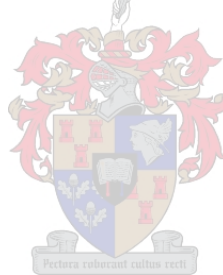# Prevention and treatment of perinatal and infant tuberculosis in the HIV era

by
Adrie Bekker

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Paediatrics and Child Health,
Faculty of Medicine and Health Sciences
at Stellenbosch University

Supervisor: Professor Anneke Catharina Hesseling
Co-supervisor: Professor Hendrik Simon Schaaf

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DECLARATION

I, the undersigned, hereby declare that the entirety of the work contained in this dissertation is my own original work, and that I have not previously in its entirety or in part submitted it for obtaining any university qualification.

Signature: Date: December 2016

DEDICATION

I dedicate this thesis to my father, Rev. Daniel Pieter Bekker, who has supported me in every important life decision. His humilty, wisdom, compassion and respect for others will always stay with me.

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SUMMARY

Infants (<12 months) born to women with tuberculosis are at high risk of *Mycobacterium tuberculosis (M. tuberculosis)* exposure, infection and disease early in life. In settings with high prevalence of human immunodeficiency virus (HIV) and tuberculosis, tuberculosis disproportionately affects women of childbearing age. The aim of this dissertation was to comprehensively investigate prevention and treatment strategies for perinatal and infant tuberculosis in a high HIV-prevalence setting. Research objectives included: 1) defining clinical and epidemiological aspects of maternal-infant tuberculosis at a large referral hospital; 2) identifying barriers and solutions to isoniazid preventive therapy (IPT) delivery in tuberculosis-exposed newborns; and 3) obtaining rigorous pharmacokinetic data to guide the dosing of first-line antituberculosis drugs in newborns and infants for the prevention and treatment of tuberculosis.

In the first retrospective study, 70 newborns (42 HIV-exposed) were investigated for tuberculosis at Tygerberg Hospital, a large provincial referral hospital in Cape Town. Newborns were mainly screened for tuberculosis because of maternal tuberculosis. Isoniazid preventive therapy (IPT) was initiated in 36/50 (72%) newborns, because of maternal tuberculosis infectious risk and exposure of infants. Few of the newborns who received IPT were traceable at one-year, and of those traced, less than half completed IPT.

To generate more rigorous clinical and epidemiological data on maternal-infant tuberculosis, a prospective cohort study was conducted in pregnant and postpartum women receiving tuberculosis treatment at Tygerberg Hospital. Over a one-year period, 74 pregnant and postpartum women, 53 (72%) HIV-infected, were consecutively enrolled. Nearly half of the women, 35 (47%) were diagnosed with tuberculosis only at delivery or postpartum, and a third of women with tuberculosis reported prior tuberculosis treatment. Tuberculosis-exposed newborns were often premature and of low birth weight (LBW; <2500 grams). All deaths occurred in HIV-infected women (n=5) and all stillbirths (n=4) and newborn deaths (n=6) were from HIV-infected women. Favourable maternal tuberculosis treatment outcomes (cure and tuberculosis treatment completion) were documented only in 41/74 (55%) women,

while 33 (45%) had unfavourable treatment outcomes (death, treatment failure and loss to follow-up). These poor observed outcomes highlight the need for earlier diagnosis and treatment of tuberculosis during pregnancy, and close follow-up to ensure maternal tuberculosis treatment completion. Improved care for pregnant women with tuberculosis, with and without HIV infection, will likely reduce morbidity and mortality in mothers and tuberculosis-exposed newborns. Delayed maternal tuberculosis diagnosis led to IPT initiation in a large number of newborns. Forty-four newborns on IPT were followed to 6 months. A hospital-based tuberculosis linkage to care intervention, led to 29/44 (66%) newborns completing IPT without a study team intervention. A further 8 infants completed IPT after study-team intervention. Appropriate tuberculosis referral and linkage to care from hospital to local tuberculosis clinic substantially improved IPT completion among tuberculosis-exposed newborns.

More pharmacokinetic data regarding the appropriate use of antituberculosis drugs are required in neonates and infants, who undergo considerable physiological changes in the first year of life. An intensive isoniazid (INH) pharmacokinetic study was therefore designed and implemented in premature and LBW infants (n=20). Relatively high median INH peak concentrations of 5.63 μg/ml were achieved in LBW infants (at an INH dose of 10 mg/kg), compared to the adult proposed target value of > 3 μg/ml. INH exposures were higher with longer half-lives in smaller infants, and among genotypically determined *N-acetyltransferase-2 (NAT2)* slow acetylators, suggesting reduced clearance of INH. This first study of isoniazid use in LBW and premature neonates showed that the INH dose in premature and LBW infants should probably not exceed 10 mg/kg/day.

