A Randomised Controlled Trial of N-acetylcysteine in the Management of Anti-tuberculosis Drug-induced Liver Injury

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Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY (PhD)

in Department of Medicine

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

January 2023

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Declaration

I, Muhammed Shiraz Moosa, do hereby declare that this thesis is based on my original work (except where acknowledgements indicate otherwise) and that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in this or any other university. I hereby grant the University of Cape Town permission to reproduce this thesis in whole or part for the purpose of research or teaching.

I do hereby declare that this thesis includes three journal manuscripts. Two (Chapters 3 & 4) of the three manuscripts included in this thesis have been published in peer reviewed journals. The last manuscript (Chapter 5) has been accepted for publication. The contents of each of these manuscripts remains unchanged from that which has been published or accepted for publication. The manuscripts are listed below, with a description of my contribution to all of the studies. The contribution of my co-authors are included in the preamble to each of the chapters 3 to 5.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co- authorships are involved, my co-authors have agreed that I may include the publication(s).

1. Moosa MS, Maartens G, Gunter H, Allie S, Chughlay MF, Setshedi M, Wasserman S, Stead D, Hickman N, Stewart A, Sonderup M, Spearman CW, Cohen K. A randomized controlled trial of intravenous n-acetylcysteine in the

management of anti-tuberculosis drug-induced liver injury. *Clin Infect Dis*. Aug 26, 2020; doi:10.1093/cid/ciaa1255 **(Chapter 3/Appendix 1)**

2. Moosa MS, Maartens G, Gunter H, Allie S, Chughlay MF, Setshedi M, Wasserman S, Stead DF and Cohen K. Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort. S Afr J HIV Med. 2022;23(1), a1376. https://doi.org/10.4102/sajhivmed.v23i1.1376 **(Chapter 4/Appendix 3)**

3. Moosa MS, Russomanno G, Dorfman JR, Gunter H, Patel C, Costello E, Carr D, Maartens G, Pirmohamed M, Goldring C, Cohen K. Analysis of serum microRNA-122 in a randomised controlled trial of N-acetylcysteine for treatment of anti-tuberculosis drug-induced liver injury. British Journal of Clinical Pharmacology doi: 10.1111/bcp.15661 (accepted for publication on 04/01/2023) **(Chapter 5/Appendix 4)**

Together with my supervisors, I conceptualised and designed the studies presented in this thesis. I led participant recruitment, data collection, prepared datasets for analysis, assisted with analyses, and was the lead author on all manuscripts. My supervisors have confirmed to the University of Cape Town Doctoral Degrees Board that the included papers all overwhelmingly reflect my own scientific work.

This thesis is presented for examination in fulfilment of the requirements for the degree of Doctor in Philosophy in Medicine.

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This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

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20 January 2023

Abstract

Background

Liver injury is the most common severe adverse effect of first-line anti-tuberculosis therapy (ATT). N-acetylcysteine (NAC) has efficacy in patients with paracetamol toxicity and may be of benefit in liver injury due to other causes, such as anti-tuberculosis drug-induced liver injury (AT-DILI). Following AT-DILI, rechallenge of first line ATT is often attempted and may result in recurrence of liver injury. Alanine transaminase (ALT) is the biomarker mainly used in AT-DILI diagnosis. MicroRNA-122 (miR-122) is a sensitive biomarker for liver injury due to paracetamol, but data on utility as a biomarker for AT-DILI are limited.

Methods

We conducted a randomised double-blind placebo-controlled trial of intravenous NAC in adult hospitalized participants with AT-DILI. Primary endpoint was time to ALT < 100 U/L; secondary endpoints included length of hospital stay and 8-week mortality. We described outcomes of ATT rechallenge following AT-DILI. We quantified miR-122 and ALT concentrations before and after infusion of NAC/placebo and explored the effect of NAC on miR-122.

Results

We enrolled 102 participants with AT-DILI, 53 randomized to NAC and 49 to placebo. Mean age was 38 (SD±10) years, 58 (57%) were female and 89 (87%) were HIV positive. Median time to ALT <100 U/L did not differ between study arms: NAC 7.5 (IQR 6–11) days and placebo 8 (IQR 5–13) days (HR, 1.03; 95% CI, 0.68-1.57). Median hospital stay was shorter in the NAC arm (9 days, IQR 6–15) than in the placebo arm (18 days IQR 10–25) (HR, 1.73; 95% CI, 1.13–2.65).

Seventy-nine participants were rechallenged with first-line ATT, of whom 14 (18%) had a positive rechallenge. Positive rechallenge was associated with pyrazinamide (p=0.005), female sex (p=0.039) and first episode of tuberculosis (p=0.032).

Median ALT and miR-122 concentrations pre- NAC/placebo infusion were 420 U/L (IQR 238- 580) and 0.58 pM (IQR 0.18-1.47) respectively. Median fold-change in ALT and miR-122 concentrations between sampling was 0.56 (IQR 0.43 - 0.69) and 0.75 (IQR 0.23 - 1.53) respectively and was similar in the NAC and placebo arms. We observed wide intra- and inter-individual variability in miR-122 concentrations

Conclusions:

NAC did not hasten time to ALT<100 U/L in our participants with AT-DILI, but reduced length of hospital stay which may indicate clinical benefit. This requires confirmation in larger studies with duration of admission as the primary endpoint. Pyrazinamide was associated with positive rechallenge and should be avoided if possible. Positive rechallenge resulted in significant delays in re-initiation of first-line ATT and ART. MiR-122 concentrations in our participants were considerably higher than previously reported in healthy volunteers and in patients on ATT without liver injury, suggesting utility of miR-122 in diagnosing AT-DILI. However, the wide intra-individual variability in miR-122 observed, may limit its use in monitoring recovery from AT-DILI. Further larger studies monitoring miR-122 before the onset of AT-DILI, and over the full course of recovery are required to characterize changes in this biomarker over time, and associations between miR-122 concentrations and outcomes.

Acknowledgements

First and foremost, I thank Almighty Allah for His Infinite Mercy and Blessings, without which I would not have been able to complete this thesis. Peace and Blessings upon His Final Messenger, Muhammed bin Abdullah and upon his family and companions.

The randomized controlled trial was supported by the South African Medical Research Council (Self-Initiated Grant) awarded to Professor Gary Maartens, and by the Academy of Medical Sciences, United Kingdom (Newton Advanced Fellowship awarded to Professor Karen Cohen). The study drug N-acetylcysteine (Paradote) was manufactured and donated by Equity Pharma Ltd.

I sincerely thank Professor Karen Cohen and Prof Gary Maartens for their continual support and guidance throughout the course of this thesis. Their expertise in critical analysis, statistics and scientific writing was invaluable and greatly appreciated. I thank them for encouraging me to present my research at various meetings, for teaching me immensely about clinical research and especially for teaching me how to summarize lengthy manuscripts. I truly would not have been able to complete this thesis without their support and friendship.

I thank Prof Jeffrey Dorfman at Department of University of Stellenbosch, for his assistance with the statistics and graphs in the miR-122 study. I also thank Prof Chris Goldring, Dr Guisy Russomanno, Chandni Patel and their team at the Department of

Pharmacology, University of Liverpool, United Kingdom for their support in running the miR-122 assays and their guidance on the interpretation of the mir-122 results.

I thank all the participants who volunteered for the study and all the colleagues who helped to recruit participants in the randomised trial (Hannah Gunter, Shaazia Allie, Mohamed F Chughlay, Mashiko Setshedi, Sean Wasserman and Dave Stead). I thank Nicole Hickman for co-ordinating the randomised trial and overseeing the various sites. I thank the study pharmacists for their tireless efforts in preparing the study infusions (Waheeda Ismail, Galiemah Karriem, Wynand Smythe and Meagan Saal). I thank Sr Bernadette Khiba at New Somerset Hospital ARV clinic for assisting with participant follow up and blood draws. I also thank Dr Yakoob Vallie for always referring patients for the trial and Prof Graeme Meintjies for independently reviewing deaths.

I thank my wife and family for their patience, sacrifices and enduring support throughout this journey. I also thank my father for his support and persistent prodding: “ When are going to submit your thesis?”, which spurred me on.

Signed by candidate

Muhammed Shiraz Moosa

20 January 2023

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Chapter 1 Introduction

1.1 The burden of tuberculosis and HIV co-infection

Tuberculosis (TB) is one of the leading causes of death worldwide with the World Health Organization (WHO) estimating 10 million people developing the disease and 1.5 million people dying from it in 2020, including 214 000 people living with HIV (1) . South Africa is among the top 8 high TB burden countries which together accounted for two thirds of the global TB burden in 2020 (1). A national survey in South Africa in 2018 estimated a prevalence of bacteriologically confirmed pulmonary TB of 852 per 100 000 adult population (2). WHO statistics give an estimated incidence of 328 000 people with active TB in South Africa in 2020, of who about 234,000 (59%) have both HIV and TB infection (1).

HIV positive patients have a higher risk of developing active TB infection than individuals without HIV infection (3). A retrospective survey of TB/HIV co-infection at a rural South African hospital found that participants without HIV had more favourable TB treatment outcomes than patients with HIV on anti-retroviral therapy (ART) (2.18 OR, 1.13-4.20 95% CI) and patients with HIV not on ART (4.98 OR, 2.07-11.25 95% CI) (4) . Some studies have shown a 2-3 times greater crude risk of mortality from TB in participants starting ART and during ART compared to HIV uninfected participants (5).

Guidelines on the treatment of drug susceptible TB recommend a 4-drug regimen comprising rifampicin, isoniazid, pyrazinamide and ethambutol for the first 2 months (intensive phase), followed by a 2-drug regimen comprising rifampicin and isoniazid for the following 4 months (6, 7, 8, 9, 10). This first-line anti-tuberculosis regimen is effective, but is not without peril, especially the risk of severe adverse drug reactions.

1.2 Anti-tuberculosis drug-induced liver injury

1.2.1. Incidence and risk of anti-tuberculosis drug-induced liver injury

Anti-tuberculosis drug-induced liver injury (AT-DILI) is the most common severe adverse effect of first line anti-tuberculosis therapy (ATT), with an estimated incidence of 2-28% depending on the definition of AT-DILI used and on the population being studied, with a higher incidence in developing countries (11). A local retrospective survey of patients with hepatitis at GF Jooste Hospital in Cape Town found that 10% of hepatitis was caused by ATT (12). Liver injury due to first-line ATT may cause prolonged hospitalization (13) and is associated with increased mortality from acute liver failure (12, 14, 15). In a prospective Indian study of 269 participants with AT-DILI, 90-day mortality was 22.7 % and was associated with jaundice, encephalopathy and ascites but not with age, sex or HIV status (only 7% of their cohort was HIV positive) (14) .

Regular alcohol intake (16), increasing age (17), HIV (18), female sex (17, 19), pre-existing viral hepatitis (18, 20, 21), low albumin (19, 22, 23) and polymorphism of the N-acetyltransferase-2 gene (slow acetylator status) (16, 24, 25, 26, 27) have been reported as risk factors for developing AT-DILI. Some studies have found male sex to be associated with AT-DILI (16, 28) and not increasing age (29).

1.2.2 Definition and mechanism of anti-tuberculosis drug-induced liver injury

Anti-tuberculosis drug-induced liver injury is thought to be an idiosyncratic drug reaction and usually occurs within 4 weeks of starting first-line ATT but can occur 2-6 months after starting ATT. Asymptomatic elevation in ALT and AST below 2 times the upper limit of normal (ULN) is referred to as "hepatic adaptation", was largely observed with isoniazid monotherapy in the 1960's and 1970's, is usually self-limiting and resolves without stopping ATT (30, 31, 32). The diagnosis of AT-DILI is based on the temporal relationship between the initiation of hepatotoxic ATT and the elevation of liver enzymes with or without symptoms, and is confirmed by the normalisation of liver enzymes after withdrawal of the offending drug. Patients with AT-DILI may present with symptoms of jaundice, nausea, vomiting and abdominal pain. Competing diagnoses such as viral hepatitis and autoimmune hepatitis need to be excluded before one can diagnose AT-DILI. Causality assessment tools such as the Roussel Uclaf Causality Assessment System (RUCAM) and the Maria and Victorino System may be used to assess the likelihood of a drug causing liver injury (33).

The pattern of liver injury with ATT is usually hepatocellular with a predominant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin (34). A widely used definition of AT-DILI is that of the American Thoracic Society which defines it as an increase in ALT more than 5 times ULN without symptoms, or an increase in ALT more than 3 times ULN with symptoms or an increase in bilirubin more than 2 times ULN with symptoms (34). The British Thoracic Society defines AT-DILI as a 5 times increase in ALT and AST or as an increase in bilirubin, and the European Respiratory Society as a 5 times increase in liver function tests or the presence of icterus (8, 9). It must be noted that the latter guidelines are more than 20 years old and likely need to be revised.

The exact mechanism of AT-DILI is not well understood but is thought to be a metabolic idiosyncratic reaction to any one of the three hepatotoxic anti-tuberculosis drugs (rifampicin, isoniazid and pyrazinamide). All three drugs can cause an asymptomatic minor increase in serum aminotransferases in 10-20 % of patients referred to as “hepatic adaptation”, or they can cause a more severe, symptomatic and even fatal liver injury (35). This suggests a host related mechanism of liver injury rather than a drug related one. There is evidence that the isoniazid metabolite, acetylhydrazine, may be toxic to liver tissue through the generation of free oxygen radicals and mitochondrial stress (31, 36). Rifampicin can increase isoniazid hydrolase activity and the production of acetylhydrazine in slow acetylators (37). A recent review suggests that there is an immune-mediated mechanism underpinning isoniazid induced liver injury supported by the detection of increased interleukin-10 and interleukin-17 levels as well as autoantibodies to isoniazid metabolites (38). Some experimental data suggests that rifampicin and isoniazid co-therapy interferes with porphyrin biosynthesis resulting in accumulation of hepatotoxic protoporphyrin IX (39). The mechanism of pyrazinamide hepatotoxicity is not clear. Hepatotoxicity due to pyrazinamide may be caused by a hepatic intermediate metabolite generated through the amidase or xanthine oxidase pathways (40) or other metabolic pathways (41, 42, 43); and it seems to occur more frequently with higher doses suggesting a partly direct toxic effect (41). However, the evidence that hepatotoxic pyrazinamide metabolites, pyrazinoic acid and 5-hydroxy-pyranoic acid, are formed through the amidase and xanthine oxidase activation of pyrazinamide, is limited to animal studies and has not been confirmed in humans (40).

1.2.3. Diagnosis of anti-tuberculosis drug-induced liver injury in HIV positive patients

The diagnosis of AT-DILI in HIV positive patients can be challenging because these patients are often taking additional drugs with overlapping hepatotoxicity such as anti-retroviral agents (e.g. nevirapine, efavirenz, protease inhibitors or integrase inhibitors) and prophylactic drugs (e.g. cotrimoxazole or fluconazole) (44). Furthermore, patients with HIV and TB co-infection who start ART are at risk of developing paradoxical TB immune reconstitution inflammatory syndrome (TB-IRIS) which can cause elevated liver enzymes and thus mimic AT-DILI (45). Patients with disseminated TB may have TB granulomas within the liver causing a mixed cholestatic and hepatocellular liver injury (typical ALT range 0-200 U/L) , and this may make it difficult to diagnose concurrent DILI (46).

1.2.4. Management of anti-tuberculosis drug-induced liver injury

There is currently no specific and proven antidote to AT-DILI. The management of suspected AT-DILI includes the cessation of all potentially hepatotoxic anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide), as well as anti-retroviral drugs (nevirapine, efavirenz, protease inhibitors) in HIV positive patients on ART. This is followed by possible initiation of alternative or background ATT, regular monitoring of liver function tests, and supportive care while awaiting liver recovery (34). In addition to supportive care, patients with acute liver failure (defined as encephalopathy and INR > 1.5), are treated with broad spectrum antibiotics, lactulose, vitamin K and possible liver transplantation (47).

Various drugs have been investigated as possible treatment of AT-DILI. Traditional herbal drugs curcumin and silymarin were shown to be protective against AT-DILI, in an in vitro model of human hepatocellular carcinoma cells (48). In a retrospective study of 71 participants with AT-DILI, participants who received ursodeoxycholic acid or glycyrrhizin, both of which are used in the treatment of chronic hepatitis, did not recover faster from liver injury than those who did not receive them (49).

N-acetylcysteine (NAC) has been widely used in the management of paracetamol-induced liver injury for decades (50) and it may offer some benefit for other causes of liver disease such as ischaemic or viral hepatitis (51, 52). There is also some evidence for the use of NAC in the treatment of non-paracetamol drug-induced liver failure (53, 54). There is limited evidence that NAC can prevent against AT-DILI in animal (48, 55) and human studies (56). These studies are discussed in further detail in the literature review or chapter 2.

We arrive at our first and main research question “Does intravenous NAC improve outcomes of AT-DILI?”. This question is explored in a randomised controlled human trial which is detailed in the first publication or chapter 3.

1.3 Drug rechallenge following anti-tuberculosis drug-induced liver injury

Following AT-DILI, patients are usually placed on background ATT which consists of ethambutol, and second-line drugs like a quinolone and linezolid. Previous background regimens included aminoglycosides but current guidelines advise against their use, firstly due to their risk of nephrotoxicity and ototoxicity which was observed mainly in the treatment of drug resistant TB (57), and secondly because newer drugs

like linezolid and bedaquiline are less toxic and are associated with better outcomes (58). When the serum ALT falls below 100 U/L, first-line ATT is usually rechallenged because second-line ATT is less effective in achieving clinical cure, longer in duration and generally more toxic (59). Rechallenge may result in positive rechallenge (recurrence of liver injury) and may prolong interruption of first-line ATT and delay initiation or re-initiation of anti-retroviral therapy (ART) in HIV positive patients. Positive rechallenge can present with liver injury that is more severe than the initial injury, and can result in mortality (60).

Our second research question explores the outcomes of drug rechallenge following AT-DILI in the cohort of participants from our randomised controlled trial. In the second publication or chapter 4 we describe the outcomes of drug rechallenge, including positive rechallenge and its associated risk factors, background and rechallenge ATT regimens used , and the impact of rechallenge on the interruption of first-line ATT and on the interruption or initiation of ART in HIV positive participants.

1.4 Biomarkers of anti-tuberculosis drug-induced liver injury

Serum alanine aminotransferase (ALT) is the current standard marker used to diagnose AT-DILI and to monitor recovery from AT-DILI. However, ALT does not increase early following liver injury, provide prognostic value, nor distinguish between liver injury and inflammation (61). Serum ALT may be increased due to mechanisms not involving hepatocyte injury such as membrane blebs which form during ischaemic injury and rupture to release aminotransferases, increased hepatic ALT gene expression due to drugs (e.g. fibrates), and circulating macroenzymes which are formed when ALT binds to immunoglobulins prolonging their half-life (61). Furthermore, ALT is not a specific marker of liver injury as it may be released from

tissues other than the liver e.g. skeletal muscle, kidney and heart (62). Hence the need for more specific and more informative biomarkers of DILI. MicroRNA-122 (miR-122) is a novel serum biomarker of DILI, with good sensitivity in the early diagnosis of paracetamol induced liver injury (63, 64) and may also provide some prognostic value in this condition (65) . There are only two reported human studies which quantify miR-122 concentrations in participants with AT-DILI; and they report varying results (66, 67). These studies are discussed in further detail in the literature review or chapter 2.

Our third research question, which is explored in the third publication and chapter 6, is: Does serum miR-122 have any utility as a biomarker in the diagnosis and management of AT-DILI? We quantify ALT and miR-122 concentrations before and after NAC/placebo infusion, describe changes in ALT and miR-122 concentrations between samples and assess correlation between ALT and miR-122 concentrations. We also explore the effect of NAC on miR-122 concentrations.

1.5 Research questions

The 3 key research questions addressed in this thesis and each of the publications are:

1. Does intravenous NAC improve outcomes of AT-DILI when compared to placebo in a randomised controlled trial?
2. What are the outcomes of drug rechallenge following AT-DILI, factors associated with positive rechallenge and impact of drug rechallenge on first-line ATT and ART interruption?
3. Does serum miR-122 have any utility as biomarker in the diagnosis and management of AT-DILI compared to ALT?

1.6 Description of the project

In March 2011, we conceptualised and drafted a protocol for a randomised controlled trial of NAC in the management of AT-DILI (appendix 6). Between 2011 and 2013 we obtained UCT HREC approval (appendix 7), MCC approval and were secured funding support from the South African MRC. In July 2014, we started screening and recruiting adult patients with suspected AT-DILI at 3 public sector hospitals in Cape Town: Groote Schuur Hospital, New Somerset Hospital and Khayelitsha District Hospital. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719) [appendix 8] and ClinicalTrials.gov (NCT02182167) [appendix 9].

Those participants who met inclusion criteria (n=102) were randomised to either intravenous N-acetylcysteine (NAC) or placebo. Our primary endpoint was time for serum alanine aminotransferase (ALT) to reach less than 100 U/L and secondary endpoints were length of hospital stay and death.

After recovery from liver injury, we described the outcomes of ATT rechallenge in 79 participants from the randomised trial in whom at least one anti-tuberculosis drug was re-introduced.

We quantified serum ALT and miR-122 concentrations in 45 participants with stored paired samples taken before and after N-acetylcysteine or placebo administration. We collaborated with the Department of Clinical Pharmacology, University of Liverpool, United Kingdom, who performed the miR-122 assays and assisted with interpretation of laboratory results.

We completed recruitment of 102 participants in January 2019 and published the first research paper “A randomised controlled trial of intravenous N-acetylcysteine in the management of anti-tuberculosis drug-induced liver injury” in *Clinical Infectious*

Diseases in August 2020. We published the second research paper "Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort" in *Southern African Journal of HIV Medicine* in June 2022. Our third research paper "Analysis of microRNA-122 concentrations in a randomized controlled trial of n-acetylcysteine in the treatment of anti-tuberculosis drug-induced liver injury" was accepted for publication on 4th January 2023 by the *British Journal of Clinical Pharmacology.*

1.7 Coherence of the thesis

First, the unifying theme is the management of patients with anti-tuberculosis drug-induced liver injury in a setting of high HIV prevalence. Second, the work stems from a single project with the same cohort of participants. Third, I am the first author and lead investigator on all the studies and manuscripts included. I initiated and led all the projects from conception to execution to publication.

1.8 Outline of the thesis

Chapter 2 is the background and literature review for the three research questions addressed in this thesis

Chapter 3 reports findings of the double-blind randomised placebo-controlled trial of NAC in 102 participants with AT-DILI. The primary endpoint was time to ALT to less than 100 U/L. Secondary endpoints were length of hospital stay and 8-week mortality.

Chapter 4 describes the outcomes of rechallenge of first-line ATT in 79 participants from the randomised clinical trial. It describes background and rechallenge regimens

used, positive rechallenge (recurrence of liver injury) and its risk factors. We also explored the impact of rechallenge on interruption of first-line ATT and on interruption and initiation of ART in HIV positive participants.

Chapter 5 explores the utility of serum microRNA-122 (miR-122), as a potential biomarker in the diagnosis and management of AT-DILI. We quantified miR-122 and ALT concentrations before and after NAC or placebo administration and describe changes in their concentrations between sampling. We assessed whether NAC administration influenced change in miR-122 concentrations between sampling, compared to placebo. We also assessed correlation between miR-122 and ALT concentrations.

Chapter 7 is a summary of the research findings, conclusion and the focus of future research .

Chapter 2 Background and Literature Review

2.1 N-acetylcysteine in the treatment of drug-induced liver injury

2.1.1 N-acetylcysteine in the treatment of paracetamol drug-induced liver injury

N-acetylcysteine (NAC) has been widely used as treatment for paracetamol drug-induced liver-injury (DILI) for decades (50) and is thought to improve liver injury by restoring the liver anti-oxidant glutathione (68). Hepatic glutathione is essential in the detoxification of N-acetyl-p-benzoquinone imine (NAPQI), the toxic metabolite of paracetamol (68).

After therapeutic doses, paracetamol is conjugated by the cytochrome P450 2E1 enzyme system to glucuronide and sulphate moieties which are mainly excreted in bile and urine (69). During paracetamol toxicity, the cytochrome P450 2E1 system is overwhelmed and paracetamol is metabolised to the highly reactive NAPQI . Detoxification of NAPQI occurs when it binds to the sulfhydryl group of glutathione (GSH) and is ultimately excreted in the urine as cysteine and mercapturic acid conjugates (70). However, when glutathione stores are depleted, oxidative liver cell injury occurs due to the direct effect of toxic unconjugated NAPQI.

In participants with paracetamol-induced liver failure, NAC was shown to improve cardiac output and oxygen delivery (71). In another study of liver failure due to various causes, NAC increased oxygen delivery and consumption, as well as improved hepatic clearance of indocyanine green (72). The exact mechanism of these beneficial effects of NAC is not completely understood, but it may involve glutathione replenishment, free radical scavenging and improvements in hepatic blood flow (71, 73).

2.1.2 N-acetylcysteine in the treatment of non-paracetamol drug-induced liver injury

N-acetylcysteine is not only beneficial in treating paracetamol DILI, but it is inexpensive, easy to administer (either orally or intravenously) and has a good safety profile. Minor adverse drug reactions to NAC like nausea, vomiting, pruritis, rash, and bronchospasm are common, occurring in 3% to 48% of patients, whereas only 0.6% to 2% of patients develop serious anaphylactic reactions (74, 75, 76, 77). N-acetylcysteine's safety and efficacy in paracetamol DILI have made it an attractive drug for the potential treatment of liver injury or inflammation due to causes other than paracetamol. N-acetylcysteine may provide benefit for non-paracetamol causes of hepatitis such as ischaemic and viral hepatitis (51, 52). In a trial of 60 patients with septic shock , those randomised to intravenous NAC showed signs of improved liver blood flow compared to those who received placebo (51). In a multicentre double-blinded placebo-controlled trial of 144 patients with chronic hepatitis B, daily administration of NAC improved the rate of decline of total bilirubin levels without a significant increase in adverse effects (52).

In a prospective Kuwaiti cohort study of 155 participants with non-paracetamol acute liver failure, 85 participants were administered intravenous NAC and were compared to 70 historical controls who did not receive NAC. The primary endpoint was mortality or need for liver transplantation, while secondary endpoints were length of ICU or hospital stay, organ failure and encephalopathy. Baseline characteristics were similar between the NAC and control groups, and participants were young (NAC 33.5 ±11 years; control 34.8 ±8.8 years). They found transplant-free survival to be 96% in the NAC group compared to 23% in the control group ($p<0.01$), and the transplantation rate was 7% in the NAC group compared to 53% in the control group, ($p<0.01$). Hospital stay was

significantly shorter in the NAC group (10 days) compared to the control group (28 days), $p<0.01$. Limitations of this study are: the use of historical controls, only 38% of participants had DILI, and abnormal creatinine, sodium and potassium were more prevalent in the control group (73%) compared to NAC group (23%). Of note, the decline in ALT did not differ significantly between groups.

Lee and colleagues conducted a randomised controlled trial of intravenous NAC in 173 participants with non-paracetamol induced acute liver failure (54). They found no difference in their primary endpoint of 3-week overall survival between NAC and placebo arms, but found improved 3-week transplant-free survival (a secondary endpoint) in those with grade 1 and 2 encephalopathy. However, this study was underpowered to assess efficacy in the small DILI subgroup which comprised 26% of participants.

A systematic review of NAC as treatment for non-paracetamol DILI published in 2016, identified only one prospective study (by Lee and colleagues mentioned above) which explored the use of NAC as treatment for non-paracetamol–induced liver injury (78). Therefore, they concluded that there was insufficient evidence to support the use of NAC in non-paracetamol–induced liver injury.

A systematic review and meta-analysis of prospective trials of NAC in non-paracetamol DILI included 672 participants from 5 studies, 2 RCTs and 3 prospective studies with historical controls (79). They conclude that NAC does improve transplant-free survival and reduce length of hospital stay, but with the caveat that outcomes based on aetiology of liver injury were uncertain.

A more recent systematic review of NAC in prevention and treatment of non-paracetamol DILI (80) included 11 studies: 3 placebo controlled RCTs (2 double blinded, 1 unknown blinded), 3 interventional studies without information on

randomisation and 5 observational studies. Five studies included participants with acute liver failure from various causes (majority were not DILI) and one study did not include encephalopathy as a inclusion criteria for acute liver failure. They conclude that NAC is a safe drug and may have benefits in non -paracetamol DILI. However, based on the limited evidence as well as the limitations across studies, the authors suggest that further larger clinical trials with better NAC dose definition and causality assessment, are needed to confirm this.

2.1.3 N-acetylcysteine in the treatment of anti-tuberculosis drug-induced liver injury

There is some pre-clinical and clinical evidence that NAC can prevent AT-DILI. N-acetylcysteine improved liver histology in a study of rats that were administered high intraperitoneal doses of isoniazid and rifampicin (55). In an vitro study using human hepatocellular carcinoma cells exposed to toxic doses of isoniazid, rifampicin, and pyrazinamide in various combinations, NAC reduced cellular and mitochondrial membrane damage, and apoptosis (48). In an Iranian study of 60 participants with newly diagnosed TB and starting on treatment, oral NAC was given to half of the participants for 2 weeks. Those participants who received NAC had no elevation in ALT and AST (56). However, this study had limitations such as small sample size, lack of placebo comparison and short duration of follow-up . Three other studies which explored oral NAC as preventative treatment for AT-DILI in humans, were included in a systematic review (80) . The results of the 3 studies favoured NAC as preventative treatment, however, the studies were either unpublished conference abstracts, very small in size or both.

In our first study and publication (Chapter 3) we report on a double-blind placebo-controlled trial of intravenous NAC in the management of AT-DILI. Our primary endpoint was time to ALT less than 100 U/L and secondary endpoints were length of hospital stay and in-hospital mortality. Following publication of this paper in *Clinical Infectious Diseases*, we responded to a letter from Hampannavar and colleagues who requested a subgroup analysis of our primary and secondary endpoints based on liver injury severity. This letter to the author was also published in *Clinical Infectious Diseases* (appendix 2).

2.2 Drug rechallenge following anti-tuberculosis drug-induced liver injury

Following AT-DILI, when the ALT falls below 100 U/L, rechallenge with hepatotoxic first-line anti-tuberculosis therapy (ATT) is recommended because second-line ATT is less effective against TB, longer in duration and cause more toxicity (59). Patients diagnosed with AT-DILI are often prescribed “background ATT” (e.g. ethambutol, a quinolone and linezolid) while waiting for the liver to recover and before rechallenging first-line ATT. In a minority of patients where the initial diagnosis of TB is found to be unlikely, background ATT is not initiated nor is first-line ATT rechallenged. Patients living with HIV may also be taking concomitant hepatotoxic drugs such as anti-retroviral agents (e.g. nevirapine, efavirenz, protease inhibitors or integrase inhibitors) and prophylactic drugs (e.g. cotrimoxazole or fluconazole); and these drugs need to be stopped in addition to ATT.

The most significant complication of ATT rechallenge is recurrence of liver injury or positive rechallenge. Positive rechallenge is the most important factor influencing choice of rechallenge regimen. An Indian study of 3 different ATT rechallenge

regimens found no difference in the proportion of positive rechallenges between 3 different rechallenge regimens viz. concomitant full dose, sequential full dose and sequential incremental dose regimens; but the study was underpowered (81). A small Turkish study found a high proportion of positive rechallenge when using the concomitant full dose regimen including pyrazinamide (6 of 25) compared to the sequential full dose regimen without pyrazinamide (0 of 20) (82). In a recent network meta-analysis of ATT rechallenge regimens in participants with AT-DILI (83), 11% of those rechallenged with a sequential full dose regimen had a positive rechallenge. Risk factors reported to be associated with positive rechallenge are female sex (82), increased age and low serum albumin (81, 84). However, a retrospective study found young age and higher total bilirubin concentrations to be associated with increased risk of positive rechallenge (85).

The South African National TB Management Guidelines (6) recommend a rechallenge regimen which is based on the American Thoracic Society (ATS) guidelines for the treatment of AT-DILI (34). These guidelines recommend that once the serum ALT has fallen to below 100 U/L, individual anti-tuberculosis drugs are re-introduced sequentially and at full dose: rifampicin at maximum dose for 3-7 days, check ALT, isoniazid at maximum dose for 3-7 days, check ALT, pyrazinamide at maximum dose for 3-7 days, check ALT. The WHO guidelines on TB treatment also recommend the sequential full dose regimen (10).

The British Thoracic Society (BTS) recommends a sequential incremental dose rechallenge regimen: isoniazid 100mg/day from day 1, maximum dose from day 4, check ALT; rifampicin 150 mg/day from day 8, maximum from day 11, check ALT; and pyrazinamide 500 mg/day from 15, maximum from day 18 and check ALT (8). This rechallenge regimen is much longer than that recommended by the ATS; and because the BTS guidelines are more than 20 years old, it probably needs revision.

Due to pyrazinamide's high hepatotoxic potential, rechallenge with pyrazinamide is not recommended if the initial liver injury was severe i.e. ALT more than 5 times ULN or bilirubin more than 2 times ULN (34). Sequential drug rechallenge regimens enable the clinician to identify the culprit drug causing positive rechallenge, unlike concomitant drug rechallenge regimens.

There are few studies on the outcomes of drug rechallenge following AT-DILI (81, 82, 84), especially in populations with high prevalence of HIV coinfection. There is limited evidence on optimal background and rechallenge ATT regimens, risk factors for positive rechallenge, anti-tuberculosis drugs most frequently implicated in positive rechallenge, and interruption and re-initiation of ATT and anti-retroviral therapy (ART) in HIV positive participants, who present with AT-DILI.

In our second study and publication (Chapter 4), we address this gap in knowledge and we describe the outcomes of rechallenge including positive rechallenge and its risk factors, background and rechallenge ATT regimens used; and we explore the impact of AT-DILI and drug rechallenge on the interruption of first-line ATT, as well as on the interruption or initiation of ART in HIV positive participants.

2.3 MicroRNA-122 as a potential biomarker of anti-tuberculosis drug-induced liver injury

2.3.1 MicroRNA-122 as a specific liver biomarker

Drug-induced liver injury is a major safety concern in the development of new drugs and is a major cause of withdrawal of approved drugs from the market (86, 87). This has prompted extensive research over the last decade, into novel biomarkers of DILI, one example of which is microRNA. MicroRNAs are small, endogenous RNAs of about

22 nucleotides in length, that do not code for any proteins but regulate about 30% of post-transcriptional gene expression (88). They are the most abundant form of RNA in the cell and are involved in cellular processes such as proliferation, differentiation, apoptosis and energy metabolism (88, 89). Serum or plasma miRNA's are stable complexes that can be quantified accurately using quantitative real time polymerase chain reaction (qRT-PCR) (90).

The canonical biogenesis of miRNA begins in the cell nucleus with the generation of a primary-miRNA transcript. Two RNAse III proteins, Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8), cleave primary miRNA to produce precursor-miRNA (91). Precursor-miRNA is exported to the cytoplasm by Exportin5/RanGTP and processed to mature miRNA duplex. Finally, either the 5p or 3p strands of the mature miRNA duplex is loaded into the Argonaute family of proteins to form a miRNA-induced silencing complex. This miRNA-induced silencing complex then binds to target mRNAs to inhibit their translation (91).

In recent years studies have shown that hepatic diseases such as hepatocellular carcinoma, chronic hepatitis B and DILI, can cause altered expression profiles of various miRNA's (92, 93, 94, 95).

MicroRNA-122 (miR-122) is a liver specific microRNA which accounts for 70% of hepatic microRNA (96) and plays an essential role in lipid metabolism, anti-inflammatory and anti-tumourigenic mechanisms, as well as in hepatitis C viral proliferation (97). MiR-122 concentrations are unaltered by impaired renal function (98, 99) and muscle injury (100), indicating miR-122's greater specificity for liver tissue than ALT.

