysis and cost-](#)

[effectiveness study that focuses on compliance and liver toxicity](#). *Clin Infect Dis*. 2009 Dec 15;49(12):1883-9

11. Page KR, Sifakis F, Montes de Oca R, Cronin WA, Doherty MC, Federline L, Bur S, Walsh T, Karney W, Milman J, Baruch N, Adalakun A, Dorman SE. Improved adherence and less toxicity with rifampin vs isoniazid for treatment of latent tuberculosis: a retrospective study. *Arch Intern Med*. 2006 Sep 25;166(17):1863-70.
12. Donald PR. Antituberculosis drug-induced hepatotoxicity in children. *Pediatr Rep* 2011;**3**:e16.
13. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov*. Jun 2005;4(6):489-499.
14. Lee WM. Drug-induced hepatotoxicity. *N Engl J Med*. Jul 31 2003;349(5):474-485.
15. Wu SS, Chao CS, Vargas JH, Sharp HL, Martin MG, McDiarmid SV, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. *Transplantation* 2007;**84**:173-9.
16. Beaudry PH, Brickman HF, Wise MB, MacDougall D. Liver enzyme disturbances during isoniazid chemoprophylaxis in children. *Am Rev Respir Dis* 1974;**110**:581-4.
17. Kopanoff DE, Snider DE, Jr., Caras GJ. Isoniazid-related hepatitis: a U.S. Public Health Service cooperative surveillance study. *Am Rev Respir Dis* 1978;**117**:991-1001.
18. Nemir RL, O'Hare D. Tuberculosis in children 10 years of age and younger: three decades of experience during the chemotherapeutic era. *Pediatrics* 1991;**88**:236-41.
19. Palusci VJ, O'Hare D, Lawrence RM. Hepatotoxicity and transaminase measurement during isoniazid chemoprophylaxis in children. *Pediatr Infect Dis J* 1995;**14**:144-8.
20. LoBue PA, Moser KS. Use of isoniazid for latent tuberculosis infection in a public health clinic. *Am J Respir Crit Care Med* 2003;**168**:443-7.
21. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* 1991;**99**:465-71.

22. Tedla Z, Nyirenda S, Peeler C, Agizew T, Sibanda T, Motsamai O, et al. Isoniazid-associated hepatitis and antiretroviral drugs during tuberculosis prophylaxis in HIV-infected adults in Botswana. *Am J Respir Crit Care Med* 2010;**182**:278-85.
23. Martinson NA, Barnes GL, Moulton LH, Msandiwa R, Hausler H, Ram M, et al. New regimens to prevent tuberculosis in adults with HIV infection. *N Engl J Med* 2011;**365**:11-20.
24. Samandari T, Agizew TB, Nyirenda S, et al. 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet*. May 7 2011;377(9777):1588-1598.
25. Gray D, Nuttall J, Lombard C, Davies MA, Workman L, Apolles P, et al. Low Rates of Hepatotoxicity in HIV-infected Children on Anti-retroviral Therapy with and Without Isoniazid Prophylaxis. *J Trop Pediatr* 2009;**56**:159-65.
26. Madhi SA, Nachman S, Violari A, Kim S, Cotton MF, Bobat R, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011;**365**:21-31.
27. ClinicalTrials.gov. Available from:
<http://clinicaltrials.gov/ct2/show/NCT00330304?term=NCT00330304&rank=1>.
(accessed 1 June 2011)
28. Zar HJ, Cotton MF, Strauss S, Karpakis J, Hussey G, Schaaf HS, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007;**334**:136.
29. Levine M, Ensom MH. Post hoc power analysis: an idea whose time has passed? *Pharmacotherapy*. 2001 Apr;**21**(4):405-9.
30. Smith AH, Bates MN. Confidence limit analyses should replace power calculations in the interpretation of epidemiologic studies. *Epidemiology*. 1992 Sep;**3**(5):449-52.
31. Zar HJ, Workman L, le Roux SM, Jennings T, Jele N, Schaaf HS, et al. A randomized controlled trial of intermittent compared with daily cotrimoxazole preventive therapy in HIV-infected children. *AIDS* 2010;**24**:2225-32.

32. le Roux SM, Cotton MF, Golub JE, le Roux DM, Workman L, Zar HJ. Adherence to isoniazid prophylaxis among HIV-infected children: a randomized controlled trial comparing two dosing schedules. *BMC Med* 2009;**7**:67.
33. National Institutes of Health. Division of AIDS (DAIDS) revised toxicity tables for grading severity of pediatric adverse experiences. US National Institutes of Health DAIDS HIV Vaccine and Research Program, version 1.0. 2004. Available from: http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/DAIDS_AEGradingTable.pdf. (accessed November 24, 2009)
34. Cook T, DeMets D. Introduction to Statistical Methods for Clinical Trials.: Chapman & Hall/CRC, 2008

B. STRUCTURED LITERATURE REVIEW

1. Summary:

INTRODUCTION: Isoniazid preventive therapy (IPT) is an effective strategy to reduce the incidence of tuberculosis in both HIV-infected and HIV-uninfected children.

However, isoniazid has been reported to cause idiosyncratic liver injury. The risk for IPT-related liver injury is considered low in HIV-negative children; its risk among HIV-infected children, especially those receiving antiretroviral therapy (ART), is not well-established.

OBJECTIVES: To summarize, evaluate and compare published data on the risk of liver injury among both HIV-infected and HIV-uninfected children receiving IPT, and to identify knowledge gaps and further research needs.

STUDY ELIGIBILITY CRITERIA (DESIGN, POPULATION & INTERVENTION): Studies presenting longitudinal data on hepatic adverse events among children (0-18 years) receiving isoniazid monotherapy as tuberculosis prevention were eligible.

STUDY APPRAISAL: Potential sources of bias evaluated: study design; *a priori* case definitions of liver injury; degree and type of toxicity monitoring; method for attribution of causality; assessment and reporting of loss to follow-up and adherence.

RESULTS: Four hundred and ninety studies were found through 3 database searches (Medline, Scopus and Web of Science; an additional 8 records were identified through reference list searches and review articles. Titles, abstracts and full-text articles were evaluated sequentially, with ineligible articles discarded at each stage. Twenty-four articles were included for review, of which 3 were in HIV-infected children. The studies

were variable in dosages, duration of IPT; definitions of liver injury; type and degree of monitoring; assessment of loss to follow-up and adherence; ages of patients, and concurrent viral hepatitis screening. Only 2 studies were prospective, randomized and placebo-controlled. The estimated risk for severe IPT-related liver injury in HIV-uninfected children ranged from none (INH given for 6 months at 5 mg/kg), to 1.78% (INH given for 6 months at 10-20 mg/kg). Risk estimates for IPT-related liver injury among HIV-infected children ranged from 0.4% to 7%; the calculation of 7% risk included cases where liver injury was ascribed to other causes. Overall, sixteen cases of hepatic failure were described, none among HIV-infected children. Viral hepatitis was excluded in very few children. Overall, intensive laboratory monitoring and higher dosage of isoniazid appeared to increase the risk for liver injury. In general, HIV-infection did not appear to increase risk of IPT-related DILI.

LIMITATIONS: The limited scope of the review methodology increases the risk of selective reporting bias, publication bias and reviewer bias. However, the reviewed publications extended over several decades of global IPT use, and included several studies with sample sizes over 1000; thus the potential impact of any missed reports is likely to be minimal. Given the heterogeneity of monitoring and liver injury definitions used, biased assessment of outcomes is highly likely, especially in the retrospective studies; misclassification bias could have influenced risk estimates in either direction. However, the monitoring and to a lesser degree the definitions of liver injury were aligned with national and international guidelines of tuberculosis prevention at the time of the research. The lack of placebo-controlled studies also increased risk for

misclassification bias due to faulty attribution of causality. However, much toxicity literature depends on post-marketing reports, and given the efficacy of IPT in children, placebo controls would have been unethical in most of the research settings.

CONCLUSIONS & IMPLICATIONS: IPT-related liver injury appears to occur infrequently among HIV-negative children; severe liver injury is extremely rare, especially with frequent monitoring and early interruption of therapy in the event of symptomatic hepatitis. Viral hepatitis should be considered in children with apparent drug-induced hepatotoxicity. The risk for IPT-related liver injury in HIV-infected children does not appear to be higher than in HIV-negative children. However insufficient numbers of older children were evaluated in the three studies reporting risk of DILI in HIV-infected children to make meaningful comparisons. . As the global ART roll-out progresses, ongoing vigilance for liver injury is imperative as larger numbers of children will be receiving ART and INH simultaneously.

2. Literature review:

2.1 INTRODUCTION

Isoniazid has been successfully used as preventive therapy against active tuberculosis (TB) since its efficacy was established by large, controlled trials in the middle of the 20th century¹⁻⁴; it remains the mainstay of treating latent tuberculosis infection (LTBI) globally⁵.

Appropriate use of preventive therapy requires a careful balance of potential benefits and risks⁶. Children, particularly immune-compromised children such as those with HIV or under the age of 5 years, are at substantial risk of progressing from LTBI to severe forms of tuberculosis, with substantial associated mortality^{7,8}. Although isoniazid preventive therapy (IPT) can significantly reduce this risk^{2,9,10}, some concerns exist regarding potential hepatotoxicity, as isoniazid can cause idiosyncratic drug-induced liver injury (DILI)^{11,12}. Although fatal IPT-related DILI has been described¹³, the risk is considered low in children not infected with HIV¹⁴. Applying current concepts of idiosyncratic liver injury processes, children infected with HIV may however be at higher risk for severe IPT-related liver injury than their HIV-uninfected counterparts.

Idiosyncratic DILI appears to result from a succession of events, described as a “multi-hit process”, where a potentially mild to moderate DILI (“upstream” insult) may be amplified or suppressed in the presence of other factors (through “downstream” pathways)^{15,16}. Liver insults can therefore wax and wane over time as environmental

and other risk factors change¹⁵. Known risk factors for DILI are viral infections such as Hepatitis A, B or C; concurrent use of other hepatotoxic agents; and malnutrition¹⁷⁻¹⁹.

Most of the world's HIV-infected children live in sub-Saharan Africa²⁰, where almost 50% of the population live in poverty²¹. In such settings, both viral hepatitis and childhood malnutrition are common^{21, 22}. Furthermore, anti-retroviral therapy, itself potentially hepatotoxic¹⁷, is increasingly available for HIV-infected children, and may amplify any IPT-initiated DILI. It is also biologically plausible that HIV infection itself may increase the risk for DILI, given the altered cytokine milieu of chronic viral infections¹⁶. As global efforts intensify towards improving child survival and the reduction of HIV-associated tuberculosis, increasing numbers of children access both ART and IPT^{20, 23}. Understanding the IPT-associated risk for liver injury in children with HIV is crucial to enable a considered weighing of the risks and benefits of this combined approach.

Objectives:

This literature review seeks to summarize, evaluate and compare published data on the risk of liver injury among both HIV-infected and HIV-uninfected children receiving IPT, and to identify knowledge gaps and further research needs.

2.2 METHODS

i. Information sources and literature search:

Three databases (Medline, Scopus and Web of Science) were searched for articles published from the earliest date through October 2013. The search strategies were adapted to each database, but combined three main themes: "isoniazid" in combination

with terms for prophylaxis, and terms for hepatotoxicity. The MEDLINE search strategy is outlined below:

1. isoniazid
2. prophylaxis OR “preventive therapy” OR chemoprevention OR treatment OR therapy
3. hepatotoxicity OR “liver injury” OR “serious adverse events” OR “side effects” OR “liver toxicity” OR toxicity
4. 1 AND 2 AND 3

Bibliographies of selected review articles were screened^{14, 24-26}, and expert opinion sought, to identify additional publications or studies. The searches were limited to English language articles reporting on children.

ii. Eligibility criteria:

Original articles reporting longitudinal data were included; thus eligible study designs were randomized controlled trials (RCTs) or quasi-RCTs, controlled interventional trials and both prospective and retrospective cohort studies. Studies reporting only adult data were excluded; the population of interest was children (≤ 18 years of age), irrespective of HIV status. Isoniazid used as monotherapy to prevent tuberculosis was the intervention of interest; studies reporting use of isoniazid in conjunction with other anti-tuberculosis agents were excluded, as were studies where isoniazid was used for other reasons. Eligible outcomes were any report (clinical or laboratory) of number of

hepatic adverse events, provided a denominator (total number of children exposed to isoniazid) was available for risk estimation.

iii. Study selection:

A single author evaluated all retrieved articles by title and abstract, retaining for full text review those without abstracts and those potentially eligible for inclusion. The same author conducted a full-text review of the retained articles, applying the *a priori* specified eligibility criteria outlined above.

iv. Data collection and analysis:

Structured, piloted forms were used to extract data including information on population ages, sample size, isoniazid dosage and duration, type and schedule of adverse event monitoring, clinical information on liver injury, study-specific definitions of hepatotoxicity, laboratory findings and risk estimates for liver injury (appendix 1).