The final study evaluated whether the revised higher 2009 World Health Organization (WHO)-recommended paediatric doses for rifampicin (RMP), INH, pyrazinamide (PZA) and ethambutol (EMB) achieved adequate drug concentrations in infants, compared to current adult pharmacokinetic target concentrations. All 39 infants enrolled achieved the minimum proposed adult target peak concentrations of > 3 μg/ml for INH at a mean dose of 12.8 mg/kg (10.3 - 15.4 mg/kg), and the minimum adult target of > 20 μg/ml for PZA at a mean dose of 33.3 mg/kg (28.5 – 38.5 mg/kg). RMP administered at mean dose of 15.4 mg/kg (10.1 - 20.5 mg/kg) resulted

in very low RMP peak concentrations for both RMP formulations used during the study. None of the infants achieved the minimum proposed adult RMP target concentration of > 8 μg/ml. Given the findings of this study, higher doses of RMP in infants should be considered especially given emerging data from adult RMP dose-escalation studies showing better efficacy at higher doses with limited toxicity for short-term use. For EMB, only 1 of 16 infants achieved the recommended adult target concentration of > 2 μg/ml when given at a mean dose of 20.2 mg/kg (15.4-24.1 mg/kg). EMB dose-dependent ocular toxicity however poses a concern regarding the recommendation of higher EMB doses in infants where vision testing is challenging. This is the largest pharmacokinetic study of first-line antituberculosis drugs performed in infants to date, which has generated valuable pharmacokinetic data to inform the effective and safe dosing of first-line antituberculosis drugs in infants.

Pregnant women in settings with a high burden of tuberculosis and HIV and their infants face a considerable burden of tuberculosis disease in HIV-endemic settings. Maternal-infant tuberculosis care can be improved by health systems strengthening interventions. Data generated from pharmacokinetic studies of antituberculosis drugs in tuberculosis-exposed infants will inform much needed dosing guidelines of first-line antituberculosis drugs for newborns and infants, who have a high risk of tuberculosis and are prone to develop severe forms of tuberculosis.

OPSOMMING

Babas (<12 maande) van vroue met tuberkulose het 'n hoë risiko vir *Mikobacterium tuberculosis (M. tuberculosis)* blootstelling na geboorte, wat kan lei tot infeksie en die ontwikkeling van tuberkulose siekte. In gebiede met 'n hoë voorkoms van menslike immuungebrek virus (MIV) word vroue van kinderdraende ouderdom tot 'n groot mate aangetas deur tuberkulose. Die doel van hierdie verhandeling was om die voorkoming en behandelingstrategieë vir tuberkulose in die perinatale en baba tydperk omvattend te ondersoek binne die konteks van 'n omgewing met 'n hoë voorkoms van MIV. Navorsingsdoelwitte het die volgende ingesluit: 1) die definïering van kliniese en epidemiologiese aspekte van tuberkulose in moeders en babas by 'n groot verwysingshospitaal, 2) die identifisering van struikelblokke en oplossings vir die gebruik van isoniasied voorkomende behandeling in babas met blootstelling aan *M. tuberculosis*; en 3) die verkryging van betroubare farmakokinetiese data wat doseringsriglyne kan verskaf vir eerste-linie antituberkulose middels vir die voorkoming en behandeling van tuberkulose in pasgeborenes en babas.

In die eerste studie is 70 pasgeborenes (waarvan 42 blootgestel was aan MIV) retrospektiewelik ondersoek vir tuberkulose by Tygerberg Hospitaal. Tygerberg Hospitaal is 'n groot provinsiale verwysingshospitaal in Kaapstad. Pasgeborenes was hoofsaaklik vir tuberkulose ondersoek as gevolg van moederlike tuberkulose. Vanweë potensiële tuberkulose blootstelling aan pasgeborenes en die risiko dat tuberkulose moeders nog aansteeklik was, is isoniasied voorkomende behandeling gegee in 36/50 (72%) pasgeborenes. Min van die pasgeborenes wat isoniasied voorkomende behandeling ontvang het kon opgespoor word na een jaar, en minder as die helfte van die babas wat opgespoor is, het isoniasied voorkomende behandeling voltooi.

'n Prospektiewe kohortstudie is onderneem in swanger en postpartum vroue op behandeling vir tuberkulose by Tygerberg Hospitaal. Die doel van hierdie studie was om meer omvattende kliniese en epidemiologiese inligting te versamel in moeders met tuberkulose en hulle babas. Gedurende die verloop van een jaar is 74 swanger en postpartum vroue, 53 (72%) met MIV-infeksie, ingesluit in die studie. Ongeveer die

helfte van die vroue, 35 (47%) was eers gediagnoseer met tuberkulose tydens verlossing of in die postpartum periode. 'n Derde van vroue met tuberkulose het 'n geskiedenis gehad van vorige tuberkulose behandeling. Tuberkulose-blootgestelde pasgeborenes was dikwels prematuur en/of gebore met 'n lae geboorte gewig (LGG; <2500 gram). Alle moederlike sterftegevalle het voorgekom in moeders met MIV-infeksie (n=5) en alle stilgeboortes (n=4) en babasterftes (n=6) was in babas van moeders met MIV-infeksie. Gunstige uitkomste van moederlike tuberkulose behandeling (genesing en voltooiïng van TB behandeling) was gedokumenteer in slegs 41/74 (55%) vroue, terwyl 33 (45%) ongunstige behandelingsuitkomste gehad het (sterfte, onsuksesvolle behandeling en verlore tydens opvolg). Hierdie ongunstige uitkomste beklemtoon die behoefte aan 'n vroeër diagnose en behandeling van tuberkulose tydens swangerskap, asook noukeurige opvolg gedurende tuberkulose behandeling ten einde voltooiïng te verseker. Verbeterde sorg vir swanger vroue met tuberkulose, ongeag van MIV-infeksie, behoort die morbiditeit en mortaliteit in moeders en hulle tuberkulose-blootgestelde pasgeborenes te verminder. Die laat diagnose van moederlike tuberkulose tydens swangerskap het daartoe aanleiding gegee dat 'n groot aantal pasgeborenes isoniasied voorkomende behandeling benodig het. Vier-en-veertig pasgeborens wat isoniasied voorkomende behandeling ontvang het, is vir 'n tydperk van 6 maande opgevolg. 'n Hospitaal-gebaseerde strategie wat die koppeling van hospitaal na plaaslike TB klinieke ingesluit het, het aanleiding gegee tot die voltooiïng van isoniasied voorkomende behandeling in 29/44 (66%) pasgeborenes. Hierdie voltooiïng van isoniasied voorkomende behandeling is bewerkstellig sonder die ingryping van die studiespan. Na ingryping deur die studiespan het 'n verdere 8 pasgeborenes isoniasied voorkomende behandeling voltooi. Toepaslike tuberkulose verwysing, wat die koppeling vanaf hospitaal na plaaslike TB klinieke verbeter het, het 'n beduidende bydrae gelewer tot die voltooiïng van isoniasied voorkomende behandeling in pasgeborenes blootgestel aan *M. tuberculosis.*