MicroRNA-122 can be quantified in serum or plasma using a reverse transcriptase polymerase chain reaction (qRT-PCR) assay, which is considered the "gold standard"

(101). After adequate centrifugation of the blood sample to avoid blood cell contamination, serum or plasma must be stored within a few hours of collection at -80^0 C to avoid miRNA degradation. MicroRNA is first chemically extracted followed by a solid-phase extraction on silica columns using a commercially available kit (e.g. miRNeasy Qiagen®) (102). A synthetic miRNA like three *C. Elegans* or cel-lin-4 may be spiked in the sample at this stage to monitor for efficiency of the extraction process (102). Amplification by RT-qPCR is then performed using a commercially available kit (e.g.TaqMan miRNA Assays ®), and data is normalized either by use of a synthetic miRNA generated curve (absolute quantification) or by a spiked in synthetic miRNA such as U6 or three *C. elegans* (relative quantification)(101) . Major challenges to comparing miRNA data between studies include the differences in sample type, handling, processing and data normalisation method (101).

2.3.2 MicroRNA-122 in paracetamol drug-induced liver injury

MicroRNA-122 has been widely studied as a novel biomarker of paracetamol drug-induced liver injury in both animal (63, 103) and human studies (95, 99, 100, 104, 105). See Table 2.1. In paracetamol-induced liver injury, miR-122 concentrations increased earlier than ALT concentrations, and were sensitive in predicting those participants who went on to develop liver injury (63, 64). In many studies peak increases in serum miR-122 concentrations correlated with later increases in serum ALT concentrations (103, 104, 106). In 2 large cohorts of participants with paracetamol-induced liver injury and other causes of DILI, miR-122 concentrations were significantly elevated, but displayed large intra- and inter-individual variability (107).

Large registries of paracetamol and non-paracetamol DILI have been established worldwide, including the Critical Path Institute's Predictive Safety Testing Consortium

(PSTC) in the United States, the Safer and Faster Evidence based Translation (SAFE-T) consortium within the Innovative Medicines Initiative (IMI) in Europe and The Drug Induced Liver Injury Network (DILIN) in the United States (108). The PSTC and SAFE-T cohorts included healthy volunteer data used for determining reference ranges of biomarkers. In a study of participants enrolled in these 3 registries, they found miR-122 concentrations to be elevated, especially in paracetamol DILI, miR-122 concentrations correlated with ALT concentrations ($r=0.66$; $p<0.0001$) and miR-122 concentrations significantly identified participants with DILI (107). Among healthy volunteers, miR-122 concentrations demonstrated high inter-individual variability: [% coefficient of variation (CV) of 90.89 and 213.51 in the PSTC and SAFE-T cohorts, respectively] and intra-individual variability in the PSTC cohort (intra-individual %CV of 93.56), especially in black individuals.

In a study of 78 participants and 40 controls from the DILIN cohort, 11 (14%) died, 10 within 6 months of DILI onset and 5 were assessed to have died due to liver disease (65). MicroRNA-122 concentrations were significantly higher in acute DILI cases than in controls. Low miR-122 concentrations together with low serum albumin were found to predict 6-month mortality with a sensitivity and specificity of 100% and 81% respectively (65).

2.3.3 MicroRNA-122 in anti-tuberculosis drug-induced liver injury

There is limited evidence on miR-122 as a biomarker of AT-DILI. In a trial of 76 Ethiopian participants who were taking hepatotoxic ART and/or ATT for 12 weeks, a subgroup of 44 participants with ALT of more than 3 times elevated from their own baseline, were found to have increased miR-122 concentrations by 1.4-fold at week 1 and 1.9-fold at week 8 compared to 22 matched participants without ALT elevation

(100). MicroRNA-122 also peaked earlier than ALT (ALT: week 2 vs miR-122: week 1) (100). The authors do not report on the number of participants on either ART or ATT, or on both.

In the ALISTER cohort study, miR-122 concentrations were quantified in healthy volunteers and in HIV negative participants with active TB, latent TB and non-tuberculous mycobacterial infection (66). In participants with elevated ALT defined as ALT > 50 U/L and < 150 U/L, miR-122 concentrations were increased 8-fold compared to those with a normal ALT < 50 U/L. Two participants in the ALISTER cohort who developed AT-DILI (defined as ALT > 150 U/L with symptoms or ALT > 250 U/L without symptoms), were found to have miR-122 concentrations increased by 15- and 20-fold from baseline respectively (66).

MicroRNA-122 concentrations were 73-75% lower in a cohort of Indian participants with AT-DILI, compared to those of healthy controls and participants on ATT without DILI (67). This study's findings are largely different from previous studies where miR-122 concentrations increase with AT-DILI, and this inconsistency is difficult to explain. Limitations of the Indian study were that miR-122 was only sampled at enrolment, participants had a mild DILI (median ALT 130 U/L) and therefore may not be comparable to other studies, and they did not report on HIV prevalence, which may have a bearing on their findings.

2.3.4 The effect of N-acetylcysteine on microRNA-122 concentrations

There is limited data on the effect of NAC on miR-122 concentrations. In a study of paracetamol DILI and ischaemic hepatitis, miR-122 concentrations were significantly increased and subsequently declined with NAC therapy (105). This decline in miR-122 following NAC therapy could be due to a direct effect of NAC, or physiological

clearance of miR-122. However, when NAC therapy was stopped, rebound increases in miR-122 were observed, suggesting either a direct effect of NAC or ongoing liver repair processes.

In our study, embodied in the third publication or Chapter 6, our aim was to determine whether miR-122 has any utility as a biomarker of liver injury in participants with AT-DILI. We quantified miR-122 concentrations before and after NAC or placebo administration in 45 participants from the randomised controlled trial. We assessed correlation between ALT and mir-122 concentrations, described changes in miR-122 and ALT concentrations between sampling and explored the effect of NAC administration on miR-122 concentrations.

Table 2.1 Studies of miR-122 expression or concentrations in drug-induced liver injury

Study	Species	DILI type	ALT (U/L)	ALT (fold increase)	miR-122 (fM)	miR-122 (fold increase[1])
Wang (63)	Mice	Paracetamol	13269 (±1253)	—	—	470
Bala **(103)**	Mice	Paracetamol	200-600	—	—	400
Wolenskia **(109)**	Rats	Paracetamol	252	—	—	112
Su **(110)**	Rats	Paracetamol	—	5	—	54
Antoine (64)	Human (n=129)	Paracetamol (normal ALT day 0); n=15	445 (IQR 224-1187)	—	—	9.8 (2.8-96)
		Paracetamol (sample < 8hrs of drug	487 (IQR 266-942)	—	—	3.69 (0.43-96)

		ingestion); n=11				
Russo (65)	Human (n=78; hc=40)	Multiple drug cause DILI	1065 (±1382)	—	—	5-12
Starkey Lewis **(104)**	Human (n=53; hc=25)	Paracetamol	5199 (IQR 2966-7984)	—	—	1265 (IQR 491-4270)
Vliegenthart **(99)**	Human (n=27; hc=27)	Paracetamol	2150 (IQR 487-4444)	—	—	68 (IQR 11-277)
Ward **(105)**	Human (n=48)	Paracetamol	6789 (±3568)	—	—	7.25
		Ischaemic hepatitis	2085 (±1325)	—	—	4.96
Rissin **(111)**	Human (n=4; hc=4)	Paracetamol	1581 (±1177)	—	20273 (±18734)	—
Ding **(112)**	Human (n=8)	Paraquat poisoning	0-800 800-2000 >2000	—	—	6 50 150
Thulin **(100)**	Human	Paracetamol (n=30)	—	1.9	—	2.2
		ART/AT-DILI (n=44)	—	> 3	—	1.9
Rupprechter (66)	Human (n=26; hc=28)	On ATT without DILI (n=24)	14-50	—	3.00 (1.31-4.82)	—
			50-150	—	23.9 (11.5-60.4)	—
		AT-DILI (n=2)	431; 194	—	60 ; 336	—
Bakshi (67)	Human (n=50)	AT-DILI	—	> 2	—	0.75 **decrease**[1]
Moosa **(113)**	Human (n=45)	AT-DILI	420 (IQR 238-580)	—	580 (IQR 180-1470)	—

Abbreviations: ALT alanine aminotransferase; AT-DILI anti-tuberculosis drug-induced liver injury DILI drug-induced liver injury; fM femtomoles; hc human controls; IQR interquartile range; n number or sample size

Notes: - [1] Bakshi reports a fold **decrease** in miR-122 concentrations

- median values are expressed with (IQR)
- mean values are expressed with (±standard deviation)

Chapter 3 A randomised controlled trial of intravenous N-acetylcysteine in the management of anti-tuberculosis drug induced liver injury

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Co-author contributions

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Study drug and site preparation: NH

Database maintenance and data analysis: AS

Critical review of manuscript: all authors

Citation

Moosa MS, Maartens G, Gunter H, Allie S, Chughlay MF, Setshedi M, Wasserman S, Stead D, Hickman N, Stewart A, Sonderup M, Spearman CW, Cohen K. A randomized controlled trial of intravenous n-acetylcysteine in the management of anti-tuberculosis drug-induced liver injury. *Clin Infect Dis*. Aug 26, 2020.

DOI: 10.1093/cid/ciaa1255

The original publication is included as Appendix 1. Supplementary materials are included in Appendix 5.

3.1 Abstract

Background: Liver injury is a common complication of first-line anti-tuberculosis therapy. N-acetylcysteine (NAC) is widely used in patients with paracetamol toxicity with limited evidence of benefit in liver injury due to other causes.

Methods: We conducted a randomized, double-blind, placebo-controlled trial to assess whether intravenous NAC hastens liver recovery in hospitalized adult patients with anti-tuberculosis drug induced liver injury (AT-DILI). The primary endpoint was the time for serum alanine aminotransferase (ALT) to fall below 100 U/L. Secondary endpoints included length of hospital stay, in-hospital mortality and adverse events.

Results: Fifty-three participants were randomized to NAC and 49 to placebo. Mean age was 38 (SD±10) years, 58 (57%) were female and 89 (87%) were HIV-positive. Median serum ALT and total bilirubin at presentation were 462 U/L (IQR 266-790) and 56 mmol/L (IQR 25-100) respectively. Median time to ALT <100 U/L was 7.5 days (IQR 6 - 11) in the NAC arm and 8 days (IQR 5 -13) in the placebo arm. Median time to hospital discharge was shorter in the NAC arm (9 days; IQR 6-15) than in the placebo arm (18 days; IQR 10-25), HR 1.73 (95% confidence interval 1.13 to 2.65). Mortality was 14% overall and did not differ by study arm. The study infusion was stopped early due to an adverse reaction in 5 participants receiving NAC [nausea and vomiting (3), anaphylaxis (1), pain at drip site (1)].

Conclusions: NAC did not shorten time to ALT <100 U/L in participants with AT-DILI, but significantly reduced length of hospital stay. NAC should be considered in management of AT-DILI

3.2 Introduction

Liver injury is the most common severe adverse drug reaction caused by first-line anti-tuberculosis therapy (ATT) with an estimated incidence of 2-28% depending on the definition of drug-induced liver injury (DILI) used and the population studied (11). Liver injury due to first-line anti-tuberculosis therapy (AT-DILI) may cause prolonged hospitalization (13), and is associated with increased mortality (12, 14). There is currently no specific therapy for AT-DILI. The management of suspected AT-DILI includes the cessation of all potentially hepatotoxic anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide), possible introduction of alternative ATT, monitoring of liver function tests, and supportive care while awaiting liver recovery. When the serum alanine aminotransferase (ALT) falls below 100 U/L, ATT rechallenge may be considered (34).

N-acetylcysteine (NAC) is widely used as treatment for paracetamol liver toxicity (50) and may provide benefit for other causes of hepatitis [7, 8]. A prospective cohort study of 155 participants with non-paracetamol acute liver failure, found improvement in transplant-free survival in those treated with NAC compared with historical controls; DILI was the cause of liver failure in 38% [9]. A randomized controlled trial of NAC in 173 participants with non-paracetamol acute liver failure found improved transplant free survival overall, but the study was under-powered to assess efficacy in the small DILI subgroup (26% of participants) [10]. A systematic review concluded that there was insufficient evidence to support the use of NAC in non-paracetamol induced liver injury [11].

There is evidence that NAC may prevent AT-DILI. NAC improved liver histology in rats exposed to high dose isoniazid and rifampicin intraperitoneally (55). NAC reduced

cellular and mitochondrial membrane damage, and apoptosis in an in vitro study using human hepatocellular carcinoma cells exposed to toxic doses of isoniazid, rifampicin and pyrazinamide in various combinations (48). Oral NAC administered to participants during the first two weeks of ATT prevented increases in ALT in a small open label randomized controlled trial (56).

We hypothesised that NAC would shorten the duration of AT-DILI and conducted a randomized, double-blind placebo-controlled trial of intravenous NAC in adults with suspected AT-DILI.

3.3 Methods

3.3.1 Study participants

We recruited adult patients with a diagnosis of AT-DILI at 3 public sector hospitals in Cape Town, South Africa: Groote Schuur Hospital (tertiary level academic hospital), New Somerset Hospital (secondary level hospital) and Khayelitsha District Hospital. To be eligible for recruitment, a patient had to meet the American Thoracic Society (ATS) criteria for AT-DILI requiring cessation of ATT (34), by either having an ALT of more than three times the upper limit of normal if symptoms of hepatitis were present, or an ALT of more than 5 times the upper limit of normal without symptoms of hepatitis. Other inclusion criteria were age 18 years or older, taking first-line ATT for the treatment of active TB and liver injury that was attributed to ATT. We included both participants who were admitted to hospital because of AT-DILI and those who developed AT-DILI while in hospital. Patients with acute liver failure, which we defined as fulminant

hepatitis resulting in coagulopathy (INR>1.5) and an altered mental status (47), were eligible for inclusion.

We excluded patients with asthma, because of risk of NAC-induced bronchospasm, pregnant patients, and patients known to have viral hepatitis at the time of screening.

The primary endpoint was the time to ALT falling below 100 U/L. This primary endpoint was chosen because it is the ALT concentration at which liver injury is considered safe to start anti-tuberculosis drug rechallenge, according to the ATS guidelines on the management of AT-DILI (34). Secondary endpoints included time to hospital discharge, in-hospital mortality and study infusion related adverse events.

3.3.2 Study procedures

This study was a pragmatic randomized controlled trial, nested within routine clinical care. Participants were investigated and managed by hospital clinicians, except for administration of the study infusion and monitoring for adverse reactions by a member of the study team. Hospital clinicians, who were blinded to treatment allocation, made the decision to discharge participants from hospital, in line with their clinical judgement.

We collected baseline demographic, clinical, pharmacological and biochemical data on all study participants at the time of randomization. We graded hepatic encephalopathy using the West Haven score [16] (Supplementary Table 3.1). As part of routine clinical work-up, participants were tested for acute viral hepatitis A (anti-hepatitis A IgM), acute viral hepatitis B (hepatitis B surface antigen and anti-core IgM) and viral hepatitis C (anti-hepatitis C total antibodies and polymerase chain reaction).

Study participants were randomized 1:1 to receive intravenous NAC or placebo. Randomization was stratified by site and performed in blocks of 10 using a computer-generated randomization schedule. Study pharmacists at each site had restricted access to the randomization schedule and prepared the study infusion according to treatment allocation. Investigators and study participants were blinded to treatment allocation.

NAC was dosed and administered according to the regimen for paracetamol overdose as per manufacturer provided guidelines: 150 mg/kg over 1 hour, 50 mg/kg over 4 hours and 100 mg/kg over 16 hours (see Supplementary Table 3.2 for the weight-based dosing schedule). We used 0.9% saline as diluent for the NAC and placebo infusions, except for participants with acute liver failure or hypoglycaemia (serum glucose < 3.5 mmol/l), for whom 5% dextrose was used. Intravenous NAC is colourless and indistinguishable from placebo when mixed with either saline or dextrose solution.

Study participants were closely monitored for adverse events during the first hour of the study infusion and at the beginning and end of each infusion bag. A study investigator reviewed participants clinically at least twice weekly during hospital admission. We graded severity of adverse events using DAIDS categories (114). All participant deaths were reviewed by an independent physician, to assess whether the study drug was implicated in the death. The serum ALT was monitored by the clinical care team at least twice weekly until it fell below 100 U/L as per standard clinical practice.

3.3.3 Analysis

We powered the study to detect a 33% reduction in time for ALT to fall below 100 U/L with 80% power and an alpha value of 0.05. We assumed a mean (SD) time for ALT to fall below 100 U/L in the placebo arm of 18 days (±10) days, based on the ALT normalization time after cessation of ATT reported in a trial of ATT rechallenge regimens (81) . We calculated that we would require 88 participants (44 in each arm); we inflated the sample size to 100 participants to allow for deaths and loss to follow-up.

Analysis was by modified intention to treat. We included all randomized participants in whom we commenced the study infusion in the analysis. Continuous variables were described using means and standard deviations if parametrically distributed or medians and ranges if non-parametrically distributed. Categorical variables were described using counts and percentages. Time to ALT <100 U/L and time to discharge from hospital were described using Kaplan-Meier analyses. For calculation of time to hospital discharge we used the interval from study consent to discharge home or to a chronic care facility. The analysis of time to hospital discharge included both patients presenting with AT-DILI before admission to hospital and those who developed AT-DILI during hospital admission. We performed a log rank test to compare survival curves. We performed a univariable Cox regression to calculate a hazard ratio (HR) for ALT falling below 100 U/L, and a HR for hospital discharge, with a 95% confidence interval (CI). Data were analysed using Stata (Version SE/15.0 Statacorp Texas USA).

3.3.4 Ethics

This study was conducted according to the guidelines of the Helsinki Declaration of 2013 and the ICH principles of Good Clinical Practice (115, 116). The protocol was approved by the Western Cape Department of Health, University of Cape Town Human Research Ethics Committee (HREC 087/2012) [appendix 7] and the South African Health Products Regulatory Authority. Participants provided written informed consent [appendix 6]. Our research ethics committee granted us permission to include patients with hepatic encephalopathy who were too sick to consent at presentation, and to seek their consent after they had recovered from encephalopathy. We were also granted permission to include the data of participants who did not recover from encephalopathy, in the analysis. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719) [appendix 8].

3.4 Results

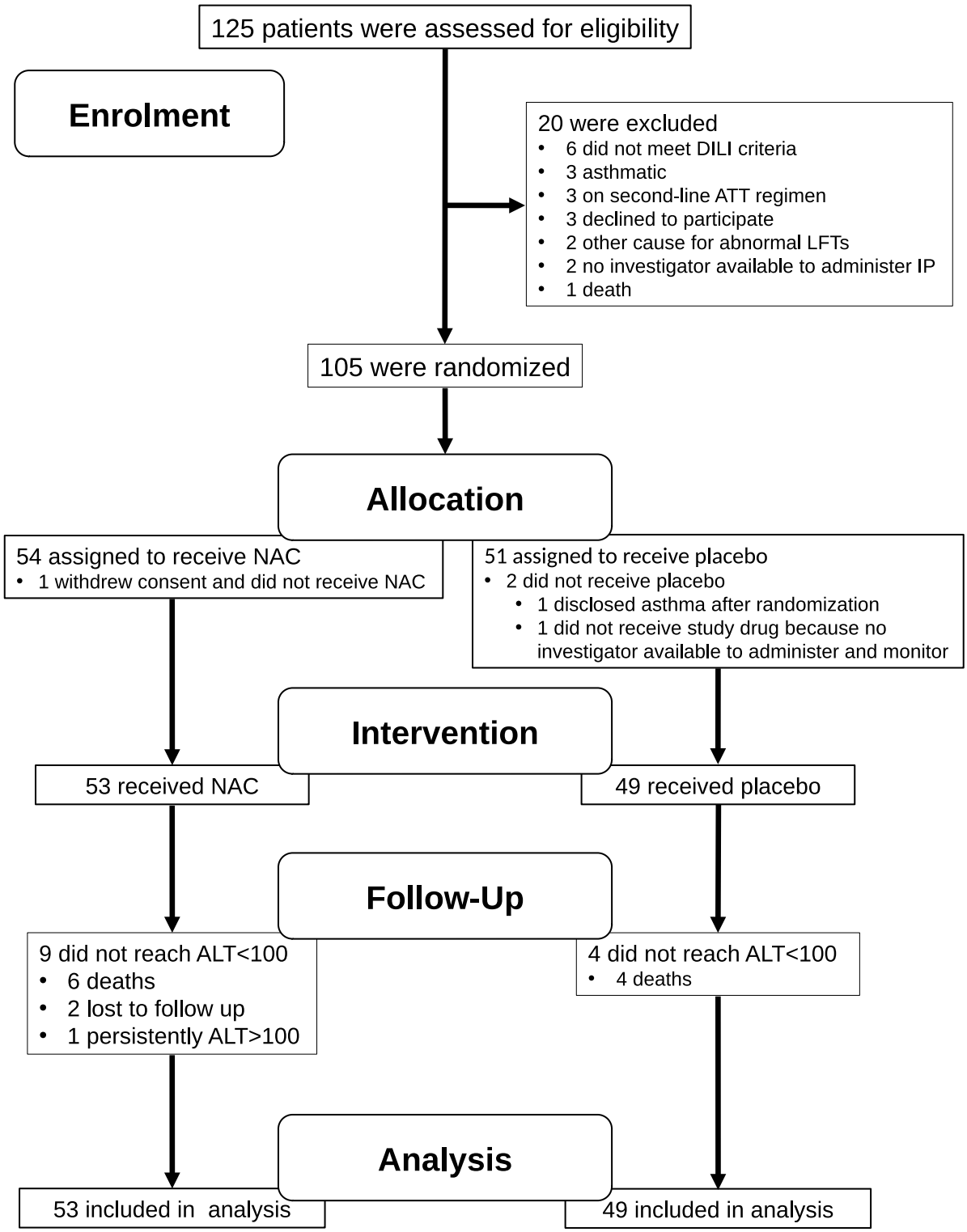
We screened 125 patients with suspected AT-DILI, 20 of whom were excluded (Figure 3.1). We enrolled 105 participants of which 54 were randomized to the NAC arm and 51 were randomized to the placebo arm. Three randomized individuals (1 in the NAC arm and 2 in the placebo arm) were not administered the study infusion and were not included in the analyses: 1 participant withdrew consent, 1 participant disclosed that they were asthmatic (an exclusion criterion), and 1 participant could not be administered the study infusion as there was no study team member available to commence and monitor the infusion (Figure 3.1).

The baseline characteristics of the 102 participants who started the study infusion were similar in the NAC and placebo arms (Table 3.1). Sixty participants (59%) had

pulmonary TB, 37 (36%) had extra-pulmonary TB and in 5 participants (5%) the site of TB was not specified. Twenty-two participants (22%) had previously received a course of ATT before this treatment episode. One hundred participants (98%) were taking intensive phase ATT (rifampicin, isoniazid, pyrazinamide and ethambutol) and 2 participants (2%) were taking continuation phase ATT (rifampicin and isoniazid) at the time of presentation with a liver injury. Eighty-nine participants (87%) were HIV positive, of whom 31 were taking an efavirenz-containing ART regimen, 9 were taking a lopinavir-ritonavir-containing ART regimen and 23 were taking cotrimoxazole at the time of presentation with a liver injury. Four participants (1 in the NAC arm and 3 in the placebo arm) reported an alcohol consumption greater than 14 units/week. Twelve of 53 (23%) participants in the NAC arm and 9 of 40 (18%) participants in the placebo arm developed the AT-DILI during a hospital admission, with no significant difference between arms (Chi squared p=0.594).

Seventy-four participants (73%) reported symptoms of AT-DILI at screening. The most commonly reported symptoms and signs were jaundice (47%), vomiting (43%), nausea (33%) and abdominal pain (25%). Eleven participants presented with hepatic encephalopathy, of whom 9 had mild encephalopathy (West Haven coma score of 1-2) and 2 had moderate encephalopathy (West Haven coma score of 3). Eighty-two participants had a prolonged INR (>1.1) and 11 had a serum sodium of less than 125 mmol/L.

Figure 3.1 Screening, randomisation and follow-up of participants in a randomised trial of n-acetylcysteine in the management of anti-tuberculosis drug-induced liver injury



Abbreviations: ALT, alanine aminotransferase; ATT, anti-tuberculosis therapy; DILI, drug-induced liver injury; LFT, liver function test; NAC, n-acetylcysteine

None of the participants had evidence of viral hepatitis based on serology at the time of enrolment. However, during the course of the trial three participants were found to have serological evidence of chronic viral hepatitis B. In addition, in two hepatitis B surface antigen-positive participants, anti-core IgM quantification was not requested by the clinical care team, so acute hepatitis B could not be excluded. One of these participants was also hepatitis A total antibody positive but hepatitis A IgM was not performed to exclude acute hepatitis A.

Five participants (1 in NAC arm and 4 in placebo arm) were assessed by study investigators as having other diseases that could have caused hepatitis (leptospirosis, disseminated Emergo mycosis, hepatocellular carcinoma, sepsis, and TB immune reconstitution inflammatory syndrome). Eight participants (5 in NAC arm and 3 in placebo arm) were assessed by study investigators as possibly having another drug implicated in the liver injury: efavirenz in 3, lopinavir-ritonavir in 2, cotrimoxazole in 2 and fluconazole in 1.

The time to ALT <100 U/L was similar in the treatment arms (Figure 3.2A), with a median of 7.5 days (interquartile range [IQR]5.5 -11 days) and 8 days (IQR 5 -13 days) in the NAC and placebo arms respectively. The hazard ratio (HR) for ALT falling below 100 U/L was 1.03 (95% CI, 0.68 to 1.57).

Time to discharge from hospital was shorter in the NAC arm than in the placebo arm (Figure 3.2B), with a median of 9 days (IQR 6-15 days) in the NAC arm, and 18 days (IQR 10-25 days) in the placebo arm. The HR for hospital discharge was 1.73 (95% CI, 1.13 to 2.65).

The overall mortality was 14% and did not differ by treatment arm. The causes of death were ATT-induced liver failure in 9, chronic lung disease in 2, sepsis in 1, *Pneumocystis*

jirovecii pneumonia in 1 and post liver biopsy haemorrhage in 1. The study drug was not implicated in any of the deaths.

There were 16 adverse events (AEs) during the study infusion, 13 in the NAC arm and 3 in the placebo arm. The study infusion was stopped early due to an AE in 5 participants, all of whom were receiving NAC (Table 3.2).

Serious AEs that occurred during study follow-up (after study drug administration) are described in Supplementary Table 3.3. None of these serious AEs were assessed by the investigators as being caused by the study drug; 19 serious AEs were assessed as possibly caused by other drugs.

Table 3.1 Baseline characteristics of participants by study arm

Baseline Characteristics	NAC (n=53)	Placebo (n=49)
Age years, mean (±SD)	37 (±10)	38 (±9)
Female, n (%)	34 (64)	24 (49)
Weight kg, median (IQR)	55 (47-67)	53 (45-63)
First time on TB treatment, n (%)	41 (77)	39 (80)
Duration of TB treatment, days, median(IQR)	18 (10-31)	25 (15-40)
HIV positive, n (%)	44 (83)	45 (92)
ART, n (%) Efavirenz, n (%) Lopinavir-ritonavir, n (%)	23 (43) 18 (34) 5 (9)	17 (35) 13 (27) 4 (8)
Cotrimoxazole, n (%)	8 (15)	15 (31)
Symptoms of DILI, n (%)	39 (74)	35 (71)
Encephalopathy, n (%)	6 (11)	5 (10)
ALT U/L, median (IQR)	448 (286-685)	384 (266-566)
Total bilirubin, mmol/L, median (IQR)	55 (19-93)	65 (30-117)
ALP U/L, median (IQR)	170 (101-248)	175 (113-253)
INR, median (IQR)	1.5 (1.2-2.2)	1.3 (1.1-2.2)
Albumin g/L, median (IQR)	26 (19-30)	23 (20-29)

Sodium mmol/L, mean (±SD)	131±5	129±5
CD4 count cells/mm^3, median (IQR) (HIV positive participants)	89 (40 -285)	75 (12-144)

Abbreviations: ALT, alanine transferase; ALP, alkaline phosphatase; ART, anti-retroviral therapy; HIV, human immunodeficiency virus; INR, international normalised ratio; IQR, interquartile range; SD, standard deviation; TB, tuberculosis

Table 3.2 Adverse events during study drug infusion

Adverse Event	**NAC (n=53)**	**Placebo (n=49)**
Nausea and/or vomiting	9 (infusion discontinued in 3)	2
Rash	1	0
Pruritis	1	0
Pain at drip site	1 (infusion discontinued in 1)	0
Hypotension	0	1
Anaphylactoid reaction	1 (infusion discontinued in 1)	0
Total	13	3

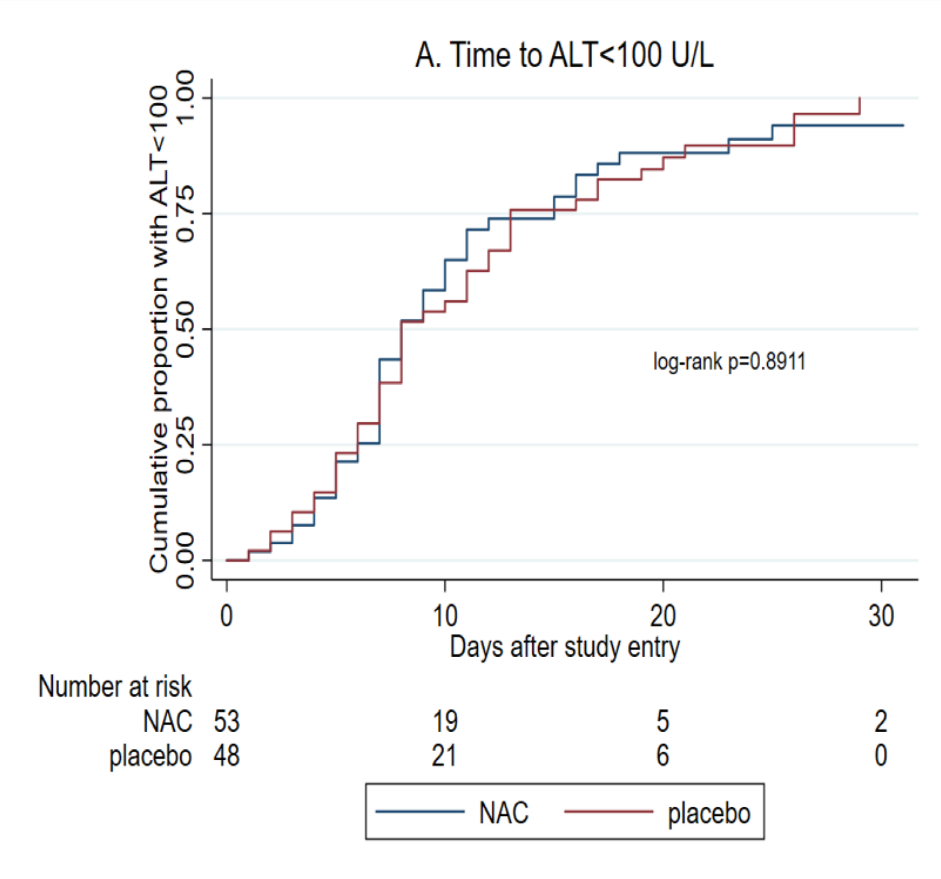


Figure 3.2A. Cumulative estimates of time to ALT < 100 U/L in participants with AT-DILI randomized to NAC or placebo

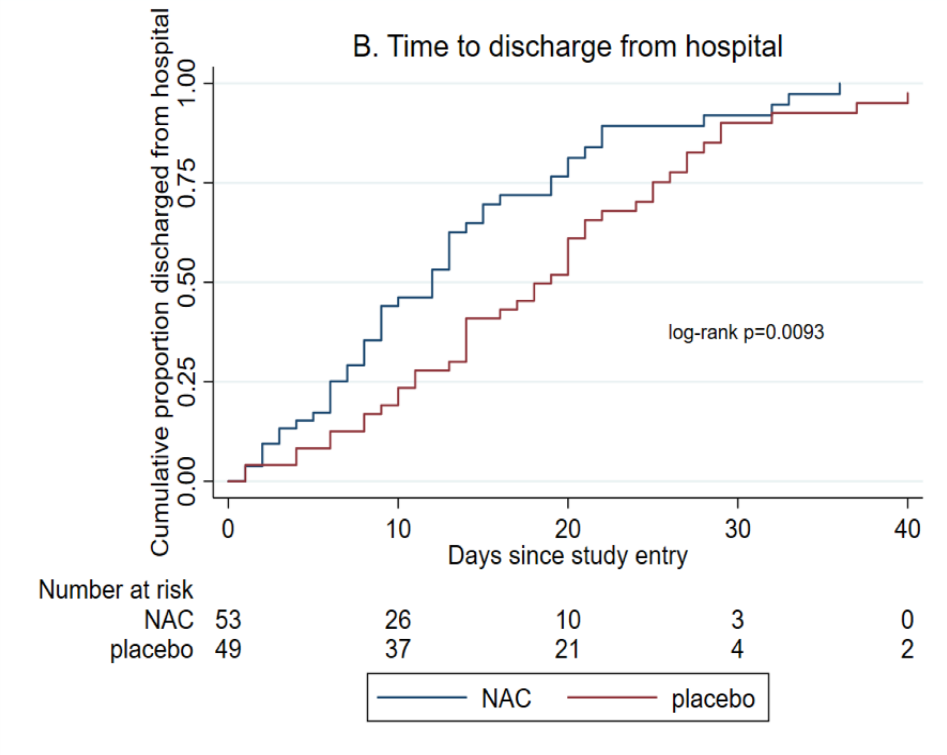


Figure 3.2B. Cumulative estimates of time to hospital discharge in participants with AT-DILI randomized to NAC or placebo

3.5 Discussion

We found no significant difference in our primary outcome (time to ALT <100 U/L) between participants with AT-DILI who received NAC or placebo. However, the median time to hospital discharge was 9 days shorter in the NAC arm. NAC was generally well tolerated, but all 5 AEs that resulted in discontinuation of the study infusion occurred in the NAC arm. N-acetylcysteine is widely used to treat patients with paracetamol overdose; however, the quality of evidence for its efficacy in this setting is limited (117). In contrast to paracetamol, which has a direct dose dependant hepatotoxic effect via intermediary metabolites, the mechanism of AT-DILI is thought to be an idiosyncratic, immune response to the covalent binding of drug metabolites to liver proteins (38). Furthermore, the duration of liver injury due to ATT is typically much longer than that due to paracetamol. Despite these differences in the pathogenesis of AT-DILI and paracetamol induced liver injury, NAC prevented increases in ALT in rats and humans exposed to ATT (55, 56) ; and intravenous NAC improved transplant-free survival in a randomized placebo-controlled trial in participants with non-paracetamol induced liver failure, some of whom had DILI (54) .

In our trial there was no difference in our primary efficacy endpoint of time to ALT <100 U/L between treatment arms. However, ALT is not a good biomarker of liver injury: ALT concentrations may be normal during the early stages of hepatocyte injury or apoptosis; ALT may be elevated due to mechanisms other than hepatocyte injury (e.g., membrane blebbing, increased hepatic expression, macroenzymes) (61) ; and ALT may be released from tissues other than liver such as skeletal muscle, kidney and heart (62) . Novel biomarkers of liver injury such as microRNA, are more specific for liver injury than ALT and increase earlier than ALT in patients with paracetamol induced liver injury (100, 118, 119).

In our trial, participants with AT-DILI who received NAC had significantly shorter hospital stays, which was a prespecified secondary endpoint of the trial. Our study was blinded, therefore the clinical decision to discharge participants from hospital could not have been biased by knowledge of treatment allocation. Furthermore, the decision to discharge participants from hospital was not made by the study investigator but by the clinical care team, who were unaware of adverse effects experienced by participants during the NAC infusion. However, unblinding could have occurred with adverse reactions to the NAC infusion. Our finding therefore suggests that NAC hastened clinical recovery, which is consistent with findings of shorter hospital stay in the NAC arm of a randomized placebo-controlled trial of NAC in participants with non-paracetamol induced liver failure (54).