Populations of patients studied prior to 1990 were presumed to be HIV negative; if authors did not comment on HIV testing, a negative status was also assumed. Data on potential threats to validity were also extracted, including proportion lost to follow up, adherence and lack of allocation concealment where applicable. No meta-analysis was conducted, as study characteristics were heterogeneous.

v. Study appraisal

As recommended in the PRISMA (Preferred Reporting Items for Systematic review and Meta-analyses) guidelines²⁷, risk of bias was considered at both the study and the outcome level. The following characteristics were systematically evaluated: the study

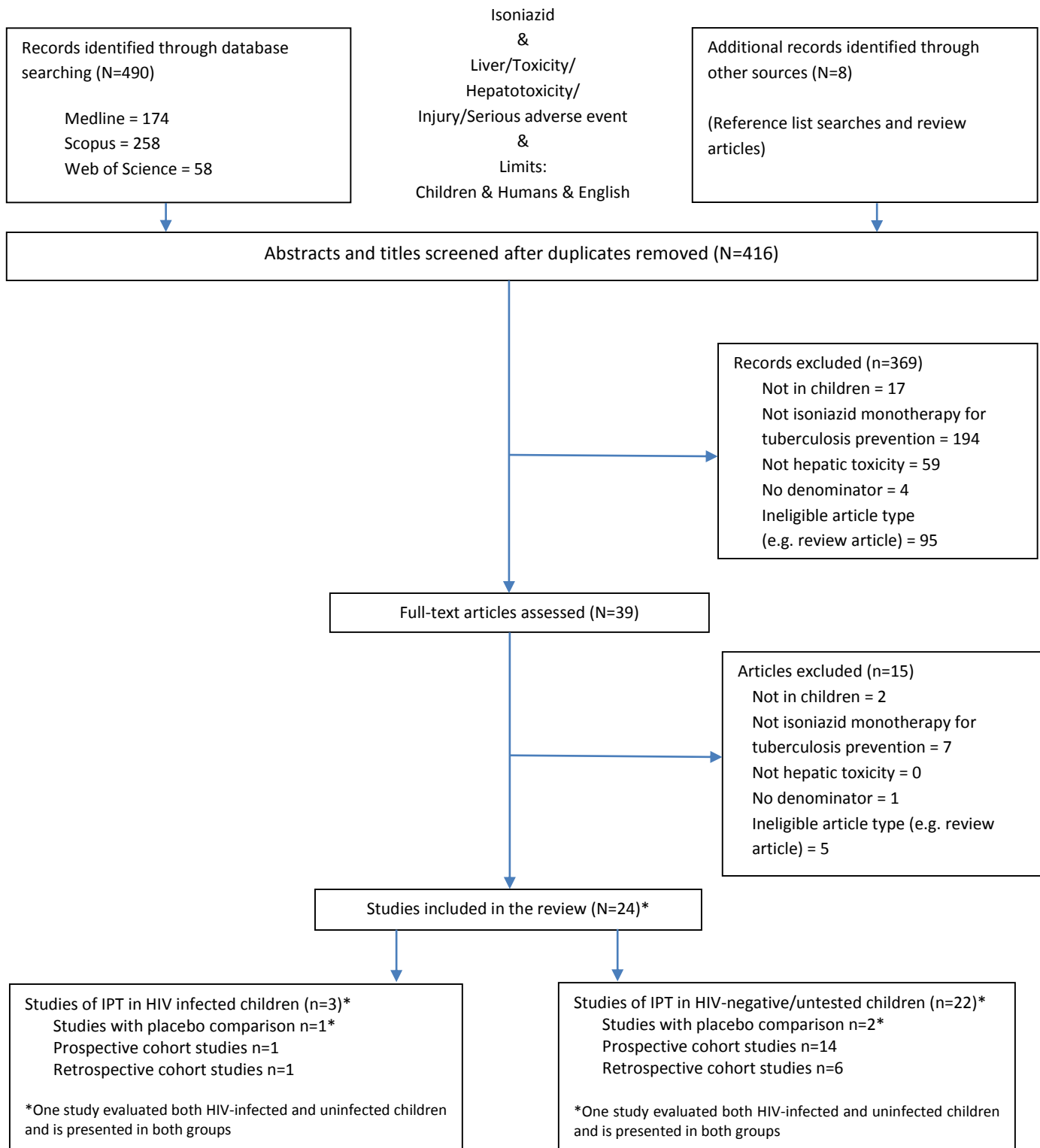
design (interventional vs. observational, prospective vs. retrospective; use of placebo; randomization and allocation concealment); whether there was an *a priori* definition of liver injury; how serious adverse events were monitored (actively or passively, by clinical parameters and/or laboratory tests); how causality was attributed (in particular, whether screening for viral hepatitis was performed); to what degree outcome ascertainment for children who were lost to follow up occurred; and whether adherence to medication was assessed and accounted for.

2.3 SUMMARY OF FINDINGS AND INTERPRETATION

i. Description of studies included

Four hundred and ninety studies were identified through database searches (Medline, Scopus and Web of Science); an additional 8 records were identified through reference list searches, discussion with an expert, and review articles (figure 1). Four hundred and sixteen titles and abstracts were screened after the removal of duplicates; of these, 369 records were excluded. Reasons for exclusion were: not providing numbers of IPT-exposed children (no denominator for risk calculation, 4 publications), not providing information on *hepatic* toxicity (59), not investigating isoniazid monotherapy for TB prevention (194), not being an eligible article type (95) or study population not including children (17). Of 39 full-text articles reviewed, 15 more articles were excluded, for the same reasons (figure 1). Twenty-four studies were included for the review, of which 21 were conducted in HIV-uninfected children, 2 in HIV-infected children and 1 in both HIV-infected and HIV-uninfected children.

FIGURE 1. Flow of information through literature review process: liver injury among children receiving isoniazid preventive therapy



The studies were variable regarding study design; population age; dosages and duration of IPT; definitions of liver injury; type and degree of monitoring; assessment of loss to follow-up and adherence; and use of viral hepatitis screening (tables 1-4). Two studies were prospective, randomized and placebo-controlled^{4, 28}; only one of these provided an *a priori* definition for liver injury and conducted standardized serious adverse event monitoring²⁸.

a. Study designs, settings and populations

Fourteen of the 22 studies in HIV-uninfected children were conducted in the United States of America (USA)^{4, 29-41}, including the 5 studies with sample size of over 1000 children. The large studies all included children younger than 5 years of age, although the proportions of very young children were usually not made explicit³⁰. Except for the randomized, placebo-controlled Public Health Service Trial⁴, the US-based studies were all observational. Smaller studies were conducted in Greece^{42, 43}, Spain⁴⁴, South Africa^{28, 45}, Iran⁴⁶, Turkey⁴⁷ and Canada⁴⁸. Of these, only one was randomized with a placebo comparison²⁸. The majority of the 20 observational studies were of prospective cohorts of children seen at tuberculosis clinics.

All three of the studies conducted among HIV-infected children were South African, with median ages of 2 years or younger^{28, 49, 50}. The study by Madhi et al²⁸ was a randomized, placebo controlled trial, whereas the other 2 studies were observational, of which one was prospective. Although the latter had originally been designed as randomized and placebo-controlled, the placebo arm was discontinued early in the study, and randomization broken; hence the majority of IPT-exposure time was accrued under observational conditions.

TABLE 1: Liver injury among children with HIV status unknown/negative receiving isoniazid preventive therapy (IPT) at 5 mg/kg

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of hepatotoxicity	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Mount & Ferebee ^{3,4} 1957 & 1961 (2 reports from one study)	Randomized, placebo controlled Multi-site Blinded & allocation concealment	4-6 mg/kg daily for 12 months	n=1394 ≥ 1 year; no upper limit given 95% ≤ 11 years	Clinical: monthly pill collection and examination	Not stated	Nausea and vomiting in 2 (0.14%); recurrence when re-challenged	Not stated	n/a	Not stated	0.14% (2/1394)	None	0.14% (2/1394)	Treatment discontinued in 2 cases No hepatic failure/death due to IPT LTFU@12m: 0.5%
Litt ³⁷ 1976	Prospective cohort, no placebo	4-6 mg/kg daily for 46 weeks	n=178 12-16 years	Laboratory: Pre-treatment and weekly liver function tests including ALT & AST	Not stated	No symptomatic cases	Unclear; treatment discontinued in one adolescent with AST > 1200 U/L	14/155 (9%) patients had increased AST overall	Most within first 10 weeks	8.4% (13/155)	0.6% (1/155)	9% (14/155)	Treatment discontinued in 1 case No hepatic failure/death due to IPT % LTFU unclear
Spyridis ⁴³ 1979	Prospective cohort, no placebo	300 mg ± 5 mg/kg daily for at least 3 months	n=239 9-14 years	Laboratory: Pre-treatment and monthly ALT and AST	Pre-treatment HBsAg; At liver injury: HBsAg repeated, EBV All negative	No symptomatic cases	Unclear; treatment discontinued for ALT/AST >100 U/L	AST>40 U/L in 36/239 (15%) ALT>30 U/L in 23/239 (9.6%)	Most within first 8 weeks	16.3% (39/239)	0.8% (2/239)	17.1% (41/239)	Treatment discontinued in 2 cases No death/hepatic failure due to IPT % LTFU unclear
Tortajada ⁴⁴ 2005	Prospective, randomized to INH or Rif/PZA No placebo, no allocation concealment	5 mg/kg daily for 6 months	n=35 1-19 years	Laboratory: Liver enzyme tests at 2, 4, 6 and 8 weeks	Not routinely done	No symptomatic cases	AST or ALT ≥ 5 times upper limit of normal	No comment on low grade rise in transaminases	No liver injury detected	None	None	None	No treatment interruptions No death/hepatic failure due to IPT % LTFU of children unclear
Marais ⁴⁵ 2006	Prospective cohort, clinic-based No placebo	5 mg/kg daily for 6 months	180 <5 years	Clinical: monthly pill collection	Not stated	No symptomatic cases	Not stated	None	No liver injury detected	None	None	None	No treatment interruptions No death/hepatic failure due to IPT LTFU: 79% lost within 4 months
Aminzadeh ⁶ 2010	Retrospective cohort No placebo	5 mg/kg daily for 3-6 months	15 <6 years	Clinical: seen 3 monthly	Not stated	No symptomatic cases	Not stated	Not tested	No liver injury detected	None	None	None	No treatment interruptions No death/hepatic failure due to IPT % LTFU unclear

Abbreviations: HIV, Human immunodeficiency virus; INH, isoniazid; n/a, not applicable; IPT, isoniazid preventive therapy; LTFU, loss to follow-up; mg/kg, milligram per kilogram; ALT, alanine transaminase; AST, aspartate aminotransferase; HBsAG, Hepatitis B surface antigen; EBV, Epstein-Barr virus; Rif, Rifampicin; PZA, pyrazinamide

TABLE 2: Liver injury among children with HIV status unknown/negative receiving isoniazid preventive therapy (IPT) at 10 mg/kg

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of Liver injury	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Beaudry³³ 1974	Prospective cohort No placebo Incomplete data for 65/434 (15%) patients;	10 mg/kg daily for 3-12 months	369 1-18 years 7 had raised AST pre-treatment	Laboratory: AST done pre-treatment and after 8 weeks; clinical visits at 2, 4, 8 and 12 months	HBSAg and EBV tested in "most" children with elevated AST; none tested positive	No symptomatic cases	AST > 95 U (2x ULN)	AST > 50 U in 25/369 (6.8%) AST > 95 U in 4/369 (1.1%)	Within first 2 months	21/369 (5.7%)	4/369 (1.1%)	25/369 (6.8%)	Treatment interrupted in 4 children; 3 restarted uneventfully. No death/hepatic failure due to IPT 27/434 (6%) non-compliant or LTFU
Nakajo³⁸ 1989	Retrospective cohort No placebo	10 mg/kg daily for 12 months	564 3 months - 18 years; mean of 8 years	Clinical: seen monthly; liver function tests done if symptomatic	Tested in child with raised trans-aminases: HAV, HBV & EBV negative	Nausea, vomiting, anorexia, malaise, and abdominal tenderness (all in 1 case)	Symptoms (specified) and ALT and/or AST > 100 U ± increased TSB	One child: AST 730 ALT 1444 Normal TSB; normalized within 4 weeks	6 weeks	39/564 (6.9%) symptoms compatible with liver injury but AST/ALT remained <100	1/564 (0.18%)	40/564 (7.1%)	Treatment interruption in 1 adolescent; failed re-challenge No death/hepatic failure due to IPT LTFU in 5%
Palusci⁴⁰ 1995	Retrospective cohort No placebo	10 mg/kg daily for 9 months	318 3 months to 18 years	Clinical: seen monthly; liver function tests done if symptomatic	For one patient (with hepatic failure), all negative: HAV, HBV, HCV; CMV & EBV	Abdominal pain (6); nausea/vomiting (2); weight loss (1); ↓appetite(1); jaundice (1)	Not stated	A child had hepatitis symptoms with AST 328 U/l and ALT 313 U/l	4 months	10/318 (3.1%) symptoms of hepatitis But AST/ALT < 50 U/l	1/318 (0.3%)	11/318 (3.4%)	Treatment interruption in 1 adolescent with hepatic failure No deaths due to IPT. % LTFU not stated
Spyridis⁴² 2007	Prospective cohort (9 months of INH vs 3-4 months of INH with Rif Quasi-randomized, no allocation concealment No placebo	10 mg/kg daily for 9 months	Total 232; < 15 years, mean age 9 years, 15% <6 years Only 200 considered "compliant"	Clinical: follow-up schedule not stated	Not stated	Nausea and epigastric pain in 13/200 (6.5%)	Symptoms with liver enzymes >3 times ULN	12/200 (6%) had increased liver enzymes, but all ≤ 3 times ULN	Not stated	13/200 (6.5%)	None	13/200 (6.5%)	No treatment interruptions No death/hepatic failure due to IPT Poor to moderate compliance in 34.5% % LTFU not stated