Verdere farmakokinetiese inligting word benodig vir effektiewe antituberkulose behandeling in pasgeborenes en babas wat aansienlike fisiologiese veranderinge in die eerste lewensjaar ondergaan. 'n Intensiewe isoniasied (INH) farmakokinetiese studie is beplan en uitgevoer in premature en LGG babas (n=20). LGG babas wat 'n dosis van 10 mg/kg INH ontvang het, het relatiewe hoë mediane INH piek konsentrasies

van 5.63 μg/ml bereik, in vergelyking met die die voorgestelde teiken waarde vir volwassenes van > 3 μg/ml. INH konsentrasies was hoër met 'n langer half-leeftyd in kleiner babas, asook in genotipies-vasgestelde *N-asetieltransferase-2 (NAT-2)* stadige asetileerders, wat daarop aandui dat daar verminderde INH opruiming was. Hierdie eerste studie van isoniasied in LGG en premature pasgeborenes het aangedui dat die dosering van INH na alle waarskynlikheid nie 10 mg/kg/dag behoort te oorskry nie.

Die finale studie het die gewysigde hoër 2009 Wêreld Gesondheidsheidsorganisasie (WGO)-aanbevole pediatriese doserings vir rifampisien (RMP), INH, pirasinamied (PZA) en etambutol (EMB) ondersoek, om te bevestig of voldoende geneesmiddelkonsentrasies in babas bereik word, in vergelyking met die huidige teikenkonsentrasies in volwassenes. Al 39 babas op die studie het die minimum voorgestelde volwasse teikenkonsentrasies van > 3 μg/ml vir INH bereik met die toediening van 'n gemiddelde dosering van 12.8 mg/kg (10.3 - 15.4 mg/kg), en die minimum volwasse teikenkonsentrasie van > 20 μg/ml vir PZA met die toediening van 'n gemidddelde dosering van 33.3 mg/kg (28.5 - 38.5 mg/kg). Rifampisien was toegedien teen 'n gemiddelde dosering van 15.4 mg/kg (10.1 - 20.5 mg/kg) en het baie lae RMP-konsentrasies tot gevolg gehad vir beide RMP formulerings wat in die studie gebruik is. Die voorgestelde volwasse RMP teikenkonsentrasie van > 8μg/ml is nie waargeneem in enige van die babas nie. Gegewe die bevindinge van hierdie studie behoort hoër doserings van RMP in babas oorweeg te word. Hoër RMP doserings is veral noodsaaklik in die lig van onlangse studies in volwassenes waar dit meer doeltreffend blyk te wees, en ook 'n beperkte toksisiteit getoon het met korttermyn toediening. Vir EMB het slegs 1 uit 16 babas die vereiste aanbeveelde volwasse teiken konsentrasie van > 2 μg/ml bereik, met 'n gemiddelde EMB toediening van 20.2 mg/kg (15.4 - 24.1 mg/kg). 'n Dosis-afhanklike risiko vir oog-toksisiteit met EMB is rede tot kommer ingevolge die gebruik van hoër EMB dosisse in babas, aangesien die bepaling van sig moeilik is in babas. Hierdie is die grootste farmakokinetiese studie van eerste-linie antituberkulose geneesmiddels wat al in babas uitgevoer is en het waardevolle farmakokinetiese inligting verskaf wat bydra tot die effektiewe en veilige doserings van hierdie geneesmiddels in babas.

Swanger vrouens, asook hulle babas, is baie vatbaar vir blootstelling aan *M. tuberculosis* infeksie en die ontwikkeling van tuberkulose in gebiede met 'n hoë

voorkoms van tuberkulose en HIV. Die sorg van moeders en babas met tuberkulose kan verbeter word deur die versterking van bestaande gesondheidssisteem strukture. Inligting verskaf van farmakokinetika studies in eerste-linie antituberkulose geneesmiddels wat in pasgeborenes en babas met tuberkulose gedoen word, sal help om die nodige doseringsriglyne te verskaf vir babas, wat n hoë risiko het vir die ontwikkeling van tuberkulose, insluitende ernstige vorms van tuberkulose.