There are several potential explanations for the shorter hospital stay in the NAC arm of our study, despite similar time to ALT resolution. First, improved recovery from AT-DILI due to NAC may not have resulted in a difference in ALT trajectory between groups, but might have been detected had we used a better biomarker of liver injury than ALT. Second, the anti-oxidant effects of NAC may be beneficial in people living with HIV (87% of our participants), who often have glutathione depletion (120) . A randomized placebo controlled trial of NAC in HIV positive participants with low glutathione concentrations reported reduced mortality in participants who received NAC, but open label NAC was offered to all participants after a short (8 week) randomized phase; a further limitation of the study was that it was conducted in the era before effective combination ART. Third, it is possible that NAC could have improved tuberculosis as there is some evidence that NAC inhibits *Mycobacterium tuberculosis* growth and augments the mycobactericidal activity of first-line ATT in vitro (121, 122). Fourth, the shorter hospital stay in the NAC arm could have been due to chance.

Our study had limitations. First, we included patients who were taking drugs other than ATT, which have also been associated with DILI (e.g., efavirenz and cotrimoxazole). Second, we did not exclude viral hepatitis A and B in 2 participants in the NAC arm; and we did not test for viral hepatitis E in our cohort as it is not part of local routine clinical care. Third, we did not monitor ALT daily but twice weekly, as per routine clinical care. Fourth, HIV prevalence was high in our cohort and therefore our findings may not be generalizable to other populations. Fourth, our primary outcome was a reduction in ALT, which has limitations as a biomarker of DILI.

Further studies are needed to determine NAC clinical benefits in AT-DILI in populations with low HIV prevalence. Future trials of NAC for AT-DILI should have a clinical primary outcome and use more appropriate markers of DILI than ALT.

3.6 Conclusion

Our randomized controlled trial did not demonstrate any effect of NAC on reducing the time to ALT <100 U/L in patients with AT-DILI. However, length of hospital stay was significantly shorter in the NAC arm. N-acetylcysteine, which is widely available, should be considered in the management of AT-DILI.

Chapter 4 Rechallenge after anti-tuberculosis drug-induced liver injury: a cohort study in a high HIV prevalence setting

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Supervision: KC, GM

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Citation:

Moosa MS, Maartens G, Gunter H, Allie S, Chughlay MF, Setshedi M, Wasserman S, Stead DF and Cohen K. Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort. S Afr J HIV Med. 2022;23(1), a1376.

DOI: https://doi.org/10.4102/sajhivmed.v23i1.1376

The original publication is included as Appendix 3.

4.1 Abstract

Background: There are limited data on the outcomes of rechallenge with anti-tuberculosis therapy (ATT) following anti-tuberculosis drug-induced liver injury (AT-DILI) in a high HIV prevalence setting.

Objectives: To describe the outcomes of rechallenge with first-line ATT.

Methods: We described the outcomes of rechallenge in hospitalised participants with AT-DILI who were enrolled into a randomized controlled trial of N-acetylcysteine in Cape Town, South Africa.

Results: Seventy-nine participants were rechallenged. Mean age was 37 years (SD±10) and 41 (52%) were female. Sixty-eight (86%) were HIV positive of whom 34 (50%) were on antiretroviral therapy (ART) at time of AT-DILI presentation. Five participants had serious adverse reactions to an aminoglycoside used in background ATT: acute kidney injury in 3, and hearing loss in 2.

First-line ATT was interrupted for a median of 29 days (IQR 23-39). ART was interrupted for a median of 32 days (IQR 17-58) among HIV-positive participants. Fourteen (18%) participants had positive rechallenge. Positive rechallenge was associated with pyrazinamide rechallenge (p=0.005), female sex (p=0.039) and first episode of TB (p=0.032).

Conclusion: Rechallenge was successful in most of our cohort. Pyrazinamide rechallenge should be carefully considered.

4.2 Introduction

Liver injury is the most frequent complication of first-line anti-tuberculosis therapy (ATT) with an estimated incidence of 2-28% (11). Following recovery from anti-tuberculosis drug-induced liver injury (AT-DILI), rechallenge with hepatotoxic first-line anti-tuberculosis drugs (rifampicin, isoniazid, and, in some circumstances, pyrazinamide) is recommended because second line ATT regimens are less effective, longer and more toxic (123). While awaiting resolution of liver injury, a background ATT regimen is given, typically consisting of ethambutol and at least two other second line anti-tuberculosis drugs.

There are few studies on rechallenge following AT-DILI in populations with high prevalence of HIV coinfection. There is limited evidence on optimal background ATT regimens, optimal ATT rechallenge protocols, risk factors for positive rechallenge, anti-tuberculosis drugs most frequently implicated in positive rechallenge, and interruption and re-initiation of anti-retroviral therapy (ART) and cotrimoxazole prophylaxis in people living with HIV (PLHIV) who present with AT-DILI.

This study is nested within our randomised placebo-controlled trial of intravenous N-acetylcysteine (NAC) in the management of AT-DILI, which has previously been reported (113). We describe the characteristics, background and rechallenge regimens, and outcomes of rechallenge in those participants who were rechallenged with ATT. Amongst HIV positive participants, we explore the impact of AT-DILI and drug rechallenge on initiation and/or interruption of ART.

4.3 Methods:

4.3.1 Study participants

Participants with AT-DILI admitted to 3 hospitals in Cape Town, South Africa were enrolled in a pragmatic randomised placebo-controlled trial of intravenous NAC. AT-DILI was defined as alanine aminotransferase (ALT) ≥ 3 times the upper limit of normal if symptoms of hepatitis were present, or an ALT ≥ 5 times the upper limit of normal without symptoms of hepatitis (34). Other trial inclusion criteria were age 18 years or older, taking first-line therapy for tuberculosis (TB) and liver injury attributed to ATT.

After completion of the NAC/placebo infusion, decisions regarding clinical management were made by clinicians at participating hospitals and outpatient clinics. This included decisions regarding background ATT initiation and regimen, whether to rechallenge ATT, choice of rechallenge regimen, and interrupting, rechallenging or initiating ART. Participants were followed up until the study primary endpoint (ALT reaching <100 U/L) was reached and ATT rechallenge was completed. We included all trial participants who were rechallenged with at least one anti-tuberculosis drug in this analysis.

4.3.2 Identification and assessment of positive rechallenge cases

We defined positive rechallenge as doubling of ALT and/or total bilirubin concentration after rechallenge of an anti-tuberculosis drug (124). A multidisciplinary causality assessment panel including a clinical pharmacologist, a pharmacist, an infectious diseases specialist and a general physician, assessed cases with a positive

rechallenge, and identified the drug that was most likely to be causative, and/or any non-drug related cause for the increase in ALT and/or bilirubin.

4.3.3 Statistical analysis

Categorical data was described using counts and percentages. Numerical data was described using means and standard deviations if normally distributed and medians and ranges if non-normally distributed. We compared parametric data using the Student's t-test, non-parametric data using the Wilcoxon Rank Sum test and categorical data using the Fisher's exact test. When comparing proportion with positive rechallenge between rechallenged drugs, we assumed that the 3 groups were independent. A p-value of <0.05 was considered to be statistically significant throughout. Data were analyzed using Stata (Version SE/15.1 Statacorp Texas USA).

We calculated "first-line ATT interruption time" as the interval from date of presentation with AT-DILI and interruption of first-line ATT to the date of completion of rechallenge. In HIV positive participants on ART, we calculated "ART interruption time" as the interval from the date of presentation with AT- DILI and ART discontinuation to the date of ART re-initiation. In HIV positive participants not on ART, we calculated "delay in ART initiation time" as the interval from date of presentation with AT-DILI to the date of ART initiation.

4.3.4 Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference for Harmonisation (115, 116). The study protocol was approved by University of Cape Town Human Research Ethics

Committee and the Western Cape Department of Health (HREC 087/2012) [appendix 7]. Participants provided written informed consent. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719) [appendix 8].

4.4 Results

Seventy-nine of 102 participants (77%) with AT-DILI enrolled into the randomized trial were rechallenged with ATT (Figure 4.1). Reasons for not rechallenging 23 participants were: 12 died before rechallenge was attempted, 8 had insufficient evidence of TB to justify rechallenge, 2 had prolonged hyperbilirubinaemia and were placed on second line ATT because the clinical team deemed first line ATT rechallenge to be unsafe, and 1 was lost to follow up before planned rechallenge could be commenced.

Baseline characteristics of the 79 rechallenged participants, grouped by positive and negative rechallenge, are described in Table 4.1. Sixty eight of the 79 (86%) participants rechallenged were HIV positive, 34 of whom were on ART at presentation with AT-DILI, (27 on an efavirenz-based regimen and 7 on a lopinavir plus ritonavir-based regimen) and 18 were on cotrimoxazole prophylaxis.

Forty-three participants commenced rechallenge during hospital admission, 10 of whom were referred to a community health centre to complete rechallenge. Twenty-five participants were rechallenged at a community health centre and 11 at a stepdown inpatient TB care facility.

Sixty-eight of 79 participants (86%) rechallenged were initiated on background ATT after first line ATT interruption prior to rechallenge (Table 4). In the remaining 11 participants, background ATT was not commenced; reasons for this decision were not

documented. All 68 participants initiated on background ATT received a fluoroquinolone, and 58 received an aminoglycoside (46 kanamycin, 11 amikacin, 1 streptomycin). Five of the participants who received an aminoglycoside (9%) had a serious adverse drug reaction: acute kidney injury in 3, and hearing loss in 2.

Most participants (96%) were rechallenged with a minimum of 2 individual drugs re-introduced sequentially and in full dosages (Table 5). Three participants completed only rifampicin rechallenge after which further rechallenge was discontinued: 1 was found to have no evidence of TB, 1 had worsening canalicular enzymes (likely due to TB-immune reconstitution inflammatory syndrome [IRIS] rather than DILI recurrence), and 1 died from sepsis and multi-organ failure before completion of rechallenge.

First-line anti-tuberculosis drugs were rechallenged at full dose. Drugs were rechallenged sequentially in 77/79 participants, with new drugs introduced at approximately 3-day intervals (Table 4.2). Rechallenge regimens differed in the sequence in which individual drugs were re-introduced: rechallenge commenced with rifampicin in 68 participants and with isoniazid in 11 (Table 4.3). The clinical care team elected not to rechallenge with pyrazinamide in 22 of 72 participants who had interrupted ATT due to DILI during the intensive phase because of the severity of the liver injury. The median ATT interruption time was 29 days (IQR 22-39).

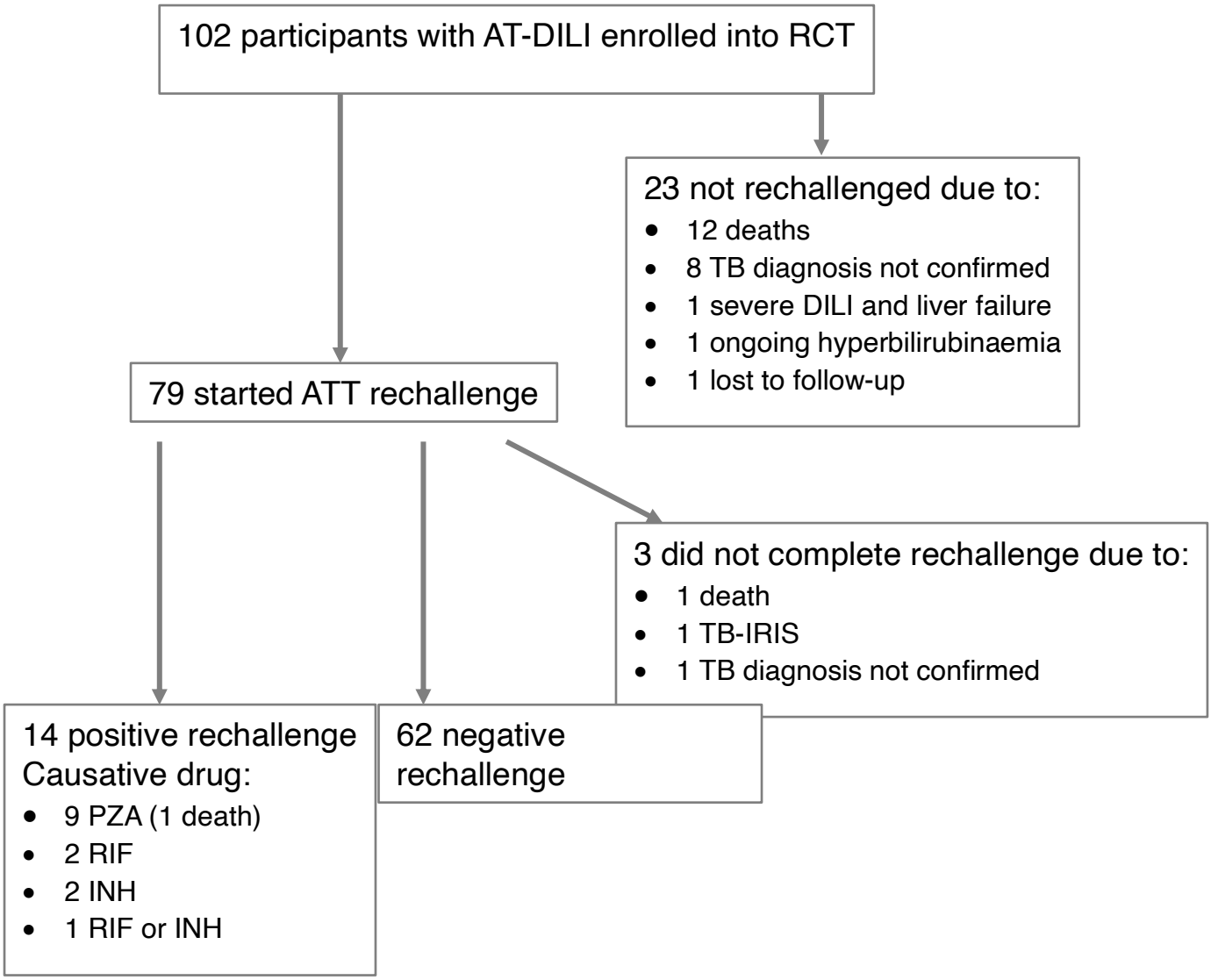

Figure 4.1 Anti-tuberculosis drug rechallenge following drug-induced liver injury in N-acetylcysteine randomised controlled trial participants.

Abbreviations: AT-DILI, anti-tuberculosis drug induced liver injury; ATT, anti-tuberculosis therapy; INH, isoniazid; IRIS, immune reconstitution inflammatory syndrome; PZA, pyrazinamide; RCT, randomised controlled trial; RIF, rifampicin; TB, tuberculosis.

Table 4.1 Baseline characteristics of participants with positive and negative rechallenge following anti-tuberculosis drug-induced liver injury.

Baseline Characteristics	Positive Rechallenge (n=14)	Negative Rechallenge (n=65)	All rechallenged participants (n=79)	P-value[1]
Age years, mean (±SD)	35 (±12)	38 (±9)	37(±10)	0.368
Female, n (%)	11 (79)	30 (46)	41 (52)	0.039
Weight kg, median (IQR)	59 (50-74)	54 (46-64)	54 (47-64)	0.418
First time on TB treatment, n (%)	14 (100)	47 (72)	61 (77)	0.032
HIV positive, n (%)	10 (71)	58 (89)	68 (86)	0.075
CD4 count cells/mm^3 (for HIV positive) median (IQR)[2]	56 (4-277)	76 (26-144)	70 (26-144)	0.646
ALT U/L, median (IQR)	255 (225-352)	385 (279-558)	357 (254-558)	0.090
Total bilirubin mmol/L, median (IQR)	44 (26-81)	49 (21-94)	47 (22-90)	0.767
ALP U/L, median (IQR)	126 (101-194)	183 (112-258)	175 (110-254)	0.245
INR, median (IQR)[3]	1.1 (1.0-2.1)	1.3 (1.1-1.8)	1.2 (1.1-1.8)	0.288
Albumin g/L, median (IQR)[4]	26 (19-35)	26 (21-30)	26 (21-30)	0.758

Abbreviations: ALT alanine transferase, ALP alkaline phosphatase, ART anti-retroviral therapy, ATT anti-tuberculosis therapy, EFV efavirenz, HIV human immunodeficiency virus, INR international normalised ratio, IQR interquartile range, LPV/r, lopinavir plus ritonavir, SD standard deviation, TB tuberculosis

[1] Fishers exact test for categorical variables, T test for parametric data, Rank sum test for non-parametric data

[2] 26 with missing data

[3] 5 with missing data

[4] 5 with missing data

Table 4.2 Background anti-tuberculosis drug regimens prescribed following anti-tuberculosis drug-induced liver injury

Background anti-tuberculosis drug regimen	Number of participants
ethambutol + moxifloxacin + aminoglycoside[†]	54
ethambutol + moxifloxacin +ethionamide	6
ethambutol + moxifloxacin	4
ethambutol + moxifloxacin + ethionamide + aminoglycoside[‡]	2
moxifloxacin + ethionamide + aminoglycoside[§]	2
no background anti-tuberculosis therapy	11

†, 42 participants on kanamycin, 11 on amikacin, one on streptomycin.

‡, 2 participants on kanamycin.

§, 2 participants on kanamycin.

Table 4.3 Sequence of anti-tuberculosis drug rechallenge

Rechallenge regimen	Participants	
	n	%
RIF → INH → PZA[†,‡]	38	50
INH → RIF → PZA	6	8
RIF → INH	26	30
INH → RIF	4	5
RIF → PZA	1	1
INH → PZA	1	1
RIF	3	5

Abbreviations: INH, isoniazid; PZA, pyrazinamide; RIF, rifampicin.

†, One participant was rechallenged with RIF and INH concomitantly followed by PZA.

‡, One participant was rechallenged with RIF, PZA and INH concomitantly.

4.4.1 Positive rechallenge

There were 14 positive rechallenges in the 79 rechallenged participants (18%). Positive rechallenge was associated with female sex (Fishers exact test p=0.039) and first episode of TB (Fishers exact test p=0.032) (Table 4.1). Rechallenge was positive in 9/46 participants rechallenged with pyrazinamide, 2/78 rechallenged with rifampicin, and 2/74 rechallenged with isoniazid. One participant had a positive rechallenge after sequential introduction of rifampicin and isoniazid. On causality assessment both drugs were potentially implicated in the positive rechallenge because the participant's serum ALT only settled after both drugs were withdrawn. The proportion of positive

rechallenges did not differ significantly between NAC (6/30) and placebo (8/35) groups; Chi-squared p= 0.822.

The proportion with a positive rechallenge was significantly higher among those rechallenged with pyrazinamide than among those rechallenged with rifampicin or isoniazid, Fisher's exact test p=0.005. One of the participants with positive pyrazinamide rechallenge developed a fatal systemic hypersensitivity reaction with rash, jaundice and acute kidney injury.

One participant had markedly increased serum canalicular liver enzymes (alkaline phosphatase and gamma-glutamyl transferase) at AT-DILI presentation which increased further after rifampicin rechallenge. The hospital clinicians assessed this as a positive rifampicin rechallenge and stopped rifampicin. However, the canalicular enzymes continued to increase after rifampicin cessation. On causality assessment, the increased canalicular enzymes were attributed to TB IRIS rather than a positive rifampicin rechallenge.

Positive rechallenge resulted in prolonged first-line ATT interruption: median first-line ATT interruption time was 42 days (IQR 35-52) in participants with positive rechallenge compared with 28 days (IQR 22-35) in those with negative rechallenge; Wilcoxon Rank Sum test p=0.0003.

ART was interrupted at presentation with liver injury in 26 of the 34 (79%) HIV positive participants who were receiving ART. At 8-weeks follow-up, 24 of these 26 participants had been re-initiated on ART: 21 recommenced their previous efavirenz-based regimen and 3 were switched from efavirenz-based to boosted protease inhibitor-based ART. The median ART interruption time was 32 days (IQR 17-58). Twenty-one of 34 (62%) HIV positive participants who were not on ART at the time of AT-DILI were initiated on

ART after ATT rechallenge, after a median of 53 days (IQR 35-91). Fifteen of 68 (22%) HIV positive participants were not yet on ART when study follow-up ended.

4.5 Discussion

In our cohort of patients with AT-DILI, the majority of whom had advanced HIV disease, rechallenge was attempted in the majority (77%). A wide variety of background regimens were used during rechallenge; aminoglycosides in the background regimen caused a high incidence of toxicity. Rechallenge was positive in 18%, which was associated with female sex and first episode of TB. Positive rechallenge was significantly higher with pyrazinamide than with isoniazid and/or rifampicin. Positive rechallenge resulted in prolonged disruption of ATT, and delays in initiating or commencing ART.

4.5.1 Risk of positive rechallenge

In a recent network meta-analysis of ATT rechallenge regimens in participants with AT-DILI (83), 11% of those rechallenged with a sequential full dose regimen had a positive rechallenge. This is lower than the 18% we observed and could be explained by the longer rechallenge regimens used in the studies included in the meta-analysis. The majority of participants in the meta-analysis were rechallenged with rifampicin on day 1, isoniazid on day 8 and pyrazinamide on day 15-18; whereas the majority of our study participants were rechallenged with rifampicin on day 1, isoniazid on day 4 and pyrazinamide on day 7. Although AT-DILI is thought to be idiosyncratic, some experts including the British Thoracic Society, consider the sequential incremental dose

regimen less likey to cause recurrence of liver injury than the sequential full dose regimen.

We found that women and participants with their first episode of TB were more likely to have a positive rechallenge. Other studies have also found female sex to be associated with AT-DILI (19, 23) as well as with positive ATT rechallenge (82) . We did not find low serum albumin or increased age to be associated with positive rechallenge, in contrast to previous studies (81, 84).

4.5.2 Pyrazinamide rechallenge

Pyrazinamide was the main cause of positive rechallenge in our study, with positive rechallenge in 20% of those rechallenged. Positive pyrazinamide rechallenge contributed to the death of one study participant. In a small randomised trial 6 of 25 (24%) participants rechallenged with a concomitant full dose regimen including pyrazinamide had a positive rechallenge compared with 0 of 20 in the sequential full dose regimen group excluding pyrazinamide (82). American Thoracic society guidelines advise against rechallenging pyrazinamide after severe AT-DILI (34). With increasing availability of effective second line anti-tuberculosis drugs including fluroquinolones, linezolid and bedaquiline, avoidance of pyrazinamide rechallenge in all cases of AT-DILI should be considered.

4.5.3 Anti-retroviral therapy interruption and re-initiation

There is little published data on impact of AT-DILI on ART in PLHIV. In our study, 79% of participants on ART at the time of AT-DILI presentation had their ART interrupted, with a median interruption of 32 days. ART interruptions may impact on efficacy of therapy and contribute to the emergence of antiretroviral resistance (125). Median delay from AT-DILI presentation to ART initiation in our cohort was 53 days, and 22% of the cohort were not yet on ART when study follow up ended. Delays in initiation of ART in patients with advanced disease have previously been shown to increase mortality (44).

4.5.4 Study limitations

Our study has limitations. Although our study was nested within a randomised control trial, it is descriptive, and was not powered to identify risk factors for positive rechallenge. Study follow up ended after rechallenge was complete, and we therefore could not quantify impact of positive rechallenge on outcomes of ATT or ART. Our study cohort had a high prevalence of HIV and the findings may not be generalizable to lower HIV prevalence settings.

4.6 Conclusion

In this cohort of patients with AT-DILI, the majority of whom were HIV positive, pyrazinamide was the most common cause of positive rechallenge. Positive rechallenge resulted in prolonged interruption of first-line ATT, and delays in initiating

or recommencing ART. Use of alternative second line anti-tuberculosis drugs should be considered as an alternative to pyrazinamide rechallenge.

Chapter 5 Analysis of serum microRNA-122 in a randomised controlled trial of N-acetylcysteine for treatment of anti-tuberculosis drug-induced liver injury

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Co-author contributions

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Study design: KC, GM, DC, MP

Oversight of miR-122 assays and interpretation of laboratory results: GR, CP, EC, CG

Data analysis strategy and analysis: CG, KC, JRD

Critical review of manuscript: all co-authors

Citation

Moosa MS, Russomanno G, Dorfman JR, Gunter H, Patel C, Costello E, Carr D, Maartens G, Pirmohamed M, Goldring C, Cohen K. Analysis of serum microRNA-122 in a randomised controlled trial of N-acetylcysteine for treatment of anti-tuberculosis drug-induced liver injury. British Journal of Clinical Pharmacology (accepted for publication on 4 January 2023). doi: 10.1111/bcp.15661

The original publication is included as Appendix 4. Supplementary Materials are included in Appendix 5.

5.1 Abstract

Aim: Serum microRNA-122 (miR-122) is a novel biomarker for drug-induced liver injury, with good sensitivity in early diagnosis of paracetamol-induced liver injury. We describe miR-122 concentrations in participants with anti-tuberculosis drug-induced liver injury (AT-DILI). We explored the relationship between miR-122 and alanine aminotransferase (ALT) concentrations, and the effect of N-acetylcysteine (NAC) on miR-122 concentrations.

Methods: We included participants from a randomised placebo-controlled trial of intravenous NAC in AT-DILI. ALT and miR-122 concentrations were quantified before and after infusion of NAC/placebo. We assessed correlations between ALT and miR-122 concentrations and described changes in ALT and miR-122 concentrations between sampling occasions.

Results: We included 45 participants; mean age (± standard deviation) was 38 (±10) years, 58% were female and 91% were HIV positive. Median (interquartile range) time between pre- and post- infusion biomarker specimens was 68 hours (47-77 hours). Median pre-infusion ALT and miR-122 concentrations were 420 U/L (238- 580) and 0.58 pM (0.18-1.47) respectively. Pre-infusion ALT and miR-122 concentrations were correlated (Spearman's ρ=0.54, p=0.0001). Median fold-change in ALT and miR-122 concentrations between sampling was 0.56 (0.43 - 0.69) and 0.75 (0.23 - 1.53) respectively and were similar between NAC and placebo groups (p=0.40 and p=0.68 respectively).

Conclusion: MiR-122 concentrations in our participants with AT-DILI were considerably higher than previously reported in healthy volunteers and in patients on anti-tuberculosis therapy without liver injury. We did not detect an effect of NAC on miR-122

concentrations. Further research is needed to determine the utility of miR-122 in the diagnosis and management of AT-DILI.

5.2 Introduction

Drug-induced liver injury (DILI) is the most frequent severe adverse effect of first-line anti-tuberculosis therapy (11). Anti-tuberculosis drug induced liver injury (AT-DILI) is primarily diagnosed by elevated serum alanine aminotransferase (ALT) concentrations, together with symptoms and signs of DILI (34). However, ALT is not a specific biomarker of DILI because it may be released from tissues other than the liver (e.g., skeletal muscle, kidney, and heart) (62). More specific biomarkers of DILI are therefore potentially important.

MicroRNAs can be detected in blood as stable complexes and quantified using quantitative real-time polymerase chain reaction (qRT-PCR) (90). Liver injury due to drug exposure, viral hepatitis, or hepatocellular carcinoma, have been shown to result in altered microRNA expression profiles (92, 93, 94, 95). MicroRNA-122 (miR-122) accounts for 70% of hepatic microRNA (96) and plays an essential role in lipid metabolism, anti-inflammatory and anti-tumorigenic mechanisms (97). MiR-122 has been explored as a novel biomarker of paracetamol-induced liver injury in pre-clinical (63, 103) and clinical studies (95, 99, 100, 104, 105). In paracetamol-induced liver injury, an increase in miR-122 concentrations occurred earlier than an increase in ALT concentrations and was a sensitive test for identifying those patients who went on to develop liver injury (63, 64). Peak increases in serum miR-122 concentrations correlated with later increases in serum ALT (103, 104, 106). MiR-122 concentrations have also been found to be elevated in patients with non-paracetamol DILI (65, 107).

MiR-122 is largely liver-specific and therefore concentrations are unaltered by impaired renal function (99) and muscle injury (100).

There are limited data on miR-122 as a biomarker of AT-DILI. For this study, we quantified miR-122 concentrations in stored specimens from a randomised placebo-controlled trial of N-acetylcysteine (NAC) for treatment of AT-DILI. We found that NAC administration shortened duration of hospitalisation, but time for ALT to fall below 100 U/L was similar for NAC and placebo groups (113).

Our aims for this analysis were to describe miR-122 concentrations in AT-DILI, to assess correlations between ALT and miR-122 concentrations, to describe changes in miR-122 concentrations over time, and to determine if repeat miR-122 quantification would be more informative than ALT in detecting a biochemical response to NAC administration.

5.3 Methods

5.3.1 Study participants

Participants were drawn from a previously reported (113), randomised placebo-controlled trial (RCT) of NAC in the management AT-DILI conducted in Cape Town, South Africa. NAC was dosed intravenously according to the regimen for paracetamol overdose: 150 mg/kg over 1 hour, 50 mg/kg over 4 hours, and 100 mg/kg over 16 hours. The study was a pragmatic randomised trial nested within routine clinical care at the participating hospitals. The study protocol specified that participants' ALT be tested before the study infusion and twice weekly during hospital follow-up. These ALT samples were generally taken by the hospital clinical care team. In addition to the monitoring of ALT concentrations for the clinical study, investigators collected blood samples for storage for future biomarker research. For this analysis, we included the

subset of participants from the RCT with paired specimens stored for biomarker quantification: the first specimen taken between 24 hours before and 30 minutes after commencement of the study infusion and the second specimen taken within 7 days after initiation of the study infusion. Blood was centrifuged within 8 hours of collection, and serum was stored at -80°C. We compared baseline characteristics of the participants with paired samples, and study participants not included in this analysis to confirm that they were similar.

5.3.2 ALT quantification

ALT was quantified in stored serum using an International Federation of Clinical Chemistry recommended method. ALT and miR-122 concentrations were determined from the same sample in each case, except for two pre-infusion samples which were unsuitable for ALT determination; in those two cases we used the ALT concentration from clinical records from an assay performed on the same day of the infusion. Both of these ALTs were from samples taken within 3 hours of the sample used for miR-122 determination.

5.3.3 MicroRNA isolation from serum

For recovery of miRNA-enriched fractions from serum, the miRNeasy Mini Kit and the RNeasy MinElute Cleanup Kit (Qiagen) were used on the QIAcube automated platform (Qiagen), following manufacturer's instructions with addition of 200 pM cel-lin-4 (Ambion, cat. 4464066, ID MC10768) as a spiked-in exogenous non-human miRNA to monitor for the efficiency of the miRNA extraction process. Freshly isolated miRNA-containing eluate (15 μL) was stored at -80˚C.

5.3.4 Reverse transcriptase and real-time quantitative PCR (RT-qPCR) reactions

TaqMan miRNA Assays for miR-122 (Assay ID 002245) and cel-lin-4 (Assay ID 000258; Thermo Fisher Scientific) were used to perform RT-qPCR. MiRNA-containing eluate was reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific) and a custom Multiplex RT Primer pool in a GeneAmp 9700 PCR System (Applied Biosystems). A fixed volume of 2 µL miRNA-containing eluate was added to an RT Master Mix containing dNTPs, RNase Inhibitor, Reverse Transcription Buffer, 75 units per reaction of MultiScribe Reverse Transcriptase, and a multiplex RT primer pool consisting of primers for miR-122 and cel-lin-4 (Thermo Fisher Scientific). A 1 nM synthetic miR-122 (mirVana miRNA mimic, Assay ID MC11012, Ambion) was reverse-transcribed together with the samples to allow the generation of a standard curve in each plate. No-template samples were included as negative controls. Reaction conditions followed the manufacturer's instructions: annealing for 30 min at 16°C, followed by cDNA synthesis for 30 min at 42°C and denaturation for 5 min at 85°C. The resulting cDNA was diluted 1:3 with RNase-free water and stored at -20°C until further processing.

Pre-amplification of cDNA was run on a GeneAmp 9700 PCR System (Applied Biosystems). Negative controls containing RNase-free water instead of cDNA were included. Reaction conditions followed the manufacturer's instructions: 95°C for 10 min, 55°C for 2 min, 72°C for 2 min, followed by 12 cycles at 95°C for 15 sec and 60°C for 4 min followed by enzyme inactivation at 99.9°C for 10 min. The pre-amplified synthetic miR-122 was serially diluted 1:10 (ranging from 1 nM to 0.1 fM) and stored at -20°C.

Two µL of the diluted pre-amplification product was combined with a qPCR Master Mix containing 5 µL of 2x TaqMan Universal MasterMix II without Uracil-N glycosylase

(Thermo Fisher Scientific) in a total reaction volume of 10µL. RT-qPCR was then performed in duplicates on an ABI ViiA 7 Thermocycler (Applied Biosystems) using a 2-step thermal cycling protocol of 95°C for 10 min followed by 40 cycles of 95°C (15 s) and 60°C (60 s) for both miR-122 and cel-lin-4. Data were analysed using QuantStudio Real-Time PCR Software v1.3. The number of copies of miR-122 in each sample was quantified using the absolute quantification method or number of threshold cycles (Ct value), with the standard curve generated with synthetic miR-122 in each plate. MiR-122 levels were normalised to the level of cel-lin-4 to account for technical variations between samples. 10% duplicates (including samples run in a separate plate) were added to each run to account for intra- and inter-plate variability, and final miR-122 copy numbers were averaged across experiments (intra- and inter-assay CV less than 5% and 10%, respectively).

5.3.5 Statistical analysis

Data were analyzed using Stata SE Version 13.1, Statacorp, College Station, Texas USA, and Microsoft Excel. We compared numerical data with a normal distribution using the Student's t-test, non-normally distributed data using the Wilcoxon rank sum test, and categorical data using the Fisher's exact test. We calculated the Spearman's rank correlation coefficient (ρ) to assess correlation between ALT and miR-122 concentrations. We calculated the coefficient of variation (CV) for ALT and miR-122 concentrations pre and post study infusion. We log transformed ALT and miR-122 concentrations for parametric comparative analyses. To explore change in miR-122 and ALT concentration between samples, we calculated the ratio of post-infusion to pre-infusion concentration and performed a Student's t-test on logged ratios to look for differences between groups. To determine the average change in ALT and miR-122 concentrations per day, we calculated the slope between the concentration before

and the concentration after study infusion. A p-value of <0.05 was considered to be statistically significant throughout.

5.3.6 Ethics

The study protocol was approved by University of Cape Town Human Research Ethics Committee (HREC 087/2012 and HREC 421/2017) [appendix 7] and the Western Cape Department of Health. Participants provided written informed consent [appendix 6]. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719) [appendix 8] and clinicaltrials.gov (NCT02182167) [appendix 9].

5.4 Results

5.4.1 Participant characteristics

We included 45 of the 102 NAC randomised control trial participants, from whom paired biomarker specimens had been collected within the specified time windows, 26 of these were from the NAC and 19 were from the placebo group (see Supplementary Figure 5.1). Baseline characteristics of the 45 included participants did not differ significantly from the 57 participants without paired specimens (see Supplementary Table 5.1). Four of the 45 participants with paired specimens died during study follow up (2 in the NAC and 2 in the placebo group); versus 10/57 participants without paired specimens (Fishers exact p=0.255).

Participant characteristics were similar between the NAC and placebo groups in those participants with biomarker samples (Table 5.1). The mean age was 38 years ± standard deviation (SD) 10, 58% were female and 91% were HIV positive. We had self-

reported ethnicity data on 31 participants, of whom 27 (87%) self-identified as Black African.