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of Liver injury	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Wu ³² 2007	National retrospective cohort using data from transplant databases, physician surveys, literature searches and the CDC	10 mg/kg daily for various durations	216 776 0-14 years	Not possible to determine; children with liver failure had had clinical monitoring only	Tested in all children with liver failure: HBV + (1) HAV + (1); CMV + (1); No child tested positive for HCV or EBV	15 children on IPT presented with fulminant hepatic failure	Hepatic failure	Not stated	Range: 6 weeks to 9 months	Cannot be estimated from the data used	3.2 per 100 000 child years	Cannot be estimated from the data used	15 cases of acute hepatic failure on IPT: 3 (20%) survived without OLT 7 (47%) survived with OLT 5 (33%) died despite OLT
Devrim ⁴⁷ 2010	Retrospective cohort No placebo	Not stated†	617 ages not stated	Not stated but most likely laboratory, based on results	Not stated	Not stated	WHO toxicity grading used; test details not stated	Grade I-II: 6 (0.9%) Grade III: 2 (0.33%); Grade IV: 9 (1.5%)	Not stated	6/617 (0.9%)	11/617 (1.78%)	17/617 (2.8%)	Not stated

Abbreviations: HIV, Human immunodeficiency virus; INH, isoniazid; n/a, not applicable; IPT, isoniazid preventive therapy; LTFU, loss to follow-up; mg/kg, milligram per kilogram; ALT, alanine transaminase; AST, aspartate aminotransferase; HBsAG, Hepatitis B surface antigen; EBV, Epstein-Barr virus; HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; CMV, cytomegalovirus; ULN, upper limit of normal; TSB, total serum bilirubin; Rif, rifampicin; CDC, Centers for Disease Control & Prevention; WHO, World Health Organization; OLT, orthoptic liver transplantation

† These children were most probably managed according to WHO treatment guidelines of 2006⁵¹ (10mg/kg daily for 6 months)⁵²

TABLE 3: Liver injury among children with HIV status unknown/negative receiving isoniazid preventive therapy (IPT) at 10-20 mg/kg daily or 20-30 mg/kg twice weekly

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of hepato-toxicity	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Hsu ³⁶ 1974	Prospective cohort No placebo	10-20 mg/kg daily for 12 months	1487 0.5 -15 years; 50% < 8 years	Clinical; seen 3 monthly "Watchful vigilance"	Not stated	Rash, vomiting and/or diarrhea	Not stated	Not stated	Not stated	4/1487 (0.27%)	None	4/1487 (0.27%)	No treatment interruptions No deaths/hepatic failure due to IPT % LTFU not stated; only those who completed therapy were included
Kopanoff³⁰ 1978	Prospective multi-site surveillance cohort No placebo	Not stated ¹	2473 <20 years	Clinical; seen monthly	Not routinely done	Not stated	Probable/ Possible cases: variable cut-off for raised ALT depending on concomitant liver disease	Not stated	Not provided separately for children	1 per 1000 persons	None	1 per 1000 persons	No information on treatment interruption in children No deaths/hepatic failure in children due to IPT % LTFU not stated; Non-compliant patients screened for signs/ symptoms of hepatitis
Rapp ⁴¹ 1978	Prospective cohort study No placebo	10-15 mg/kg daily for 12 months	116 <21 years; 14/116 <3 years of age	Clinical and laboratory: ALT and TSB done at completion of treatment ± pre-treatment ± at 6-20 weeks	Not stated	No symptomatic cases	Not stated	Increased ALT > ULN for age in 5/118 (4.3%) tests done during treatment	Not stated	5/118 (4.3%)	None	5/118 (4.3%)	No information regarding treatment interruption No deaths/hepatic failure due to IPT LTFU: 14 patients not included in study due to non-compliance (14/130, 10.8%)

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of hepato-toxicity	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Byrd ³⁴ 1979	Prospective cohort No placebo	Not stated ¹	19 <19 years	Clinical and laboratory at monthly intervals	Not stated	Not stated	AST > 5X ULN (defined as 100 IU)	No child had AST > 5X ULN	No cases among children	Unclear	None	Unclear	Six treatment interruptions unrelated to liver function tests; reasons unclear No deaths/hepatic failure due to IPT % LTFU not stated
Dash ³⁵ 1980	Prospective cohort No placebo	Not stated ¹	636 <15 years	Clinical: monthly visits; liver function tests done when indicated clinically	Not stated	Rash and malaise "most frequently reported"	Mild, moderate and severe not differentiated; Cases were either "probable" or "possible"	Not provided	Not stated for children	4 possible cases of hepatitis: 4/636 (0.6%)	No probable cases of hepatitis in children	4/636 (0.6%)	31 treatment interruptions (5%) No deaths/hepatic failure due to IPT LTFU % not stated but "dropout rate was... the least for those under 15..."
Nolan ³⁹ 1999	Prospective cohort No placebo	Not stated ¹	1468 <15 years	Clinical: monthly structured questionnaire	For patients with symptoms: minimally, HAV, HBV and HCV tested (all negative)	No symptomatic cases among those < 15 years old	Symptoms (specified); AST ≥ 5 times ULN ± raised TSB; and, clinical resolution after withdrawal of IPT	No cases among children	No cases among children	No cases among children	No cases among children	No cases among children	No treatment interruptions No death/hepatic failure due to IPT Overall LTFU: 35%; not available for paediatric subgroup. Hospitals' discharge databases reviewed for data on outcomes
LoBue ³¹ 2003	Prospective cohort No placebo	Not stated ¹	1277 <14 years	Clinical: monthly structured questionnaire Transaminases measured in high-risk patients	Not stated	No symptomatic cases among children	Any signs/symptoms previously associated with INH, onset after IPT initiation, with no alternative cause found AND increased liver enzymes: AST/ALT > 5x ULN if asymptomatic OR AST/ALT > 3x ULN if symptomatic	No cases among children	No cases among children	No cases among children	No cases among children	No cases among children	No treatment interruptions in children due to hepatotoxicity No deaths/hepatic failure due to IPT 26% of children did not complete the prescribed IPT course; no reasons stated

Authors	Study design and attributes	INH dosage and duration	Patient numbers and ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of hepato-toxicity	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Minodier ⁴⁸ 2010	Prospective cohort No placebo	10-15 mg/kg daily for 6-9 months	545 Mostly 10-12 years old	Clinical: seen at 1, 3, 6 and 9 months	Not stated	Abdominal pain (4.7%), weight loss (2.8%), vomiting (1.9%)	Not stated	Not stated	Not stated	Not stated	None	Not stated	Treatment interruptions: none recorded 38.7% non-adherence; reasons not stated
Madhi ²⁸ 2011	Randomized controlled trial with placebo; allocation concealment double-blinded	10-20 mg/kg daily for 2 years	403 HIV-negative children in IPT group; Median (IQR) age 96 (91-120) days	Clinical and laboratory at three-monthly study visits including AST and ALT	Not stated	Not stated	Grade III/IV increase in liver enzymes (> 5x ULN)	Not stated	Not stated	Not stated	ALT: 3/403 (0.7%) AST: 8/403 (2%)	ALT: 3/403 (0.7%) (Placebo group, 8/401 (2%))	No treatment interruptions for hepatotoxicity No death/hepatic failure due to IPT LTFU: 56/403 (13.9%); placebo group had 15% LTFU
Cruz ²⁹ 2013	Retrospective cohort No placebo comparison	20-30 mg/kg bi-weekly for 3-9 months	1337; 0-18 years	Clinical; visit schedule not stated. Transaminase levels done for "abdominal complaints"	Not stated	Abdominal pain and/or vomiting in 30 cases (2.2%)	Transaminases > 3 times the ULN	Transaminases done in 27 children; 3/27 had increases, ranges: AST 415-788 ALT 408 - 884	Mean time to onset of symptoms: 1.6 months	Not stated	3/1337 (0.22%)	Not stated	Treatment interruptions: 22/1337 (1.6%) No death/hepatic failure due to IPT 19/1337 (1.42%) did not complete treatment

¹ Children in these studies were most likely managed according to recommendations of the Medical Section of the American National Tuberculosis and Respiratory Disease Association⁵³, and would have received an INH dosage of 10-20 mg/kg or 10-15 mg/kg, for 9-12 months, as recommended in later documents of the American Thoracic Society and Centers for Disease Control and Prevention^{14, 54}

Abbreviations: HIV, human immunodeficiency virus; INH, isoniazid; n/a, not applicable; IPT, isoniazid preventive therapy; LTFU, loss to follow-up; mg/kg, milligram per kilogram; ALT, alanine transaminase; AST, aspartate aminotransferase; HBsAG, Hepatitis B surface antigen; EBV, Epstein-Barr virus; HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; CMV, cytomegalovirus; ULN, upper limit of normal; TSB, total serum bilirubin; IQR, inter-quartile range

TABLE 4: Liver injury among HIV-infected children receiving isoniazid preventive therapy (IPT) at 10mg/kg daily:

Authors	Study design and attributes	INH dosage and duration	Patient numbers, ages	Monitoring	Viral hepatitis testing	Clinical indicators of liver injury	Study definition of hepatotoxicity	Summary of laboratory findings	Timing of liver injury detection	Risk of mild-moderate liver injury	Risk of severe liver injury	Total risk of liver injury	Outcomes
Gray ⁵⁰ 2010	Retrospective cohort No placebo	10mg/kg daily for up to 24 months	112 children median (IQR) age 22 (10-53) months	Laboratory: ALT levels at 1, 3, 6 months and 6 months thereafter	Tested in those with raised ALT: HAV+ (1) Details of other tests not stated	No symptomatic cases	Pediatric division of AIDS Grade III/IV raised ALT (>5 times ULN)	Peak ALT level range (346-1467 U/l)	Within first 12 months	Not stated	Grade III/IV elevation in 8/112 (7.1%)	Not stated	Treatment interruptions in 4 children; all re-challenged successfully No death/hepatic failure due to IPT % LTFU not stated
Madhi ²⁸ 2011	Randomized controlled trial with placebo; allocation concealment, double-blinded Included HIV+ and HIV- children	10-20 mg/kg daily for 2 years	273 HIV-infected children in IPT group; Median (IQR) age 97 (91-120) days	Clinical and laboratory at three-monthly study visits including AST and ALT	Not stated	Not stated	Grade III/IV increase in liver enzymes (>5x ULN)	Not stated	Not stated	Not stated	ALT: 1/273 (0.4%) AST: 11/273 (4%)	ALT: 1/273 (0.4%) (Placebo group, 5/274 (1.8%))	No treatment interruptions for hepatotoxicity No death/hepatic failure due to IPT LTFU: 34/273 (12.4%); placebo group had 7.6% LTFU
Le Roux ⁴⁹ 2013	Prospective cohort Limited initial placebo	10 mg/kg daily for up to 5 years	297 children, Median (IQR) age 23 (9.5-48.6) months	Laboratory: ALT levels six-monthly; Clinical: three-monthly visits	Tested in most (12/19) children with severe liver injury: HAV+ (5) CMV+ (2) EBV+ (1) No child tested positive for HBV or HCV	6/16 cases were asymptomatic Vomiting, diarrhea, anorexia, malaise, fever, cough 2/16 cases were jaundiced	ALT >10x ULN	Peak ALT range: 293-1761 U/l	Range: 4 weeks to 4 years; median onset 6 months	Not stated	All-cause, while on IPT: 16/297 (5.4%) IPT-related: 5/297 (1.7%)	All-cause, while on IPT: 16/297 (5.4%) IPT-related: 5/297 (1.7%)	Treatment interruptions in 11/297 (3.7%); 10 successfully re-challenged No death/hepatic failure due to IPT % LTFU on IPT not stated; overall LTFU 28/324 (8.6%)