LIST OF ABBREVIATIONS

AFB	Acid-fast bacilli
ART	Antiretroviral therapy
AUC	Area under the time-concentration curve
C_{max}	Maximum serum concentration
cART	Combination antiretroviral therapy
CYP450	Cytochrome P450
EMB	Ethambutol
FDC	Fixed dose combination.
GA	Gestational age
HIV	Human immunodeficiency virus (type 1)
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography/ mass spectrometry
INH	Isoniazid
IPT	Isoniazid preventive therapy
IQR	Interquartile range
LBW	Low birth weight (< 2500 grams)
LPV/r	Lopinavir and ritonavir
M. tuberculosis	*Mycobacterium tuberculosis*
NAT2	N-acetyltransferase 2
NCA	Non-compartmental analysis
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non- nucleoside reverse transcriptase inhibitor
NTP	National tuberculosis programme
PK	Pharmacokinetic
PI	Protease inhibitor
PMTCT	Prevention of mother to child transmission
PZA	Pyrazinamide
RMP	Rifampicin
SD	Standard deviation
$t_{\frac{1}{2}}$	Half-life
T_{max}	Time to maximum concentration
TB	Tuberculosis
TBH	Tygerberg Hospital
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Epidemiology of tuberculosis and human immunodeficiency virus

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*) and typically affects the lungs (pulmonary TB), but can affect any other site in the body (extrapulmonary TB). Of the 2-3 billion people globally estimated to be infected with *M. tuberculosis*, only a small proportion (5-10%) will develop TB disease during their lifetime (1). TB and human immunodeficiency virus (HIV) control however remains a global challenge, especially in sub-Saharan Africa (2). HIV-infected individuals are at higher risk of developing TB disease (3). In 2014, the World Health Organization (WHO) estimated there to be 9.6 million new TB cases (3.2 million in women and 1 million in children) worldwide, of which 1.2 million (12%) were HIV-infected (1). Twenty-eight percent of the world's TB cases were located in the African region in 2014 (1). During this period, the notification rate of TB in South Africa was 834 per 100 000 population and 61% of all TB cases were HIV-infected (1).

Impact of TB/HIV on women of reproductive age

TB and HIV among pregnant women is of great concern. Both TB and HIV disproportionately affect young adults (20-39 years), including women of childbearing age (4, 5). Women of reproductive age experience TB rates 1.5-2 times higher than men of the same age group in settings with high HIV prevalence (6). As most countries do not report the pregnancy status of female TB cases, it is unclear how many women with TB are pregnant. Sugarman et al. estimated that there were 216 500 TB cases in pregnant women worldwide in 2011, of which the African region (89 400 cases; 41%) had the largest number (7). This number may be an underestimation of the true burden. The diagnosis of TB during pregnancy can be challenging, partly due to the non-specific nature of early TB symptoms in pregnancy (8) and lack of routine TB screening in pregnancy. Traditional symptom-based screening for TB does not perform well in HIV-infected pregnant women, with two recent studies reporting a sensitivity of between 28-54.5%, and a specificity of 84-90.9% in this population (9, 10). HIV-infected women however, are at dramatically

increased risk of TB with reported TB prevalence during pregnancy ranging from 1-11%, compared to the 0.06-0.53% documented in HIV-uninfected pregnant women (11). Two studies from Soweto, South Africa, indicated a high burden of TB amongst HIV-infected pregnant and postpartum women. The first, conducted amongst post-partum HIV-infected South African women prior to the roll-out of combination antiretroviral therapy (cART), reported that 11% of HIV-infected women with a positive tuberculin skin test (TST) also had undiagnosed TB disease (12). The second study, conducted within the context of the routine Prevention of Mother to Child HIV Transmission (PMTCT) programme, showed that 2.16% (2 160 per 100 000 population) of HIV-infected pregnant women had TB disease, identified through simple symptom-based TB screening (13). In the Western Cape province, South Africa, the peak TB notification rate amongst young adults (25-45 years) exceeded 1 400 per 100 000 population in 2009 (14). At this time the antenatal HIV prevalence was 16.9% in women attending public antenatal clinics (15). However, the true burden of TB disease during pregnancy, and the attributable effect of HIV infection, remains undefined.

Maternal and infant mortality related to TB/HIV

TB/HIV co-infection poses a serious threat to women and their offspring. A 2.5 to 7-fold increase in maternal mortality has been reported in TB/HIV co-infected mothers (16-18). With the emergence of the HIV epidemic in South Africa, a dramatic increase in the caseload of TB in pregnant women was observed in Durban during 1996-1998, prior to the national roll-out of cART (19). TB became a leading cause of maternal mortality during this period, with rates of up to 323.3 per 100 000 deaths reported in HIV-infected pregnant women, compared to 148.6 per 100 000 live births in HIV-uninfected pregnant women (20). Since then, antiretroviral therapy (ART) uptake in HIV-infected pregnant women in South Africa has increased to 83%, as documented by a study conducted among 9803 HIV-infected pregnant women at 580 nationally representative public health facilities in 2010 (21). However, non-pregnancy related infections (mainly deaths in HIV-infected women complicated by TB and pneumonia) were still the single most common cause of maternal mortality between 2011-2013 in South Africa, and accounted for more than 35% of all South African maternal deaths (22). Adverse pregnancy outcomes for women with TB/HIV also include an increased risk of infant mortality, i.e. deaths in children less than one

year of age. A study from Pune, India, reported an almost 4-fold increase in infant mortality in the presence of maternal HIV-associated TB. Women with incident TB and their infants had an adjusted 2.2- and 3.4-fold increased probability of death, respectively, compared to women-infant pairs without TB (23). Furthermore, between 2003 to 2005 with limited cART availability, a mortality of 24% was observed in infants less than 3 months of age with culture-confirmed TB, with or without HIV in Cape Town, South Africa (24).