Table 5.1 Baseline characteristics and biomarker concentrations before and after study infusion in participants with anti-tuberculosis drug-induced liver injury randomised to intravenous n-acetylcysteine or placebo

Baseline Characteristics	**NAC (n=26)**	**Placebo (n=19)**	**Total (n=45)**	**p-value***
Age, years, mean (±SD)	38 (±12)	38 (±7)	38 (±10)	0.897
Female, n (%)	17 (65)	9 (47)	26 (58)	0.360
Weight, kg, median (IQR)	55 (49-64)	57 (45-63)	56 (48-64)	0.980
HIV positive, n (%)	22 (85)	19 (100)	41 (91%)	0.126
CD4 count (HIV positive) cells/mm^3, median (IQR)	93 (54-277)	54 (7-132)	74 (19-161)	0.222
Enrolment Total bilirubin, μmol/L, median (IQR)	50 (17-91)	74 (35-191)	52 (28-117)	0.112
Pre-infusion ALT, U/L, median (IQR) CV (%)	361 (238-580) 91	427 (238-612) 64	420 (238-580) 81	0.899
Post-infusion ALT, U/L, median (IQR) CV (%)	194 (119-293) 93	250 (111-419) 98	221 (119-342) 95	0.550
Pre-infusion mir-122, pM, median (IQR) CV (%)	0.44 (0.17-1.47) 167	0.84 (0.31-2.79) 152	0.58 (0.18-1.47) 169	0.251
Post-infusion mir-122, pM, median (IQR) CV(%)	0.18 (0.77-0.94) 214	0.33 (0.16-0.86) 145	0.26 (0.12-0.86) 207	0.113

5.4.2 Time intervals between AT-DILI presentation, biomarker sampling and study infusion.

The median time from presentation with AT-DILI to collection of the pre-infusion sample was 2.7 days (interquartile range (IQR) 1.5-4.1 days). This first sample was taken at a median of 0.6 hours (IQR 0.2-11 hours) before study infusion commenced, and the second sample was taken at a median of 50 hours (IQR 26-73 hours) after commencement of study infusion (supplementary Figure 5.1). The median time interval between paired specimens was 68 hours (IQR 47-77 hours) and was similar between NAC and placebo groups, (Wilcoxon rank sum test p=0.08).

5.4.3 ALT and miR-122 concentrations before study infusion

ALT and miR-122 concentrations and CVs are summarized in Table 5.1. The median ALT concentration before intravenous NAC/placebo infusion was 420 U/L (IQR 238-580 U/L) and the median miR-122 concentration was 0.58 pM (IQR 0.18 -1.47 pM), Figure 5.1 . MiR-122 and ALT concentrations before study infusion were correlated (Spearman's $\rho = 0.54$, p=0.0001), Figure 5.2. The median Ct pre-infusion was 23.1 (IQR 20.8-24.7).

5.4.4 ALT and miR-122 concentrations after study infusion

Post-infusion ALT and miR-122 concentrations were similar in the NAC and placebo groups see Table 5.1 and Figure 5.1. Post-infusion miR-122 concentrations were correlated with ALT concentrations in both the NAC and placebo groups (Spearman's ρ=0.42, p=0.031 and Spearman's ρ=0.53, p=0.020 respectively) and when participants from both groups were analyzed together (Spearman's ρ=0.45, p=0.002, Figure 5.2). The median Ct post-infusion was 23.7 (IQR 22.0-24.7).

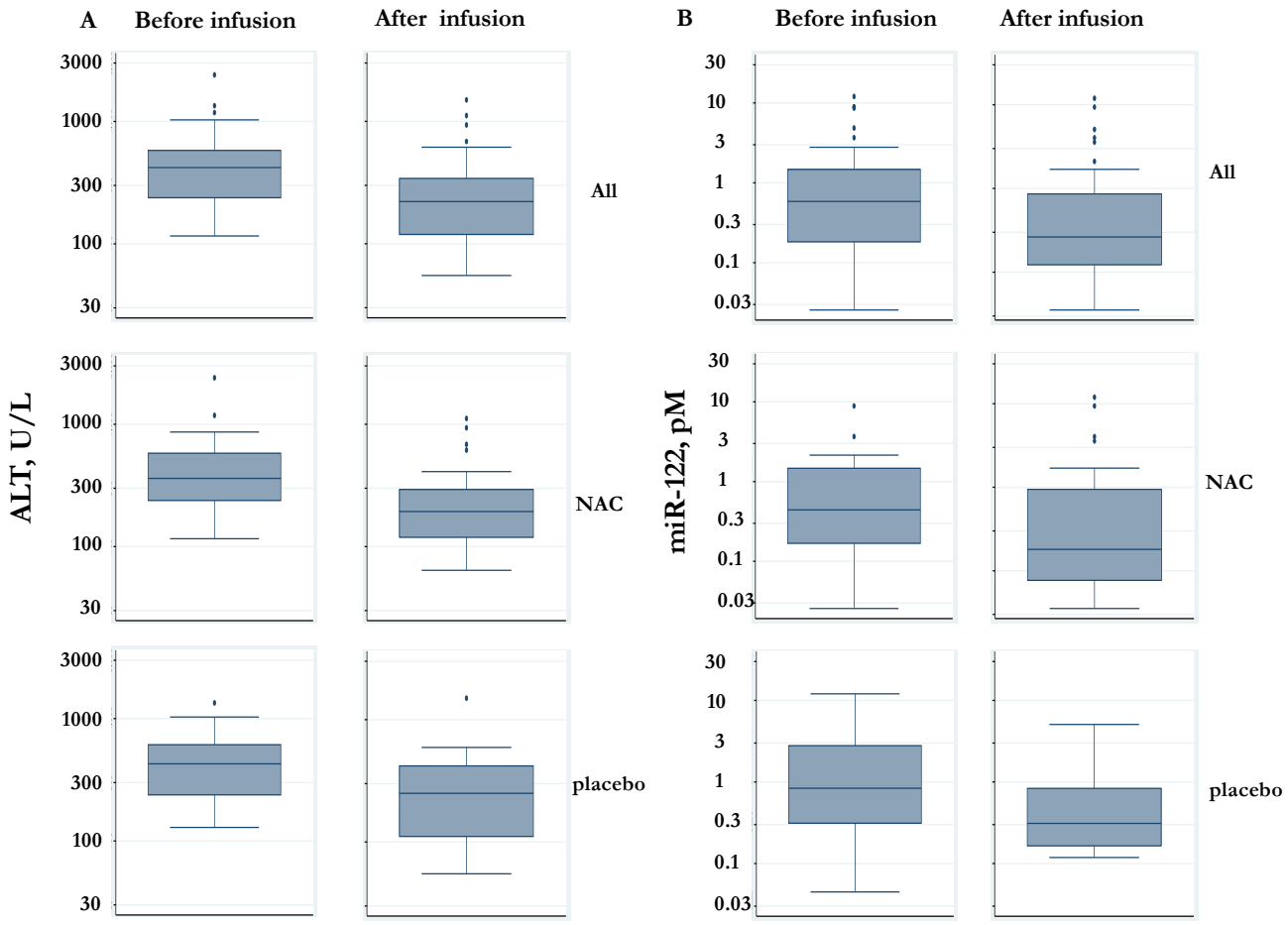

Figure 5.1. Box and whisker plots of (A) alanine aminotransferase (ALT) and (B) microRNA-122 (miR-122) serum concentrations before and after study infusion in 45 participants in a randomised placebo-controlled trial of intravenous n-acetylcysteine (NAC) in the management of anti-tuberculosis drug-induced liver injury

5.4.5 Change in ALT and miR-122 concentrations

ALT concentrations decreased between samples in most 43 (96%) participants. In contrast, the magnitude and direction of change in miR-122 concentrations varied substantially between participants, and miR-122 concentrations increased in 16 (36%) participants (10/26 in NAC group and 6/19 in placebo group, Figure 5.3).

The median fold-change in ALT concentrations between samples was 0.56 overall (IQR 0.43 - 0.69) and was similar in the NAC and placebo groups: median 0.55 (0.39 - 0.78) and 0.60 (0.46 - 0.69), respectively (Wilcoxon ranksum-test p=0.40), Supplementary Figure 5.2. The median fold change in miR-122 concentrations was 0.75 (IQR 0.23 - 1.53) overall and was similar in NAC and placebo groups; median 0.78 (IQR 0.23 - 1.53) and 0.54 (IQR 0.20 - 1.62) respectively, (Wilcoxon ranksum test p=0.68), Supplementary Figure 5.2; note that fold change value greater than 1 corresponds to an increase.

5.4.6 Change in ALT and miR-122 concentrations per day

ALT concentrations changed a median of 0.79-fold per day (IQR 0.75 - 0.83-fold) between the two biomarker samples. MiR-122 concentrations changed a median of 0.89-fold per day (IQR 0.49-1.17-fold) between the two biomarker samples.

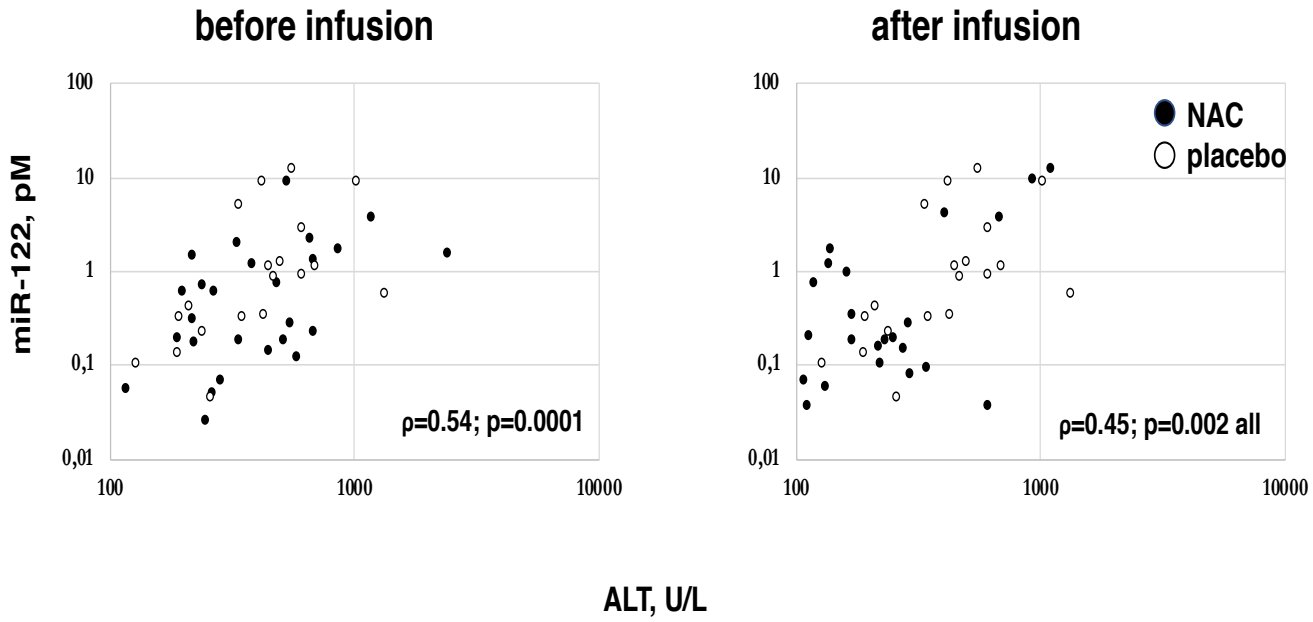

Figure 5.2. Correlation of pre-and post-study infusion serum alanine aminotransferase (ALT) and microRNA-122 (miR-122) concentrations in 45 participants with anti-tuberculosis drug-induced liver injury randomised to intravenous n-acetylcysteine (NAC) or placebo

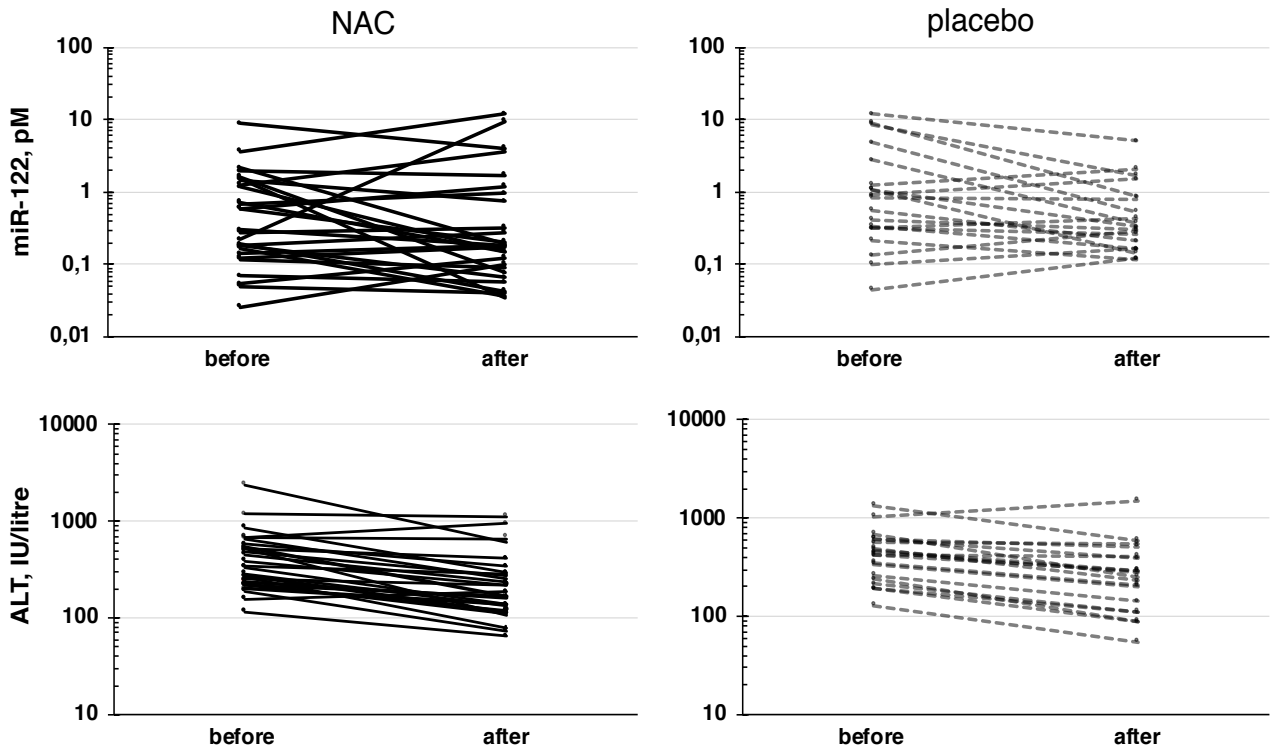

Figure 5.3. Change in serum alanine aminotransferase (ALT) and microRNA-122 (miR-122) concentrations after study infusion in 45 participants with anti-tuberculosis drug-induced liver injury randomised to intravenous n-acetylcysteine (NAC) or placebo

5.5 Discussion

The miR-122 concentrations observed in our participants with AT-DILI were much higher than those previously observed in healthy volunteers (107), or participants on anti-tuberculosis therapy without DILI (66). We found that serum ALT and miR-122 concentrations were correlated at both sampling occasions in participants with AT-DILI. ALT concentrations declined in almost all participants at the post-infusion sampling occasion. In contrast, changes in miR-122 concentrations were more variable, showing an increase in over a third of participants. The fold change in miR-122 concentrations between samples did not differ between NAC and placebo arms.

The median miR-122 concentration before study infusion in our cohort was 0.58 pM, which is 10 times higher than the upper limit of normal (ULN) of the reference range for healthy volunteers derived from the European Safer and Faster Evidence based Translation (SAFE-T) cohort, and 20 times the ULN from the Critical Path Institute's Predictive Safety Testing Consortium (PSTC) in the United States (107). Only two of our participants had concentrations below the ULN derived from the SAFE-T cohort, and none were below the PSTC ULN.

In the ALISTER study cohort in Scotland, participants starting anti-tuberculosis therapy without liver injury had similar miR-122 concentrations to healthy volunteers. MiR-122 concentrations increased slightly after starting anti-tuberculosis therapy but remained considerably lower (median 0.004pM) than the miR-122 concentrations observed in our participants with AT-DILI. (66).

There are limited data on miR-122 concentrations in patients with AT-DILI. Two participants in the ALISTER cohort developed DILI with ALTs of 431 and 194 U/L and miR-122 concentrations of 0.06 and 0.34 pM respectively. In both these participants, MiR-122 concentrations increased considerably from baseline, by 15- and 20-fold respectively. A recent Indian study by Bakshi et al found miR-122 expression to be 75% lower in participants with AT-DILI compared to healthy controls (67), but miR-122 was only sampled at enrolment and participants had less severe DILI than in our study, with a median ALT of 120 U/L. Despite these differences, the reason for the conflicting results is unclear and requires further exploration.

MiR-122 concentrations varied widely between participants in our study, as did the intra-individual change in miR-122 between sampling occasions. In the absence of other clinical evidence, it is unclear whether these changes in miR-122 concentrations indicate worsening or improvement of the liver injury, or merely reflect high intra- and inter-individual variability. In the PSTC healthy volunteer cohort who were sampled

repeatedly over 3 weeks, both inter-subject and intra-subject coefficients of variation for miR-122 were high, at 91% and 94% respectively (107). Intra-individual variability was particularly high in black participants (107). A North American healthy volunteer cohort also found high intra and inter-donor variability in miR-122 expression, particularly in participants who identified as non-Caucasian (126). Inter-individual variability poses challenges to defining cut-offs for abnormal elevation of miR-122. Intra-individual variability complicates interpretation of changes observed in miR-122 concentration over time. Inter-individual and intra-individual variability may therefore limit utility of miR-122 as a biomarker in diagnosing AT-DILI and monitoring AT-DILI progression or recovery.

We observed an increase in miR-122 concentrations between the two sampling occasions in a third of our study participants. The hepatocellular injury in AT-DILI (38) may be more sustained than injury due to oxidative stress injury in paracetamol overdose; and miR-122 may therefore decline more slowly in AT-DILI. Even among studies of paracetamol-induced hepatotoxicity, there are some that describe increased miR-122 concentrations from 3 to 14 days after DILI onset (99, 100).

Our study has limitations. We only included approximately half of the cohort with two biomarker samples, which may introduce selection bias to our study. However, baseline characteristics and liver biochemistry did not differ substantially between those participants included in this study and those not included. HIV prevalence was high in our cohort and therefore our findings may not be generalizable to other populations with lower HIV prevalence. We only quantified miR-122 at 2 time points. We did not have a control group without AT-DILI drawn from the same population to allow for comparison. Different quantification and normalization methods used in different studies may influence comparisons between our results and results from other studies.

5.6 Conclusion

MiR-122 concentrations were markedly higher in our cohort of AT-DILI than previously observed in healthy controls, and in participants on anti-tuberculosis therapy without liver injury. MiR-122 may therefore be a useful biomarker to diagnose AT-DILI in this population. However, high intra-individual and inter-individual variability in miR-122 concentrations may limit its utility to monitor recovery from AT-DILI. To characterize miR-122 concentrations prior to liver injury onset and in early AT-DILI, a large prospective cohort study collecting repeated samples for biomarker quantification from patients on anti-tuberculosis therapy, with frequent clinical review, would be required, as AT-DILI occurs in a small subset of patients on anti-tuberculosis therapy. Further larger studies monitoring miR-122 over the full course of recovery from AT-DILI are required to characterize changes in this biomarker over time, and associations between concentrations observed and outcomes.

Chapter 6 Summary, Conclusions and Future Research

6.1 Summary and conclusions

In summary, my 3 distinct research objectives were: First, does N-acetylcysteine (NAC) improve outcomes, more specifically resolution of liver injury, in participants with anti-tuberculosis drug-induced liver injury (AT-DILI)? I addressed this objective by conducting a randomized double-blind placebo-controlled trial of NAC in hospital participants diagnosed with AT-DILI, which was the focus of my first publication. My second objective was to describe outcomes of drug rechallenge following AT-DILI in a high HIV prevalence setting, and this is addressed in the descriptive study or second publication. Third, I wanted to explore the utility of microRNA-122 (miR-122) as a biomarker of AT-DILI, and the effect of NAC on miR-122; this objective is addressed in the third publication.

In **Chapter 3** we reported on the double-blind placebo-controlled randomised trial of intravenous NAC in the management of AT-DILI. To our knowledge this is the first published RCT investigating NAC as a therapy for AT-DILI. We included 102 participants from 3 public sector hospitals in Cape Town, who were diagnosed with AT-DILI. We included HIV positive participants (87% of our cohort) of whom less than half were taking concurrent ART. Our primary end point was time for ALT to reach less than 100 U/L and our prespecified secondary endpoints were length of hospital stay and in hospital mortality. We found no difference in our primary endpoint between NAC and placebo groups: median time to ALT less than 100 U/L was 7.5 days (IQR 6–11 days) in the NAC group and 8 days (IQR 5–13 days) in the placebo group, hazard ratio (HR) 1.03 (95% CI, 0.68–1.57). Median time to hospital discharge was shorter in the NAC group: 9 days (IQR 6–15 days) than in the placebo group 18 days (IQR 10–25

days) (HR, 1.73; 95% CI, 1.13–2.65) and there was no difference in mortality between the groups. In response to a letter to the editor by Hampannavar et al, we did a further subgroup analysis based on liver injury severity (severe liver injury being defined as INR > 1.5 and/or encephalopathy), and we found no difference in our primary and secondary endpoints when participants were stratified according to liver injury severity (127). However, we recognise the following caveats; this subgroup analysis according to liver injury severity was not prespecified in our research protocol, randomisation was not stratified by liver injury severity and our study was not powered for this sub-group analysis.

In my literature review, I found studies that describe how NAC prevented elevation in serum ALT in animals and humans exposed to ATT (55, 56). Furthermore, intravenous NAC improved 3-week transplant-free survival in a randomized placebo-controlled trial of participants with grade 1-2 non-paracetamol-induced liver failure, 26% of whom had DILI (54). In that trial, the NAC group had a shorter hospital stay than the placebo group, but the rate of decline in ALT concentrations was not reported.

Possible explanations for NAC not hastening biochemical liver recovery in our study are: First, the mechanism of the liver injury associated with ATT may differ from that of paracetamol, where NAC may have some benefit. Anti-tuberculosis drug-induced liver injury is thought to be an idiosyncratic immune response to the covalent binding of drug metabolites to liver proteins (38), as compared to paracetamol's direct dose dependant hepatotoxic effect. The onset of liver injury due to ATT is typically more delayed and liver injury persists for longer than that due to paracetamol. Second, ALT is not a sensitive biomarker of liver injury. ALT concentrations may be normal during the early stages of hepatocyte injury or apoptosis, and ALT may be released from tissues other than liver such as skeletal muscle, kidney, and heart (62). I considered that, perhaps if we had used a more sensitive and liver specific biomarker such as

microRNA-122, we might detect a difference between the treatment groups in our trial. This is one of the hypotheses that we tested in the smaller miR-122 study embodied in chapter 5, where we explored the effect of NAC on miR-122 concentrations in less than half of our participants.

In our trial, participants with AT-DILI who received NAC had significantly shorter hospital stays, similar to what Lee and colleagues observed in their study of NAC in non-paracetamol liver failure (54). This suggests that NAC hastened clinical recovery from AT-DILI. Length of hospital stay was a prespecified secondary endpoint and was not biased by the clinician's decision to discharge patients, because treatment allocation was blinded. There are two possible explanations for the shorter hospital stay in the NAC group, despite the similar time to ALT < 100 U/L between groups.

There is evidence that NAC's antioxidant effects can be beneficial to HIV infected individuals (87% of our participants), who often have glutathione depletion (120). In a trial conducted in the United States of America in the late 1990's, 246 HIV positive participants were randomised to oral NAC or placebo for 8 weeks, followed by an open label phase where NAC was offered to all participants for 2 -3 years. Survival improved in the NAC group during 2–3-year follow-up (RR 1.8, 95% CI 1.1-3.0, p=0.02), however, no HIV viral load nor CD4 count data were collected and this trial was performed in the era before effective ART was available.

It is also possible that NAC could have a direct therapeutic effect against TB. There is some evidence that NAC inhibits *Mycobacterium tuberculosis (M.Tb)* growth and augments the mycobactericidal activity of first-line ATT in vitro (121, 122). N-acetylcysteine reduced oxidative stress and *M.Tb* growth in vitro, as well as *M.Tb* growth in vivo in animals. However, there is no reported trial in humans to date to confirm any clinically relevant antimycobacterial activity of NAC.

In **Chapter 4**, which is a study nested within the RCT, we describe the rechallenge of first-line ATT following recovery from AT-DILI. We included 79 of the 102 participants from the RCT who were rechallenged with at least one first-line anti-tuberculosis drug. Most participants (86%) were started on background ATT and most (96%) were rechallenged with a minimum of two individual drugs re-introduced sequentially and in full dosages. Of fifty-eight participants who received an aminoglycoside as part of background ATT, 5 (9%) had a serious adverse drug reaction: acute kidney injury in 3 participants and hearing loss in 2.

There were 14 positive rechallenges in the 79 rechallenged participants (18%). Positive rechallenge was associated with pyrazinamide rechallenge (p=0.005), female sex (Fisher's exact test p = 0.039) and first episode of TB (Fisher's exact test p = 0.032). One participant died due to a fatal hypersensitivity reaction related to pyrazinamide rechallenge. A study of different rechallenge regimens found a high proportion of positive rechallenge in the group that received a concomitant drug regimen with pyrazinamide compared to the group that received a sequential regimen without pyrazinamide (82). The high risk of positive rechallenge associated with pyrazinamide found in our study and other studies should encourage guidelines to completely omit pyrazinamide from rechallenge regimens, including in mild forms of DILI, and especially when less toxic second line anti-tuberculosis drugs like levofloxacin, linezolid and bedaquiline are available. Furthermore, these newer MDR-TB regimens could be used instead of rechallenge altogether, but they are more toxic – there is a need for a trial of rechallenge versus a new regimen.

Some studies have also found female sex to be a risk factor for AT-DILI (19, 23) as well as for positive ATT rechallenge (82). In contrast to other studies, we did not find low serum albumin or increased age to be associated with positive rechallenge. (81, 84).

We found that ATT rechallenge had a major impact on the interruption of ATT and ART. The median time from first-line ATT interruption to start of rechallenge was 13 days (IQR 8–18 days). During this period patients are usually placed on less effective second-line ATT and this may theoretically increase the risk of morbidity associated with TB. However, there is no evidence to support this assumption. Anti-retroviral therapy was interrupted at presentation with liver injury in 79% of HIV-positive participants who were receiving ART with a median interruption time of 32 days (IQR 17-58 days). Interruption of ART may impact negatively on the efficacy of therapy and contribute to the emergence of anti-retroviral drug resistance (125). The median delay from AT-DILI presentation to ART initiation in our cohort was 53 days, and 22% of our cohort were not yet on ART when study follow-up ended. There is evidence that delays in the initiation of ART in patients with advanced HIV disease have caused increased mortality (44).

This study had limitations. First, it was descriptive and therefore not powered to identify risk factors associated with positive rechallenge. Second, follow-up was short-term and ended after rechallenge was completed. We therefore could not quantify the impact of positive rechallenge on long term outcomes of ATT or ART. Again, our cohort comprised majority HIV positive participants so our findings cannot be generalised to other populations with low HIV prevalence.

In Chapter 5, we investigate the utility of blood biomarker microRNA-122 (miR-122) as a marker in the diagnosis and management of AT-DILI. We compare miR-122 to the current standard of care biomarker for DILI, alanine aminotransferase (ALT). The rationale behind this study is that ALT is not a specific marker of liver injury and may be increased due to mechanisms other than hepatocyte death (61). We quantified serum miR-122 and ALT concentrations in our participants with AT-DILI at 2 sampling occasions: before and after NAC/placebo infusion. We described correlations between

ALT and miR-122 concentrations as well as changes in ALT and miR-122 concentrations between sampling occasions. We explored the effect of NAC infusion on miR-122 concentrations.

We included 45 participants from the RCT who had paired pre- and post-infusion samples collected within the pre-specified time windows. The median time between pre- and post-infusion biomarker specimens was 68 hours (IQR 47-77 hours). Median pre-infusion ALT and miR-122 concentrations were 420 U/L (IQR 238- 580) and 0.58 pM (IQR 0.18-1.47) respectively, were similar between NAC and placebo groups, and were correlated (Spearman's ρ=0.54, p=0.0001). ALT concentrations decreased between samples in most (96%) participants compared to miR-122 concentrations, which unexpectedly increased in over a third of participants. The median fold-change in ALT and miR-122 concentrations between sampling were similar between NAC and placebo groups.

First, the median miR-122 concentration in our participants with AT-DILI were 10 times higher than those previously reported in healthy volunteers from the SAFE-T cohort and 20 times more than that of the PSTC cohort , both from The United States (107). In the ALISTER study cohort in Scotland, miR-122 concentrations increased slightly in participants after starting anti-tuberculosis therapy but remained considerably lower (median 0.004pM) than the miR-122 concentrations observed in our participants with AT-DILI (66).

There are limited data on miR-122 concentrations in patients with AT-DILI, albeit with inconsistent results. In two participants in the ALISTER cohort who developed AT-DILI, miR-122 concentrations increased considerably from baseline, by 15- and 20-fold respectively. However, an Indian study found miR-122 concentrations to be 75% lower in participants with AT-DILI compared to healthy controls (67).

The reason for the increase in miR-122 concentrations in our cohort of AT-DILI is uncertain. One possible explanation could be that the mechanism of AT-DILI is different from that of paracetamol DILI where most of the studies of miR-122 profiles have been done. We know that ATT causes an idiosyncratic and immune mediated injury which may be more sustained than the direct and dose dependant oxidative stress injury caused by paracetamol.

We observed wide intra and inter-individual variability in the miR-122 concentrations in our study. Intra- and inter-assay co-efficient of variation (CV) for miR-122 were less than 5% and 10%, respectively (intra-plate, mean 1.31%, min 0.07%, max 3.39%; inter-plate, mean 2.82, min 0.16%, max 8.5%), suggesting good reproducibility of our results. For this reason, we doubt that the variability in miR-122 expression observed amongst participants in our cohort, is due to inherent variability in the qT-PCR test, but rather is likely due to hepatic pathophysiology.

Furthermore, the wide intra- and inter-individual variability in miR-122 concentrations that we observed, has been previously reported in other studies (107). Inter-individual variability poses challenges to defining normal ranges of miR-122 and intra-individual variability complicates interpretation of changes observed in miR-122 concentration over time. Inter-individual and intra-individual variability may therefore limit the utility of miR-122 as a biomarker in diagnosing AT-DILI and monitoring AT-DILI progression or recovery.

We did not detect any effect of NAC on miR-122 concentrations between samples. In our RCT we found that NAC did not hasten decline in the conventional DILI biomarker, ALT. It is possible that NAC has a direct effect on both these biomarkers and further studies exploring the mechanistic effects of NAC on these biomarkers would be useful.

Our miR-122 study has limitations. We only included approximately half of the RCT cohort with two biomarker samples, which may introduce selection bias to our study. However, baseline characteristics and liver biochemistry did not differ substantially between those participants included in this study and those not included. See supplementary table 3. We only quantified miR-122 at 2 time points, thus offering only a snapshot of miR-122 profiles following AT-DILI. We did not include a control group without AT-DILI drawn from the same population to allow for comparison. Again, HIV prevalence was high in our cohort and therefore our findings may not be generalizable to other populations with lower HIV prevalence. Different quantification and normalization methods used in different studies may influence comparisons between our results and results from other studies

In conclusion, miR-122 concentrations were increased in our study cohort and may therefore suggest its potential as a biomarker to diagnose AT-DILI. However, the evidence as a whole is limited, as well as inconsistent in terms of this finding. We observed wide intra- and inter-individual variability in miR-122 concentrations suggesting that miR-122 may not be a reliable marker to monitor recovery from AT-DILI. We did not detect an effect of NAC on miR-122 concentrations.

6.2 Future research

Our randomised controlled trial demonstrated that intravenous NAC did not improve biochemical recovery from AT-DILI but NAC did reduce length of hospital stay significantly by 9 days. This clinical benefit of NAC needs to be confirmed in further larger trials of AT-DILI with longer participant follow-up, and with clinical outcomes used as the primary endpoint. A potential reduction in hospital costs associated with reduced hospital stays, also needs to be evaluated in any future clinical trials of NAC in AT-DILI. The majority of our study participants were HIV positive (87%) of whom 45% were taking concomitant and potentially hepatotoxic ART, which could have contributed to DILI. In future larger studies, sub-group analyses of primary outcomes in HIV negative participants as well as HIV positive participants, both taking and not taking potentially hepatotoxic ART need to be pre-specified. Future studies would be encouraged to pre-specify analysis of primary and secondary outcomes within strata of liver injury severity. Randomisation to treatment or placebo would have be stratified accordingly.

We found that aminoglycosides used as background ATT in our participants, were associated with severe adverse effects. Soon after the completion of our trial, local guidelines were changed to exclude aminoglycosides from background anti-tuberculosis regimens. We found pyrazinamide to be significantly associated with positive rechallenge. Therefore, future clinical guidelines on anti-tuberculosis drug rechallenge need to reconsider pyrazinamide rechallenge, including in mild grades of liver injury, especially with the availability of alternative and less hepatotoxic drugs such as fluoroquinolones, linezolid and bedaqiline. Future studies should also evaluate the proportion of positive rechallenges with regimens that exclude pyrazinamide, and

compare this to the proportion of positive rechallenges reported in our study and others, which included pyrazinamide, to see if there are significant differences.

Drug rechallenge following AT-DILI caused prolonged interruption of first-line ATT and ART, as well as delays in initiating anti-retroviral therapy in ART naïve participants. Further studies with longer participant follow-up (> 8 weeks) are needed to evaluate the effects of drug rechallenge on clinical outcomes including the emergence of anti-tuberculosis and anti-retroviral drug resistance, morbidity and mortality. Rechallenge can also cause prolonged hospitalisation and need for outpatient care which can potentially increase healthcare costs. Such potential additional costs need to be evaluated in future studies.

MicroRNA-122 concentrations were significantly elevated in our participants with AT-DILI compared to healthy volunteers and controls from other studies. However, we observed wide intra- and inter-individual variability in miR-122 concentrations. Future trials of miR-122 in AT-DILI should include controls from the same population. Future larger prospective cohort studies should consider frequent sampling of miR-122 in patients starting ATT, to better characterise miR-122 concentrations prior to liver injury and in early AT-DILI, because AT-DILI occurs in a small subset of patients on ATT. Further larger studies over the full course of recovery from AT-DILI are required to characterise changes in this biomarker over time and evaluate any associations between concentrations observed and clinical outcomes.

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8. Appendices

Appendix 1: A randomized controlled trial of intravenous n-acetylcysteine in the management of anti-tuberculosis drug-induced liver injury.

Clinical Infectious Diseases

MAJOR ARTICLE

A Randomized Controlled Trial of Intravenous N-Acetylcysteine in the Management of Anti-tuberculosis Drug–Induced Liver Injury

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Background. Liver injury is a common complication of anti-tuberculosis therapy. N-acetylcysteine (NAC) used in patients with paracetamol toxicity with limited evidence of benefit in liver injury due to other causes.

Methods. We conducted a randomized, double-blind, placebo-controlled trial to assess the efficacy of intravenous NAC in hospitalized adult patients with anti-tuberculosis drug–induced liver injury (AT-DILI). The primary endpoint was time for serum alanine aminotransferase (ALT) to fall below 100 U/L. Secondary endpoints included length of hospital stay, in-hospital mortality, and adverse events.

Results. Fifty-three participants were randomized to NAC and 49 to placebo. Mean age was 38 (SD±10) years, 58 (57%) were female, 89 (87%) were HIV positive. Median (IQR) serum ALT and bilirubin at presentation were 462 (266–790) U/L and 56 (25–100) µmol/L, respectively. Median time to ALT <100 U/L was 7.5 (6–11) days in the NAC arm and 8 (5–13) days in the placebo arm. Median time to hospital discharge was shorter in the NAC arm (9 [6–15] days) than in the placebo arm (18 [10–25] days) (HR, 1.73; 95% CI, 1.13–2.65). Mortality was 14% overall and did not differ by study arm. The study infusion was stopped early due to an adverse reaction in 5 participants receiving NAC (nausea and vomiting [3], anaphylaxis [1], pain at drip site [1]).

Conclusions. NAC did not shorten time to ALT <100 U/L in participants with AT-DILI, but significantly reduced length of hospital stay. NAC should be considered in management of AT-DILI.

Clinical Trials Registration. South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719).

Keywords. anti-tuberculosis therapy; drug-induced liver injury; N-acetylcysteine; tuberculosis.

Liver injury is the most common severe adverse drug reaction caused by first-line anti-tuberculosis therapy (ATT), with an estimated incidence of 2–28% depending on the definition of drug-induced liver injury (DILI) used and the population studied [1]. Liver injury due to first-line anti-tuberculosis therapy (AT-DILI) may cause prolonged hospitalization [2] and is associated with increased mortality [3, 4]. There is currently no specific therapy for AT-DILI. The management of suspected AT-DILI includes the cessation of all potentially hepatotoxic anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide), possible introduction of alternative ATT, monitoring of liver function tests, and supportive care while awaiting liver recovery. When the serum alanine aminotransferase (ALT) falls below 100 U/L, ATT rechallenge may be considered [5].