Abbreviations: HIV, human immunodeficiency virus; INH, isoniazid; ART, antiretroviral therapy; IQR, inter-quartile range; U/l, units per liter; IPT, isoniazid preventive therapy; LTFU, loss to follow-up; mg/kg, milligram per kilogram; ALT, alanine transaminase; AST, aspartate aminotransferase; HBsAG, Hepatitis B surface antigen; EBV, Epstein-Barr virus; HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; CMV, cytomegalovirus; ULN, upper limit of normal; TSB, total serum bilirubin

b. Intervention & comparison

Isoniazid was administered at a dose of 5 mg/kg/day in 6 studies^{4, 37, 43-46} (Table 1), 10 mg/kg/day in 6 studies^{32, 33, 38, 40, 42, 47} (table 2) and 10-20 mg/kg/day in 10 studies^{28, 30, 31, 34-36, 41, 48, 55} of IPT in HIV-uninfected children (table 3). One retrospective cohort study from the US reported outcomes among HIV-uninfected children receiving 20-30 mg/kg twice weekly²⁹ (table 3). All studies including HIV-infected children provided IPT at a dose of 10 mg/kg/day^{28, 49, 50} (table 4). Duration of IPT varied according to the local tuberculosis prevention protocols at the time of the study: the most commonly reported duration was 12 months of IPT (10 studies)^{4, 30, 31, 33-36, 38, 39, 41}, followed by 9 months (5 studies)^{29, 37, 40, 42, 48}, 3-6 months (depending on indication for IPT, 4 studies)⁴⁴⁻⁴⁷ and one study observed children for the first 3 months of an unspecified duration of therapy. All three South African studies conducted among HIV-infected children reported on prolonged IPT use, with between 2 and 5 years of IPT exposure^{28, 49, 50}.

ii. Evaluation of outcomes: Incidence of liver injury

a. Definition of liver injury

The definition of “clinically significant” liver injury has been debated and redefined several times since the 1970s^{6, 52, 56-59}. Early efficacy studies of IPT in HIV-uninfected children reported an extremely low incidence of hepatotoxicity, based on clinical assessments⁴. After wide-spread introduction of IPT in the USA, case reports surfaced demonstrating cases of severe hepatitis and even death from hepatic failure among both adults and children receiving IPT^{60, 61}. A large and intensive national isoniazid

surveillance study (using structured, monthly clinical monitoring) followed in 1972, as published by Kopanoff et al in 1974³⁰. The same period saw an increased use of serial liver enzyme testing to monitor for IPT-related liver injury^{33, 34, 37, 41, 43, 59}. Several studies in both adults and children reported what became known as “transient transaminase elevation” (TTE), where liver enzymes increase then normalize without any clinical symptoms or intervention^{33, 37, 41, 43, 62}. Studies that defined liver injury only on the basis of an increase in serum liver enzymes necessarily reported higher overall rates of liver injury than those studies in which liver injury was diagnosed on only clinical outcomes. However, as reported by both Palusci⁴⁰ and Wu³², non-specified or unstructured clinical monitoring alone may miss subtle early signs of liver dysfunction, to the detriment of the patient.

Taking into account the likely under-diagnosis of liver injury when using non-structured clinical monitoring (using a passive surveillance system, awaiting spontaneous report of symptoms by parents) and the likely over-diagnosis of liver injury when using repeated laboratory measures in asymptomatic individuals, international guidelines^{51, 54, 58} now recommend structured clinical monitoring (specifically inquiring about symptoms at regular clinical contact, preferably using a structured questionnaire), with laboratory testing only in children who are at high risk or have symptoms suggestive of hepatitis. In these cases liver injury is defined as a symptomatic case with significantly increased liver enzyme tests; a “significant” increase cut off point is commonly set at 3, 5 or 10-fold the upper limit of normal (ULN), using either ALT and/or AST, whilst also testing serum bilirubin^{51, 54, 58}.

Hepatic failure and death are however undisputed indicators of severe liver injury. Only two of the reviewed publications reported death or hepatic failure; both cohorts of children had been monitoring clinically only, in an unspecified manner^{32, 40} (table 2). One of 318 (0.3%) patients developed hepatic failure in the retrospective cohort reported by Palusci et al; the child survived following liver transplantation⁴⁰. Wu et al calculated the overall risk of IPT-induced liver failure by extracting data from national transplant databases, and using the CDC registry of childhood IPT to provide a denominator; the estimated risk for IPT-related hepatic failure was 3.2 per 100 000 child-years³². There were 5 deaths among the 15 children identified with liver failure, despite receiving liver transplants (case fatality ratio, 33%). Despite the outcome assessment bias inherent to this calculation, it is notable that all the reports of liver failure and death found in this review were among children who received only non-specified clinical monitoring³².

b. Monitoring of liver injury

Three broad approaches to toxicity monitoring were identified in the reviewed studies (table 5). Two approaches involved primarily clinical monitoring without routine liver enzyme testing. In the first approach, identification of clinical hepatitis relied on spontaneous parental report of symptoms, without closely spaced clinical visits and/or use of structured questionnaires asking about specific symptoms (“unstructured” clinical monitoring)^{4, 32, 36, 45, 46, 48}. The second broad approach was to maintain regular patient contact with structured, specific toxicity screening questionnaires; additionally, liver enzyme tests were conducted in all children with symptoms suggestive of hepatitis

(“structured” clinical monitoring, with targeted liver enzyme testing)^{29-31, 35, 38-40, 42}.

Routine laboratory testing comprised a third approach to monitoring, and was utilized in 10 studies, including all three studies conducted among HIV-infected children^{28, 33, 34, 37, 41, 43, 44, 47, 49, 63}. Liver enzyme tests were conducted at pre-specified intervals during IPT exposure, irrespective of symptoms. Generally, higher proportions of children with severe liver injury were identified when adverse event monitoring involved either a structured clinical approach and/or regular laboratory liver enzyme testing.

TABLE 5. Liver injury risk estimates for 24 studies by dosage and type of toxicity monitoring:

ISONIAZID DOSE	TYPE OF MONITORING USED PER STUDY					
	Unspecified or unstructured clinical monitoring		Specific clinical monitoring with active symptom screening		Routine laboratory monitoring	
	Severe liver injury	Mild-moderate liver injury	Severe liver injury	Mild-moderate liver injury	Severe liver injury	Mild-moderate liver injury
5 mg/kg/day	None ⁴ None ⁴⁵ None ⁴⁶	0.14% ⁴ None ⁴⁵ None ⁴⁶	-	-	0.6% ³⁷ 0.8% ⁴³ None ⁴⁴	8.4% ³⁷ 16.3% ⁴³ None ⁴⁴
10 mg/kg/day	3.2/100 000 PY ³²	Not stated ³²	0.18% ³⁸ 0.3% ⁴⁰ None ⁴²	6.9% ³⁸ 3.1% ⁴⁰ 6.5% ⁴²	1.1% ³³ 1.78% ⁴⁷ 7.1% ⁶³ 1.7% ⁴⁹	5.7% ³³ 0.9% ⁴⁷ Not stated ⁶³ Not stated ⁴⁹
10-20 mg/kg/day	None ³⁶ None ⁴⁸	0.27% ³⁶ Not stated ⁴⁸	None ³⁵ None ³⁹ None ³¹ None ³⁰	0.6% ³⁵ None ³⁹ None ³¹ 1 per 1000 PY ³⁰	None ⁴¹ None ³⁴ 0.7% ^{28,†} 0.4% ^{28,‡}	4.3% ⁴¹ Not stated ³⁴ Not stated ^{28,†} Not stated ^{28,‡}
20-30 mg/kg twice weekly	-	-	0.22% ²⁹	Not stated ²⁹	-	-
Total	One of 6 (16%) studies describe severe liver injury (approximate proportion, 0.003%)		Three of 8 (37.5%) studies describe severe liver injury, range of risk proportion estimates (0.18%-0.3%)		Eight of 10 (80%) studies describe severe liver injury, range of risk proportion estimates (0.4% - 7.1%)	

Numbers are expressed as proportion of children exposed to isoniazid preventive therapy, or as incidence rate per person year of exposure, as provided by authors

† HIV-uninfected group, receiving isoniazid preventive therapy; ‡ HIV-infected group, receiving isoniazid preventive therapy. The two groups were enrolled in the same study, reference 28. Abbreviations: mg/kg, milligram per kilogram; PY, person-years

c. Dosage and duration of isoniazid

Among children monitored in an approximately similar manner, those receiving the lowest dose (5 mg/kg/day) appeared to have the lowest risk of severe liver injury^{4, 37, 43-46} (table 1, table 5). Nonetheless, the relatively higher risks seen in cohorts receiving higher doses of IPT were still low in absolute terms (table 2, table 3). Among HIV-uninfected children, all durations of IPT (3-6 months, 9 months and 12 months) were associated with low and similar estimates of risk, and in each category of IPT duration there were at least two studies reporting no cases of severe liver injury (table 5). The HIV-infected cohorts all received isoniazid therapy for longer than 12 months^{28, 49, 63}. In each of these studies some cases of severe hepatotoxicity occurred, but without hepatic failure or mortality (table 4); in twelve of the fourteen cases defined as IPT-related hepatotoxicity through Grade III/IV elevations in ALT, IPT was successfully restarted without further incidence of hepatotoxicity despite close monitoring^{49, 63}. These studies were also similar in that all utilized repeated measures of transaminases, despite many of the children being asymptomatic. In the absence of randomized comparisons of longer vs shorter durations of IPT, it is not possible to reasonably assess the potentially increased risk associated with longer isoniazid exposure time. However, in keeping with international causality assessment guidelines⁶⁴⁻⁶⁶, liver dysfunction is most likely to be drug-induced if it occurs soon after the introduction of the drug. Thus it is possible that most cases of DILI will appear in the first few months of IPT, with only some sporadic cases in the later months, possibly due to some changes in environmental (“downstream”) factors¹⁶. As evidence of this, the majority of cases described in the

reviewed studies occurred within the first 4 months of IPT^{29, 32, 33, 37, 38, 40, 41, 43, 49, 50} (tables 1-4).

d. Comparison of liver injury risk in HIV-infected vs. HIV uninfected children

Estimates of severe liver injury risk varied between the three studies conducted in HIV-infected children. The retrospective cohort reported by Gray et al described the highest overall estimate of severe liver injury (7%), but this included several cases where liver dysfunction was caused by infectious agents⁵⁰. While 1.7% of children developed severe liver injury reasonably related to IPT in the cohort study reported by le Roux et al, not all children were screened for viral hepatitis, and all but one child subsequently tolerated IPT; thus the true incidence was likely lower⁴⁹. The RCT conducted by Madhi et al found no difference in incidence of severe liver injury comparing HIV-infected children receiving IPT to those receiving placebo, with an overall 0.4% estimate of severe liver injury based on serial measurements of ALT²⁸. Ninety-eight percent of these HIV-infected children were also receiving ART. This study further compared risk of severe liver injury between HIV-infected and HIV-uninfected children receiving IPT (0.4% and 0.7% respectively), and found no significant difference between these groups either²⁸. Thus although there are limited study numbers, with relatively small sample sizes, in general HIV does not appear to confer a higher risk of IPT-related DILI, even in the presence of ART.

iii. Potential threats to validity

Apart from one study set in a juvenile detention setting³⁷, *selection bias* is likely to have occurred in the observational studies (21 of 24), where participants who were choosing to attend clinical care, were mostly adherent, and not lost to follow-up, contributed the largest proportion of exposure time. Apart from the studies by Nolan³⁹ and Cruz²⁹, proportions of and reasons for loss to follow-up were either not stated or not evaluated. It is possible that those children with higher incidence of hepatitis may have been more likely to stop attending care. Similarly, poor adherence is often the result of unpleasant side effects; in these instances selection bias due to informative censoring would have resulted in an underestimation of the true risk of liver injury. Although it can be argued that the children who were more adherent had longer IPT-exposure time and therefore should represent those at highest risk, thereby implying that the risk estimates could have been overestimations, this is less likely given the increased likelihood of DILI in the early weeks of therapy, when most children were still in follow-up^{15, 66}.

Information bias due to misclassification of children who did/did not experience liver injury is highly likely in the studies where monitoring was inadequate (under-estimation of risk among children monitored clinically in an unspecified way, or conversely, overestimation among asymptomatic children with mild liver enzyme elevations). Similarly, as liver injury may wax and wane, studies employing infrequent monitoring are also likely to underestimate liver injury, incorrectly classifying children who had experienced liver injury in the interim period as not having had a liver injury.

Attribution of causality is notoriously difficult in DILI^{15, 66}. In the absence of placebo comparison, attributing liver dysfunction to isoniazid exposure is complicated by other (often undiagnosed) causes of hepatitis. Of particular interest in these studies was the high incidence of viral hepatitis in vulnerable children. In studies where viral causes were not excluded, misclassification bias due to inappropriate attribution of causality would falsely inflate the true risk of IPT-induced hepatitis.

iv. Limitations of the review

The use of only published literature, in only English, limits the quality of the literature search. As with any review limited to published studies, publication bias is a concern. Early trials of IPT were focused on addressing the urgent public health need to reduce incident tuberculosis. Hepatotoxicity was considered a minor concern, and data regarding safety and tolerability may not have been published readily given the optimism regarding IPT. However, as the risk has appeared consistently low over several decades, it is unlikely that a wider search would have yielded results to drastically change the conclusions. Case reports are usually included in review of interventional harms; however, the main aim of this review was to summarize estimated risks, for which case reports are not suitable, given the lack of denominator. Literature reviews of harm are also prone to selective reporting bias; it is possible that other trials of IPT chose not to report on liver injuries. A gray literature search would have allowed better estimation of selective reporting. This review does however include the most prominent national surveillance estimates, where harm was specifically looked

for; selective underreporting of other, smaller and less well-conducted trials would be unlikely to alter the conclusions of this review. Given the heterogeneity of monitoring and liver injury definitions used, outcome assessment bias is highly likely, especially in the retrospective studies. However, the monitoring and to a lesser degree the definitions of liver injury were aligned with national and international guidelines of tuberculosis prevention at the time of the research. The lack of placebo-controlled studies, and heterogeneity of methods precluded meaningful meta-analysis. However, much toxicity literature depends on post-marketing reports, and given the efficacy of IPT in children, placebo controls would have been unethical in most of the research settings.