Epidemiology of perinatal TB

Congenital TB, a rare disease entity, occurs when *M. tuberculosis* is transmitted to the newborn, either haematogenously (*in utero)* from the mother to the foetus, or during the birth process, through ingestion/aspiration of infected amniotic fluid. Postnatal TB, which is more common, is acquired during the first weeks of life, mainly by inhalation of airborne TB bacilli from the mother or close contact with another infectious TB source case. Because of the difficulty in clinically distinguishing between true congenital TB and postnatal transmission, it has been proposed to combine these two disease entities, using the term "perinatal TB" (25-27). In summary, perinatal TB manifests when *M. tuberculosis* is transmitted to a foetus or newborn infant early in life (27) and is extremely rare if the mother is effectively treated for TB during pregnancy (25). The burden of perinatal TB in South Africa is not well documented, however a 16% incidence of vertical TB transmission to infants was reported in a study of 82 HIV-infected and 25 non-infected pregnant mothers at a tertiary hospital in KwaZulu-Natal, South Africa (1997-1999), prior to the availability of PMTCT or cART in HIV-infected pregnant women (28). At Tygerberg Hospital (TBH) in the Western Cape province, routine surveillance data on all cases of culture-confirmed childhood TB (<13 years of age) from March 2003 - February 2009, post cART rollout for HIV-infected pregnant women, found that 72 (8%) of 905 of culture-confirmed paediatric TB cases were younger than 3 months of age at diagnosis; at least 12 (1.3%) of these infants had confirmed congenital TB (27).

TB/HIV related morbidity in neonates

The increased frequency of prematurity (gestational age <37 weeks) and low birth weight (LBW; < 2500g) amongst infants contribute to the poor observed outcome of neonates born to mothers with TB, irrespective of maternal and infant HIV co-

infection. Women with TB are twice as likely to have a premature and/or LBW infant (29). In a study from Tanzania, TB was associated with LBW amongst infants, and HIV-infected women were twice as likely to give birth to LBW infants compared to HIV-uninfected women (30). Maternal TB in HIV-infected women is also a risk factor for vertical HIV transmission. Gupta et al. have shown that infants born to HIV-infected mothers with TB had a 2.51-fold (95% CI, 1.05-6.02; p=0.04) increased adjusted odds of acquiring HIV infection compared to infants born to HIV-infected mothers without TB (31).

Impact of maternal TB/HIV on infants

Infants born to women living in high-burden TB and HIV settings are at high risk of exposure to *M. tuberculosis* and infection early in life, with a high subsequent risk of TB disease progression (Figure 1). In the absence of appropriate preventive therapy, up to 50% of infants infected with *M. tuberculosis* will develop disease, of which 30% will develop progressive pulmonary or disseminated disease (32). In the Western Cape province, South Africa, more than 10% of infants born to HIV-infected women already had reported contact with a TB source case by 3-4 months of age in a trial which investigated the efficacy of isoniazid versus placebo in HIV-exposed and infected infants (33). Furthermore, a South African population-based study found that HIV-infected infants were 24-fold more likely to be diagnosed with culture-confirmed TB than their HIV-uninfected counterparts in the absence of cART access (34). These findings not only emphasize the high risk of *M. tuberculosis* exposure and disease progression early in life in TB/HIV endemic settings, but also the importance of TB preventive and curative therapy for the infected and diseased neonate and infant.
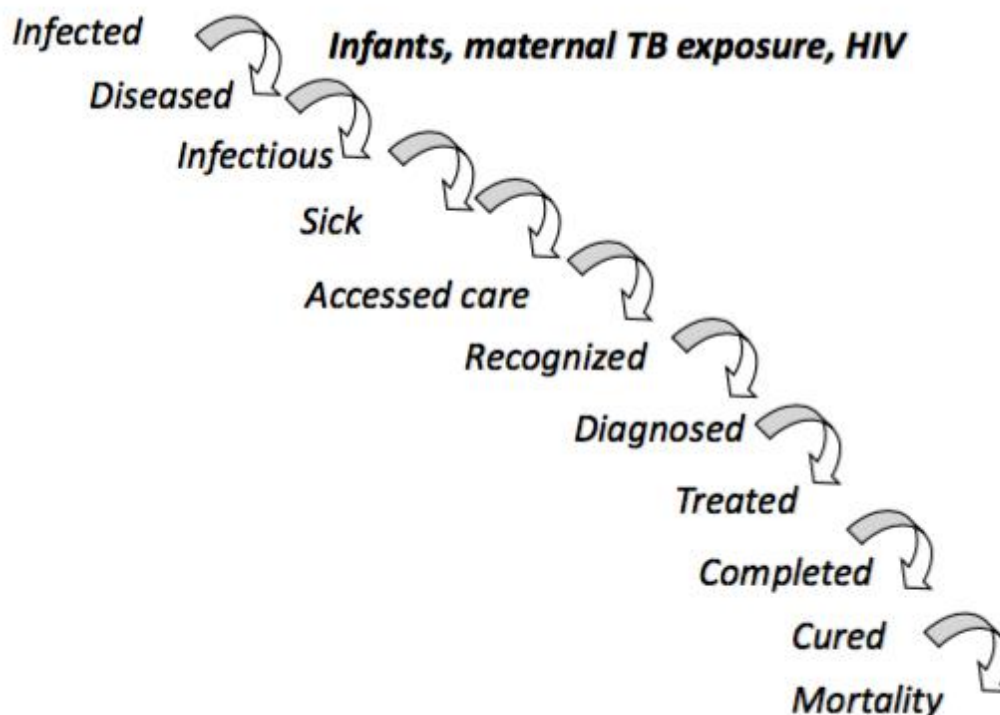
Figure 1. adapted from Don Enarson, The Union