N-acetylcysteine (NAC) is widely used as treatment for paracetamol liver toxicity [6] and may provide benefit for other causes of hepatitis [7, 8]. A prospective cohort study of 155 participants with non-paracetamol acute liver failure found improvement in transplant-free survival in those treated with NAC compared with historical controls; DILI was the cause of liver failure in 38% [9]. A randomized controlled trial of NAC in 173 participants with non-paracetamol acute liver failure found improved transplant-free survival overall, but the study was underpowered to assess efficacy in the small DILI subgroup (26% of participants) [10]. A systematic review concluded that there was insufficient evidence to support the use of NAC in non-paracetamol–induced liver injury [11].

There is evidence that NAC may prevent AT-DILI. N-acetylcysteine improved liver histology in rats exposed to high-dose isoniazid and rifampicin intraperitoneally [12] and reduced cellular and mitochondrial membrane damage, and apoptosis, in an in vitro study using human hepatocellular

Received 7 May 2020; editorial decision 11 August 2020; published online 26 August 2020.

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Clinical Infectious Diseases® 2021;73(9):e3377–83

DOI: 10.1093/cid/ciaa1255

carcinoma cells exposed to toxic doses of isoniazid, rifampicin, and pyrazinamide in various combinations [13]. Oral NAC administered to participants during the first 2 weeks of ATT prevented increases in ALT in a small, open-label, randomized controlled trial [14].

We hypothesized that NAC would shorten the duration of AT-DILI and conducted a randomized, double-blind, placebo-controlled trial of intravenous NAC in adults with suspected AT-DILI.

METHODS

Study Participants

We recruited adult patients with a diagnosis of AT-DILI at 3 public sector hospitals in Cape Town, South Africa: Groote Schuur Hospital (tertiary-level academic hospital), New Somerset Hospital (secondary-level hospital), and Khayelitsha District Hospital. To be eligible for recruitment, a patient had to meet the American Thoracic Society criteria for AT-DILI requiring cessation of ATT [5], by either having an ALT of more than 3 times the upper limit of normal if symptoms of hepatitis were present, or an ALT of more than 5 times the upper limit of normal without symptoms of hepatitis. Other inclusion criteria were age 18 years or older, taking first-line ATT for the treatment of active tuberculosis (TB), and liver injury that was attributed to ATT. We included both participants who were admitted to hospital because of AT-DILI and those who developed AT-DILI while in hospital. Patients with acute liver failure, which we defined as fulminant hepatitis resulting in coagulopathy (international normalized ratio [INR] >1.5) and an altered mental status [15], were eligible for inclusion.

We excluded patients with asthma, because of the risk of NAC-induced bronchospasm; pregnant patients; and patients known to have viral hepatitis at the time of screening.

The primary endpoint was the time to ALT falling below 100 U/L. Secondary endpoints included time to hospital discharge, in-hospital mortality, and study infusion–related adverse events.

Study Procedures

This study was a pragmatic randomized controlled trial, nested within routine clinical care. Participants were investigated and managed by hospital clinicians, except for the administration of the study infusion and monitoring for adverse reactions by a member of the study team. Hospital clinicians, who were blinded to treatment allocation, made the decision to discharge participants from the hospital, in line with their clinical judgement.

We collected baseline demographic, clinical, pharmacological, and biochemical data on all study participants at the time of randomization. We graded hepatic encephalopathy using the West Haven score [16] (Supplementary Table 1). As part of routine clinical workup, participants were tested for acute viral hepatitis A (anti–hepatitis A immunoglobulin M [IgM]), acute viral hepatitis B (hepatitis B surface antigen and anti-core IgM), and viral hepatitis C (anti–hepatitis C total antibodies and polymerase chain reaction).

Study participants were randomized 1:1 to receive intravenous NAC or placebo. Randomization was stratified by site and performed in blocks of 10 using a computer-generated randomization schedule. Study pharmacists at each site had restricted access to the randomization schedule and prepared the study infusion according to treatment allocation. Investigators and study participants were blinded to treatment allocation.

N-acetylcysteine was dosed and administered according to the regimen for paracetamol overdose as per the manufacturer's provided guidelines: 150 mg/kg over 1 hour, 50 mg/kg over 4 hours, and 100 mg/kg over 16 hours (see Supplementary Table 2 for the weight-based dosing schedule). We used 0.9% saline as diluent for the NAC and placebo infusions, except for participants with acute liver failure or hypoglycemia (serum glucose <3.5 mmol/L), for whom 5% dextrose was used. Intravenous NAC is colorless and indistinguishable from placebo when mixed with either saline or dextrose solution.

Study participants were closely monitored for adverse events during the first hour of the study infusion and at the beginning and end of each infusion bag. A study investigator reviewed participants clinically at least twice weekly during hospital admission. We graded the severity of adverse events using Division of AIDS (DAIDS) categories [17]. All participant deaths were reviewed by an independent physician, to assess whether the study drug was implicated in the death. Serum ALT was monitored by the clinical care team at least twice weekly until it fell below 100 U/L, as per standard clinical practice.

Statistical Analysis

We powered the study to detect a 33% reduction in time for ALT to fall below 100 U/L, with 80% power and an α value of 0.05. We assumed a mean (SD) time for ALT to fall below 100 U/L in the placebo arm of 18 (±10) days, based on the ALT normalization time after cessation of ATT reported in a trial of ATT rechallenge regimens [18]. We calculated that we would require 88 participants (44 in each arm); we inflated the sample size to 100 participants to allow for deaths and loss to follow-up.

Analysis was by modified intention to treat. We included all randomized participants in whom we commenced the study infusion in the analysis. Continuous variables were described using means and SDs if parametrically distributed or medians and ranges if non–parametrically distributed. Categorical variables were described using counts and percentages. Time to ALT <100 U/L and time to discharge from hospital were described using Kaplan-Meier analyses. For calculation of time to hospital discharge we used the interval from study consent to discharge home or to a chronic care

facility. The analysis of time to hospital discharge included both patients presenting with AT-DILI before admission to the hospital and those who developed AT-DILI during hospital admission. We performed a log-rank test to compare survival curves. We performed a univariable Cox regression to calculate a hazard ratio (HR) for ALT falling below 100 U/L and an HR for hospital discharge, with a 95% confidence interval (CI). Data were analyzed using Stata (version SE/15.0; StataCorp, College Station, TX).

Ethics

This study was conducted according to the guidelines of the Helsinki Declaration of 2013 and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) principles of Good Clinical Practice [19, 20]. The protocol was approved by the Western Cape Department of Health, University of Cape Town Human Research Ethics Committee (HREC 087/2012), and the South African Health Products Regulatory Authority. Participants provided written informed consent. Our research ethics committee granted us permission to include patients with hepatic encephalopathy who were too sick to consent at presentation, and to seek their consent after they had recovered from encephalopathy. We were also granted permission to include the data of participants who did not recover from encephalopathy in the analysis. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719).

RESULTS

We screened 125 patients with suspected AT-DILI, 20 of whom were excluded (Figure 1). We enrolled 105 participants of whom 54 were randomized to the NAC arm and 51 were randomized to the placebo arm. Three randomized individuals (1 in the NAC arm and 2 in the placebo arm) were not administered the study infusion and were not included in the analyses: 1 participant withdrew consent, 1 participant disclosed that they were asthmatic (an exclusion criterion), and 1 participant could not be administered the study infusion as there was no study team member available to commence and monitor the infusion (Figure 1).

The baseline characteristics of the 102 participants who started the study infusion were similar in the NAC and placebo arms (Table 1). Sixty participants (59%) had pulmonary TB, 37 (36%) had extrapulmonary TB, and in 5 participants (5%) the site of TB was not specified. Twenty-two participants (22%) had previously received a course of ATT before this treatment episode. One hundred participants (98%) were taking intensive-phase ATT (rifampicin, isoniazid, pyrazinamide, and ethambutol) and 2 participants (2%) were taking continuation-phase ATT (rifampicin and isoniazid) at the time of presentation with a liver injury. Eighty-nine participants (87%) were human immunodeficiency virus (HIV) positive, of whom 31 were taking an efavirenz-containing antiretroviral therapy (ART) regimen, 9 were taking a lopinavir-ritonavir–containing ART regimen, and 23 were taking cotrimoxazole at the time of presentation with a liver injury. Four participants (1 in the NAC arm and 3 in the placebo arm) reported alcohol consumption greater than 14 units/week. Twelve participants in the NAC arm and 9 participants in the placebo arm developed the AT-DILI during a hospital admission. Seventy-four participants (73%) reported symptoms of AT-DILI at screening. The most commonly reported symptoms and signs were jaundice (47%), vomiting (43%), nausea (33%), and abdominal pain (25%). Eleven participants presented with hepatic encephalopathy, of whom 9 had mild encephalopathy (West Haven coma score of 1–2) and 2 had moderate encephalopathy (West Haven coma score of 3). Eighty-two participants had a prolonged INR (>1.1) and 11 had a serum sodium of less than 125 mmol/L.

None of the participants had evidence of viral hepatitis based on serology at the time of enrollment. However, during the course of the trial, 3 participants were found to have serological evidence of chronic viral hepatitis B. In addition, in 2 participants with positive hepatitis B surface antigen, anti-core IgM quantification was not requested by the clinical care team, so acute hepatitis B could not be excluded. One of these participants was also hepatitis A total antibody positive, but hepatitis A IgM was not performed to exclude acute hepatitis A.

Five participants (1 in the NAC arm and 4 in the placebo arm) were assessed by study investigators as having other diseases that could have caused hepatitis (leptospirosis, disseminated Emergo mycosis, hepatocellular carcinoma, sepsis, and TB immune reconstitution inflammatory syndrome). Eight participants (5 in the NAC arm and 3 in the placebo arm) were assessed by study investigators as possibly having another drug implicated in the liver injury: efavirenz in 3, lopinavir-ritonavir in 2, cotrimoxazole in 2, and fluconazole in 1.

The time to ALT <100 U/L was similar in the treatment arms (Figure 2*A*), with a median of 7.5 days (interquartile range [IQR], 5.5–11 days) and 8 days (IQR, 5–13 days) in the NAC and placebo arms, respectively. The hazard ratio (HR) for ALT falling below 100 U/L was 1.03 (95% CI, .68–1.57).

Time to discharge from hospital was shorter in the NAC arm than in the placebo arm (Figure 2*B*), with a median of 9 days (IQR, 6–15 days) in the NAC arm and 18 days (IQR, 10–25 days) in the placebo arm. The HR for hospital discharge was 1.73 (95% CI, 1.13–2.65).

The overall mortality was 14% and did not differ by treatment arm. The causes of death were ATT-induced liver failure in 9, chronic lung disease in 2, sepsis in 1, *Pneumocystis jirovecii* pneumonia in 1, and post–liver biopsy hemorrhage in 1. The study drug was not implicated in any of the deaths.

There were 16 adverse events (AEs) during the study infusion, 13 in the NAC arm and 3 in the placebo arm. The study

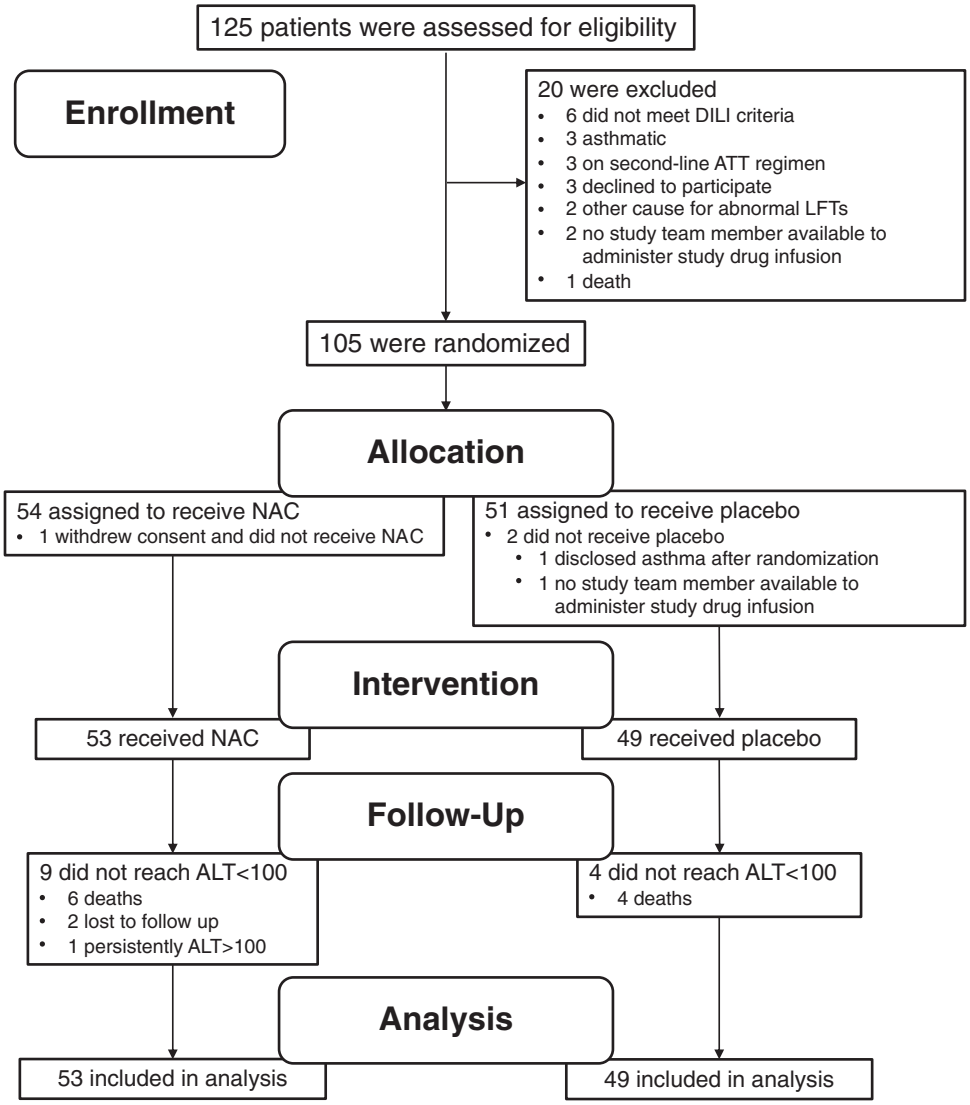

Figure 1. Screening, randomization, and follow-up. Abbreviations: ALT, alanine aminotransferase; ATT, anti-tuberculosis therapy; DILI, drug-induced liver injury; LFT, liver function test; NAC, N-acetylcysteine.

infusion was stopped early due to an AE in 5 participants, all of whom were receiving NAC (Table 2).

Serious AEs that occurred during study follow-up (after study drug administration) are described in Supplementary Table 3. None of these serious AEs were assessed by the investigators as being caused by the study drug; 19 serious AEs were assessed as possibly caused by other drugs.

DISCUSSION

We found no significant difference in our primary outcome (time to ALT <100 U/L) between participants with AT-DILI who received NAC or placebo. However, the median time to hospital discharge was 9 days shorter in the NAC arm. N-acetylcysteine was generally well tolerated, but all 5 AEs that resulted in discontinuation of the study infusion occurred in the NAC arm.

N-acetylcysteine is widely used to treat patients with paracetamol overdose; however, the quality of evidence for its efficacy in this setting is limited [21]. In contrast to paracetamol, which has a direct dose-dependent hepatotoxic effect via intermediary metabolites, the mechanism of AT-DILI is thought to be an idiosyncratic immune response to the covalent binding of drug metabolites to liver proteins [22]. Furthermore, the duration of liver injury due to ATT is typically much longer than that due to paracetamol. Despite these differences in the pathogenesis of AT-DILI and paracetamol-induced liver injury, NAC prevented increases in ALT in rats and humans exposed to ATT [12, 14], and intravenous NAC improved transplant-free survival in a randomized placebo-controlled trial in participants with non–paracetamol-induced liver failure, some of whom had DILI [10].

In our trial there was no difference in our primary efficacy endpoint of time to ALT <100 U/L between treatment arms.

Table 1. Baseline Characteristics of Participants by Study Arm

Baseline Characteristics	NAC (n = 53)	Placebo (n = 49)
Age, mean ± SD, years	37 ± 10	38 ± 9
Female, n (%)	34 (64)	24 (49)
Weight, median (IQR), kg	55 (47–67)	53 (45–63)
First time on TB treatment, n (%)	41 (77)	39 (80)
Duration of TB treatment, median (IQR), days	18 (10–31)	25 (15–40)
HIV positive, n (%)	44 (83)	45 (92)
ART, n (%)	23 (43)	17 (35)
Efavirenz	18 (34)	13 (27)
Lopinavir-ritonavir	5 (9)	4 (8)
Cotrimoxazole, n (%)	8 (15)	15 (31)
Symptoms of DILI, n (%)	39 (74)	35 (71)
Encephalopathy, n (%)	6 (11)	5 (10)
ALT, median (IQR), U/L	448 (286–685)	384 (266–566)
Total bilirubin, median (IQR), µmol/L	55 (19–93)	65 (30–117)
ALP, median (IQR), U/L	170 (101–248)	175 (113–253)
INR, median (IQR)	1.5 (1.2–2.2)	1.3 (1.1–2.2)
Albumin, median (IQR), g/L	26 (19–30)	23 (20–29)
Sodium, mean ± SD, mmol/L	131 ± 5	129 ± 5
CD4 count, median (IQR), cells/mm^3 (participants with HIV)	89 (40–285)	75 (12–144)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ART, antiretroviral therapy; DILI, drug-induced liver injury; HIV, human immunodeficiency virus; INR, international normalized ratio; IQR, interquartile range; NAC, N-acetylcysteine; TB, tuberculosis.

Table 2. Adverse Events During Study Drug Infusion

Adverse Event	NAC (n = 53)	Placebo (n = 49)
Nausea and/or vomiting	9 (infusion discontinued in 3)	2
Rash	1	0
Pruritis	1	0
Pain at drip site	1 (infusion discontinued in 1)	0
Hypotension	0	1
Anaphylactoid reaction	1 (infusion discontinued in 1)	0
Total	13	3

Abbreviation: NAC, N-acetylcysteine.

However, ALT is not a good biomarker of liver injury: ALT concentrations may be normal during the early stages of hepatocyte injury or apoptosis, ALT may be elevated due to mechanisms other than hepatocyte injury (eg, membrane blebbing, increased hepatic expression, macroenzymes) [23], and ALT may be released from tissues other than liver such as skeletal muscle, kidney, and heart [24]. Novel biomarkers of liver injury, such as microRNA, are more specific for liver injury than ALT and increase earlier than ALT in patients with paracetamol-induced liver injury [25–27].

In our trial, participants with AT-DILI who received NAC had significantly shorter hospital stays, which was a prespecified secondary endpoint of the trial. Our study was blinded; therefore, the clinical decision to discharge participants from the hospital could not have been biased by knowledge of treatment allocation. Our finding therefore suggests that NAC hastened clinical recovery, which is consistent with findings of shorter hospital stay in the NAC arm of a randomized placebo-controlled trial of NAC in participants with non–paracetamol-induced liver failure [10].

There are several potential explanations for the shorter hospital stay in the NAC arm of our study, despite similar time to ALT resolution. First, improved recovery from AT-DILI due to NAC may not have resulted in a difference in ALT trajectory between groups but might have been detected had we used a better biomarker of liver injury than ALT. Second, the antioxidant effects of NAC may be beneficial in people living with HIV (87% of our participants), who often have glutathione depletion [28]. A randomized placebo-controlled trial of NAC in participants with HIV with low glutathione

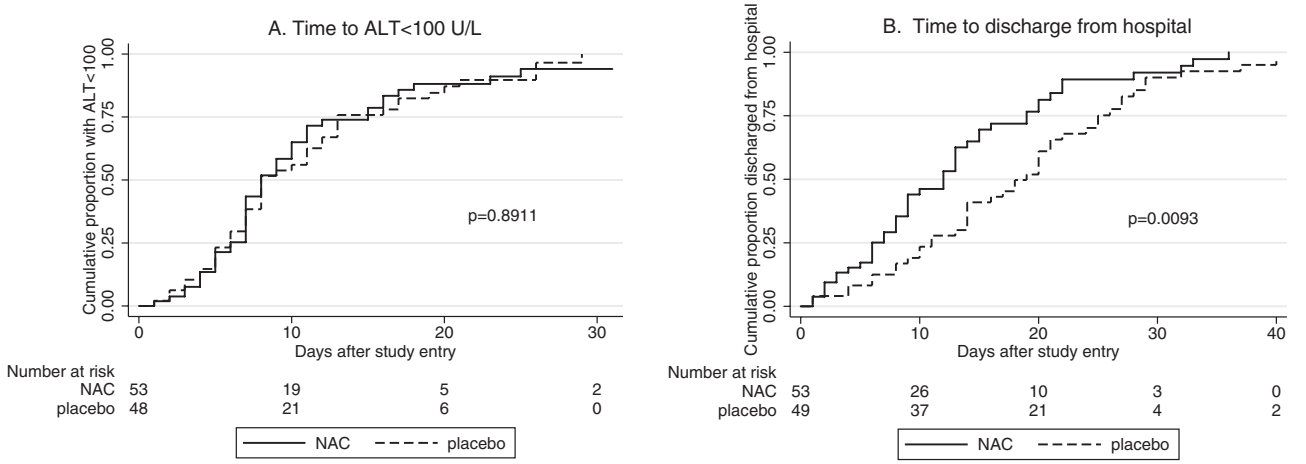

Figure 2. Cumulative estimates of time to ALT <100 U/L (*A*) and time to hospital discharge (*B*) in participants with anti-tuberculosis drug–induced liver injury randomized to NAC or placebo. Abbreviations: ALT, alanine aminotransferase; NAC, N-acetylcysteine.

concentrations reported reduced mortality in participants who received NAC, but open-label NAC was offered to all participants after a short (8-week) randomized phase; a further limitation of the study was that it was conducted in the era before effective combination ART [28]. Third, it is possible that NAC could have improved TB, as there is some evidence that NAC inhibits *Mycobacterium tuberculosis* growth and augments the mycobactericidal activity of first-line ATT in vitro [29, 30]. Fourth, the shorter hospital stay in the NAC arm could have been due to chance.

Our study had limitations. First, we included patients who were taking drugs other than ATT, which have also been associated with DILI (eg, efavirenz and cotrimoxazole). Second, we did not exclude viral hepatitis A and B in 2 participants in the NAC arm, and we did not test for viral hepatitis E in our cohort as it is not part of local routine clinical care. Third, HIV prevalence was high in our cohort and therefore our findings may not be generalizable to other populations. Fourth, our primary outcome was a reduction in ALT, which has limitations as a biomarker of DILI.

Further studies are needed to determine NAC clinical benefits in AT-DILI in populations with a low HIV prevalence. Future trials of NAC for AT-DILI should have a clinical primary outcome and use more appropriate markers of DILI than ALT.

Conclusions

Our randomized controlled trial did not demonstrate any effect of NAC on reducing the time to ALT <100 U/L in patients with AT-DILI. However, length of hospital stay was significantly shorter in the NAC arm. N-acetylcysteine, which is widely available, should be considered in the management of AT-DILI.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank the study pharmacists Waheeda Ismail and Galiemah Karriem, New Somerset Hospital, Cape Town; Meagan Saal, Khayelitsha District Hospital, Cape Town; Wynand Smythe, Groote Schuur Hospital, Cape Town; Dr Yakoob Vallie, Department of Medicine, New Somerset Hospital, Cape Town; and Sr Bernadette Khiba, ARV Clinic, New Somerset Hospital, Cape Town for their contributions. Professor Graeme Meintjes of the Clinical Infectious Diseases Research Initiative, University of Cape Town, served as an independent reviewer for deaths.

Disclaimer. Equity Pharma Ltd had no role in the study design or data analysis.

Financial support. This work was supported by the South African Medical Research Council (Self-Initiated Grant) and the Academy of Medical Sciences, United Kingdom (Newton Advanced Fellowship). The study drug N-acetylcysteine (Paradote) was manufactured and donated by Equity Pharma Ltd.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Appendix 2 Reply to Author (First Publication)

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Clinical Infectious Diseases® 2021;73(7):e1779–80

DOI: 10.1093/cid/ciaa1776

N-Acetyl Cysteine in Antitubercular Drug–Induced Liver Injury: In Search of Homogenous Evidence

To the Editor—We read with great interest the paper entitled "A randomized controlled trial of intravenous N-acetylcysteine (NAC) in the management of anti-tuberculosis drug–induced liver injury" by Moosa et al [1]. The authors have explored an avenue that is commonly used but lacks quality evidence. However, some key issues need to be addressed.

Most importantly, the authors have chosen a population with suspected antitubercular drug–induced liver injury (ATT-DILI), of whom 87% have tuberculosis–human immunodeficiency virus (TB-HIV) coinfection. This, in itself, could potentially influence the applicability of the results in multiple ways. First, the independent hepatic effects of HIV infection itself, as well as the effect of antiretroviral therapy and cotrimoxazole, need to be taken into account. Second, the antiretroviral drug interactions with antitubercular drugs may influence the DILI pharmacodynamics. Third, there is some evidence on the role of antimycobacterial and anti-inflammatory properties of NAC in tuberculosis, irrespective of its role in DILI [2, 3]. This may influence the achieved secondary endpoint of the study of shorter duration of hospital stay.

On technical grounds, the study population, besides having a predominant HIV-TB cohort, is rather heterogeneous with possibilities of few patients having tropical febrile syndromes, sepsis, acute hepatitis B virus, and hepatitis A virus. Furthermore, the hepatitis E virus serology status has not been assessed.

Fourth, the authors have chosen time to alanine aminotransferase (ALT) normalization as the primary endpoint. However, what would have also been of interest is to know the subgroup analysis of patients who fit into the definition of acute liver injury with a prolonged international normalised ratio (INR) >1.5 and those having hepatic encephalopathy [4].

The total duration of usage of NAC will also be of interest to know, as will be the dynamics of ALT, bilirubin, and INR changes with respect to duration of therapy. With regard to the duration of NAC, a previous study has found a 5-day course in alcoholic hepatitis as possibly insufficient [5]. Last, the authors conclude that NAC should be considered in the management of ATT-DILI, whereas only a secondary endpoint of reduction in hospital-stay duration was achieved in a rather heterogeneous population. Evaluating the role in a more homogenous population without concomitant HIV and potential confounders will perhaps guide us to more robust evidence for the use of NAC in ATT-induced DILI.

Notes

Authors' contributions. M. S. H.: writing; A. R.: writing and critical revision; V. S.: critical revision.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Clinical Infectious Diseases® 2021;73(7):e1780

DOI: 10.1093/cid/ciaa1683

Reply to Author

To the Editor—We thank Hampannavar and colleagues for their letter, in which they asked what outcomes of treatment with N-acetylcysteine (NAC) were in the subgroup of participants with severe antituberculosis drug-induced liver injury (AT-DILI) [1]. We have performed this subanalysis; however, there are important caveats: this was not prespecified in our protocol, randomization was not stratified by liver injury severity, and our study was not powered to detect effect modification by liver injury severity.

In total, 42/102 study participants had severe liver injury (defined as coagulopathy (INR > 1.5) and/or encephalopathy) at baseline: 24/53 (45%) in the NAC arm and 18/49 (37%) in the placebo arm. For those with severe injury, median time to alanine aminotransferase (ALT) <100 U/L was 10.5 days (interquartile range [IQR] 7–15) with NAC and 8 days (interquartile range [IQR] 6–12.5) for placebo, hazard ratio (HR) 0.72 (95% confidence interval [CI] .37 to 1.43). For those without severe injury median time to ALT <100 U/L was 7 days (IQR 4–9) with NAC and 8 days (IQR 5–13) with placebo, HR 1.41 (95% CI .82 to 2.41). There was no evidence for effect modification by liver injury severity (likelihood ratio test $P = .133$). There was thus no significant difference between groups for our primary endpoint when stratified by liver injury severity.

We also explored duration of hospital stay in the 2 strata of liver injury severity. In those with severe injury, median duration was 12 days (IQR 8–19) for NAC and 20 days (IQR 14–25) for placebo. The HR for hospital discharge for NAC versus placebo in the severe injury stratum was 1.69 (95% CI .82 to 3.48). In those without severe injury median hospital stay was 7 days (IQR 3–13) for NAC and 15.5 days (IQR 8.5–23) for placebo, HR 2.08 (95% CI 1.21 to 3.59). There was no evidence for effect modification by liver injury severity (likelihood ratio test $P = .345$). Liver injury severity is a potential confounder as it was associated with hospitalization duration and distribution was not balanced between the 2 arms. Adjusting for injury severity in a multivariable cox regression strengthened the association of NAC with shorter hospital stays overall, adjusted HR 1.90 (95% CI 1.23 to 2.94). These analyses suggest that the effect of NAC on hospitalization duration may be more marked in those without severe liver injury. However, results of subanalyses, unless prespecified and adequately powered, are only hypothesis generating.

With respect to the other comments by Hampannavar et al, we acknowledged the high prevalence of human immunodeficiency virus (HIV) infection in our study population and the fact that many were on other hepatotoxic drugs as limitations, and the NAC dosing schedule we used is described in our paper. We agree that further studies will be needed to explore the efficacy of NAC in treating AT-DILI in different populations and to explore alternative dosing schedules.

We suggest that future studies of NAC in AT-DILI include severe liver injury as a prespecified subgroup, and that randomization be stratified by liver injury severity.

Notes

Acknowledgments. The authors thank Dr Maxwell Chirehwa for his contribution.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Clinical Infectious Diseases® 2021;73(7):e1780–1

DOI: 10.1093/cid/ciaa1687

The Low Case Fatality Rate of Coronavirus Disease 2019 (COVID-19) in Hong Kong Could Be Deceptive

To the Editor—We read with interest the article by Lui et al [1] reporting a low coronavirus disease 2019 (COVID-19) case fatality rate (CFR) of only 0.4% in Hong Kong until 14 April 2020.

The authors speculate that "accessibility to medical care and national strategies for testing and case identification are possibly the major factors causing differences in reported CFRs." They also postulate a lower burden on surge capacity of the Hong Kong healthcare system.

We do not agree. The main reason for the low CFR observed in Hong Kong during the first weeks of the epidemic was the low number of older persons among the confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases. We have recently shown a strong association between the proportion of older age groups and country-specific CFRs in Europe, the United States, and Canada [2]. On 14 April, only 4.6% (47/1013) of confirmed cases in Hong Kong were older than 70 years [3], a rate well below those of many other countries. Consequently, the CFR was very low. However, the situation in Hong Kong as of April 2020 could be deceptive. As shown in Figure 1, the overall CFR in Hong Kong has increased since then, paralleling the increasing proportion of older persons among confirmed cases. It is probable that the differences between other countries

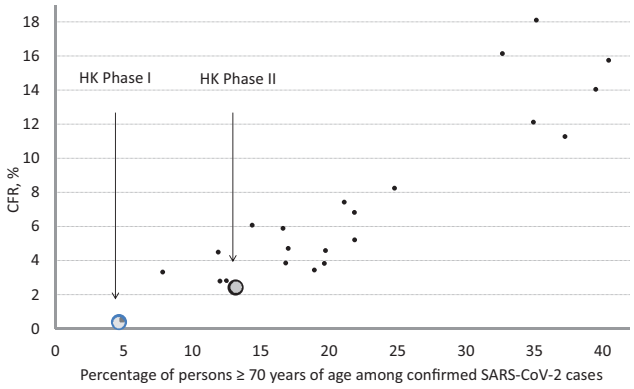

Figure 1. Case fatality rate and the proportion of persons over 70 years of age among all confirmed SARS-CoV-2 cases for the 20 most affected European countries, the United States, Canada, and for Hong Kong. Age distributions of confirmed cases and deaths were extracted from national health authority websites by 6 July 2020 (for details see [2]). The light-gray bubble indicates values in Hong Kong from 23 January to 15 April (HK Phase I, n = 1023); the darker bubble indicates values in the time period from 16 April to 6 October 2020 (HK Phase II, n = 4120). Note: The black dot within the light-gray bubble indicates Iceland, which, among European countries, had the lowest CFR and the lowest proportion of older people among confirmed cases, with rates very similar to Hong Kong during phase I. Abbreviations: CFR, case fatality rate; HK, Hong Kong; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Appendix 3: Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort

Southern African Journal of HIV Medicine
ISSN: (Online) 2078-6751, (Print) 1608-9693

Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort

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Dates:
Received: 01 Feb. 2022
Accepted: 22 Mar. 2022
Published: 14 June 2022

Background: There are limited data on the outcomes of rechallenge with anti-tuberculosis therapy (ATT) following anti-tuberculosis drug-induced liver injury (AT-DILI) in a high HIV prevalence setting.

Objectives: To describe the outcomes of rechallenge with first-line ATT.

Method: Hospitalised participants with AT-DILI who were enrolled into a randomised controlled trial of N-acetylcysteine in Cape Town, South Africa, were followed up until completion of ATT rechallenge. We described rechallenge outcomes, and identified associations with recurrence of liver injury on rechallenge (positive rechallenge).

Results: Seventy-nine participants were rechallenged of whom 41 (52%) were female. Mean age was 37 years (standard deviation [s.d.] ±10). Sixty-eight (86%) were HIV-positive, of whom 34 (50%) were on antiretroviral therapy (ART) at time of AT-DILI presentation. Five participants had serious adverse reactions to an aminoglycoside included in the alternate ATT regimen given after first-line ATT interruption: acute kidney injury in three and hearing loss in two. The median time from first-line ATT interruption to start of first-line ATT rechallenge was 13 days (interquartile range [IQR]: 8–18 days). Antiretroviral therapy was interrupted for a median of 32 days (IQR: 17–58) among HIV-positive participants on ART before AT-DILI. Fourteen participants had positive rechallenge (18%). Positive rechallenge was associated with pyrazinamide rechallenge (P = 0.005), female sex (P = 0.039) and first episode of tuberculosis (TB) (P = 0.032).

Conclusion: Rechallenge was successful in most of our cohort. Pyrazinamide rechallenge should be carefully considered.

Keywords: tuberculosis; anti-tuberculosis drugs; drug-induced liver injury; positive rechallenge; pyrazinamide; treatment interruption.

Introduction

Liver injury is the most frequent complication of first-line anti-tuberculosis therapy (ATT) with an estimated incidence of 2% – 28%.[1] Following recovery from anti-tuberculosis drug-induced liver injury (AT-DILI), rechallenge with hepatotoxic first-line anti-tuberculosis drugs (rifampicin, isoniazid and, in some circumstances, pyrazinamide) is recommended because second-line ATT regimens are less effective, longer and more toxic.[2] While awaiting resolution of liver injury, a background ATT regimen is given, typically consisting of ethambutol and at least two other second-line anti-tuberculosis drugs.

There is limited evidence on rechallenge following AT-DILI in populations with high prevalence of HIV coinfection. There is limited evidence on optimal background ATT regimens, optimal ATT rechallenge protocols, risk factors for positive rechallenge, anti-tuberculosis drugs most frequently implicated in positive rechallenge, and interruption and re-initiation of antiretroviral therapy (ART) in people living with HIV (PLHIV) who present with AT-DILI.

This study is nested within our randomised placebo-controlled trial of intravenous N-acetylcysteine (NAC) in the management of AT-DILI, which has previously been reported.[3] We describe the characteristics, background ATT regimens (alternate ATT regimens initiated after first-line ATT interruption), rechallenge regimens, and outcomes of rechallenge in those participants who were rechallenged with ATT. Among HIV-positive participants, we explore the impact of AT-DILI and drug rechallenge on initiation or interruption of ART.