2.4 CONCLUSIONS AND IMPLICATIONS

Although great heterogeneity exists between studies, a large amount of data is available on IPT-related DILI in HIV-negative children, for a variety of dosages and schedules. Overall, severe liver injury occurs infrequently, especially in the very young children who are at highest risk for TB disease progression and death.

Data on IPT-related liver injury in children with HIV is however limited. Three papers taken together indicate a low incidence, with no deaths or hepatic failure. However, these three publications report comparatively small numbers of children. Two of the three studies were clinical trials; there are no data from routine operational settings where monitoring for adverse events is less intense than in clinical trials. There is suggestive evidence that IPT in HIV-infected children, even in combination with ART, is

not associated with substantially higher risk of DILI compared to HIV-uninfected children; however risk-benefit evaluation of long-term IPT in children with advanced HIV disease who commence ART is not yet possible. There is a need for larger, prospective evaluations of the impact and risks of IPT in addition to ART, given under routine clinical conditions.

2.5 REFERENCES

1. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* 1970; **26**: 28-106.
2. Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. *The American review of respiratory disease* 1967; **95**(6): 935-43.
3. Ferebee S, Mount FW, Anastasiades A. Prophylactic effects of isoniazid on primary tuberculosis in children; a preliminary report. *American review of tuberculosis* 1957; **76**(6): 942-63.
4. Mount FW, Ferebee SH. Preventive effects of isoniazid in the treatment of primary tuberculosis in children. *The New England journal of medicine* 1961; **265**: 713-21.
5. WHO. Treatment of Tuberculosis guidelines. 4th ed. Geneva, Switzerland: World Health Organization; 2012.
6. Comstock GW, Edwards PQ. The competing risks of tuberculosis and hepatitis for adult tuberculin reactors. *The American review of respiratory disease* 1975; **111**(5): 573-7.
7. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2004; **8**(4): 392-402.
8. Walters E, Cotton MF, Rabie H, Schaaf HS, Walters LO, Marais BJ. Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on anti-retroviral therapy. *BMC pediatrics* 2008; **8**: 1.

9. Zar HJ, Cotton MF, Strauss S, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ (Clinical research ed)* 2007; **334**(7585): 136.
10. Frigati LJ, Kranzer K, Cotton MF, Schaaf HS, Lombard CJ, Zar HJ. The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011; **66**(6): 496-501.
11. From the Centers for Disease Control and Prevention. Severe isoniazid-associated hepatitis--New York, 1991-1993. *JAMA : the journal of the American Medical Association* 1993; **270**(7): 809.
12. Molleston JP, Fontana RJ, Lopez MJ, Kleiner DE, Gu J, Chalasani N. Characteristics of idiosyncratic drug-induced liver injury in children: results from the DILIN prospective study. *Journal of pediatric gastroenterology and nutrition* 2011; **53**(2): 182-9.
13. Gal AA, Klatt EC. Fatal isoniazid hepatitis in a child. *Pediatric infectious disease* 1986; **5**(4): 490-1.
14. Donald PR. Antituberculosis drug-induced hepatotoxicity in children. *Pediatr Rep* 2011; **3**(2): e16.
15. Lee WM. Drug-induced hepatotoxicity. *The New England journal of medicine* 2003; **349**(5): 474-85.
16. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 2005; **4**(6): 489-99.
17. Chalasani N, Bjornsson E. Risk factors for idiosyncratic drug-induced liver injury. *Gastroenterology* 2010; **138**(7): 2246-59.
18. Russmann S, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. *Current medicinal chemistry* 2009; **16**(23): 3041-53.
19. Mehta S. Malnutrition and drugs: clinical implications. *Developmental pharmacology and therapeutics* 1990; **15**(3-4): 159-65.
20. UNAIDS. Global report: UNAIDS Report on the global AIDS epidemic 2012.
21. World Development Indicators. 2010. <http://data.worldbank.org/topic/poverty> (accessed 13 February 2014).

22. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 2010; **28**(41): 6653-7.
23. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource constrained settings. 2011. http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf (accessed 1 June 2011).
24. Frydenberg AR, Graham SM. Toxicity of first-line drugs for treatment of tuberculosis in children: review. *Tropical medicine & international health : TM & IH* 2009; **14**(11): 1329-37.
25. Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *Journal of gastroenterology and hepatology* 2008; **23**(2): 192-202.
26. Yew WW, Leung CC. Antituberculosis drugs and hepatotoxicity. *Respirology (Carlton, Vic)* 2006; **11**(6): 699-707.
27. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Annals of internal medicine* 2009; **151**(4): W65-94.
28. Madhi SA, Nachman S, Violari A, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *The New England journal of medicine* 2011; **365**(1): 21-31.
29. Cruz AT, Starke JR. Twice-weekly therapy for children with tuberculosis infection or exposure. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2013; **17**(2): 169-74.
30. Kopanoff DE, Snider DE, Jr., Caras GJ. Isoniazid-related hepatitis: a U.S. Public Health Service cooperative surveillance study. *The American review of respiratory disease* 1978; **117**(6): 991-1001.
31. LoBue PA, Moser KS. Use of isoniazid for latent tuberculosis infection in a public health clinic. *American journal of respiratory and critical care medicine* 2003; **168**(4): 443-7.
32. Wu SS, Chao CS, Vargas JH, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. *Transplantation* 2007; **84**(2): 173-9.
33. Beaudry PH, Brickman HF, Wise MB, MacDougall D. Liver enzyme disturbances during isoniazid chemoprophylaxis in children. *The American review of respiratory disease* 1974; **110**(5): 581-4.

34. Byrd RB, Horn BR, Solomon DA, Griggs GA. Toxic effects of isoniazid in tuberculosis chemoprophylaxis. Role of biochemical monitoring in 1,000 patients. *JAMA : the journal of the American Medical Association* 1979; **241**(12): 1239-41.
35. Dash LA, Comstock GW, Flynn JP. Isoniazid preventive therapy: Retrospect and prospect. *The American review of respiratory disease* 1980; **121**(6): 1039-44.
36. Hsu KH. Isoniazid in the prevention and treatment of tuberculosis. A 20-year study of the effectiveness in children. *JAMA : the journal of the American Medical Association* 1974; **229**(5): 528-33.
37. Litt IF, Cohen MI, McNamara H. Isoniazid hepatitis in adolescents. *The Journal of pediatrics* 1976; **89**(1): 133-5.
38. Nakajo MM, Rao M, Steiner P. Incidence of hepatotoxicity in children receiving isoniazid chemoprophylaxis. *The Pediatric infectious disease journal* 1989; **8**(9): 649-50.
39. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. *JAMA : the journal of the American Medical Association* 1999; **281**(11): 1014-8.
40. Palusci VJ, O'Hare D, Lawrence RM. Hepatotoxicity and transaminase measurement during isoniazid chemoprophylaxis in children. *The Pediatric infectious disease journal* 1995; **14**(2): 144-8.
41. Rapp RS, Campbell RW, Howell JC, Kendig EL, Jr. Isoniazid hepatotoxicity in children. *The American review of respiratory disease* 1978; **118**(4): 794-6.
42. Spyridis NP, Spyridis PG, Gelesme A, et al. The effectiveness of a 9-month regimen of isoniazid alone versus 3- and 4-month regimens of isoniazid plus rifampin for treatment of latent tuberculosis infection in children: results of an 11-year randomized study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007; **45**(6): 715-22.
43. Spyridis P, Sinaniotis C, Papadea I, Oreopoulos L, Hadjiyiannis S, Papadatos C. Isoniazid liver injury during chemoprophylaxis in children. *Archives of disease in childhood* 1979; **54**(1): 65-7.
44. Tortajada C, Martinez-Lacasa J, Sanchez F, et al. Is the combination of pyrazinamide plus rifampicin safe for treating latent tuberculosis infection in persons not infected by the human

- immunodeficiency virus? *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2005; **9**(3): 276-81.
45. Marais BJ, van Zyl S, Schaaf HS, van Aardt M, Gie RP, Beyers N. Adherence to isoniazid preventive chemotherapy: a prospective community based study. *Archives of disease in childhood* 2006; **91**(9): 762-5.
 46. Aminzadeh Z, Asl RT. A six months follow-up on children less than 6 years old in contact with smear positive tuberculosis patients, varamin city, tehran, iran. *International journal of preventive medicine* 2011; **2**(2): 79-81.
 47. Devrim I, Olukman O, Can D, Dizdarer C. Risk factors for isoniazid hepatotoxicity in children with latent TB and TB: difference from adults. *Chest* 2010; **137**(3): 737-8.
 48. Minodier P, Lamarre V, Carle ME, Blais D, Ovetchkine P, Tapiero B. Evaluation of a school-based program for diagnosis and treatment of latent tuberculosis infection in immigrant children. *Journal of infection and public health* 2010; **3**(2): 67-75.
 49. le Roux SM, Cotton MF, Myer L, et al. Safety of long-term isoniazid preventive therapy in children with HIV: a comparison of two dosing schedules. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2013; **17**(1): 26-31.
 50. Gray D, Nuttall J, Lombard C, et al. Low rates of hepatotoxicity in HIV-infected children on anti-retroviral therapy with and without isoniazid prophylaxis. *Journal of tropical pediatrics* 2010; **56**(3): 159-65.
 51. Guidance for national tuberculosis programmes on the management of tuberculosis in children. Chapter 2: anti-tuberculosis treatment in children. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2006; **10**(11): 1205-11.
 52. Byrd RB, Horn BR, Griggs GA, Solomon DA. Isoniazid chemoprophylaxis. Association with detection and incidence of liver toxicity. *Arch Intern Med* 1977; **137**(9): 1130-3.
 53. Durfee ML, Nemir RL, Sewell EM, Smith MH, Warren BJ, Hsu KH. The treatment of tuberculosis in children. *The American review of respiratory disease* 1969; **99**(2): 304-7.
 54. CDC. Targeted tuberculin testing and treatment of latent tuberculosis infection. 2000. p. 1-54.

55. Nolan RJ, Jr. Childhood tuberculosis in North Carolina: a study of the opportunities for intervention in the transmission of tuberculosis to children. *American journal of public health* 1986; **76**(1): 26-30.
56. Thompson NP, Caplin ME, Hamilton MI, et al. Anti-tuberculosis medication and the liver: dangers and recommendations in management. *The European respiratory journal* 1995; **8**(8): 1384-8.
57. O'Brien RJ, Long MW, Cross FS, Lyle MA, Snider DE, Jr. Hepatotoxicity from isoniazid and rifampin among children treated for tuberculosis. *Pediatrics* 1983; **72**(4): 491-9.
58. Saukkonen JJ, Cohn DL, Jasmer RM, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. *American journal of respiratory and critical care medicine* 2006; **174**(8): 935-52.
59. Atuk NO, Hart AD, Hunt EH. Close monitoring is essential during isoniazid prophylaxis. *Southern medical journal* 1977; **70**(2): 156-9.
60. Garibaldi RA, Drusin RE, Ferebee SH, Gregg MB. Isoniazid-associated hepatitis. Report of an outbreak. *The American review of respiratory disease* 1972; **106**(3): 357-65.
61. Vanderhoof JA, Ament ME. Fatal hepatic necrosis due to isoniazid chemoprophylaxis in a 15-year-old girl. *The Journal of pediatrics* 1976; **88**(5): 867-8.
62. Mitchell JR, Long MW, Thorgeirsson UP, Jollow DJ. Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. *Chest* 1975; **68**(2): 181-90.
63. Gray D, Nuttall J, Lombard C, et al. Low Rates of Hepatotoxicity in HIV-infected Children on Anti-retroviral Therapy with and Without Isoniazid Prophylaxis. *Journal of tropical pediatrics* 2009; **56**(3): 159-65.
64. Danan G, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *Journal of clinical epidemiology* 1993; **46**(11): 1323-30.
65. Garcia-Cortes M, Stephens C, Lucena MI, Fernandez-Castaner A, Andrade RJ. Causality assessment methods in drug induced liver injury: strengths and weaknesses. *J Hepatol* 2011; **55**(3): 683-91.

66. Kaplowitz N. Causality assessment versus guilt-by-association in drug hepatotoxicity. *Hepatology* 2001; **33**(1): 308-10.

C. JOURNAL MANUSCRIPT

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Safety of long-term isoniazid preventive therapy in children with HIV: a comparison of two dosing schedules

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Running head: Safety of long-term IPT in children with HIV

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1. Summary

SETTING: Two pediatric hospitals in Cape Town, South Africa

OBJECTIVE: To investigate the incidence, predictors and outcomes of severe liver injury in HIV-infected children receiving long-term isoniazid preventive therapy (IPT).