TB prevention through chemotherapy

Post-exposure isoniazid preventive therapy (IPT) taken for 6-9 months has been shown to reduce the risk of progression to TB disease by 60-65% (35-37). Even though no benefit has been demonstrated for primary (pre-exposure) IPT against TB in HIV-exposed and infected children (38), six months of daily isoniazid (INH) remains the most widely accepted agent for post-exposure TB preventive therapy in children and is recommended by WHO (39). The delivery of post-exposure IPT in children is however, not prioritized by TB control programmes in countries with high burden of TB (40). Despite the WHO recommendation for contact investigation and treatment of *M. tuberculosis* exposure/infection in children < 5 years in countries with a high TB-burden (41), many countries including South Africa (42) face operational challenges in IPT implementation for young children (43-45). Studies in low- and middle-income settings have reported low but variable uptake of IPT among young child contacts. A clinic in Timor-Leste documented 18% IPT uptake (46), evidence from South India ranged from 19-33% in child contacts (47, 48), and in West Java, Indonesia, an IPT uptake of 50% have been reported (49). From the Western Cape,

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South Africa, figures ranging from 5 – 40 % have been reported for unsupervised daily INH monotherapy in child contacts < 5 years of age (44, 45, 50, 51). Improved delivery of IPT should be prioritized in TB-exposed infants who have a high risk of disease progression and who are prone to develop serious forms of TB disease. Early recognition of infants requiring IPT, and optimal chemotherapeutic treatment are both essential for improved outcomes. There are limited data on the pharmacokinetic characteristics and safety profile of INH and other TB drugs in neonates and infants. Although IPT is the preferred choice of preventive therapy in TB-exposed newborns, current WHO and South African National TB programme (SANTP) dosing guidelines for INH are based on limited evidence in very young children. To our knowledge, only one newborn study has been published on elimination kinetics of INH, with data from only in two newborns, reporting an increased half-life of 19 hours in one infant, suggesting a reduced elimination rate of INH (52). No studies have focused on LBW infants, who are specifically relevant in the context of maternal TB/HIV co-infection.

Therapeutic considerations of TB in neonates and infants

INH, rifampicin (RMP) and pyrazinamide (PZA) with or without ethambutol (EMB), form the mainstay of treatment of drug-susceptible TB in children, which represents more than 90% of paediatric TB cases globally. First-line antituberculosis drugs are routinely recommended in children and infants with TB disease (39, 53). To ensure optimal curative treatment in infants, a sound understanding of the pharmacokinetic properties of the currently used antituberculosis drugs is required. Evidence on the optimal paediatric dosage and chemotherapeutic treatment for efficacy and safety in this group of children however remains lacking. Efficacy studies for antituberculosis drugs were traditionally performed in healthy adult volunteers and/or adults with TB disease where treatment response could be measured and bacteriological load documented. Treatment response indicators, i.e. reduction of bacterial load in sputum and/or culture negativity, have been used in adults typically with a high bacillary load to establish "adequate" pharmacokinetic target concentrations for antituberculosis drugs. These values were then extrapolated to children, where the frequently paucibacillary nature of paediatric TB, prohibited bacteriological, and thus pharmacodynamic monitoring. Furthermore, the optimal use and efficacy of first-line antituberculosis drugs may require further investigation, with existing data suggesting greater pharmacokinetic variability with INH and RMP than previously thought, and

efficacy that may be poorer than traditionally reported (54). Higher RMP dosing strategies (35 mg/kg/day for two weeks) in adults have achieved an up to ten-fold higher average plasma exposure with a greater estimated fall in bacterial load (55). These high RMP doses were safe and well tolerated for the given study duration, and had the objective to establish the maximal tolerable RMP dose in terms of safety and tolerability (55). It remains unclear what the "adequate" pharmacokinetic antituberculosis drug target concentrations for adults and children with TB disease should be. The optimal dose required dose for infants requires further research.

Unique developmental and physiological changes may influence the absorption, metabolism and excretion of specific drugs in young children (56), making it essential to characterize the pharmacokinetic profile of antituberculosis drugs in infants. Enzyme maturation, drug-drug interaction and increased drug clearance for body weight are all important factors determining drug exposure in young children (57). Limited pharmacokinetic data in young children is available for the first-line antituberculosis drugs. Furthermore, in 2009 the WHO recommended higher doses of first-line antituberculosis drugs for children (58).

WHO antituberculosis dosing guidelines for children, 2006 versus 2009 (58)

Drugs	2006 mg/kg	2009 mg/kg	Increase
Isoniazid	5 (4-6)	10 (7-15)	↑ 100%
Rifampicin	10 (8-12)	15 (10-20)	↑ 50%
Pyrazinamide	25 (20-30)	35 (30-40)	↑ 40%

These WHO recommendations are based on evidence from pharmacokinetic studies involving mainly older children, which showed that higher mg/kg dosing was necessary to achieve target adult drug concentrations, which were thought to be correlated with cure in adults. Few pharmacokinetic studies of TB drugs at the new recommended WHO doses have been performed in children < 2 years of age (59, 60) and none specifically in infants (< 12 months of age). Of the 20 children included in the study performed by Thee et al. amongst South African young children, only two children were < 3 months of age (personal communication: S Thee). The therapeutic and safety profiles of antituberculosis drugs given at these higher doses, therefore

currently remain largely unknown in both neonates (< 28 days of age) and infants. It is essential to obtain information regarding appropriate and safe dosing of antituberculosis drugs in this vulnerable paediatric group, where an adequate scientific knowledge base is necessary to guide dosing of TB regimens, and ultimately prevent and treat perinatal and infant TB.