How to cite this article: Moosa MS, Maartens G, Gunter H, et al. Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort. S Afr J HIV Med. 2022;23(1), a1376. https://doi.org/10.4102/sajhivmed.v23i1.1376

Methods

Study participants

Participants with AT-DILI admitted to three hospitals in Cape Town, South Africa, were enrolled in a pragmatic randomised placebo-controlled trial of intravenous NAC. Anti-tuberculosis drug-induced liver injury was defined as an alanine aminotransferase (ALT) ≥ 3 times the upper limit of normal if symptoms of hepatitis were present, or an ALT ≥ 5 times the upper limit of normal without symptoms of hepatitis.[4] Other trial inclusion criteria were age 18 years or older, taking first-line therapy for tuberculosis (TB), and liver injury attributed to ATT.

After completion of the NAC or placebo infusion, decisions regarding clinical management were made by clinicians at participating hospitals and outpatient clinics. This included decisions regarding background ATT initiation and regimen, whether to rechallenge ATT, choice of rechallenge regimen, and interrupting, rechallenging, or initiating ART. Participants were followed up until the study primary endpoint (ALT reaching < 100 U/L) was reached and ATT rechallenge was completed. We included all trial participants who were rechallenged with at least one anti-tuberculosis drug in this analysis.

Identification and assessment of positive rechallenge cases

'Positive rechallenge' is recurrence of liver injury on drug rechallenge. For this analysis, we defined positive rechallenge as doubling of ALT or total bilirubin concentration after rechallenge of an anti-tuberculosis drug.[5] A multidisciplinary causality assessment panel including a clinical pharmacologist, a pharmacist, an infectious diseases specialist and a general physician assessed cases with a positive rechallenge, and identified the drug that was most likely to be causative, or any non-drug related cause for the increase in ALT or bilirubin.

Statistical analysis

Categorical data were described using counts and percentages. Numerical data were described using means and standard deviations if normally distributed and medians and ranges if non-normally distributed. We compared parametric data using the Student's *t*-test, non-parametric data using the Wilcoxon rank sum test and categorical data using the Fisher's exact test. When comparing proportion with positive rechallenge between rechallenged drugs, we assumed that the three groups were independent. A *P*-value of < 0.05 was considered to be statistically significant throughout. Data were analysed using Stata (Version SE/15.1 Statacorp, College Station, Texas, United States).

We calculated 'time from first-line ATT interruption to start of rechallenge' as the interval from the date of AT-DILI presentation and first-line ATT discontinuation to the date that the first rechallenged drug was introduced. In HIV-positive participants on ART, we calculated 'ART interruption time' as the interval from the date of presentation with AT-DILI and ART discontinuation to the date of ART re-initiation. In HIV-positive participants not on ART, we calculated 'delay in ART initiation time' as the interval from date of presentation with AT-DILI to the date of ART initiation.

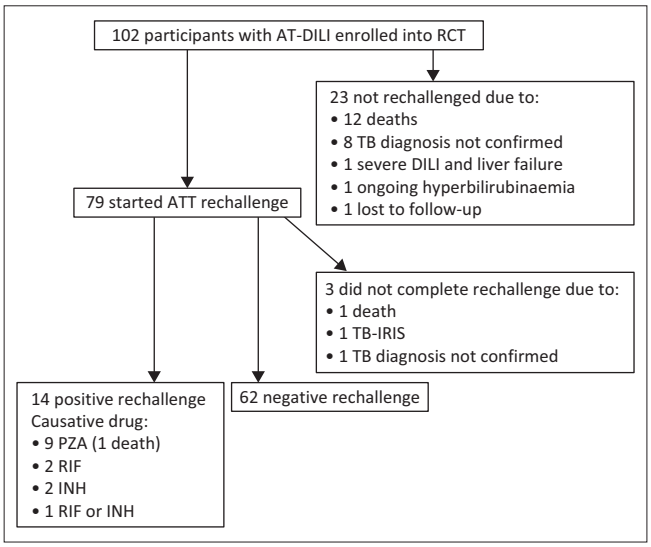
Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference for Harmonisation.[6,7] The study protocol was approved by University of Cape Town Human Research Ethics Committee and the Western Cape Department of Health (HREC 087/2012). Participants provided written informed consent. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719).

Results

Seventy-nine of 102 participants (77%) with AT-DILI enrolled into the randomised trial were rechallenged with ATT (Figure 1). Reasons for not rechallenging 23 participants were: 12 died before rechallenge was attempted, 8 had insufficient evidence of TB to justify rechallenge, 2 had prolonged hyperbilirubinaemia and were placed on second-line ATT because the clinical team deemed first-line ATT rechallenge to be unsafe, and 1 was lost to follow-up before planned rechallenge could be commenced.

Baseline characteristics of the 79 rechallenged participants, grouped by positive and negative rechallenge, are described in Table 1. Sixty-eight of the 79 (86%) participants rechallenged were HIV-positive, 34 of whom were on ART at presentation with AT-DILI, (27 on an efavirenz-based regimen and 7 on a lopinavir plus ritonavir-based regimen) and 18 were on cotrimoxazole prophylaxis.



AT-DILI, anti-tuberculosis drug-induced liver injury; ATT, anti-tuberculosis therapy; INH, isoniazid; IRIS, immune reconstitution inflammatory syndrome; PZA, pyrazinamide; RCT, randomised controlled trial; RIF, rifampicin; TB, tuberculosis.

FIGURE 1: Anti-tuberculosis drug rechallenge following drug-induced liver injury in N-acetylcysteine randomised controlled trial participants.

Forty-three participants commenced rechallenge during hospital admission, 10 of whom were referred to a community health centre to complete rechallenge. Twenty-five participants were rechallenged at a community health centre and 11 at a stepdown inpatient TB care facility.

Sixty-eight of 79 participants (86%) rechallenged were initiated on background ATT after first-line ATT interruption prior to rechallenge (Table 2). In the remaining 11 participants, background ATT was not commenced; reasons for this decision were not documented. All 68 participants initiated on background ATT received a fluoroquinolone, and 58 received an aminoglycoside (46 kanamycin, 11 amikacin, 1 streptomycin). Five of the participants who received an aminoglycoside (9%) had a serious adverse drug reaction: acute kidney injury in 3, and hearing loss in 2.

Most participants (96%) were rechallenged with a minimum of two individual drugs re-introduced sequentially and in full dosages (Table 3). Three participants completed only rifampicin rechallenge after which further rechallenge was discontinued: one was found to have no evidence of TB, one had worsening canalicular enzymes (likely due to TB-immune reconstitution inflammatory syndrome [IRIS] rather than AT-DILI recurrence), and one died from sepsis and multi-organ failure before completion of rechallenge.

First-line anti-tuberculosis drugs were rechallenged at full dose. Drugs were rechallenged sequentially in 77 of 79 participants, with new drugs introduced at approximately 3-day intervals (Table 3). Rechallenge regimens differed in the sequence in which individual drugs were re-introduced: rechallenge commenced with rifampicin in 68 participants and with isoniazid in 11 (Table 3). The clinical care team elected not to rechallenge with pyrazinamide in 22 of 72 participants who had interrupted ATT due to liver injury during the intensive phase, because of the severity of the liver injury. The median time from first-line ATT interruption to start of rechallenge was 13 days (interquartile range [IQR]: 8–18 days).

TABLE 1: Baseline characteristics of participants with positive and negative rechallenge following anti-tuberculosis drug-induced liver injury.

Baseline characteristics	Positive rechallenge (*n* = 14)	Negative rechallenge (*n* = 65)	All rechallenged participants (*n* = 79)	*P**
Age (years)				0.368
Mean ± s.d.	35 ± 12	38 ± 9	37 ± 10	
Female				0.039
n	11	30	41	
%	79	46	52	
Weight (kg)				0.418
Median	59	54	54	
IQR	50–74	46–64	47–64	
First time on TB treatment				0.032
n	14	47	61	
%	100	72	77	
HIV-positive				0.075
n	10	58	68	
%	71	89	86	
CD4 count (cells/mm³) (for HIV-positive)†				0.646
Median	56	76	70	
IQR	4–277	26–144	26–144	
ALT (U/L)				0.090
Median	255	385	357	
IQR	225–352	279–558	254–558	
Total bilirubin (mmol/L)				0.767
Median	44	49	47	
IQR	26–81	21–94	22–90	
ALP (U/L)				0.245
Median	126	183	175	
IQR	101–194	112–258	110–254	
INR‡				0.288
Median	1.1	1.3	1.2	
IQR	1.0–2.1	1.1–1.8	1.1–1.8	
Albumin g/L§				0.758
Median	26	26	26	
IQR	19–35	21–30	21–30	

ALT, alanine transferase; ALP, alkaline phosphatase; INR, international normalised ratio; IQR, interquartile range; s.d., standard deviation; TB, tuberculosis.

*, Fisher's exact test for categorical variables, *t*-test for parametric data, rank sum test for non-parametric data.

†, 26 with missing data. ‡, 5 with missing data. §, 5 with missing data.

Positive rechallenge

There were 14 positive rechallenges in the 79 rechallenged participants (18%). Positive rechallenge was associated with female sex (Fisher's exact test $P = 0.039$) and first episode of TB (Fisher's exact test $P = 0.032$) (Table 1). The median time from first-line ATT interruption to start of rechallenge was similar between those with positive and negative rechallenge: median 12 days (IQR: 8–16 days) and 13 days (IQR: 9–18) respectively, Wilcoxon rank sum $P = 0.719$.

Rechallenge was positive in 9/46 participants rechallenged with pyrazinamide, 2/78 rechallenged with rifampicin, and 2/74 rechallenged with isoniazid. One participant had a

TABLE 2: Background anti-tuberculosis drug regimens prescribed following anti-tuberculosis drug-induced liver injury.

Background anti-tuberculosis drug regimen	Number of participants
Ethambutol + moxifloxacin + aminoglycoside†	54
Ethambutol + moxifloxacin +ethionamide	6
Ethambutol + moxifloxacin	4
Ethambutol + moxifloxacin + ethionamide + aminoglycoside‡	2
Moxifloxacin + ethionamide + aminoglycoside§	2
No background anti-tuberculosis therapy	11

†, 42 participants received kanamycin, 11 amikacin, 1 streptomycin. ‡, Both participants received kanamycin. §, Both participants received kanamycin.

TABLE 3: Sequence of anti-tuberculosis drug rechallenge.

Rechallenge regimen	Participants	
	n	%
RIF → INH → PZA†‡	38	50
INH → RIF → PZA	6	8
RIF → INH	26	30
INH → RIF	4	5
RIF → PZA	1	1
INH → PZA	1	1
RIF	3	5

INH, isoniazid; PZA, pyrazinamide; RIF, rifampicin.

†, One participant was rechallenged with RIF and INH concomitantly, followed by PZA. ‡, One participant was rechallenged with RIF, PZA and INH concomitantly.

positive rechallenge after sequential introduction of rifampicin and isoniazid. On causality assessment, both drugs were potentially implicated in the positive rechallenge because the participant's serum ALT only settled after both drugs were withdrawn.

The proportion with a positive rechallenge was significantly higher among those rechallenged with pyrazinamide than among those rechallenged with rifampicin or isoniazid, Fisher's exact test $P = 0.005$. One of the participants with positive pyrazinamide rechallenge developed a fatal systemic hypersensitivity reaction with rash, jaundice and acute kidney injury.

One participant had markedly increased serum canalicular liver enzymes (alkaline phosphatase and gamma-glutamyl transferase) at AT-DILI presentation which increased further after rifampicin rechallenge. The hospital clinicians assessed this as a positive rifampicin rechallenge and stopped rifampicin. However, the canalicular enzymes continued to increase after rifampicin cessation. On causality assessment, the increased canalicular enzymes were attributed to TB IRIS rather than a positive rifampicin rechallenge.

Antiretroviral therapy was interrupted at presentation with liver injury in 26 of the 34 (79%) HIV-positive participants who were receiving ART. At 8 weeks' follow-up, 24 of these 26 participants had been re-initiated on ART: 21 recommenced their previous efavirenz-based regimen and three were switched from efavirenz-based to boosted protease inhibitor-based ART. The median ART interruption time was 32 days (IQR: 17–58). Twenty-one of 34 (62%) HIV-positive participants who were not on ART at the time of AT-DILI were initiated on ART after ATT rechallenge, after a median of 53 days (IQR: 35–91). Fifteen of 68 (22%) HIV-positive participants were not yet on ART when study follow-up ended.

Discussion

In our cohort of patients with AT-DILI, the majority of whom had advanced HIV disease, rechallenge was attempted in the majority (77%). A wide variety of background regimens were used during rechallenge; adverse reactions to aminoglycosides in the background regimen were common. Rechallenge was positive in 18%, and was associated with female sex and first episode of TB. Positive rechallenge was significantly more common with pyrazinamide rechallenge than with isoniazid or rifampicin rechallenge. Positive rechallenge resulted in delays in initiating or commencing ART.

Risk of positive rechallenge

In a recent network meta-analysis of ATT rechallenge regimens in participants with AT-DILI,[8] 11% of those rechallenged with a sequential full dose regimen had a positive rechallenge. This is lower than the 18% we observed and could be explained by the longer rechallenge regimens used in the studies included in the meta-analysis. The majority of participants in the meta-analysis were rechallenged with rifampicin on day 1, isoniazid on day 8 and pyrazinamide on day 15–18, whereas the majority of our study participants were rechallenged with rifampicin on day 1, isoniazid on day 4 and pyrazinamide on day 7.

We found that women and participants with their first episode of TB were more likely to have a positive rechallenge. Other studies have also found female sex to be associated with AT-DILI[9,10] as well as with positive ATT rechallenge.[11] We did not find low serum albumin or increased age to be associated with positive rechallenge, in contrast to previous studies.[12,13]

Pyrazinamide rechallenge

Pyrazinamide was the main cause of positive rechallenge in our study, with positive rechallenge in 20% of those rechallenged. Positive pyrazinamide rechallenge contributed to the death of one study participant. In a small randomised trial, 6 of 25 (24%) participants rechallenged with a concomitant full dose regimen including pyrazinamide had a positive rechallenge compared with 0 of 20 in the sequential full dose regimen group excluding pyrazinamide.[11] American Thoracic Society guidelines advise against rechallenging pyrazinamide after severe AT-DILI.[4] With increasing availability of effective second-line anti-tuberculosis drugs including fluroquinolones, linezolid and bedaquiline, avoidance of pyrazinamide rechallenge in all cases of AT-DILI should be considered.

Antiretroviral therapy interruption and re-initiation

There is little published data on the impact of AT-DILI on ART in PLHIV. In our study, 79% of participants on ART at the time of AT-DILI presentation had their ART interrupted, with a median interruption of 32 days. Antiretroviral therapy interruptions may impact on efficacy of therapy and contribute to the emergence of antiretroviral resistance.[14] Median delay from AT-DILI presentation to ART initiation in our cohort was 53 days, and 22% of the cohort were not yet on ART when study follow-up ended. Delays in initiation of ART in patients with advanced disease have previously been shown to increase mortality.[15]

Study limitations

Our study has limitations. Although our study was nested within a randomised control trial, it is descriptive, and was not powered to identify risk factors for positive rechallenge. Study follow-up ended after rechallenge was complete, and we therefore could not quantify the impact of positive rechallenge on outcomes of ATT or ART. Our study cohort had a high prevalence of HIV, and the findings may not be generalisable to lower HIV prevalence settings.

Conclusion

In this cohort of patients with AT-DILI, the majority of whom were HIV-positive, pyrazinamide was the most common cause of positive rechallenge. Positive rechallenge resulted in delays in initiating or recommencing ART. Use of second-line anti-tuberculosis drugs should be considered as an alternative to pyrazinamide rechallenge.

Acknowledgements

This study was nested within a randomised placebo-controlled trial of intravenous N-acetylcysteine in management of patients hospitalised with liver injury due to anti-tuberculosis drugs (https://doi.org/10.1093/cid/ciaa1255). We thank Anri Uys for contributing to causality assessment.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

M.S.M., K.C. and G.M. conceived and designed the study. M.S.M. and K.C. performed all the statistical analyses. M.S.M., K.C., G.M., H.G., S.A., M.F.C., M.S., S.W. and D.F.S. discussed the results and contributed to the final manuscript.

Funding information

This study was supported by the South African Medical Research Council (Self-Initiated Grant) and the Academy of Medical Sciences, United Kingdom (Newton Advanced Fellowship).

Data availability

The data that support the findings of this study are stored in a controlled access repository and are not openly available due to reasons of sensitivity and patient confidentiality. Data are available from the corresponding author, K.C., upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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Appendix 4: Analysis of serum microRNA-122 in a randomised controlled trial of N-acetylcysteine for treatment of anti-tuberculosis drug-induced liver injury.

Received: 24 October 2022 | Revised: 30 December 2022 | Accepted: 4 January 2023

DOI: 10.1111/bcp.15661

ORIGINAL ARTICLE

Analysis of serum microRNA-122 in a randomized controlled trial of N-acetylcysteine for treatment of antituberculosis drug-induced liver injury

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Funding information
South African Medical Research Council Self-Initiated Research Grant awarded to Gary Maartens and Academy of Medical Sciences, United Kingdom - Newton Advanced Fellowship awarded to Karen Cohen.

Aim: Serum microRNA-122 (miR-122) is a novel biomarker for drug-induced liver injury, with good sensitivity in the early diagnosis of paracetamol-induced liver injury. We describe miR-122 concentrations in participants with antituberculosis drug-induced liver injury (AT-DILI). We explored the relationship between miR-122 and alanine aminotransferase (ALT) concentrations and the effect of N-acetylcysteine (NAC) on miR-122 concentrations.

Methods: We included participants from a randomized placebo-controlled trial of intravenous NAC in AT-DILI. ALT and miR-122 concentrations were quantified before and after infusion of NAC/placebo. We assessed correlations between ALT and miR-122 concentrations and described changes in ALT and miR-122 concentrations between sampling occasions.

Results: We included 45 participants; mean age (± standard deviation) 38 (±10) years, 58% female and 91% HIV positive. The median (interquartile range) time between pre- and post-infusion biomarker specimens was 68 h (47-77 h). The median pre-infusion ALT and miR-122 concentrations were 420 U/L (238-580) and 0.58 pM (0.18-1.47), respectively. Pre-infusion ALT and miR-122 concentrations were correlated (Spearman's $\rho = .54$, $P = .0001$). Median fold-changes in ALT and miR-122 concentrations between sampling were 0.56 (0.43-0.69) and 0.75 (0.23-1.53), respectively, and were similar in the NAC and placebo groups ($P = .40$ and $P = .68$ respectively).

Conclusions: miR-122 concentrations in our participants with AT-DILI were considerably higher than previously reported in healthy volunteers and in patients on antituberculosis therapy without liver injury. We did not detect an effect of NAC on miR-122 concentrations. Further research is needed to determine the utility of miR-122 in the diagnosis and management of AT-DILI.

Christopher Goldring and Karen Cohen are joint senior authors.

The authors confirm that the PI for this paper is Karen Cohen and that she had direct clinical responsibility for study participants.

KEYWORDS
antituberculosis drugs, biomarkers, liver injury, microRNA-122

1 | INTRODUCTION

Drug-induced liver injury (DILI) is the most frequent severe adverse effect of first-line antituberculosis therapy.[1] Antituberculosis drug-induced liver injury (AT-DILI) is primarily diagnosed by elevated serum alanine aminotransferase (ALT) concentrations, together with symptoms and signs of DILI.[2] However, ALT is not a specific biomarker of DILI because it may be released from tissues other than the liver (eg, skeletal muscle, kidney and heart).[3] More specific biomarkers of DILI are therefore potentially important.

MicroRNAs are small, single-stranded RNAs that regulate gene expression at a post-transcriptional level.[4] MicroRNAs can be detected in blood as stable complexes and quantified using quantitative real-time polymerase chain reaction (qRT-PCR).[5] Liver injury due to drug exposure, viral hepatitis or hepatocellular carcinoma has been shown to result in altered microRNA expression profiles.[6–9] MicroRNA-122 (miR-122) accounts for 70% of hepatic microRNA[10] and plays an essential role in lipid metabolism, anti-inflammatory and antitumorigenic mechanisms.[11] miR-122 has been explored as a novel biomarker of paracetamol-induced liver injury in pre-clinical[12,13] and clinical studies.[9,14–17] In paracetamol-induced liver injury, an increase in miR-122 concentrations occurred earlier than an increase in ALT concentrations and was a sensitive test for identifying those patients who went on to develop liver injury.[12,18] Peak increases in serum miR-122 concentrations correlated with later increases in serum ALT.[13,14,19] miR-122 concentrations have also been found to be elevated in patients with nonparacetamol DILI.[20,21] miR-122 is largely liver-specific and therefore concentrations are unaltered by impaired renal function[15] and muscle injury.[17]

There are limited data on miR-122 as a biomarker of AT-DILI. For this study, we quantified miR-122 concentrations in stored specimens from a randomized placebo-controlled trial of N-acetylcysteine (NAC) for treatment of AT-DILI. We found that NAC administration shortened the duration of hospitalization, but time for ALT to fall below 100 U/L was similar for NAC and placebo groups.[22]

Our aims for this analysis were to describe miR-122 concentrations in AT-DILI, to assess correlations between ALT and miR-122 concentrations, to describe changes in miR-122 concentrations over time and to determine if repeat miR-122 quantification would be more informative than ALT in detecting a biochemical response to NAC administration.

What is already know about this subject?

- Serum microRNA (miR)-122 is a sensitive biomarker for early detection of paracetamol-induced liver injury.
- Serum miR-122 concentrations increase slightly in participants taking antituberculosis treatment without liver injury.
- Serum miR-122 concentrations increased markedly in participants with antituberculosis drug-induced liver injury (AT-DILI) in one study but decreased in another.
- In a randomized controlled trial of intravenous N-acetylcysteine (NAC) in participants with AT-DILI, NAC did not hasten the decline of alanine aminotransferase (ALT) concentrations. The effect of N-acetylcysteine on miR-122 concentrations in this setting has not previously been studied.

What this study adds?

- In our cohort drawn from a randomized controlled trial of intravenous NAC in participants with AT-DILI, miR-122 concentrations were markedly higher than those seen in healthy volunteers and in patients on antituberculosis treatment without liver injury.
- We did not detect an effect of NAC on the change in miR-122 concentrations observed during the first week after infusion of NAC/placebo.
- We found high intra-individual and inter-individual variability in serum microRNA-122 concentrations following AT-DILI.

2 | METHODS

2.1 | Study participants

Participants were drawn from a previously reported[22] randomized placebo-controlled trial (RCT) of NAC in the management of AT-DILI conducted in Cape Town, South Africa. NAC was dosed intravenously according to the regimen for paracetamol overdose: 150 mg/kg over 1 h, 50 mg/kg over 4 h and 100 mg/kg over 16 h. The study was a pragmatic randomized trial nested within routine clinical care at the participating hospitals. The study protocol specified that participants' ALTs be tested before the study infusion and twice weekly during hospital follow-up. These ALT samples were generally taken by the hospital clinical care team. In addition to the monitoring of ALT concentrations for the clinical study, investigators collected blood samples for storage for future biomarker research. For this analysis, we included the subset of participants from the RCT with paired specimens stored for biomarker quantification: the first specimen taken between 24 h before and 30 min after

commencement of the study infusion and the second specimen taken within 7 days after initiation of the study infusion. Blood was centrifuged within 8 h of collection, and serum was stored at −80°C. We compared baseline characteristics of the participants with paired samples, and study participants not included in this analysis to confirm that they were similar.

2.2 | ALT quantification

ALT was quantified in stored serum using an International Federation of Clinical Chemistry recommended method. ALT and miR-122 concentrations were determined from the same sample in each case, except for two pre-infusion samples which were unsuitable for ALT determination; in those two cases, we used the ALT concentration from clinical records from an assay performed on the same day of the infusion. Both of these ALTs were from the samples taken within 3 h of the sample used for miR-122 determination.

2.3 | MicroRNA isolation from serum

For recovery of miRNA-enriched fractions from serum, the miRNeasy Mini Kit and the RNeasy MinElute Cleanup Kit (Qiagen, Venlo, Netherlands) were used on the QIAcube automated platform (Qiagen), following manufacturer's instructions with an addition of 200 pM cel-lin-4 (cat. 4464066, ID MC10768; Ambion, Thermo Fisher Scientific, Waltham, MA, USA) as a spiked-in exogenous nonhuman miRNA to monitor for the efficiency of the miRNA extraction process. Freshly isolated miRNA-containing eluate (15 µL) was stored at −80°C.

2.4 | Reverse transcriptase and RT-qPCR reactions

TaqMan miRNA Assays for miR-122 (Assay ID 002245) and cel-lin-4 (Assay ID 000258; Thermo Fisher Scientific) were used to perform real-time quantitative PCR (RT-qPCR). miRNA-containing eluate was reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) and a custom Multiplex RT Primer pool in a GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA, USA). A fixed volume of 2 µL miRNA-containing eluate was added to an RT Master Mix containing dNTPs, RNase Inhibitor, Reverse Transcription Buffer, 75 units per reaction of MultiScribe Reverse Transcriptase and a multiplex RT primer pool consisting of primers for miR-122 and cel-lin-4 (Thermo Fisher Scientific). A 1 nM synthetic miR-122 (mirVana miRNA mimic, Assay ID MC11012; Ambion) was reverse-transcribed together with the samples to allow the generation of a standard curve in each plate. No-template samples were included as negative controls. Reaction conditions followed the manufacturer's instructions: annealing for 30 min at 16°C, followed by cDNA synthesis for 30 min at 42°C and denaturation for 5 min at 85°C. The resulting cDNA was diluted 1:3 with RNase-free water and stored at −20°C until further processing.

Pre-amplification of cDNA was run on a GeneAmp 9700 PCR System (Applied Biosystems). Negative controls containing RNase-free water instead of cDNA were included. Reaction conditions followed the manufacturer's instructions: 95°C for 10 min, 55°C for 2 min, 72°C for 2 min, followed by 12 cycles at 95°C for 15 s and 60°C for 4 min followed by enzyme inactivation at 99.9°C for 10 min. The pre-amplified synthetic miR-122 was serially diluted 1:10 (ranging from 1 nM to 0.1 fM) and stored at −20°C.

Two microlitres of the diluted pre-amplification product was combined with a qPCR Master Mix containing 5 µL of 2x TaqMan Universal MasterMix II without Uracil-N glycosylase (Thermo Fisher Scientific) in a total reaction volume of 10 µL. RT-qPCR was then performed in duplicates on an ABI ViiA 7 Thermocycler (Applied Biosystems) using a two-step thermal cycling protocol of 95°C for 10 min followed by 40 cycles of 95°C (15 s) and 60°C (60 s) for both miR-122 and cel-lin-4. Data were analysed using QuantStudio Real-Time PCR Software v1.3. The number of copies of miR-122 in each sample was quantified using the absolute quantification method with the standard curve generated with synthetic miR-122 in each plate. miR-122 levels were normalized to the level of cel-lin-4 to account for technical variations between samples. Then, 10% duplicates (including samples run in a separate plate) were added to each run to account for intra- and inter-plate variability, and final miR-122 copy numbers were averaged across experiments (intra- and inter-assay coefficient of variation (CV) less than 5% and 10%, respectively).

2.5 | Statistical analysis

Data were analysed using Stata SE Version 13.1 (Statacorp, College Station, TX, USA) and Microsoft Excel. We compared numerical data with a normal distribution using the Student's *t*-test, non-normally distributed data using the Wilcoxon rank sum test and categorical data using the Fisher's exact test. We calculated the Spearman's rank correlation coefficient (ρ) to assess correlation between ALT and miR-122 concentrations. We calculated the CV for ALT and miR-122 concentrations pre- and post-study infusion. We log transformed ALT and miR-122 concentrations for parametric comparative analyses. To explore change in miR-122 and ALT concentration between samples, we calculated the ratio of post-infusion to pre-infusion concentration and performed a Student's *t*-test on logged ratios to look for differences between groups. To determine the average change in ALT and miR-122 concentrations per day, we calculated the slope between the concentration before and the concentration after study infusion. A *P* value of <.05 was considered to be statistically significant throughout.

2.6 | Ethics

The study protocol was approved by University of Cape Town Human Research Ethics Committee (HREC 087/2012 and HREC 421/2017) and the Western Cape Department of Health. Participants provided written informed consent. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719) and clinicaltrials.gov (NCT02182167).

3 | RESULTS

3.1 | Participant characteristics

We included 45 of the 102 NAC randomized controlled trial participants from whom paired biomarker specimens had been collected within the specified time windows, 26 of these were from the NAC and 19 were from the placebo group (see Supporting Information Figure S1). Baseline characteristics of the 45 included participants did not differ significantly from the 57 participants without paired specimens (see Supporting Information Table S1). Four of the 45 participants with paired specimens died during study follow-up (two in the NAC group and two in the placebo group) *vs* 10/57 participants without paired specimens (Fisher's exact $P = .255$).

Participant characteristics were similar between the NAC and placebo groups in those participants with biomarker samples (Table 1). The mean age was 38 years ± standard deviation (SD) 10, 58% were female and 91% were HIV positive. We had self-reported ethnicity data on 31 participants, of whom 27 (87%) self-identified as Black African.

3.2 | Time intervals between AT-DILI presentation, biomarker sampling and study infusion

The median time from presentation with AT-DILI to collection of the pre-infusion sample was 2.7 days (interquartile range [IQR] 1.5-4.1 days). This first sample was taken at a median of 0.6 h (IQR 0.2-11 h) before study infusion commenced, and the second sample was taken at a median of 50 h (IQR 26-73 h) after commencement of study infusion. The median time interval between paired specimens was 68 h (IQR 47-77 h) and was similar between the NAC and placebo groups (Wilcoxon rank sum test $P = .08$).

3.3 | ALT and miR-122 concentrations before study infusion

The ALT and miR-122 concentrations and CVs are summarized in Table 1. The median ALT concentration before intravenous NAC/placebo infusion was 420 U/L (IQR 238-580 U/L) and the median miR-122 concentration was 0.58 pM (IQR 0.18-1.47 pM) (Figure 1). miR-122 and ALT concentrations before study infusion were correlated (Spearman's $\rho = .54$, $P = .0001$); Figure 2. The median cycle threshold (Ct) pre-infusion was 23.1 (IQR 20.8-24.7).

3.4 | ALT and miR-122 concentrations after study infusion

Post-infusion ALT and miR-122 concentrations were similar in the NAC and placebo groups (see Table 1 and Figure 1).Post-infusion miR-122 concentrations were correlated with ALT concentrations in both the NAC and placebo groups (Spearman's $\rho = .42$, $P = .031$ and Spearman's $\rho = .53$, $P = .020$, respectively) and when participants from both groups were analysed together (Spearman's $\rho = .45$, $P = .002$) (Figure 2).The median Ct post-infusion was 23.7 (IQR 22.0-24.7).

TABLE 1 Baseline characteristics and biomarker concentrations before and after study infusion in participants with antituberculosis drug-induced liver injury randomized to intravenous N-acetylcysteine or placebo

	NAC (n = 26)	Placebo (n = 19)	Total (n = 45)	*P* value[a]
Age, years, mean (±SD)	38 (±12)	38 (±7)	38 (±10)	.897
Female, *n* (%)	17 (65)	9 (47)	26 (58)	.360
Weight, kg, median (IQR)	55 (49-64)	57 (45-63)	56 (48-64)	.980
HIV positive, *n* (%)	22 (85)	19 (100)	41 (91%)	.126
CD4 count (HIV positive) cells/mm^3, median (IQR)	93 (54-277)	54 (7-132)	74 (19-161)	.222
Enrolment total bilirubin, μmol/L, median (IQR)	50 (17-91)	74 (35-191)	52 (28-117)	.112
Pre-infusion ALT, U/L, median (IQR)	361 (238-580)	427 (238-612)	420 (238-580)	.899
CV (%)	91	64	81	
Post-infusion ALT, U/L, median (IQR)	194 (119-293)	250 (111-419)	221 (119-342)	.550
CV (%)	93	98	95	
Pre-infusion miR-122, pM, median (IQR)	0.44 (0.17-1.47)	0.84 (0.31-2.79)	0.58 (0.18-1.47)	.251
CV (%)	167	152	169	
Post-infusion miR-122, pM, median (IQR)	0.18 (0.77-0.94)	0.33 (0.16-0.86)	0.26 (0.12-0.86)	.113
CV (%)	214	145	207	

Abbreviations: ALT, alanine aminotransferase; CV, coefficient of variation; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

[a]Fisher's exact test for categorical variables, Student's *t*-test for parametric data, Wilcoxon rank sum test for nonparametric data.

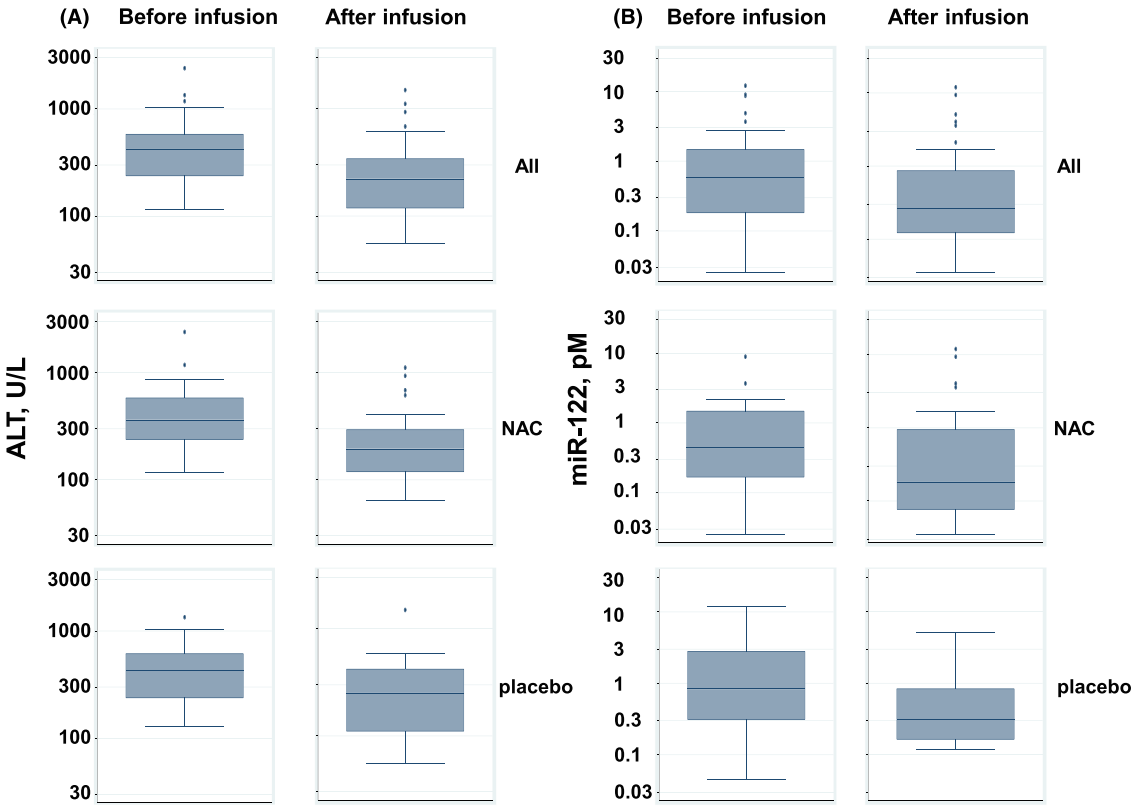

FIGURE 1 Box and whisker plots of (A) alanine aminotransferase (ALT) and (B) microRNA-122 (miR-122) serum concentrations before and after study infusion in 45 participants in a randomized placebo-controlled trial of intravenous N-acetylcysteine (NAC) in the management of antituberculosis drug-induced liver injury

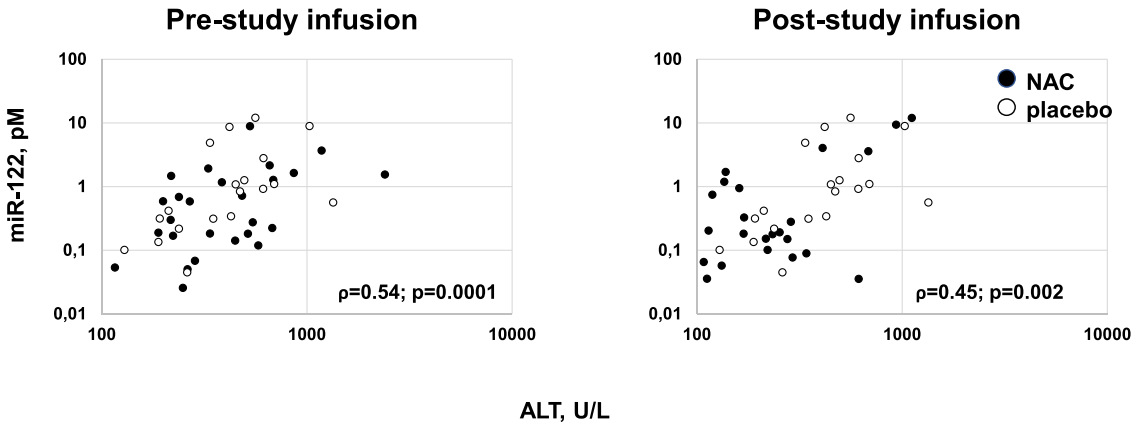

FIGURE 2 Correlation of pre- and post-study infusion serum alanine aminotransferase (ALT) and microRNA-122 (miR-122) concentrations in 45 participants with antituberculosis drug-induced liver injury randomized to intravenous N-acetylcysteine (NAC) or placebo

3.5 | Change in ALT and miR-122 concentrations

ALT concentrations decreased between samples in 43 (96%) participants. In contrast, the magnitude and direction of change in miR-122 concentrations varied substantially between participants, and miR-122 concentrations increased in 16 (36%) participants (10/26 in NAC group and 6/19 in placebo group) (Figure 3).