DESIGN: Randomized trial of IPT or placebo given daily or thrice-weekly to HIV-infected children aged ≥ 8 weeks; placebo was discontinued early. Alanine transaminase (ALT) was measured at baseline, six-monthly and during illness: an increase ≥ 10 -fold the upper limit of normal defined severe liver injury.

RESULTS: Of 324 children enrolled, 297 (91.6%) received IPT (559.1 person-years). Baseline median age was 23 months (interquartile range, IQR 9.5-48.6) and median CD4%, 20% (IQR 13.6-26.9). Two-hundred-and-seven children (63.9%) received antiretroviral therapy (ART). Nineteen developed severe liver injury, sixteen while receiving IPT. Among these were eight cases of viral hepatitis (five with hepatitis A), two antiretroviral-induced liver injuries and a case of abdominal tuberculosis. IPT-related severe liver injury occurred in 1.7% (5/297, 0.78/100 person-years). No child developed hepatic failure; one died of an unrelated cause. All surviving children subsequently tolerated IPT.

CONCLUSIONS: This study suggests that long-term IPT has a low toxicity risk in HIV-infected children. In the absence of chronic viral hepatitis, IPT can be safely reintroduced following recovery from liver injury.

2. Manuscript

2.1 INTRODUCTION

The World Health Organization (WHO) has identified isoniazid preventive therapy (IPT), intensified case finding and infection control as key to reducing tuberculosis (TB) incidence among people living with HIV¹. Current guidelines recommend six months of IPT for HIV-infected children older than one year without TB disease, even in the absence of a known TB contact; up to three years is recommended in high TB prevalence areas^{2, 3}.

However, isoniazid may cause idiosyncratic drug-induced liver injury (DILI)⁴. Its predominant metabolizing pathway is acetylation by N-acetyl transferase 2 (NAT2); toxicity is mostly attributed to metabolites⁴. Idiosyncratic DILI is thought to result from a complex “multi-hit” process, where drug-specific upstream injury is exacerbated or ameliorated by less specific downstream factors^{5, 6}. Drug-specific risk factors for IPT-related liver injury include higher dosages and polymorphisms of NAT2^{4, 7}. During TB treatment, daily dosing appears to confer higher risk than intermittent dosing⁴. Downstream factors include malnutrition, other hepatotoxic drugs (such as nevirapine and pyrazinamide), viral hepatitis, and in adults, age and alcoholism. Although widely considered a risk factor, the independent effect of HIV-infection is not yet fully established⁸. Severe hepatitis occurred in only 1.1% of HIV-infected adults receiving six months of IPT in Botswana⁹; this estimate is comparable to the 0.1%-6.4% risk described in large studies of HIV-uninfected adults⁹⁻¹¹. Nonetheless, it is biologically plausible that the altered cytokine milieu of chronic viral infection may predispose to idiosyncratic DILI⁵.

In keeping with this, a recent study observed severe liver injury in 5.5% of HIV-infected adults receiving 6 months of IPT¹². Children generally tolerate isoniazid better than adults. Although death and hepatic failure can occur¹³, only 0.8% or less of HIV-uninfected children experience moderate to severe liver injury^{4, 9, 14-16}. Data for HIV-infected children are limited. One retrospective study reported severe liver injury in 5% of 112 HIV-infected children receiving IPT, mostly ascribed to acute viral hepatitis¹⁷. In a recent South African trial, HIV-infected infants received IPT for up to two years; 0.4% developed significantly raised alanine transaminase (ALT), and 4.0% significantly raised aspartate aminotransferase (AST)¹⁸. However, there are no published, prospective long-term data on liver injury in HIV-infected children older than one year or in symptomatic HIV-infected children who commence IPT. As some risk factors are dynamic, the cumulative risk for IPT-related liver injury may increase over longer exposure periods, as was observed among HIV-infected adults receiving three to six years of IPT^{12, 19}. We investigated the incidence, predictors and outcome of severe liver injury in a cohort of HIV-infected infants and children receiving long-term IPT.

2.2 METHODS

A prospective study of opportunistic infection prevention strategies in HIV-infected children commenced in December 2002, at Red Cross and Tygerberg Children's Hospitals in Cape Town, South Africa. The trial had a factorial design with two levels of randomization: participants were randomized to receive either isoniazid with trimethoprim-sulphamethoxazole (TMP-SMX) or placebo with TMP-SMX and again randomized to either a daily or thrice-weekly dosing schedule, figure 1. Placebo was discontinued in May 2004, due to a marked survival benefit

among children receiving IPT²⁰. Thereafter, all children receiving placebo were switched to IPT and followed until December 2007 to assess potential differences in efficacy, safety and adherence.

i. Participants and allocation

HIV-infected children aged \geq eight weeks were enrolled. Exclusion criteria, previously described^{20, 21}, included clinical hepatitis. Children receiving antiretroviral therapy (ART) were eligible once stable on treatment for at least two months. Enrolment, allocation and randomization processes are described elsewhere^{20, 21}.

ii. Medication

Isoniazid tablets (100 mg, Be-Tabs Pharmaceuticals, Johannesburg, South Africa) were given at 10 mg/kg per dose (range of 8-12mg/kg, maximum 300mg), for both dosing schedules. Allocation was unblinded in children with TB disease. Those receiving placebo commenced standard anti-TB treatment for children (WHO regimen 3, isoniazid/rifampicin/pyrazinamide); those randomized to IPT also started ethambutol or ethionamide. Treatment was modified according to antimicrobial susceptibility. The South African government commenced antiretroviral roll-out in 2004 where after ART became available to all.

iii. Measurements

HIV status was assessed by enzyme-linked immuno-sorbent assay (Abbott AxSYM HIV antibody/antigen ELISA) in those older than 15 months, and by polymerase chain reaction (PCR, Amplicor HIV-1, Roche Diagnostic Systems) in those younger. Alanine aminotransferase (ALT)

was measured at baseline and six-monthly; children on ART also had measurements one and three months after enrolment. Other regular investigations have been described^{20, 21}. Tests were repeated whenever clinically indicated. Children were not routinely screened for viral hepatitis at enrolment. All deaths were investigated by accessing clinical records, or if unavailable, by verbal autopsy where feasible.

Toxicity events were graded according to the Division of AIDS (DAIDS) toxicity criteria²². For liver injury, ALT 10-15 times the upper limit of normal (ULN) was considered grade three; above 15-fold increase was considered grade four. Children with grade three or four events were urgently recalled and assessed, with repeat ALT, aspartate transaminase (AST) and total bilirubin testing. Isoniazid was discontinued and further investigations were guided by clinical condition on advice from gastroenterology and/or infectious diseases specialists. Additional tests included alkaline phosphatase, gamma-glutamyltransferase, albumin, conjugated bilirubin, prothrombin time and lactate dehydrogenase. Etiological investigations included screening for hepatitis A (HAV, IgM and IgG), hepatitis B (HBV, antigen and antibodies) and hepatitis C virus (HCV, antibodies); Epstein-Barr virus (EBV, IgM and IgG) and/or cytomegalovirus (CMV IgM, IgG antibodies, pp65 assay and/ or urine CMV culture). Abdominal ultrasound was available when indicated. For clinically severe or life-threatening events, participants were hospitalized and ART was interrupted. Most participants were managed as outpatients, with repeat ALT after 48 hours, two to four-weekly for a month then monthly until the levels were less than 3 times the ULN. Caregivers were requested to return immediately if children became symptomatic. After clinical and biochemical recovery, drugs were systematically reintroduced: isoniazid was restarted after the successful reintroduction of ART and TMP-SMX. Rechallenge with IPT

occurred at full dose, with two-to four-weekly follow-up and instructions to return immediately if symptomatic. Anti-TB therapy and/or ART were reintroduced in hospital, according to hospital protocol: antiretrovirals were started simultaneously and anti-TB drugs introduced systematically. Each event was assessed as definitely, probably, possibly or unrelated to study drugs, and notified to the relevant ethics committees.

iv. Ethics

Written, informed consent was obtained from a parent or legal guardian. The Research and Ethics Committees of the Universities of Cape Town and Stellenbosch approved the study. The trial is registered as Clinical Trials NCT00330304.

v. Statistical methods

The main outcome was severe liver injury, defined as a grade three or four elevation in ALT during follow-up. The Kaplan-Meier method was used to analyze time to severe liver injury. Person-time was censored at the first event or last known time alive, if event-free.

Crude incidence rate ratios (IRR) were used to compare dosing schedule (intent-to-treat analysis) and categories of drug exposure (as-treated analysis). Potential predictors were analyzed with Cox proportional hazards regression. Baseline age was dichotomized at one year. Exposure to ART and anti-TB drugs (placebo, IPT or anti-TB treatment) were modeled as time-varying covariates. Standard statistical methods were used for model building and checking. Analyses were done in Stata version 10.0 (Stata Corporation, College Station, Texas, USA).

Statistical tests were two-sided at $\alpha=0.05$. Sample size calculations for mortality, the primary outcome of the main study, have been described elsewhere^{20, 21}.

2.3 RESULTS

Of 339 children randomized, 15 were excluded from analysis (10 tested HIV-negative, five were lost to follow-up within the first month). Three-hundred-and-twenty-four were followed for 641 person-years. Baseline characteristics are shown in table 1. Two hundred and ninety-seven received IPT (559.1 person-years, table 2): 99 had been switched from placebo, figure 1.

Adherence was excellent and similar in the groups, as previously reported²³.

i. Incidence rates and clinical presentation

Overall, nineteen (6%) of 324 children had an episode of severe liver injury (2.96 first episodes per 100 person-years), table 2. Most were young (median baseline age 14 months, IQR 5.00-36.18). The median baseline CD4% was 23% (IQR 19.02-31.00). Ten (53%) had symptoms typically associated with hepatitis. Vomiting was the most frequent complaint; two children were jaundiced. No child developed hepatic failure. Details are provided in appendix 1.

One event occurred on placebo (incidence rate, IR 1.7 per 100 person-years), 16 on IPT (IR 2.86 per 100 person-years; 16/297, 5.4%) and two on anti-TB treatment (IR 8.64 per 100 person-years; 2/34, 5.9%), table 2. The IRR comparing IPT and ART to only ART was 0.29 (95% CI 0.038–12.87). There was a trend towards decreased risk in the thrice-weekly arm, table 2. The median time to severe liver injury was 16.4 months (IQR 5.2-23.6); only four events occurred within the first four months of IPT exposure.

Fourteen (74%) episodes of severe liver injury were considered unrelated to IPT: eight children had acute viral hepatitis (five HAV, one EBV and two CMV). Five children had severe liver injury within 3 months of starting ART or anti-TB treatment; one was diagnosed with abdominal TB. Thus only five (1.7%) of the 297 children who received IPT did not have another compelling cause for liver injury identified. All five had received daily IPT.

ii. Outcomes

The average time to normalization of ALT (\leq 3-fold ULN) was 36 days (range 6-84 days). In eight children, the repeat ALT was lower than 10-fold ULN; IPT was continued under close supervision. All improved with no recurrence. IPT was discontinued in 11 children: ten were successfully rechallenged. One child died from hypoxic pneumonia before IPT was restarted. No further episodes of severe liver injury were experienced, except for one child who subsequently contracted and recovered from hepatitis A.

iii. Cox proportional hazards regression

Baseline ALT and nutritional status were not associated with the outcome. Younger baseline age [adjusted hazard ratio (aHR) 3.47 (95% CI 1.27-9.47)] and higher baseline CD4% [aHR 1.06 (95%CI 1.01-1.12)] were significantly associated with severe liver injury, table 3.

2.4 DISCUSSION

Whereas a large number of children (16/297, 5.4%) developed severe liver injury whilst receiving IPT, only a few cases (5/297, 1.7%) were reasonably related to IPT. Non-drug causes were found in nine and rechallenge was uneventful in all survivors. Causality assessment in DILI

is notoriously difficult^{6, 24}; several algorithms and clinical scales have been proposed to aid diagnosis²⁵. Most clinical scales strongly weigh exclusion of non-drug causes, temporal association with starting the drug in question and response to rechallenge in the probability scoring for diagnosis of DILI^{24, 26, 27}. Response to rechallenge is considered the closest there is to a “gold standard”^{6, 26}. Therefore it is possible that we overestimated the true incidence of IPT-induced liver injury.

Similar or higher rates of liver injury were observed in two recent studies of HIV-infected adults receiving IPT^{9, 13, 20}. The only other published, prospective study of pediatric IPT, given to HIV-infected infants for up to two years, observed severe liver injury in 0.4% of infants¹⁸. The data therefore indicate that, as seen in HIV-uninfected populations, risk for IPT-related severe liver injury is lower in HIV-infected children than in HIV-infected adults. Although not negligible, this degree of risk has been considered acceptable in the risk-benefit assessment of IPT for adults at high risk for TB disease progression³. Quantitative risk-benefit analyses are lacking, but effective tuberculosis prevention via IPT offers substantial benefit to HIV-infected children, who have a particularly high risk for TB disease progression, morbidity and mortality, even in the context of ART^{2, 20, 28}. This study further demonstrates that IPT can be safely re-introduced after severe liver injury has resolved.