Purpose and scope of research

Comprehensively characterizing the clinical burden and epidemiological features of maternal and infant TB, including outcomes, will inform prevention and treatment modalities for infants in the context of maternal HIV infection. This includes targeted delivery of post-exposure IPT in infants and investigating optimal methods for the programmatic implementation of IPT. Knowledge gained from comprehensively studying the epidemiological aspects of maternal-infant TB sets the scene against which the study of pharmacokinetic properties of antituberculosis drugs in infants becomes possible and relevant. Effective and safe antituberculosis drug therapy is important in the prevention and treatment of TB in neonates and infants. Representative pharmacokinetic data on INH, and other first-line antituberculosis drugs in neonates and infants, will be generated from rigorously designed intensive pharmacokinetic sampling studies. Together, this research will contribute significantly to our scientific understanding of maternal and infant TB in the context of HIV, with the ultimate aim of improved prevention and treatment of TB in infants.

Overall objective

In my thesis I comprehensively investigate strategies to prevent and treat perinatal and infant TB in the context of maternal HIV infection.

Hypotheses

The following complementary hypotheses were proposed to investigate strategies to control maternal and infant TB in the context of HIV. These hypotheses were tested in four studies, and each one is discussed in detail in the five relevant chapters:

- *Study 1: H1*: There is a considerable burden of maternal TB and TB-exposed infants related to maternal HIV infection in a tertiary care setting in Cape Town, South Africa.

- *Study 2: H2:* The TB disease presentation and outcome in the HIV-infected mother and its consequences for her infant differs from that observed in HIV-uninfected mothers and their infants.

- *Study 2: H3:* The completion of IPT and curative TB treatment in TB-exposed neonates is dependent on health system, socio-economic and maternal determinants.

- *Study 3: H4:* The current WHO-recommended dose of INH of 10 mg/kg/day achieves INH concentrations in TB-exposed neonates comparable to those recommended for adults.

- *Study 4: H5:* The current dosing recommendations for first-line antituberculosis drugs in the paediatric population achieve drug concentrations in infants comparable to current recommended adult target values.

As part of the introductory chapters to the above studies, chapter 2.1 describes the burden, pathogenesis and clinical presentation of TB in neonates and infants. Chapter 2.2 addresses the current knowledge on pharmacokinetic considerations for the use of first-line antituberculosis drugs in infants. In chapters 3-7, each of the above studies is presented as an introduction and key findings followed by published manuscripts. Chapter 8 summarizes the conclusions and future considerations.

CHAPTER 2.1

MATERNAL AND INFANT TUBERCULOSIS: DISEASE BURDEN, PATHOGENESIS AND CLINICAL PRESENTATION

Early tuberculosis (TB) diagnosis and effective treatment are of critical importance in pregnant women with TB, irrespective of maternal HIV status. The successful management of TB disease in pregnant women can prevent transmission of *M. tuberculosis* to the foetus and newborn, and improve outcomes for this population. Women of reproductive age are at high risk of developing TB in pregnancy in high-burden TB and HIV settings (2, 28). HIV infection is also an important risk factor for progression from latent TB infection to TB disease (61). The improved availability and uptake of combination antiretroviral treatment (cART) may contribute towards reduced TB disease progression in HIV co-infected individuals (62), however the morbidity and mortality associated with both of these conditions in pregnant women and their infants remains high. A better understanding of the pathogenesis and clinical presentation of maternal-infant TB, with or without HIV infection, will assist in improved TB control for women and infants affected by TB.

In the following section, different aspects of TB during pregnancy are addressed, including risk factors for developing TB disease. I briefly describe the role of the tuberculin skin test and the commercial interferon-gamma release assays in pregnancy, and the traditional symptom-based TB screening tool during pregnancy in HIV-infected women (9). TB diagnosis during pregnancy can be improved by obtaining a directed history, thorough clinical examination, with or without shielded chest radiology, and appropriate special investigations. Unfortunately, TB diagnosis remains difficult in pregnancy and is often delayed until the postpartum period. Findings from a recent large United Kingdom cohort showed that women in the early postpartum period were twice as likely to develop TB than non-pregnant women (63). All of these factors contribute to high TB exposure in newborns from TB and HIV endemic settings, with untreated maternal TB posing a significant infectious risk to the foetus and newborn.

Transmission modes for perinatal TB, from the antepartum period through the postpartum period, are discussed. I refer to how primary- and disseminated maternal TB is more likely to be associated with congenital TB, compared to pulmonary maternal TB that is more likely associated with postnatal TB acquisition. A clinical approach to the TB-exposed newborn, which is based both on the infectious risk posed by the mother with TB, and the "wellness" of the newborn is provided. A flow diagram illustrates the appropriate TB investigations to be performed in "well and high-risk" and "unwell" TB-exposed newborns, and includes prevention and treatment guidelines, as well as a specific management follow-up plan for the TB-exposed newborn. As the symptoms and signs of early presentation of TB in infancy versus late presentation of TB in infancy often overlap, a detailed clinical and radiological overview is provided to highlight specific features of each. The last section of the chapter refers to the use of antituberculosis drugs in pregnancy and infants, and includes a section on infection control measures applicable to congregate neonatal settings, such as "kangaroo" care facilities.