The median fold-change in ALT concentrations between samples was 0.56 overall (IQR 0.43-0.69) and was similar in the NAC and placebo groups: median 0.55 (0.39-0.78) and 0.60 (0.46-0.69), respectively (Wilcoxon rank sum test $P = .40$) (Supporting Information Figure S2). The median fold change in miR-122 concentrations was 0.75 (IQR 0.23-1.53) overall and was similar in the NAC and placebo groups; median 0.78 (IQR 0.23-1.53) and 0.54 (IQR 0.20-1.62),

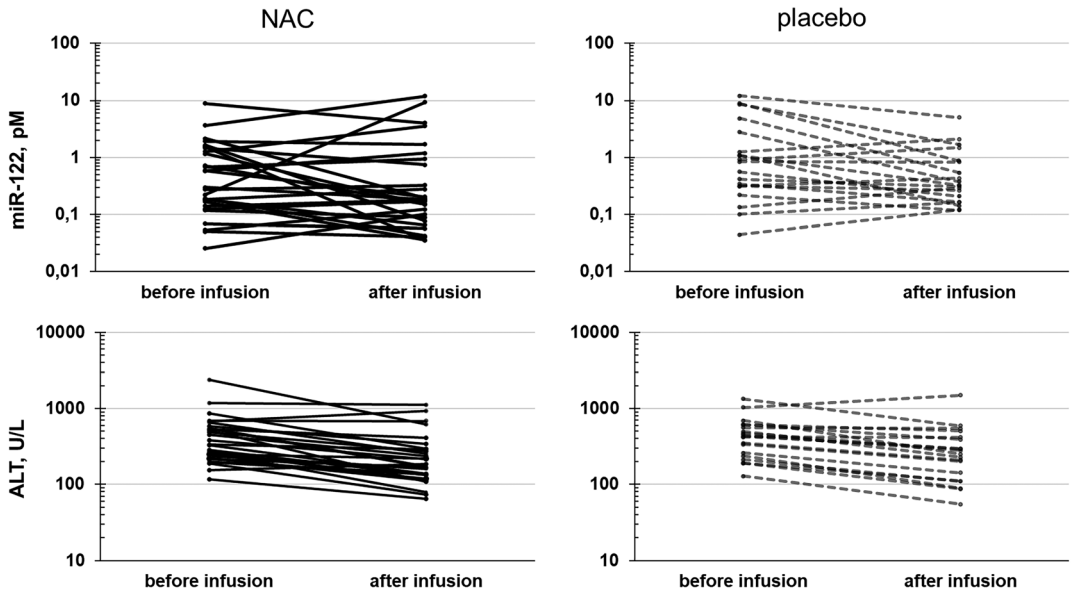

FIGURE 3 Change in serum alanine aminotransferase (ALT) and microRNA-122 (miR-122) concentrations after study infusion in 45 participants with antituberculosis drug-induced liver injury randomized to intravenous N-acetylcysteine (NAC) or placebo

respectively (Wilcoxon rank sum test $P = .68$) (Supporting Information Figure S2; note that fold change value greater than 1 corresponds to an increase).

3.6 | Change in ALT and miR-122 concentrations per day

ALT concentrations changed a median of 0.79-fold per day (IQR 0.75-0.83-fold) between the two biomarker samples. miR-122 concentrations changed a median of 0.89-fold per day (IQR 0.49-1.17-fold) between the two biomarker samples.

4 | DISCUSSION

The miR-122 concentrations observed in our participants with AT-DILI were much higher than those previously observed in healthy volunteers[20] or participants on antituberculosis therapy without DILI.[23] We found that serum ALT and miR-122 concentrations were correlated at both sampling occasions in participants with AT-DILI. ALT concentrations declined in almost all participants at the post-infusion sampling occasion. In contrast, changes in miR-122 concentrations were more variable, showing an increase in over a third of participants. The fold change in miR-122 concentrations between samples did not differ between NAC and placebo arms.

The median miR-122 concentration before study infusion in our cohort was 0.58 pM, which is 10 times higher than the upper limit of normal (ULN) of the reference range for healthy volunteers derived from the European Safer and Faster Evidence based Translation (SAFE-T) cohort, and 20 times the ULN from the Critical Path Institute's Predictive Safety Testing Consortium (PSTC) in the United States.[20] Only two of our participants had concentrations below the ULN derived from the SAFE-T cohort, and none were below the PSTC ULN.

In the ALISTER study cohort in Scotland, participants starting antituberculosis therapy without liver injury had similar miR-122 concentrations to healthy volunteers. miR-122 concentrations increased slightly after starting antituberculosis therapy but remained considerably lower (median 0.004 pM) than the miR-122 concentrations observed in our participants with AT-DILI.[23]

There are limited data on miR-122 concentrations in patients with AT-DILI. Two participants in the ALISTER cohort developed DILI with ALTs of 431 and 194 U/L, and miR-122 concentrations of 0.06 and 0.34 pM, respectively. In both these participants, miR-122 concentrations increased considerably from baseline, by 15- and 20-fold, respectively. A recent Indian study by Bakshi et al found miR-122 expression to be 75% lower in participants with AT-DILI compared to healthy controls,[24] but miR-122 was only sampled at enrolment and participants had less severe DILI than in our study, with a median ALT of 120 U/L. Despite these differences, the reason for the conflicting results is unclear and requires further exploration.

miR-122 concentrations varied widely between participants in our study, as did the intra-individual change in miR-122 between sampling occasions. In the absence of other clinical evidence, it is unclear whether these changes in miR-122 concentrations indicate worsening or improvement of the liver injury or merely reflect high intra- and inter-individual variability. In the PSTC healthy volunteer cohort who were sampled repeatedly over 3 weeks, both inter-subject and intra-subject coefficients of variation for miR-122 were high, at 91% and 94%, respectively.[20] Intra-individual variability was particularly high in Black participants.[20] A North American healthy volunteer

cohort also found high intra- and inter-donor variability in miR-122 expression, particularly in participants who identified as non-Caucasian.[25] Inter-individual variability poses challenges to defining cut-offs for abnormal elevation of miR-122. Intra-individual variability complicates interpretation of changes observed in miR-122 concentration over time. Inter-individual and intra-individual variability may therefore limit utility of miR-122 as a biomarker in diagnosing AT-DILI and monitoring AT-DILI progression or recovery.

We observed an increase in miR-122 concentrations between the two sampling occasions in a third of our study participants. The hepatocellular injury in AT-DILI[26] may be more sustained than injury due to oxidative stress injury in paracetamol overdose, and miR-122 may therefore decline more slowly in AT-DILI. Even among studies of paracetamol-induced hepatotoxicity, there are some that describe increased miR-122 concentrations from 3 to 14 days after DILI onset.[15,17]

Our study has limitations. We only included RCT participants with two biomarker samples (approximately half of the cohort), which may have introduced selection bias. However, baseline characteristics and liver biochemistry did not differ substantially between those RCT participants included in this study and those not included. HIV prevalence was high in our cohort and therefore our findings may not be generalizable to other populations with lower HIV prevalence. We only quantified miR-122 at two time points. We did not have a control group without AT-DILI drawn from the same population to allow for comparison. Different quantification and normalization methods used in different studies may influence comparisons between our results and results from other studies.

5 | CONCLUSION

miR-122 concentrations were markedly higher in our cohort of AT-DILI than previously observed in healthy controls and in participants on antituberculosis therapy without liver injury. miR-122 may therefore be a useful biomarker to diagnose AT-DILI in this population. However, high intra-individual and inter-individual variability in miR-122 concentrations may limit its utility. To characterize miR-122 concentrations prior to liver injury onset and in early AT-DILI, a large prospective cohort study collecting repeated samples for biomarker quantification from patients on antituberculosis therapy, with frequent clinical review, would be required because AT-DILI occurs in a small subset of patients on antituberculosis therapy. Further larger studies monitoring miR-122 over the full course of recovery from AT-DILI are required to characterize changes in this biomarker over time, and associations between concentrations observed and outcomes.

COMPETING INTERESTS

All authors declare that they have no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work. MP has received partnership funding for the following: MRC Clinical Pharmacology Training Scheme (co-funded by MRC and Roche, UCB, Eli Lilly and Novartis) and a PhD studentship jointly funded by EPSRC and AstraZeneca. He also has unrestricted educational grant support for the UK Pharmacogenetics and Stratified Medicine Network from Bristol-Myers Squibb. He has developed an HLA genotyping panel with MC Diagnostics but does not benefit financially from this. He is part of the IMI Consortium ARDAT (www.ardat.org). None of this funding has been used for the current paper.

CONTRIBUTORS

Muhammed Shiraz Moosa, Karen Cohen, Gary Maartens, Dan Carr and Munir Pirmohamed conceived and designed the study. Giusy Russomanno, Chandni Patel, Eithne Costello and Christopher Goldring were responsible for performance and oversight of miR-122 assays and interpretation of laboratory results. Christopher Goldring, Karen Cohen and Jeffrey R. Dorfman formulated the data analysis strategy. Muhammed Shiraz Moosa and Jeffrey R. Dorfman analysed data. Muhammed Shiraz Moosa wrote the first draft of the manuscript. All authors reviewed the manuscript and contributed to revising and finalizing the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are stored in a controlled access repository and are not openly available due to reasons of sensitivity and patient confidentiality. Data are available from the corresponding author (Karen Cohen) upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Moosa MS, Russomanno G, Dorfman JR, et al. Analysis of serum microRNA-122 in a randomized controlled trial of N-acetylcysteine for treatment of antituberculosis drug-induced liver injury. *Br J Clin Pharmacol*. 2023;1-8. doi:10.1111/bcp.15661

Appendix 5 Supplementary Material

SUPPLEMENTARY MATERIAL

Supplementary Table 3.1 West Haven Criteria for Grading of Hepatic Encephalopathy

Grade	Criteria
1	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impaired performance of addition
2	Lethargy or apathy Minimal disorientation to time or place Subtle personality changes Inappropriate behaviour
3	Somnolence to semi-stupor but responsive to verbal stimuli Confusion Gross disorientation
4	Coma (unresponsive to verbal or noxious stimuli)

Supplementary Table 3.2. Dosing Schedule for N-Acetylcysteine

Each N-acetylcysteine ampoule contains 200mg/mL N-acetylcysteine									
Regimen	Dose 1			Dose 2			Dose 3		
Fluid	200 mLs 5% glucose or sodium chloride 0.9%			500 mLs 5% glucose or sodium chloride 0.9%			1000 mLs 5% glucose or sodium chloride 0.9%		
Duration of infusion	60 minutes			4 hours			16 hours		
Drug dose	150 mg/kg N-acetylcysteine			50 mg/kg N-acetylcysteine			100 mg/kg N-acetylcysteine		
Patient Weight[1]	Dose	Ampoule volume[2]	Infusion Rate	Dose	Ampoule volume[2]	Infusion Rate	Dose	Ampoule volume[2]	Infusion Rate
kg	mg	mL	mL/h	mg	mL	mL/h	mg	mL	mL/h
40-49	6750	34	234	2250	12	128	4500	23	64
50-59	8250	42	242	2750	14	129	5500	28	64
60-69	9750	49	249	3250	17	129	6500	33	65
70-79	11250	57	257	3750	19	130	7500	38	65
80-89	12750	64	264	4250	22	131	8500	43	65
90-99	14250	72	272	4750	24	131	9500	48	66
100-109	15750	79	279	5250	27	132	10500	53	66
>110-Max dose	16500	83	283	5500	28	132	11000	55	66

[1] Dose calculations are based on the weight in the middle of each band.
[2] Ampoule volume has been rounded up to the nearest whole

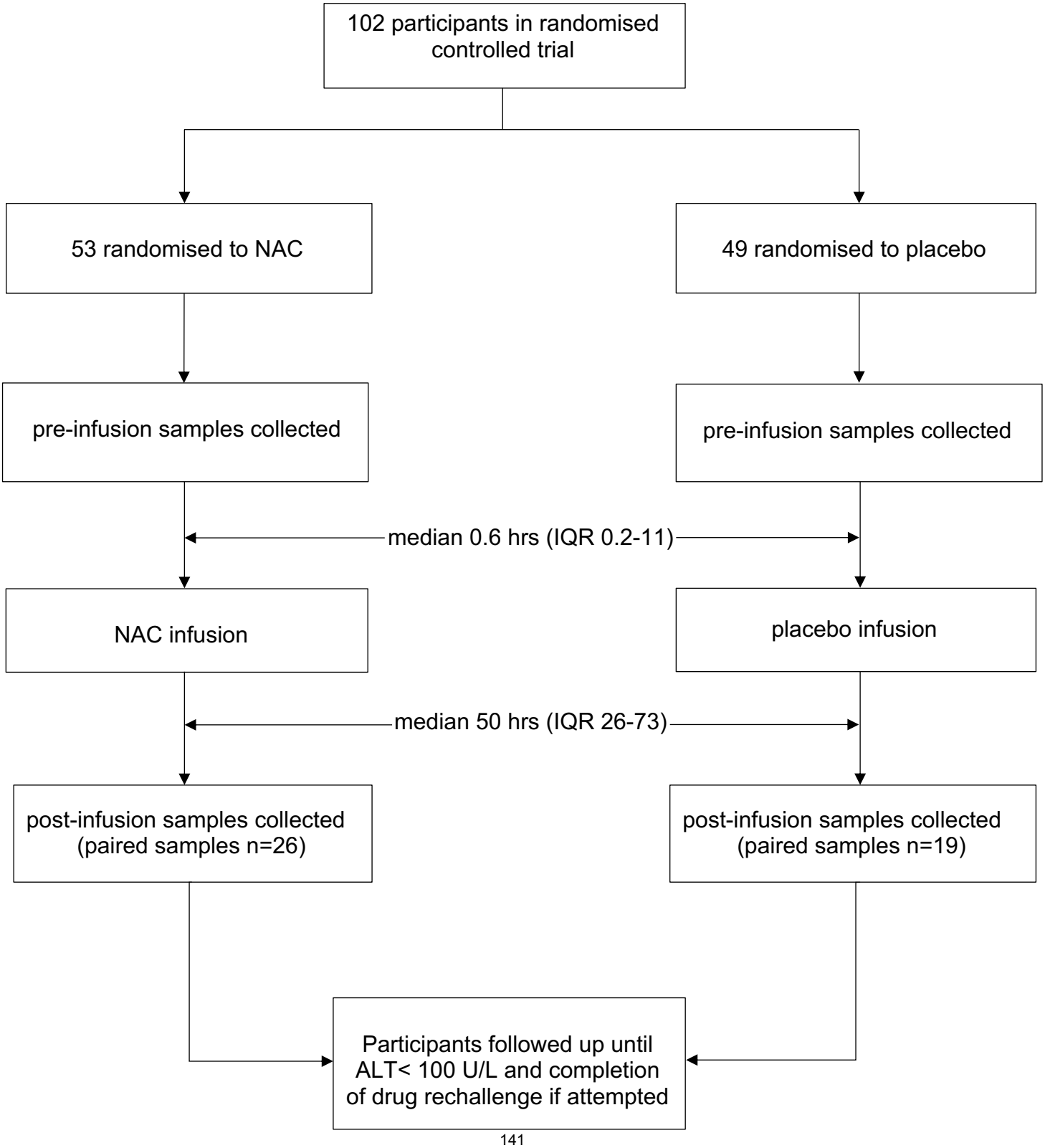
Supplementary Table 3.3. Serious Adverse Events during Participant Follow-up, by Study Arm*

Serious Adverse Event		NAC	Placebo
Death			
	Liver failure	5	4
	Sepsis/multi-organ failure	0	1
	Chronic lung disease	2	0
	Pneumocystis Jirovecii	0	1
	Haemorrhage post liver biopsy	0	1
Recurrent liver injury		5	9
Acute kidney injury		1	2
Hearing loss		1	1
Bacterial sepsis		0	2
Ongoing liver injury [#]		1	1
Disseminated intravascular coagulation		0	1
Disseminated Emergo mycosis		0	1
Hepatocellular carcinoma		0	1
Anaemia		1	0
Hypernatraemia		1	0
Hypoglycaemia		1	0
Splenic haematoma		1	0
TOTAL		19	25

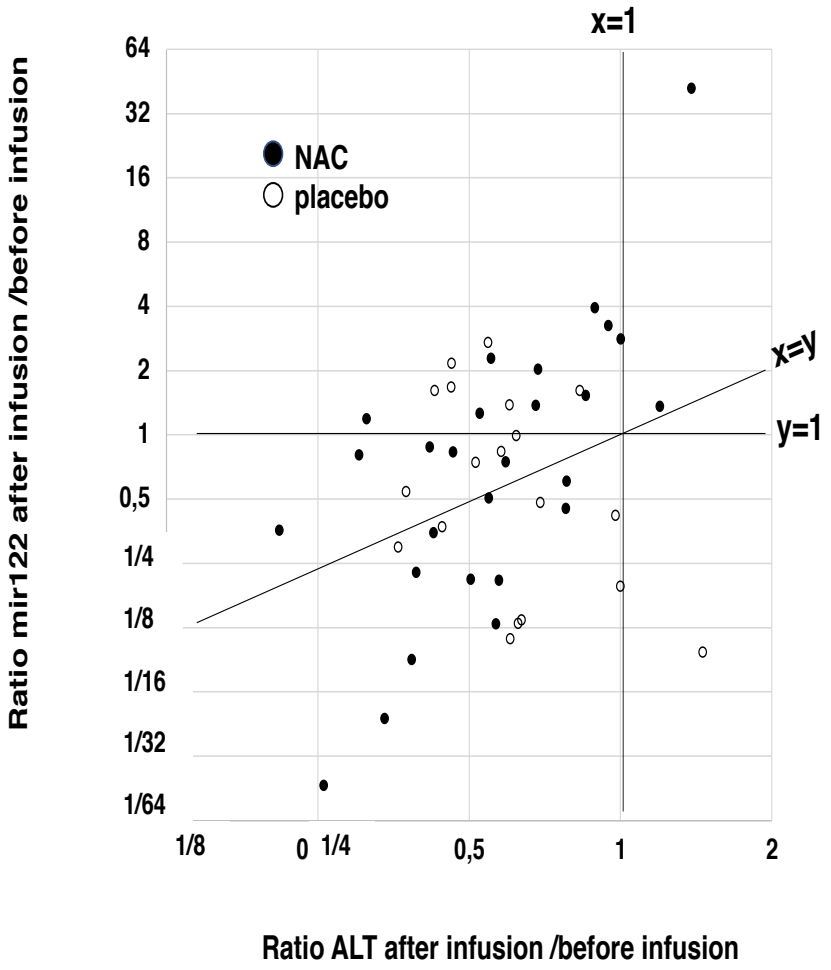
*Some participants had more than 1 AE

[#] Differential diagnosis included TB of the liver and TB immune reconstitution inflammatory syndrome

Supplementary Figure 5.1 Diagram showing study design and timing of biomarker sample storage for ALT and miR-122 quantification in a randomised placebo controlled trial of n-acetylcysteine in the management of anti-tuberculosis drug-induced liver injury



Supplementary Figure 5.2 Ratio of post-study infusion to pre-study infusion serum alanine aminotransferase and microRNA-122 concentrations in 46 participants with anti-tuberculosis drug-induced liver injury randomised to n-acetylcysteine (NAC) or placebo



Appendix 6 Research Protocol

UNIVERSITY OF CAPE TOWN

A RANDOMISED CONTROLLED TRIAL OF INTRAVENOUS N-ACETYLCYSTEINE IN THE MANAGEMENT OF ANTITUBERCULOUS DRUG-INDUCED HEPATITIS

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STUDY SYNOPSIS

South Africa has a huge tuberculosis (TB) disease burden, with 948 per 100 000 people diagnosed with TB in 2008. TB drug induced hepatitis (DIH) is a common adverse effect of TB therapy that causes significant patient morbidity and prolonged hospital stays. N-Acetylcysteine (NAC) has been extensively studied and used for many years in the treatment of paracetamol-induced hepatotoxicity, with good evidence of efficacy and safety. NAC has also been used in other forms of liver injury and drug toxicity. It has not previously been used in the management of TB DIH.

We will conduct a randomized placebo controlled trial to determine whether administration of intravenous (IV) NAC to participants with TB DIH, in dosages similar to that used in paracetamol poisoning, can improve recovery from hepatotoxicity. We will screen all patients with clinical hepatitis on TB treatment admitted to New Somerset and Groote Schuur hospitals. We aim to recruit 100 participants over 3 years. Patients with acute viral hepatitis will be excluded.

We will randomise 50 participants to receive an IV loading dose of 150mg/kg of NAC over 60 minutes followed by 50mg/kg IV over 4 hours by continuous infusion and finally 100mg/kg IV over 16 hours. Fifty participants will be randomised to receive placebo. The primary outcome will be time to normalisation of liver function (ALT<100). We will also determine the effect of NAC on duration of hospitalization, rate of recovery from liver failure, all-cause mortality, and describe adverse effects of IV NAC in this patient population.

The only study interventions will be the administration of IV NAC, daily visits in hospital by a member of the study team, a blood draw per week (over and above the standard of care blood draws done every 3-5 days) and weekly outpatient follow-up visits for 8 weeks. All other management, including the decision to rechallenge with TB drugs, will be by the admitting team.

Demographic and clinical information will be captured using standardized data capture sheets. A study number will identify individuals in the study database. Identifying information will be recorded in a logbook, stored securely and separately from the database containing the results. The database will be password protected.

Currently the standard of care for TB DIH is supportive management. Hepatotoxic TB drugs are stopped, and rechallenge is commenced once the liver functions have improved (ALT<100). As part of routine care blood for serum transaminases are drawn every 5-7 days and more frequently in patients with liver failure. Our schedule of blood draws of every 3 days will amount to an additional draw per week above the standard of care for stable patients. In addition participants will need to come for outpatient follow up visits weekly for 8 weeks after discharge. Participants will be compensated for travel costs for outpatients study follow up visits. They will receive R50 per visit. Participants will be insured against any serious adverse event by UCT's no-fault insurance policy.

We plan to use an IV formulation of NAC called Parvolex® manufactured by GlaxoSmithKline South Africa. Serious adverse effects due to IV NAC are rare. Study participants will be inpatients while receiving NAC, and will be closely monitored for adverse effects.

Contents

INTRODUCTION

TB is responsible for a death approximately every 20 seconds — nearly 5,000 deaths every day, or 1.8 million in 2008 alone, according to the latest estimates from the World Health Organization (WHO).[1] TB is second only to HIV as the leading infectious killer of adults worldwide. The WHO estimates that two billion people — one third of the world's population — are infected with *Mycobacterium tuberculosis*.

In South Africa in 2008, 948 per 100 000 people were diagnosed with TB.[1] The case detection rate has remained above target since 2003; however, treatment success rates have remained low, with high default and death rates. [1] South Africa reports the highest number of confirmed MDR-TB and XDR-TB cases in the region.

The drug treatment of TB is not without peril. TB drug induced hepatitis (DIH), not an uncommon adverse effect, can have catastrophic outcomes. Isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA) which are first line drugs for TB chemotherapy are associated with hepatotoxicity. There are no reliable statistics on the incidence of TB DIH in South Africa. The rate of hepatotoxicity varies from 2-28% depending on the definition of TB DIH, with higher incidences in developing Asian and African countries.[2] A recent retrospective survey of patients with hepatitis GF Jooste Hospital in Cape Town found that 10% of hepatitis was caused by TB drugs.[3] In this study another 10% of patients who developed DIH were on both antituberculous and antiretroviral therapy (ART). It is our estimation that at any given time there at least 2 patients with TB DIH in all secondary and tertiary hospitals in South Africa.

The management of TB DIH requires prolonged hospital admission with regular liver function monitoring until the liver function normalises. In a recent Indian study the median time to normalization of liver function was 18 days.[4] Prolonged hospital stay is associated with increased risk of nosocomial infections, increased hospital and laboratory costs as well as an increased burden on already stretched medical and nursing resources.

Once the liver function has normalized, patients are rechallenged with the TB drugs individually as per local and international guidelines. (See appendix 3) If this rechallenge is successful they are discharged to the community health clinics for further follow up.

Risk factors for and mechanism of TB DIH

Alcohol abuse, increasing age, pre-existing liver disease, slow acetylator status, and severe malnutrition have been reported as risk factors for TB DIH.[5, 6] Recent studies suggest that polymorphism and reduced activity of hepatic *N*-acetyl-transferase-2 genes (slow acetylator status) and glutathione-*S*-transferase contribute to DIH .[7]

The exact mechanism of TB DIH is not well understood but thought to be mainly a synergistic toxic effect of isoniazid (INH) and rifampicin (RIF). Pyrazinamide (PZA) causes hepatotoxicity less commonly.

INH is first acetylated and then hydrolysed to acetylhydrazine. Reactive metabolites of acetylhydrazine are probably toxic to tissues through free radical generation.[8] In rats, the free radical scavenger glutathione-related thiols, and antioxidant glutathione peroxidase and catalase activities, are diminished by INH. The antioxidant N-acetyl-cysteine, a substrate for glutathione synthesis, inhibits INH-induced liver injury in pretreated rats.[9, 10]

The role of N-acetylcysteine (NAC) in DIH

Intravenous NAC has been registered with the FDA for use in paracetamol poisoning since the early 1990's.There have been no safety issues nor any need for close safety surveillance by the FDA since its registration.[11]

Other approved indications for NAC include its use as a mucolytic in cystic fibrosis and bronchiectasis; as well as its use intravenously for the prevention of radiocontrast induced nephropathy.[12] More recently IV NAC has been recommended for treating liver failure due to all causes.[38]

<u>NAC in paracetamol overdose</u>

NAC has been used in the treatment of paracetamol overdose for decades, with good evidence of efficacy**.** In paracetamol overdose, NAC is thought to prevent hepatic injury primarily by restoring hepatic glutathione.[13] In addition, in patients with paracetamol-induced liver failure, acetylcysteine improves hemodynamics and oxygen use, [14] increases clearance of indocyanine green (a measure of hepatic clearance), [15] and decreases cerebral oedema.[16] The exact mechanism of these effects is not clear, but it may involve scavenging of free radicals or changes in hepatic blood flow. [14, 17]

Acetylcysteine was first suggested as an antidote for paracetamol toxicity in 1974.[18] Subsequently, several case series described good outcomes for patients with paracetamol overdose who were treated on the basis of the presence of a toxic blood concentration with either intravenous (IV)[19,20] or oral[21-24] acetylcysteine. The largest of these was a study involving 2540 patients and oral acetylcysteine.[21]

Cattermole in his published literature review of 2009, concluded that there were no significant differences in efficacy and rates of hepatotoxicity between oral and intravenous NAC when used in the treatment of paracetamol overdose.[25]

<u>N-Acetylcysteine in treatment and preventions of other causes of hepatitis</u>

Le Moine *et al*.[26] presented the results of a pilot study of 16 patients with biopsy-proven active ***alcoholic hepatitis*** who were treated with N-acetylcysteine. All patients had a

Maddrey's discriminant function of >32 (indicative of severe liver dysfunction), and had had no improvement in liver function tests in the previous 1 week. N-Acetylcysteine was administered in a dose of 300 mg/kg/day for 7 days (five patients) or 14 days (11 patients). The 30-day survival rate was 56% in patients treated with N-acetylcysteine. All the patients had a significant decrease in aspartate transaminase, alkaline phosphatase and prothrombin time. The authors concluded that N-acetylcysteine could be safely administered in cirrhotic patients with alcoholic hepatitis, with an improvement in some biological parameters. This uncontrolled study did not examine survival benefit

In multicenter double blinded placebo controlled trial of 144 patients, Shi and colleagues found that the daily administration of 8mg of IV NAC in patients with ***chronic hepatitis B***, improved the rate of decline of total bilirubin levels without a significant increase in adverse effects.[27] No serious adverse effects were reported.
In the ICU setting, another trial by Rank examined the effect of NAC on hepatosplanchnic blood flow and liver function in septic patients with ***ischaemic hepatitis.***[28]Patients received either a bolus of 150 mg/kg IV NAC over 15 minutes and a subsequent continuous infusion of 12.5 mg/kg/hour NAC over 90 minutes (n = 30) or placebo (n = 30). They found a significant improvement in both hepatic blood flow and hepatic function without any adverse effects in those who received NAC.
Baniasadi et al showed that NAC might have a role in preventing ***TB DIH.*** [29] In a small randomised controlled trial of 60 patients, oral NAC was administered in a dosage of 400mg twice daily to half of the patients starting on antituberculous therapy. After one week, there were no cases of TB DIH in the NAC group as compared to 12 (38%) cases of TB DIH in the control group. ($p<0.05$) This study however has limitations like its small size, its short follow-up period of 2 weeks and no placebo being administered to the control group.

There is thus some evidence of N-Acetylcysteine's protective and therapeutic benefit in various forms of hepatitis including that due to antituberculous therapy.

Route of Administration and Safety of IV NAC

Justification for intravenous administration

We have chosen the IV route above the oral route of administration because it is easier, better tolerated and serious adverse effects are rare. The duration of the IV NAC regimen as used in paracetamol poisoning is also shorter (24 hours) when compared to the oral NAC regimen (72 hours). Intravenous NAC can be run via an infusion pump thus requiring very little effort from the study nurse or doctor whereas oral NAC must be mixed with soft drinks and given every 4 hours for a total of 18 doses.

Oral NAC is often not well tolerated because of its offensive odour. Vomiting is a common adverse effect requiring repetition of the missed doses. High dosage antiemetics may have to be administered prophylactically or as rescue therapy, and may cause adverse reactions.

In participants who are obtunded, oral NAC would have to be administered via a nasogastric tube to prevent lung aspiration. The absorption of oral NAC via a nasogastric tube is an unknown variable that will need to be evaluated before this route can be utilized effectively.

Safety of intravenous NAC

Most of the safety data is derived from trials of IV NAC used in paracetamol poisoning. Serious adverse drug reactions (ADR) from IV NAC in these trials are rare.

In a RCT by Kerr and colleagues comparing a short 15 minute loading infusion of IV NAC to a longer 60 minute loading infusion, the overall rate of anaphylactoid reactions due to histamine release, like itching, flushing, urticaria and gastrointestinal side effects, was 17%.[31] Of 180 participants only 2 developed anaphylaxis requiring termination of IV NAC and there were no reported fatalities.

In case series review by Kanter the rate of ADR's from IV NAC ranged from 3-48% of patients. Most ADRs were due to histamine release, including pruritus, rash, nausea and vomiting, angioedema and bronchospasm.[32]

Merl et al noted that ADR rates were higher (42-48%) in prospective studies than in retrospective studies (4-9%). In their own study of 470 cases of paracetamol poisoning, the rate of ADR's was 11%, with major adverse drug reaction in 2 patients 0.6% (hypotension and angioedema). The most common ADR's were urticaria (56%), flushing (42%) and bronchospasm (33%).[33]

In a Cochrane review of pooled data from 9 case-series including 1614 PO patients and 637 IV patients, mortality was very low, with no deaths at all in those treated with NAC within 10 hours of paracetamol ingestion, and only 1% (PO) and 2% (IV) between 10-24 hours. Mortality overall was 0.6% (PO) and 0.9% (IV).[34]

In a meta-analysis by Buckley et al, adverse reactions to NAC occurred in 12/205 patients (6%). These were all related to the initial loading dose given over 15 minutes and ranged from flushing and urticaria to 2 anaphylactoid reactions. Reintroduction of NAC at a lower rate of infusion allowed successful completion of the treatment course in all these patients.[35]

A systematic review of adverse events with N-acetylcysteine administration found that the incidence of ADRs reported varied considerably between studies, ranging from 9% to 77% in prospective studies [39]. "Anaphylactoid" reactions, with clinical features including nausea, vomiting, rash, pruritus, bronchospasm, tachycardia, hypotension and angioedema, are described. Of these, nausea and vomiting is the commonest clinical feature, with incidence in prospective studies ranging from 5-70% [39]. Flushing occurred in 15-30%, rash in 4-32% and pruritus in 6-20% [39]. Bronchospasm was the most common systemic reaction, reported in 6-26%; hypotension was reported in 16% [39]. There are case reports of angioedema, but this adverse event was not reported in any of the prospective

studies included in this systematic review[39].Severe systemic reactions were uncommon, with an incidence of 1%[31,39]

Inclusion of HIV infected participants on antiretroviral therapy (ART) and/or cotrimoxazole

More than half of new TB patients in South Africa are HIV-infected. Besides being on TB therapy, many of them will be on cotrimoxazole and ART, both of which can cause or contribute to DIH.

Our aim is to determine whether NAC is useful in treating DIH in the population of TB patients developing DILI in South Africa. We therefore do not plan to exclude HIV-infected patients. We accept that DILI in patients on ART could be due to antiretrovirals or other medication commonly used in HIV infection such as cotrimoxazole. NAC has been used for DILI from many drugs and therefore may be of some benefit even if another drug is responsible.

Inclusion of severely ill and/or encephalopathic patients

Although NAC has not previously been specifically studied in this patient group, there is a large body of evidence for its use in paracetamol poisoning. It is unlikely to put patients with more severe DIH at risk of adverse events, and they are the participant group most likely to benefit from this intervention. We have therefore not excluded more severely ill participants from eligibility for recruitment.

STUDY AIM AND OBJECTIVES

Aim

To determine whether IV NAC shortens duration and severity of antituberculous drug-induced hepatitis.

Objectives

1. To determine the effect of IV NAC on the time to normalization of liver function in patients with TB DIH
2. To determine the effect of IV NAC on duration of hospitalization
3. To determine the effect of IV NAC on the rate of recovery from liver failure
4. To determine the effect of IV NAC on all-cause mortality in patients with TB DIH
5. To determine the adverse event profile of IV NAC when administered to patients with TB DIH
6. To determine the effect of IV NAC on success of TB drug rechallenge.
7. To determine the effect of IV NAC on duration of rechallenge
8. To bank serum, to enable us to conduct future sub studies exploring biomarkers of TB DIH if funding becomes available.

METHODS

Study setting

Participants will be enrolled at the New Somerset and Groote Schuur Hospitals.

Study design

A prospective randomised placebo controlled study.

Sample size

Our sample size calculation is based on data from a randomised controlled trial of 3 regimens for antituberculous drug rechallenge in Indian patients with TB DIH.[4] The median time to normalization of liver functions (defined as ALT<100) in this study was 18 days (IQR 14-28). Our planned study is powered to detect a 33% reduction in time to normalisation of liver function, from 18 to 12 days.

The sample size is calculated assuming a normal distribution. The standard deviation (SD) is approximated from Sharma et al's data by the formula IQR/1.35, giving an estimated SD of 10 days.