We found a high incidence of viral hepatitis, particularly HAV. South Africa is highly endemic for HAV, with an estimated seroprevalence of 83% among infants^{29, 30}. Unfortunately, HAV vaccine is not in the national immunization program. South African children have been routinely immunized against HBV since 1995, and the prevalence of HCV is thought to be low³¹. As these

viruses share transmission routes with HIV, the impact of viral hepatitis on the care of HIV-infected children from other resource-limited settings might be even greater³².

Most study participants received ART during the trial. In keeping with adult data from Botswana³³, we did not observe a higher incidence of liver injury among children receiving both ART and IPT compared to ART only. Unfortunately, our estimates lack precision, and data from larger cohorts will be required to confirm this finding.

Transient rises in ALT commonly occur during treatment with isoniazid, without apparent clinical significance^{6, 8}; consistent with this, some children with grade three or four liver injury rapidly improved to grade two or less before IPT could be interrupted, without further increases in ALT. Previous guidelines on IPT recommend biochemical monitoring for high-risk groups only⁸. Our study has identified two potentially high risk pediatric groups, namely the very young and those with high CD4%.

Generally, children are at low risk for isoniazid-related liver injury, and studies of pediatric IPT have seldom examined differential risk across age subgroups. However, one pediatric study described higher risk for TB-drug-induced liver injury among children younger than 5 years³⁴. As young children have immature enzyme systems, it is biologically plausible that infants are at increased risk of isoniazid-induced liver injury. In HAV-endemic areas, infants may also be at highest risk for HAV infection.

We found higher baseline CD4% to be associated with liver injury. Both adaptive and innate immune responses are involved in the processes that can lead to isoniazid-induced liver injury⁵. Higher CD4% might be a surrogate marker for overall better preserved immunity, paradoxically

placing healthier individuals at higher risk for toxicity. Furthermore, isoniazid has been shown to cause cell death via apoptosis³⁵; the effectiveness of cytotoxic T-cell function might influence the extent of final liver injury.

Our study has limitations. The discontinuation of placebo restricts the evaluation of isoniazid-attributable hepatotoxicity. By definition, idiosyncratic DILI is uncommon; with only 19 events, statistical power was limited. While point estimates indicated higher risk for IPT compared to placebo, and daily IPT compared to thrice-weekly, the results are inconclusive due to imprecision.

Although we explored a range of risk factors, NAT2 genotyping was not performed; possibly those with liver injury were slow acetylators^{7, 35}.

The continuation of isoniazid despite symptomatic, severe liver injury can be fatal¹³. We were able to diagnose hepatotoxicity early with prompt interruption of IPT and careful re-introduction where indicated; we also re-assessed patients frequently, and did extensive viral hepatitis screening. This level of risk management may be challenging in operational settings.

2.5 CONCLUSION

This study suggests that long-term IPT has a low hepatic toxicity risk in HIV-infected children. In the absence of chronic viral hepatitis, IPT can be safely reintroduced following recovery.

2.6 ACKNOWLEDGEMENTS

Dr Stanzi le Roux participated in data collection and patient care, conducted the statistical analysis and wrote the manuscript. Dr Carl Lombard was the trial statistician, and supervised

the statistical analysis along with Professor Landon Myer. Professors Mark F Cotton and Heather J Zar conceived the parent study, wrote the protocol and grant applications, obtained funding and supervised the study. Professor H Simon Schaaf obtained funding, and assisted with study design, supervision and co-ordination. Dr Dave le Roux assisted with statistical analysis and the journal manuscript's literature review.

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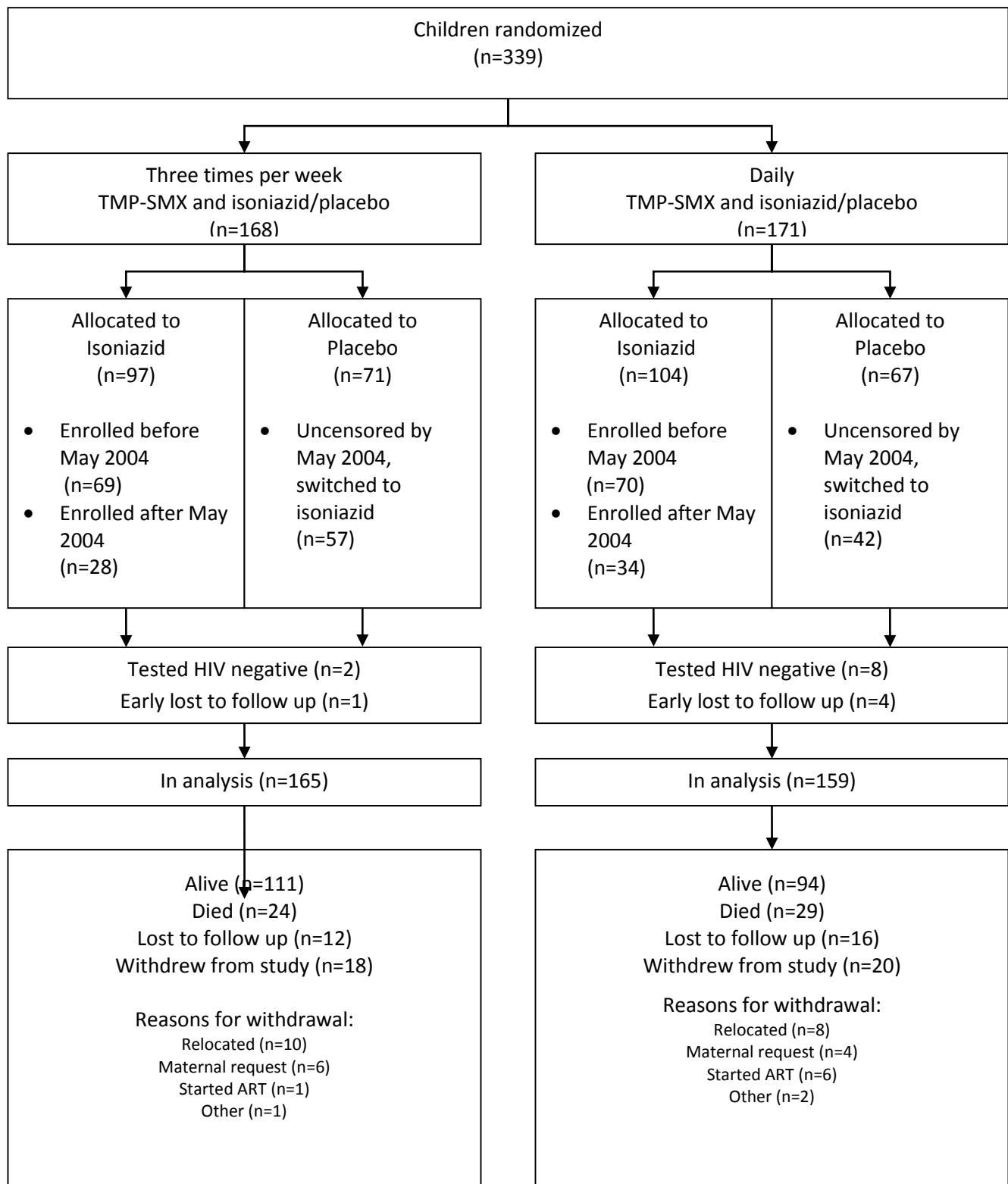
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Data and Safety Monitoring Committee – Dr J Kaplan (chair), Dr W El Sadr, Prof P Donald, Prof N Beyers; local DSMB Prof P Donald (chair), Prof N Beyers, Prof M Klein.

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2.7 COMPETING INTERESTS: None declared.

FIGURE 2. Flow of participants through trial

TMP-SMX, trimethoprim-sulphamethoxazole; ART, anti-retroviral therapy

TABLE 6. Baseline characteristics of children randomized to thrice weekly or daily isoniazid/placebo and trimethoprim-sulphamethoxazole

	Thrice weekly (n=165)	Daily (n=159)	Total (n=324)
Allocated to placebo¹	42.4% (70)	40.0% (62)	40.7% (132)
Allocated to isoniazid			
Before May 2004	40.6% (67)	40.2% (64)	40.4% (131)
After May 2004	17.0% (28)	20.8% (33)	18.8% (61)
Age (months)	21.8 (9.5 – 52.3)	24.6 (9.7 – 45.6)	23.0 (9.5 – 48.6)
Boys	56.9% (94)	54.7% (87)	55.8% (181)
Weight for age (z-score)	-1.3 (-1.8 to -1.0)	-1.6 (-1.9 to -1.2)	-1.5 (-1.8 to -1.2)
ALT (U/l)	28 (20.5 – 41.5)	27 (17 – 43)	28 (18 – 42)
CD4 (% lymphocytes)	20.4 (14.3 – 28)	19.0 (12.1 – 25.2)	20.0 (13.6 – 26.9)
ART			
At enrolment	8.5% (14)	8.8% (14)	8.6% (28)
Ever during study	63.0% (104)	64.8% (103)	63.9% (207)

Figures are % (number), or median (interquartile range); Abbreviations: ALT, alanine transaminase; ART, antiretroviral therapy

¹Children were only allocated to placebo until May 2004

TABLE 7. Incidence rates of severe liver injury by group and per drug exposure category

	Not on ART			On ART			TOTAL			
	Number of events	Time (years)	Incidence rate per 100 child-years	Number of events	Time (years)	Incidence rate per 100 child-years	Number of events	Follow-up time (years)	Incidence rate per 100 child-years	Incidence rate ratio (95%CI)
Dosing schedule										
Daily dosing	4	135.0	2.96	8	164.0	4.88	12	299.0	4.01	1
Thrice weekly dosing	5	168.8	2.96	2	173.2	1.15	7	341.96	2.04	0.51 (0.2-1.4)
Drug exposure category										
Placebo	0	47.3	0	1	11.5	8.71	1	58.8	1.7	1
IPT	8	242.7	3.30	8	316.4	2.53	16	559.1	2.86	1.68 (0.3-70.6)
Anti-TB treatment	1	13.8	7.24	1	9.3	10.7	2	23.1	8.64	5.09 (0.3-299.8)
Total	9	303.8	2.96	10	337.2	2.97	19	641.0	2.96	-

Abbreviations: CI, confidence interval; IPT, isoniazid preventive therapy; TB, tuberculosis; ART, combination antiretroviral therapy

TABLE 8. Factors associated with severe liver injury: unadjusted and adjusted hazard ratios from Cox proportional hazards regression

Variable	Unadjusted			Adjusted*		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Dosing schedule						
Daily	1.00	-	-	1.00		
Thrice weekly	0.50	0.20-1.28	0.15	0.44	0.17-1.15	0.09
Age at baseline						
> 12 months	1.00	-	-	1.00	-	-
≤ 12 months	3.20	1.29-7.95	0.01	3.47	1.27-9.47	0.02
CD4% at baseline[§]	1.04	1.00-1.09	0.05	1.06	1.01-1.12	0.01

* Adjusted for dosing schedule, baseline age, baseline CD4%, study site, current exposure to ART and current TB drug exposure

[§] Missing data for 7 children

ART, antiretroviral therapy; TB, tuberculosis

2.8 REFERENCES

1. WHO Three I's Meeting: Intensified Case Finding (ICF), Isoniazid Preventive Therapy (IPT) and TB Infection Control (IC) for people living with HIV. Report of a Joint World Health Organization HIV/AIDS and TB Department Meeting. 2-4 April 2008 2008.
http://www.who.int/hiv/pub/meetingreports/WHO_3Is_meeting_report.pdf (accessed 1 June 2011).
2. World Health Organization. Antiretroviral therapy for HIV infection in infants and children: Towards universal access. Recommendations for a public health approach: 2010 revision. Geneva 2010.
<http://www.who.int/hiv/pub/paediatric/infants2010/en/index.html> (accessed 1 June 2011).
3. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource constrained settings. 2011.
http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf (accessed 1 June 2011).
4. Donald PR. Antituberculosis drug-induced hepatotoxicity in children. *Pediatr Rep* 2011; **3**(2): e16.
5. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 2005; **4**(6): 489-99.
6. Lee WM. Drug-induced hepatotoxicity. *The New England journal of medicine* 2003; **349**(5): 474-85.
7. Huang YS, Chern HD, Su WJ, et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. *Hepatology* 2002; **35**(4): 883-9.
8. Saukkonen JJ, Cohn DL, Jasmer RM, et al. An official ATS statement: hepatotoxicity of antituberculosis therapy. *Am J Respir Crit Care Med* 2006; **174**(8): 935-52.
9. Kopanoff DE, Snider DE, Jr., Caras GJ. Isoniazid-related hepatitis: a U.S. Public Health Service cooperative surveillance study. *The American review of respiratory disease* 1978; **117**(6): 991-1001.
10. LoBue PA, Moser KS. Use of isoniazid for latent tuberculosis infection in a public health clinic. *Am J Respir Crit Care Med* 2003; **168**(4): 443-7.
11. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* 1991; **99**(2): 465-71.
12. Martinson NA, Barnes GL, Moulton LH, et al. New regimens to prevent tuberculosis in adults with HIV infection. *The New England journal of medicine* 2011; **365**(1): 11-20.
13. Wu SS, Chao CS, Vargas JH, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. *Transplantation* 2007; **84**(2): 173-9.