We calculate that, with 80% power and alpha=0.05, we would need at least 44 participants in each group. If we expect the mortality in each group to be 10% we will need an additional 5 subjects in each arm. We therefore estimate that we will need to recruit at 50 participants in each arm.

Diagnosis of TB DIH

Using the American Thoracic Society guidelines[36] we have defined TB DIH as:

A rise in ALT > 5X ULN with/without symptoms of hepatitis
A rise in ALT> 3X ULN with symptoms of hepatitis
A rise in bilirubin > 2X ULN

Symptoms and signs of clinical hepatitis include fatigue, nausea, vomiting, jaundice and right upper quadrant pain and tenderness. Acute liver failure is defined as fulminant hepatitis resulting in coagulopathy (INR>1.5) and an altered mental status.[37]

Study participants

Inclusion Criteria:

- Adults > 18 years old
- Diagnosed with pulmonary or extrapulmonary tuberculosis based on symptoms, radiological features and/or laboratory evidence.
- On first line antituberculous therapy
- Diagnosed with TB DIH

Exclusion criteria:

- Patients with a diagnosis of acute viral hepatitis based on a positive anti-HAV, IgM, anti- HBcIgM, or confirmed hepatitis C infection will be excluded.
- Patients known to be asthmatic will be excluded

Dosage and administration of IV NAC

Participants will receive IV NAC (Parvolex ^{R}) manufactured by GlaxoSmithKline SA or placebo. The dosing regimen is based on the regimens used in paracetamol poisoning [19-21, 23, 24]. IV NAC will be dosed as follows:

Initial dose: 150 mg/kg body mass of N-acetylcysteine infused in 200 mL of 5% dextrose intravenously over 60 minutes, followed by continuous infusion: 50 mg/kg body mass in 500 mL of 5% dextrose over next 4 hours, followed by 100 mg/kg body mass in 1 litre of 5% dextrose over 16 hours.

Administration of the IV NAC to study participants will be done by either a study nurse or a co-investigator who will be present during the entire initial loading dose to monitor the study participant for any ADR's. Reactions due to histamine release including urticaria, flushing and bronchospasm will be treated by temporarily halting the IV study medication infusion. A dose of 50 mg of IV hydrocortisone and 25 mg of IV promethazine will be administered. If a participant develops bronchospasm an inhaled beta-2 adrenergic agonist may be administered in addition to the IV promethazine and hydrocortisone.
Once the participant's symptoms have settled, the NAC infusion may be resumed at a slower rate.

Participants who develop nausea and/or vomiting due to IV NAC will be treated with 10 mg of IV metoclopramide stat. If nausea persists a second dose of metoclopramide will be administered. If the second dose of metoclopramide fails to control the nausea and/or vomiting, the IV NAC infusion will not be recommenced.

If a participant develops anaphylaxis or angioedema the IV study medication infusion will be stopped, and nebulized adrenaline will be administered, in addition to stat doses of 50 mg IV hydrocortisone and 25mg IV promethazine.

Any participant who develops a serious ADR such as anaphylaxis or angioedema will not be administered any further study medication. However, they will continue to be followed up as study participants.

Table 1 PARVOLEX INTRAVENOUS INFUSION DOSAGE GUIDE

PARTICIPANTS BODY MASS	INITIAL	SECOND	THIRD	TOTAL **PARVOLEX**
(kg)	150 mg/kg in 200 mL of 5% dextrose over 60 minutes	50 mg/kg in 500 mL of 5% dextrose over 4 hours	100 mg/kg in 1 litre of 5% dextrose over 16 hours	(mL)
	PARVOLEX (mL)	PARVOLEX (mL)	PARVOLEX (mL)	
50	37,5	12,5	25	75
60	45,0	15,0	30	90
70	52,5	17,5	35	105
80	60,0	20,0	40	120
90	67,5	22,5	45	135
x	0,75x	0,25x	0,5x	1,5x

Research procedures and data collection

Screening

All patients admitted to New Somerset and Groote Schuur Hospitals with clinical hepatitis who are currently taking antituberculous treatment will be screened. The study nurse or one of the investigators will perform screening and consenting.

If patients fulfilling eligibility criteria are too ill to give consent, they will be randomised before consent is taken. Consent will be taken as soon as they recover sufficiently to understand the informed consent process (see discussion under "Ethical issues" below).

Randomisation

Participants will be randomised using a computer generated random number table to receive either NAC or matching placebo. A study pharmacist will perform randomisation. The study pharmacist will maintain allocation concealment. The investigators, nurses and study participants will all be blinded to treatment allocation.

Baseline In-Hospital Assessment:

A study investigator at each site will record baseline demographic data of enrolled participants including age, sex, weight, BMI and alcohol use. They will also record HIV

status at admission, if known, history of previous hepatitis, method of TB diagnosis, length of TB treatment and a detailed drug history. They will perform a baseline clinical assessment on all participants with TB DIH. This will include an assessment for symptoms and signs of hepatitis and liver failure.

Study Interventions

- Study drug (NAC or placebo) will be administered to participants as per the dosing schedule above. Participants will be closely monitored for ADRs during administration of the loading dose, when ADRs are most likely to occur. ADR's will be managed appropriately. (see the discussion of ADR management under "Dosage and administration of IV NAC" above)
- An extra blood draw per week (above the routine standard of care blood draws) for liver function tests will be done. All results of additional blood tests performed for study purposes will be reported back to the clinicians responsible for patient care, as these may influence their management decisions

Ongoing In -Hospital Assessment

A study investigator will assess each study participant clinically on a daily basis and record clinical findings. Any findings of clinical concern will be discussed with the hospital medical team. The medical team will make decisions regarding management of the participant according to local guidelines.

Out of Hospital Assessment

After discharge, participants will be assessed weekly by one of the study investigators for a further 8 weeks. Blood will be drawn for liver functions (AST, ALT, Bilirubin, and INR) at these visits. If clinical or laboratory findings indicate recurrence of hepatitis or any other new medical problem, that participant will be referred back to the hospital medical team for care.

Routine Care by Admitting Medical Team

The admitting medical team is responsible for medical care of study participants. The usual management protocol for TB DIH at the study sites is outlined below. *These are not study procedures*:

- On admission of patients with TB DIH, the admitting medical doctor will draw bloods for: serum transaminases, alkaline phosphastase, bilirubin, albumin and INR, white cell count ,anti-HAV IgM, HBsAg, anti-HBcIgM, anti-HCVIgM and HIV ELISA antibody testing (in those patients who give consent to HIV testing).

- The admitting medical team will perform serial serum transaminases and bilirubin. These will be performed every 3-5 days until the ALT is <100 or bilirubin has normalised as defined by the ATS guidelines for TB treatment rechallenge.[37]

The decisions around whether to rechallenge with TB treatment and timing of rechallenge as well as all other clinical decisions pertaining to the management of study patients with TB DIH will be made by the admitting medical team.

Data management

Demographic and clinical information will be captured using standardized data capture sheets. A study number will identify individuals in the study database. Range and consistency checks will be applied. Incompatible data will be referred back to the study physician for verification. Identifying information will be recorded in a logbook, stored securely and separately from the database containing the results. A study number will identify blood specimens only.

Analysis

Data will be analysed using Stata™ (Version 11, College station, TX, USA). Participants will be followed up from the time of randomisation and treatment initiation until normalisation of liver function tests. Participant demographics will be described using means and standard deviations if normally distributed, medians and ranges if non-normally distributed, and counts and percentages for categorical data. Time to normalisation of liver functions will be described using Kaplan Meyer analyses. Associations will be modelled using Cox proportional hazards regression. Covariates will be included in a multivariate model if found to be associated on univariate analysis, or thought *a priori* to be associated with the outcome. Confidence intervals will be calculated for all summary statistics and parameter estimates. Observed p values will be reported, but in general statistical significance will be assessed at the 5% level, with correction for multiple comparisons where appropriate.

ETHICAL ISSUES

This study will be conducted according to the guidelines of the Helsinki declaration of 2008 [44] and according to the ICH principles of Good Clinical Practise [39]. Approval of the protocol and informed consent documents will be sought from the research ethics committee of the University of Cape Town before the study commences.

Informed consent

Written or witnessed oral consent will be taken from all participants (see annexure 1) Subject information and consent documents will be available in English, Xhosa and Afrikaans. A witness independent of the study will witness consent being taken from illiterate participants giving witnessed oral consent. The participant will be given a copy of the signed consent document.

Consent in obtunded or encephalopathic patients

We plan to include patients too unwell to give immediate consent at presentation. Such patients will be randomised and may be administered the study intervention. Informed consent will be sought as soon as they recover sufficiently to be cognitively capable of consenting.

Data from any patient who refuses consent after the study has been explained to them will not be used in the analysis. However, we will request permission from the research ethics committee to use data from patients who die before regaining consciousness, in whom we never manage to get consent. DIH is an important clinical problem with great public health relevance in South Africa. The benefit to the community of a successful intervention would be large, and understanding the value of this intervention in more severely ill patients is of great potential value to the community. Excluding data from more severely ill patients who die would result in biased results, and may bias study conclusions, and it is therefore important that these data be included in the analysis. The IRB will be informed of any such patients, and we will request the IRB's permission to use the data in the analysis.

Privacy and confidentiality

A study number will identify participants in the study database. Identifying information will be recorded in a logbook, stored securely and separately from the database containing the results.

MEDICINES CONTROL COUNCIL

NAC has not been used for the treatment of TB DIH before and an application for its use in this condition will be submitted to the South African Medicines Control Council (MCC). Approval will be obtained from the MCC before the study commences

EMERGENCY CARE AND INSURANCE

Study participants are hospitalized during the period when NAC is administered. They will therefore be closely monitored as part of routine clinical care, and will

have access to immediate care in the event of an emergency. Insurance will be covered by UCT's no fault insurance policy.

RESEARCH PROJECT TIMELINE

(Refer to APPENDIX 4 GRANT CHART OF PLANNED RESEARCH ACTIVITIES)

First 6 months:

During this period we will train all relevant research staff with respect to the project protocol, screening, consenting, phlebotomy and data capturing. A study pharmacist will also be trained in randomisation and treatment allocation. During this period, data capture sheets and a study database will be devised and tested.

Period 6 -42 months:

The trial will commence with enrollment of study participants after the first 6 months and continue to do so until 100 participants are enrolled (which we expect will take another 36 months)

Period 42 – 48 months

During this period the trial data will be analyzed and prepared for publication.

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APPENDIX 1.a Participant information

Participant information

A randomised controlled trial of intravenous N-acetylcysteine in the management of antituberculous drug- induced hepatitis

RESEACHERS:
LEAD INVESTIGATOR: Dr. M.S.Moosa, Somerset Hospital*,
PRINCIPAL INVESTIGATOR: Dr.K.Cohen, Division of Clinical Pharmacology*
CO-INVESTIGATORS: Prof. G.Maartens, Division of Clinical Pharmacology*,
Prof.W.Spearman, Division of Hepatology*
Dr.Mark Sonderup, Division of Hepatology*
*Department of Medicine, University of Cape Town

We are approaching you to invite you to participate in this study because you have been admitted to hospital with hepatitis (liver injury) caused by the drugs that you are taking to treat your TB.

Why are we doing this study?

Currently there is no drug treatment for TB drug-induced hepatitis. Sometimes patients with TB drug-induced hepatitis get very ill, and need to stay in hospital for a long time.

This study is trying to work out whether a drug called N-acetyl cysteine (NAC) will speed up recovery of the liver. We will also see if NAC shortens the time that people with TB drug induced hepatitis need to stay in hospital.

NAC is a drug used in the treatment of liver damage due to paracetamol overdose. It has not previously been used in the treatment of TB drug-induced hepatitis

What is the usual treatment for TB drug hepatitis?

The usual treatment for TB drug hepatitis is to admit you to hospital. Your doctor will draw blood to test for liver function, for liver viruses (hepatitis A, B & C) and for HIV infection (if you have given consent). These tests are done on all patients with TB drug induced hepatitis.

Your doctor will do liver function tests every five to seven days, which will tell us if there is ongoing damage and inflammation to the liver. These tests might be done more frequently if you became more unwell. Your doctor will wait until the liver function test results show that your liver has recovered, and will then start to reintroduce your TB medicines one by one, with daily blood tests to check that the liver damage is not recurring.

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What will happen if I participate in the study?

You will receive the usual treatment for TB drug-induced hepatitis. In addition you will be given the study medicine, which will be either NAC or placebo. Placebo looks exactly like NAC but is inactive. You will be allocated to NAC or placebo randomly (like flipping a coin). You will not be told whether you are getting NAC or placebo.

The study medicine will be given to you by intravenous infusion, in a drip. You will get the first dose over 60 minutes, a second dose over 4 hours, and a third dose over 16 hours. You will need to have a drip up for the study medicine for a total of at least 21 hours. The dosing schedule is as follows: 150 mg/kg body mass of N-acetylcysteine infused in 200 mL of 5% dextrose intravenously over 60 minutes, followed by continuous infusion: 50 mg/kg body mass in 500 mL of 5% dextrose over next 4 hours, followed by 100 mg/kg body mass in 1 litre of 5% dextrose over 16 hours

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You will have some additional blood tests for the study, in addition to those done for your routine medical care. We will draw 5ml of blood every 3 days (as compared to every 5-7 days with standard care) to assess your liver function until they normalise. This takes on average 2-3 weeks. This study will therefore require an extra blood draw per week until your liver has recovered. You will be seen by a study doctor every day to assess your medical condition until you are ready for discharge. Thereafter you will be seen once a week for study visits as an outpatient at our clinic for 8 consecutive weeks.

You will be compensated R50 per outpatient clinic visit to cover your travel expenses.

What are the side effects of N-Acetylcysteine?

Intravenous NAC commonly causes minor side effects, including nausea, vomiting, facial flushing and skin rashes. Serious side effects like bronchospasm (wheezing chest) and severe allergic reactions do occur but are very uncommon.

You will be monitored carefully for any side effects while you receive the study drug. Less serious side effects, like flushing or a tight chest, will be treated by slowing down the rate that the drip is running, and you may be given hydrocortisone, salbutamol in a nebuliser (a medicine that you breathe in from a face mask, to open your chest) or an antihistamine. The study drug will be stopped if any serious side effect occurs.

Another side effect that may occur is itching and redness at the drip site. This is not a side effect of the study drug, and may occur with any IV drip.

What are the benefits of participating in the study?

We do not know if you will receive any benefit from treatment with the study drug, Results of the study may benefit other people like you who develop TB drug induced hepatitis in the future.

What will happen if you decide to leave the study?
You can leave the study at any time if you wish. This will not affect your care in hospital in any way.

What will happen with information collected about me in the study?
All information collected during the course of this study will be stored securely. Dr Cohen and Dr Moosa are responsible for this. Reports about the study and results that may be published in scientific journals will not include any information, which identifies you personally.

Important contacts
The committee giving ethical approval for this study is the Human Research Ethics Committee of the University of Cape Town If you have any problem with this study please contact the University of Cape Town Ethics committee directly, telephone number 021 4066492. You can also contact the lead investigator Dr.M.S.Moosa on 083 3140711.

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). If, after you have consulted your doctor or the ethics committee, they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar
SA Medicines Control Council
Department of Health
Private Bag X828
PRETORIA, 0001.
Fax: (012) 312 3105
e-mail: labusa@health.goc.za

APPENDIX 1.b Participant consent form

Participant consent form
A randomised controlled trial of intravenous N-acetylcysteine in the management of antituberculous drug- induced hepatitis

I have fully understood the above information about this study, which I have read, or which has been read or translated to me. I understand what will be required of me if I take part in the study.

My questions concerning this study have been answered by

……..... (name of study staff member)

I agree to take part in the study: **YES / NO** (answer to be circled)

I understand that I may withdraw from this study at any time without giving a reason and without affecting my normal care and management.

Participant's signature: …….

Participant's name:……

Date: ……

If the information sheet and consent form were translated or explained to the participant, please enter the name of the translator here and their signature:

Translator's signature: …….

Translator's name: ……..

Date: ……..

If the participant gave verbal consent, please enter the name of the person who witnessed the consent here and their signature:

Witness' signature: ……

Witness' name: ……..

Date: ……

Name and signature of person taking consent

Name: ……..

Signature …….

Date: ……

APPENDIX 2.a Participant information

Participant information: **A randomised controlled trial of intravenous N-Acetylcysteine in the management of antituberculous drug- induced hepatitis- storage of specimens for future studies**

RESEACHERS:
LEAD INVESTIGATOR: Dr. M.S.Moosa, Somerset Hospital*,
PRINCIPAL INVESTIGATOR: Dr.K.Cohen, Division of Clinical Pharmacology*
CO-INVESTIGATORS: Prof. G.Maartens, Division of Clinical Pharmacology*,
Prof.W.Spearman, Division of Hepatology*
Dr.Mark Sonderup, Division of Hepatology*
*Department of Medicine, University of Cape Town

You have been admitted to hospital with hepatitis (liver injury) caused by the drugs that you are taking to treat your TB, and have been recruited into a study looking at whether N-Acetylcysteine is of benefit in treating liver injury in TB patients.

We request your permission to store blood specimens for future analysis.

Why do we want to store your specimens?

Currently very little is known about the mechanism of liver injury due to TB drugs. We would like to store your specimens so that we can analyse them in the future to learn more about how TB drugs damage the liver, and what characteristics put people taking TB medicines at risk of developing liver inflammation and liver damage.

If you consent to storage of your specimens, blood left over after we have performed the study tests will be stored the University of Cape Town. We will apply to The University of Cape Town ethics committee for approval before analysing your stored samples.

Important contacts

The committee giving ethical approval for this study is the Human Research Ethics Committee of the University of Cape Town If you have any problem with this study please contact the University of Cape Town Ethics committee directly, telephone number 021 4066492. You can also contact the lead investigator Dr.M.S.Moosa on 083 3140711.

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). If, after you have consulted your doctor or the ethics committee, they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar
SA Medicines Control Council
Department of Health
Private Bag X828
PRETORIA, 0001.
Fax: (012) 312 3105
e-mail: labusa@health.goc.za

APPENDIX 2.b Participant consent for storage of specimens

<u>Participant consent for storage of specimens for future studies:</u> A randomised controlled trial of intravenous N-acetylcysteine in the management of antituberculous drug- induced hepatitis:

I have fully understood the above information about sample storage, which I have read, or which has been read or translated to me. I understand what will take place if I agree to allow my samples to be stored.

My questions concerning specimen storage have been answered by

……..... (name of study staff member)

I agree that my blood samples can be stored: **YES / NO** (circle answer)

Participant's signature: ……

Participant's name: ……

Date: ……..

If the information sheet and consent form were translated or explained to the participant/next of kin please enter the name of the translator here and their signature:

Translator's signature: …….

Translator's name: ……

Date: …….

If the participant/next of kin gave verbal consent, please enter the name of the person who witnessed the consent here and their signature:

Witness' name: ……..

Witness' name: ……..……...…

Date. ……..

Name and signature of person taking consent

Name: ……..

Signature: ……

Date ……

APPENDIX 3. GUIDELINES FOR TB DRUG RECHALLENGE

NHLS and WESTERN CAPE ACADEMIC HOSPITALS ANTIMICROBIAL RECOMMENDATIONS 2012 -GUIDELINES FOR TB DRUG RECHALLENGE

TB drug induced liver injury is defined as a more than 5-fold increase in the transaminases or more than a 3-fold increase with symptoms or jaundice. Antituberculous therapy should be discontinued. The basis for the TB diagnosis should be reviewed. If the grounds for diagnosing TB were reasonable then commence three antituberculous drugs with low/no hepatotoxic potential (see background therapy below) Selected patients may then be rechallenged once symptoms of hepatitis have resolved, bilirubin levels have returned to normal and transaminases have decreased to less than 100 IU. Rechallenge is not recommended for those who have had fulminant hepatitis (defined as hepatic encephalopathy with coagulopathy). Rechallenge with PZA was previously not recommended, but a recent trial has shown that most patients tolerate it. PZA rechallenge should be considered in patients with severe TB (e.g. military TB, TB meningitis) or with drug resistance. Transaminase levels especially ALT, should be monitored frequently (e.g. three times weekly) during rechallenge and every two weeks for a month following rechallenge.

The rechallenge regimen of the American Thoracic Society have been followed as these are simple and quick.[37]

Rechallenge regimen:	Background therapy	Ethambutol, streptomycin and Moxifloxacin
	Day 1	Rifampicin 450mg or 600mg daily depending on weight
	Day 3	Check ALT
	Day 4-6	Add INH 300mg daily
	Day 7	Check ALT
	Day 8	Consider PZA rechallenge

Duration of therapy should be individualized after rechallenge. The following are guidelines:
Pyrazinamide not rechallenged/not tolerated: stop moxifloxacin and streptomycin, continue isoniazid, rifampicin and ethambutol for total duration of 9 months
Rifampicin not tolerated: continue streptomycin (for 2 months) and moxifloxacin, isoniazid and ethambutol for 18 months.

Isoniazid not tolerated: stop moxifloxacin and streptomycin, add ethionamide to rifampicin and ethambutol for total duration of 12 months.

APPENDIX 4 GRANT CHART OF PLANNED RESEARCH ACTIVITIES

Tasks	Task Lead	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1.Develop research protocol	Project leader																
2.Obtain UCT IRB and MCC approval	Project leader and team																
3.Develop case record forms and SOP's	Project leader																
4.Enrollment of patients at each site	Research sister																
5.Yearly reports of adverse drug reactions	Project leader																
6. Data analysis and statistics	Project leader and statistician																
7. Publication of results.	Project leader																

Appendix 7 University of Cape Town Human Research Ethics Committee Approval Letters (current and original)

HUMAN RESEARCH ETHICS COMMITTEE
04 APR 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.04.2023
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate	Date Signed	4/4/2022

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	1 April 2022		
HREC REF Number	087/2012	Current Ethics Approval was granted until	30/04/2022
Protocol title	A randomised controlled trial of intravenous N-Acetylcysteine in the management of patients with antituberculous drug-induced hepatitis		
Protocol number (if applicable)	Version 2.4		
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Karen Cohen		
Department / Office Internal Mail Address	Division of Clinical Pharmacology, Department of Medicine K45 Old Main Building, Groote Schuur Hospital		

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

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		
1.3 Annual Approval for **full committee** review	- R 3420 (inclusive of vat)	
For invoicing purposes, please provide:		
Sponsor's name		
Contact person		
Address		
Telephone number		
Email Address		

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
✓	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	102
Number of participants enrolled, since last HREC Progress report (continuing review)	0
Additional number of participants still required	0

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	2

6. Cumulative summary of participants

Total number of participants who provided consent	99
Note: three participants demised prior to recovering sufficiently for us to seek informed consent for study participation. In line with our approved protocol, we requested permission from UCT HREC to use their information and stored specimens. Approval was granted on 15 Feb 2019	
Number of participants determined to be ineligible (i.e. after screening)	18
Number of participants currently active on the study	0
Number of participants completed study (without events leading to withdrawal)	102
Number of participants withdrawn at participants' request (i.e. changed their mind)	2
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	3
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	4
Patients were discharged from hospital and did not return for follow up visits, despite our repeat efforts to contact them to attend follow up visits	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
Enrolment is now complete as we have reached our enrolment target. No participants are currently being followed-up. Fourteen participants demised. All of these deaths were deemed unlikely to be related to the Investigational Product. We are in the analysis phase of the study.

8. Protocol violations and exceptions (tick ✓ all that apply)

☐	No prior violations or exceptions have occurred since the original approval

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

✓	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐	No prior amendments have been made since the original approval
✓	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.

SAE	Causality	Outcome

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
Yes	☐ No	☐ X Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	✓ No	☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	✓ No	☐ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	✓ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
✓	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓ No
If yes, please explain and if necessary attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

14. Signature

My signature certifies that the above is complete and correct.

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Signature of PI	Signed by candidate	Date	1 April 2022

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	27.3.2015
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate	te Signed	26/3/2014

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	26 March 2014		
HREC REF Number	087/2012	Current Ethics Approval was granted until	27 March 2014
Protocol title	A randomised controlled trial of intravenous N-Acetylcysteine in the management of patients with antituberculous drug-induced hepatitis		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes X No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Karen Cohen		
Department / Office Internal Mail Address	Division of Clinical Pharmacology, K floor, Old Main Building		

1.1 Does this protocol receive US Federal funding?	☐ Yes	X No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	X No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	X No

2. List of documentation for approval

3. Protocol status (tick ✓)

X	Open to enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	0
Number of participants enrolled, since last HREC Progress report (continuing review)	0
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	0

6. Cumulative summary of participants

Total number of participants who provided consent	0
Number of participants determined to be ineligible (i.e. after screening)	0
Number of participants currently active on the study	0
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	0
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up (please comment below on reasons for loss of follow-up)	0

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 8 South African National Clinical Trials Registry Application

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Application ID:	3719	DOH Number	DOH-27-0414-4719	Page:	1/2

Applicant Details

Organisation :	University of Cape Town
Applicant Type :	Academic Investigator
Contact Name :	Muhammed Shiraz Moosa
Address :	Dept. of Medicine New Somerset Hospital Portswood Road Greenpoint 8001 Cape Town
Telephone :	0027 21 4026911
Fax :	0027 21 4026411
E-mail :	shiraz.moosa@gmail.com
Responsible Contact person (for public)	Muhammed Shiraz Moosa
Telephone :	0027 83 3140711
Research contact person	Muhammed Shiraz Moosa
Telephone :	0027 83 3140711

Trial Application Details

Issue Date :	2014/04/15
Sponsors :	University of Cape Town
Primary Sponsor :	University of Cape Town
FundingType :	Grant Funded
Research Site Names :	Groote Schuur Hospital, Cape Town New Somerset Hospital, Cape Town
Primary Research Site Name :	n/a
Total National Budget for Trial :	R 600 000,00
Protocol / Grant Reference Number	087/2012

Study Descriptive Information

Brief Tiltle of Study :	IV NAC in TB DIH
Full Title of Study :	A Randomised Controlled Trial of Intravenous N-Acetylcysteine in the Management of Antituberculous Drug Induced Hepatitis
Anticipated Start Date :	2014/05/01
Anticipated End Date :	2015/12/31
Target Sample Size :	110
Study Phase :	Phase 4
Study Scope :	Multiple Site, RSA Only
Study Type :	Interventional
Disease Type Heading :	Bacterial and Fungal Diseases
Disease Type Condition :	Actinomycetales Infections
Intervention Name (Generic) :	N-Acetylcysteine
Intervention Duration :	No. Type 21 Hours

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

Application ID:	3719	DOH Number	DOH-27-0414-4719	Page:	2/2

Interventional

Intervention Type :	Registered Drug New Application
Purpose :	Treatment
Allocation :	Randomised
Masking :	Double Blind
Control :	Placebo
Assignment :	Single Group
Endpoints :	Safety/Efficacy

Study Descriptive Information

Recruitment Status as at Date:	2014/03/31
Recruitment Status :	Not Yet Recruiting
Gender :	Males
Ethnicity :	All
Age :	From Years To Years
Qualifying Disease Condition for Inclusion :	Drug sensitive Tuberculosis
Major Exclusion Criteria :	Viral Hepatitis Asthma MDR TB
Key Primary Outcome :	Time to ALT < 100
Key Secondary Outcomes :	Mortality from TB DIH Length of hospital stay due to TB DIH Rate of successful TB drug rechallenge

Committees

Ethics Committee :	Approval Status	Ethics Number	Ethics Date
University of Cape Town	Approved	087/2012	2013/03/28
Medicine Control Council	Approved	N2/19/8/2(2700)	2014/02/11

Appendix 9 ClinicalTrials.gov Registration

COMPLETED

ClinicalTrials.gov Identifier: NCT02182167

A Trial Of Intravenous N-Acetylcysteine In The Management Of Antituberculous Drug-Induced Hepatitis (NAC in TB DIH)

Information provided by Dr Karen Cohen, University of Cape Town (Responsible Party)

Last Update Posted: 2019-02-19

Study record dates

These dates track the progress of study record and summary results submissions to ClinicalTrials.gov. Study records and reported results are reviewed by the National Library of Medicine (NLM) to make sure they meet specific quality control standards before being posted on the public website.

Study Registration Dates

FIRST SUBMITTED	FIRST SUBMITTED THAT MET QC CRITERIA	FIRST POSTED (ESTIMATE)
2014-06-18	2014-07-07	2014-07-08

Study Record Updates

LAST UPDATE SUBMITTED THAT MET QC CRITERIA	LAST UPDATE POSTED	LAST VERIFIED
2019-02-18	2019-02-19	2019-02

Study Tab

Study Overview

Brief Summary:

We will conduct a randomized placebo controlled trial to determine whether administration of intravenous (IV) NAC to participants with TB DIH, in dosages similar to that used in paracetamol poisoning, can improve recovery from hepatotoxicity.

Detailed Description:

South Africa has a huge tuberculosis (TB) disease burden, with 948 per 100 000 people diagnosed with TB in 2008. TB drug induced hepatitis (DIH) is a common adverse effect of TB therapy that causes significant patient morbidity and prolonged hospital stays. N-Acetylcysteine (NAC) has been extensively studied and used for many years in the treatment of paracetamol-induced hepatotoxicity, with good evidence of efficacy and safety. NAC has also been used in other forms of liver injury and drug toxicity. It has not previously been used in the management of TB DIH.

We will screen all patients with clinical hepatitis on TB treatment admitted to New Somerset and Groote Schuur hospitals and aim to recruit 100 participants over 3 years. We will randomise 50 participants to receive an IV loading dose of 150mg/kg of NAC over 60 minutes followed by 50mg/kg IV over 4 hours by continuous infusion and finally 100mg/kg IV over 16 hours. Fifty participants will be randomised to receive placebo. The primary outcome will be time to normalisation of liver function (ALT<100). We will also determine the effect of NAC on duration of hospitalization, rate of recovery from liver failure, all cause mortality, and describe adverse effects of IV NAC in this patient population.

OFFICIAL TITLE

A Randomised Controlled Trial of Intravenous N-acetylcysteine in the Management of Antituberculous Drug-induced Hepatitis

CONDITIONS	STUDY TYPE	ENROLLMENT (ACTUAL)
Drug-Induced Liver Injury	Interventional	102

INTERVENTION / TREATMENT	PHASE	OTHER STUDY ID NUMBERS
Drug: IV N-acetylcysteine (NAC)	Phase 2	20130808
Drug: Water	Phase 3	DOH-27-0414-4719. (Registry Identifier) (Registry Identifier: SANCTR)

STUDY START (ACTUAL)	PRIMARY COMPLETION (ACTUAL)	STUDY COMPLETION (ACTUAL)
2014-05	2019-02	2019-02

Resource links provided by the National Library of Medicine NIH NLM

MedlinePlus (https://medlineplus.gov/) related topics: Hepatitis (https://medlineplus.gov/hepatitis.html) Medicines (https://medlineplus.gov/medicines.html)

Drug Information (https://druginfo.nlm.nih.gov/drugportal) available for: Acetylcysteine (https://druginfo.nlm.nih.gov/drugportal/name/Acetylcysteine)

Other U.S. FDA Resources (https://clinicaltrials.gov/ct2/info/fdalinks)

Contacts and Locations

This section provides the contact details for those conducting the study, and information on where this study is being conducted.

South Africa

Western Province Locations

Cape Town, Western Province, South Africa, 7925
Groote Schuur Hospital

Cape Town, Western Province, South Africa, 8005
New Somerset Hospital

Participation Criteria

Researchers look for people who fit a certain description, called eligibility criteria. Some examples of these criteria are a person's general health condition or prior treatments.

For general information about clinical research, read Learn About Studies (https://beta.clinicaltrials.gov/about-studies).

Eligibility Criteria

AGES ELIGIBLE FOR STUDY	ACCEPTS HEALTHY VOLUNTEERS	SEXES ELIGIBLE FOR STUDY
18 Years and older (Adult, Older Adult)	No	All

DESCRIPTION

Inclusion Criteria:

- Adults > 18 years old
- Diagnosed with pulmonary or extrapulmonary tuberculosis based on symptoms, radiological features and/or laboratory evidence.
- On first line antituberculous therapy
- Diagnosed with TB DIH

Exclusion Criteria:

- Patients with a diagnosis of acute viral hepatitis based on a positive anti-HAV, IgM, anti- HBcIgM, or confirmed hepatitis C infection
- Patients known to be asthmatic

Study Plan

This section provides details of the study plan, including how the study is designed and what the study is measuring.

How is the study designed?

DESIGN DETAILS

Primary Purpose: Treatment
Allocation: Randomized
Interventional Model: Single Group Assignment
Masking: Quadruple

NUMBER OF ARMS

2

ARMS AND INTERVENTIONS

Participant Group/Arm	Intervention/Treatment
Experimental: IV NAC Participants will receive IV N-acetylcysteine or placebo. The dosing regimen is based on the regimens used in paracetamol poisoning . Initial dose: 150 mg/kg body mass of N-acetylcysteine/WFI infused in 200 mL of 5% dextrose intravenously over 60 minutes, followed by continuous infusion: 50 mg/kg body mass in 500 mL of 5% dextrose over next 4 hours, followed by 100 mg/kg body mass in 1 litre of 5% dextrose over 16 hours.	Drug: IV N-acetylcysteine (NAC) Other Name: • Paradote
Placebo Comparator:	Drug: Water

Placebo Water	

What is the study measuring?

PRIMARY OUTCOME MEASURES

Outcome Measure	Measure Description	Time Frame
ALT normalisation	To determine the effect of IV NAC on the time to normalization of liver function in patients with TB DIH	up to 8 weeks

SECONDARY OUTCOME MEASURES

Outcome Measure	Measure Description	Time Frame
Duration of hospitalization	To determine the effect of IV NAC on duration of hospitalization	up to 8 weeks
Recovery from liver failure	To determine the effect of IV NAC on the rate of recovery from liver failure	up to 8 weeks
All-cause mortality	To determine the effect of IV NAC on all-cause mortality in patients with TB DIH	up to 8 weeks
Adverse Events	To determine the adverse event profile of IV NAC when administered to patients with TB DIH	up to 8 weeks
TB Drug Rechallenge	To determine the effect of IV NAC on success of TB drug rechallenge.	up to 8 weeks
Rechallenge duration	To determine the effect of IV NAC on duration of rechallenge	up to 8 weeks

OTHER OUTCOME MEASURES

Outcome Measure	Measure Description	Time Frame
Biomarkers	To store blood, urine and biopsy specimens (if biopsies were taken as part of patient management),bank serum, to enable us to conduct future sub studies exploring mechanisms, predictors and biomarkers of TB DIH, genetic associations with TB DIH and improved diagnostic strategies	

Collaborators and Investigators

This is where you will find people and organizations involved with this study.

SPONSOR

University of Cape Town

COLLABORATORS	INVESTIGATORS
Medical Research Council, South Africa	Principal Investigator: Karen Cohen, University of Cape Town

Publications

The person responsible for entering information about the study voluntarily provides these publications. These may be about anything related to the study.

GENERAL PUBLICATIONS

No publications available

*** Find Publications about Study Results and related Pubmed Publications in the "Results" section of the study record.**

More Information

Terms related to this study

KEYWORDS PROVIDED BY DR KAREN COHEN, UNIVERSITY OF CAPE TOWN

Drug Induced Hepatitis (DIH)
Toxic Liver
Drug Induced Liver Injury (DILI)

ADDITIONAL RELEVANT MeSH TERMS

Liver Diseases
Digestive System Diseases
Drug-Related Side Effects and Adverse Reactions
Chemically-Induced Disorders
Poisoning
Hepatitis
Chemical and Drug Induced Liver Injury
Antiviral Agents
Anti-Infective Agents
Expectorants
Respiratory System Agents
Free Radical Scavengers
Antioxidants
Molecular Mechanisms of Pharmacological Action
Protective Agents
Physiological Effects of Drugs
Antidotes
Acetylcysteine
N-monoacetylcystine

Drug and device information, study documents, and helpful links

STUDIES A U.S. FDA-REGULATED DRUG PRODUCT

No

STUDIES A U.S. FDA-REGULATED DEVICE PRODUCT

No

STUDY DOCUMENTS

No study documents available