14. Beaudry PH, Brickman HF, Wise MB, MacDougall D. Liver enzyme disturbances during isoniazid chemoprophylaxis in children. *Am Rev Respir Dis* 1974; **110**(5): 581-4.
15. Nemir RL, O'Hare D. Tuberculosis in children 10 years of age and younger: three decades of experience during the chemotherapeutic era. *Pediatrics* 1991; **88**(2): 236-41.
16. Palusci VJ, O'Hare D, Lawrence RM. Hepatotoxicity and transaminase measurement during isoniazid chemoprophylaxis in children. *Pediatr Infect Dis J* 1995; **14**(2): 144-8.
17. Gray D, Nuttall J, Lombard C, et al. Low Rates of Hepatotoxicity in HIV-infected Children on Anti-retroviral Therapy with and Without Isoniazid Prophylaxis. *Journal of tropical pediatrics* 2009; **56**(3): 159-65.
18. Madhi SA, Nachman S, Violari A, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011; **365**(1): 21-31.
19. Samandari T, Agizew TB, Nyirenda S, et al. 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **377**(9777): 1588-98.
20. Zar HJ, Cotton MF, Strauss S, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007; **334**(7585): 136.
21. Zar HJ, Workman L, le Roux SM, et al. A randomized controlled trial of intermittent compared with daily cotrimoxazole preventive therapy in HIV-infected children. *AIDS (London, England)* 2010; **24**(14): 2225-32.
22. National Institutes of Health. Division of AIDS (DAIDS) revised toxicity tables for grading severity of pediatric adverse experiences. US National Institutes of Health DAIDS HIV Vaccine and Research Program, version 1.0. 2004. <http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/DAIDSAEGradingTable.pdf> (accessed November 24, 2009.)
23. le Roux SM, Cotton MF, Golub JE, le Roux DM, Workman L, Zar HJ. Adherence to isoniazid prophylaxis among HIV-infected children: a randomized controlled trial comparing two dosing schedules. *BMC medicine* 2009; **7**: 67.

24. Danan G, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *Journal of clinical epidemiology* 1993; **46**(11): 1323-30.
25. Garcia-Cortes M, Stephens C, Lucena MI, Fernandez-Castaner A, Andrade RJ. Causality assessment methods in drug induced liver injury: strengths and weaknesses. *J Hepatol* 2011; **55**(3): 683-91.
26. Kaplowitz N. Causality assessment versus guilt-by-association in drug hepatotoxicity. *Hepatology* 2001; **33**(1): 308-10.
27. Maria VA, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997; **26**(3): 664-9.
28. Frigati LJ, Kranzer K, Cotton MF, Schaaf HS, Lombard CJ, Zar HJ. The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011; **66**(6): 496-501.
29. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 2010; **28**(41): 6653-7.
30. Solomons R, Rabie H, Nel E, Cotton M. An overview of hepatitis A at Tygerberg Children's Hospital. *South African Journal of Child Health* 2008; **2**: 43-5.
31. Madhava V, Burgess C, Drucker E. Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *The Lancet infectious diseases* 2002; **2**(5): 293-302.
32. Kumar A, Misra PK, Mehotra R, Govil YC, Rana GS. Hepatotoxicity of rifampin and isoniazid. Is it all drug-induced hepatitis? *The American review of respiratory disease* 1991; **143**(6): 1350-2.
33. Tedla Z, Nyirenda S, Peeler C, et al. Isoniazid-associated hepatitis and antiretroviral drugs during tuberculosis prophylaxis in HIV-infected adults in Botswana. *American journal of respiratory and critical care medicine* 2010; **182**(2): 278-85.
34. Ohkawa K, Hashiguchi M, Ohno K, et al. Risk factors for antituberculous chemotherapy-induced hepatotoxicity in Japanese pediatric patients. *Clinical pharmacology and therapeutics* 2002; **72**(2): 220-6.
35. Bhadauria S, Mishra R, Kanchan R, et al. Isoniazid-induced apoptosis in HepG2 cells: generation of oxidative stress and Bcl-2 down-regulation. *Toxicol Mech Methods* 2010; **20**(5): 242-51.

D. APPENDICES



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HREC REF: 057/2002

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Paediatrics, Red Cross Children's Hospital

Dear Prof Zar

PROJECT TITLE: STRATEGIES FOR PREVENTION OF OPPORTUNISTIC INFECTIONS IN HIV-INFECTED SOUTH AFRICAN CHILDREN: COMPARISON OF 2 TRIMETHOPRIM-SULPHAMETHOXAZOLE PROPHYLAXIS REGIMENS WITH AND WITHOUT CONCOMITANT ISONIAZIDE-IMPACT ON MORBIDITY, MORTALITY, BACTERIAL RESISTANCE AND INCIDENCE OF TUBERCULOSIS.

Thank you for your e-mail to the Faculty of Health Sciences Human Research Ethics Committee, dated 19th June 2010.

This letter is to confirm that Dr Stanza Le Roux has permission to access data on HREC REF NO 057/2002 in order to conduct further analysis and an MMed dissertation.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely,

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

First author & publication year:				Number	
Study design:	Placebo controlled?		INH dose, duration:		
Numbers & Ages:	HIV status:		Type of monitoring used:		
Viral hepatitis screening?	Clinical indicators of liver injury:		Definition of hepatotoxicity/liver injury:		
Laboratory findings:	Timing of injury:	Mild-moderate injury: Denominator: Numerator: Rate provided?	Severe liver injury: Denominator: Numerator: Rate given?	Total injury: Denominator: Numerator: Rate given?	
Outcomes: treatment interruptions	Outcomes: Hepatic failure or death? (liver transplants?)		Outcomes: numbers & comments on loss to follow up and/or adherence:		



The International Journal of Tuberculosis and Lung Disease

INSTRUCTIONS FOR AUTHORS

THE INTERNATIONAL JOURNAL OF TUBERCULOSIS AND LUNG DISEASE

(IJTLD), the Official Journal of the International Union Against Tuberculosis and Lung Disease (The Union), publishes:

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Details of ethics approval (or a statement that it was not required) should be provided in the Methods section of all research studies submitted to the Journal.

As of 1 November 2013, all articles must be submitted in English (US/UK). If the quality of the English requires professional help, authors will be informed. The summaries of all articles are published in French and Spanish.

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Summary: An informative structured abstract of **not more than 200 words** should be provided that can be understood **without reference to the text** (see *Ann Intern Med* 1990; 113: 69-76). For optimal clarity, the author should use the headings Setting, Objective, Design, Results and Conclusion. Abstracts will be translated into the two other languages on acceptance for publication (authors are welcome to provide translations). Unstructured summaries may be submitted for review articles (250 words) and Short Communications (100 words).

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Conclusions

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Acknowledgements: Acknowledge only persons who have made substantial contributions to the study, with their consent, all sources of support in the form of grants, and author contributions.

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e.g., Gordon J B, Bennett A M. Tuberculosis in reindeer. *Scand Rev Respir Dis* 1978; 96 (Suppl): 217-219.

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e.g., Girling D J. The chemotherapy of tuberculosis. In: Ratledge C, Stanford J, Grange J M, eds. *Biology of the mycobacteria*. London, UK: Academic Press, 1989: pp 285-323.

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Tables should be referred to consecutively in the text and placed after the references. They should be numbered in Arabic numerals which are used for reference in the text. A short descriptive title should appear above the table. Each column should have a short or abbreviated title. All abbreviations should be explained in a clear legend below the table. The number and size of the tables should be

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Nr	Study drugs*	Dosing	Time exposed to isoniazid (months)	Other drugs	Time on ART (months)	Clinical presentation	Highest ALT (U/l)	Viral hepatitis ⁺ screen performed	Diagnosis ⁺	Outcome	Time to resolution/rechallenge (days)
1	Isoniazid	Daily	3.64	None	0	Anorexia, malaise & cough	1575	Not done	Isoniazid DILI (possible)	Recovered	23
2	Isoniazid	Daily	11.74	None	0	Asymptomatic	490	Not done	Isoniazid DILI (probable)	Recovered	39
3	Isoniazid	Daily	17.1	3TC/D4T/ LPV/r	21.11	Asymptomatic	857	HAV, HBV	Isoniazid DILI (possible)	Recovered	73
4	Isoniazid	Daily	24.03	None	0	Asymptomatic	413	HAV, HBV, HCV, EBV, CMV	Isoniazid DILI (possible)	Recovered	28
5	Isoniazid	Daily	52.79	3TC/D4T/NVP	33.44	Vomiting	1306	Not done	Isoniazid DILI (possible)	Recovered	84
6	Isoniazid	3xWk	5.64	None	0	Vomiting, diarrhoea & fever	1761	HAV	Hepatitis A ¹	Recovered	32
7	Isoniazid	3xWk	10.65	None	0	Jaundice	3138	HAV, HBV, HCV	Hepatitis A ¹	Recovered	53
8	Isoniazid	Daily	21.57	3TC/AZT/ LPV/r	6.26	Cough & fever	482	HAV, HBV	Hepatitis A ¹	Recovered	14
9	Isoniazid	Daily	23.57	3TC/AZT/RTV	15.64	Asymptomatic	633	HAV, HBV	Hepatitis A ¹	Recovered	21
10	Isoniazid	Daily	23.77	3TC/D4T/EFV	19.5	Asymptomatic	872	HAV, HBV, HCV CMV, EBV	Hepatitis A ¹	Recovered	9
11	Placebo	Daily	0	3TC/AZT/RTV	2.20	Cough	293	Not done	ART DILI	Recovered	56
12	Isoniazid	Daily	4.72	3TC/AZT/EFV	3.08	Vomiting	651	Not done	ART DILI	Recovered	62
13	Isoniazid	3xWk	9.44	3TC/AZT/EFV	1.25	Vomiting, diarrhoea	405	Not done	ART DILI	Recovered	30
14	Isoniazid	Daily	6.45	None	0	Asymptomatic	373	HAV, HBV, HCV CMV, EBV	EBV hepatitis ²¹	Recovered	28
15	Isoniazid	3xWk	2.62	3TC/AZT/RTV Fluconazole	3 days	Vomiting, anorexia, failure to thrive, jaundice, fever, diarrhoea & cough	557	HAV, HBV, HCV CMV, EBV	Disseminated CMV with hepatitis ³¹	Recovered	14
16	Isoniazid	3xWk	1.02	Erythromycin	0	Vomiting, jaundice cough & fever	356	HAV, HBV CMV	CMV hepatitis ⁴¹	Died: CMV pneumonia	-
17	(TB treatment)	Daily	5.04	TB treatment ⁵ 3TC/D4T/EFV Fluconazole	1.08	Vomiting & fever	421	HAV, HBV, HCV	Anti-TB DILI	Recovered	6

Nr	Study drugs*	Dosing	Time exposed to isoniazid (months)	Other drugs	Time on ART (months)	Clinical presentation	Highest ALT (U/l)	Viral hepatitis [†] screen performed	Diagnosis [‡]	Outcome	Time to resolution/rechallenge (days)
18	(TB treatment)	3xWk	8.20	TB treatment [#]	0	Asymptomatic	398	Not done	Anti-TB DILI	Recovered	7
19	Isoniazid	3xWk	3.64	Omeprazole	0	Vomiting, diarrhoea & fever	1290	HAV, HBV, HCV EBV	Abdominal TB	Recovered	68

Abbreviations: 3XWk – Thrice weekly; ALT – alanine aminotransaminase; cART – combination antiretroviral therapy; DILI – drug induced liver injury; 3TC – lamivudine; D4T – stavudine; AZT – zidovudine; Lopinavir/ritonavir – LPV/r; RTV – Ritonavir; NVP – nevirapine; EFV – efavirenz; HAV – Hepatitis A virus; HBV – Hepatitis B virus; HCV – Hepatitis C virus; CMV – cytomegalovirus; EBV – Epstein Barr virus; TB – tuberculosis

*All received trimethoprim-sulphamethoxazole according to the dosing schedule for isoniazid/placebo

[†]Viral hepatitis screening: unless otherwise indicated, screening tests were – IgM and IgG antibodies for HAV, EBV and CMV; surface antigen for HBV; antibodies for HCV

[‡]Diagnosis based on:

1. Positive Hepatitis A IgM antibody; 2. Positive EBV Viral capsid antigen IgM;
3. Positive pp65, positive CMV viral culture from urine; clinical CMV retinitis; 4 Positive pp65, positive CMV IgM and IgG with CMV pneumonia on lung biopsy

§Isoniazid/rifampicin/pyrazinamide/ethionamide (hepatotoxicity occurred after 90 days)

#Isoniazid/rifampicin/pyrazinamide/ethionamide (hepatotoxicity occurred after 53 days)