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TUBERCULOSIS IN NEONATES AND INFANTS

Adrie Bekker

HIGHLIGHTS OF THIS CHAPTER

- Any unwell tuberculosis-exposed infant, regardless of the mother's infectious status, needs urgent evaluation for tuberculosis disease.
- Treatment initiation is urgent in any infant with tuberculosis. If an infant is symptomatic, start treatment as soon as appropriate specimens have been obtained for culture and determination of drug-susceptibility.
- If at all possible, an infant with tuberculosis should remain in close contact with the mother and every effort made to enable her to continue breastfeeding.
- Remain vigilant for drug toxicity in the very young infant where enzyme immaturity and varying body constitution may affect antituberculosis and antiretroviral drug exposure and toxicity.
- Undiagnosed infectious tuberculosis may occur in any health facility, but it is particularly dangerous in congregate neonatal settings such as "kangaroo" care units; continual vigilance for symptoms and signs of tuberculosis in patients and staff is essential.

TUBERCULOSIS IS a global health problem and adversely affects both pregnant women and their offspring. In 2013, the World Health Organization (WHO) estimated there were 3.3 million new cases of tuberculosis in women, resulting in 510,000 deaths, of which 180,000 (35%) were in HIV-infected women.[1] Non-obstetric infection-related deaths, including tuberculosis, now account for 28% of maternal deaths worldwide.[2]

Tuberculosis in pregnancy is associated with unfavorable perinatal and infant outcomes. Increases in pre-eclampsia and vaginal bleeding have been observed, as well as a twofold risk of delivering premature and low birthweight infants, and a sixfold increase in perinatal infant deaths.[3,4] Table 13.1 refers to definitions for maternal-infant tuberculosis used in this chapter. Tuberculosis in pregnant and post-partum women, especially if untreated, can

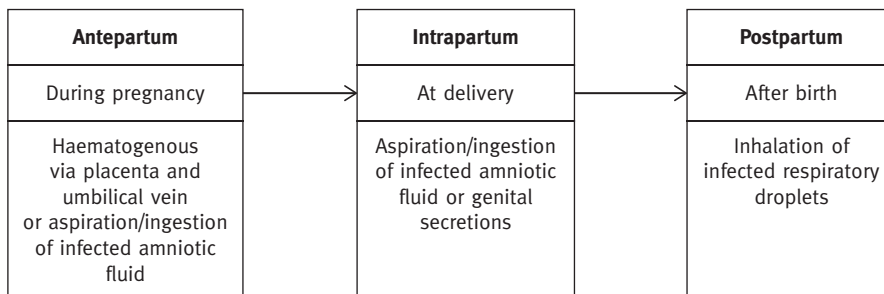| Antepartum	Intrapartum	Postpartum
During pregnancy	At delivery	After birth
Haematogenous via placenta and umbilical vein or aspiration/ingestion of infected amniotic fluid	Aspiration/ingestion of infected amniotic fluid or genital secretions	Inhalation of infected respiratory droplets

FIGURE 13.1   Transmission modes for perinatal tuberculosis.

result in transmission of *Mycobacterium tuberculosis* to the fetus and newborn (Figure 13.1). *In utero* transmission, hematogenously via the placenta or aspiration, or ingestion of infected amniotic fluid before or during birth, results in *congenital* tuberculosis; respiratory droplet spread from an infectious source case, usually the mother, after birth results in *postnatal* tuberculosis. *Perinatal tuberculosis* is the preferred term, combining the entities of congenital (ante- and intra-partum transmission) and postnatal (post-partum transmission) tuberculosis.

Tuberculosis disease progression is highest in the first year following infection, affecting particularly the very young, when immune immaturity is present. Without appropriate treatment, up to 50% of infected infants will develop tuberculosis disease, 30% of whom will have progressive pulmonary or disseminated disease.[5] In Pune, India, a fourfold increase in mortality was reported among infants with maternal HIV-associated tuberculosis,[6] while among South African infants, a 24% mortality was observed in those aged less than three months with culture-confirmed tuberculosis.[7] The outcome of isolated pulmonary tuberculosis can be good in the young, however, if treatment is initiated early.[8] Increased awareness and early diagnosis and treatment are vital to improve outcomes of tuberculosis in infants.

A high index of suspicion by health care providers for tuberculosis in pregnancy and post-partum is imperative in order to identify and treat the disease early. Appropriate assessment of the tuberculosis-exposed newborn is essential, considering the high risk of progression to disease following infection. Signs and symptoms of tuberculosis in the newborn and infant must be recognized early and acted upon rapidly. In infants, the time elapsing between infection and disease can be of shorter duration and disease presentation more acute than in older children. Optimal treatment in newborns

and infants is essential for improved outcomes. In high-burden tuberculosis/HIV settings, HIV testing should be offered to all persons with suspected tuberculosis, including mothers and infants, and combination antiretroviral therapy (ART) initiated if indicated. Integrated maternal and infant tuberculosis care strategies are key to control this disease and improve overall outcomes in these vulnerable populations.

TUBERCULOSIS IN PREGNANCY

The global burden of tuberculosis disease among pregnant women remains undefined, but 216,500 cases of tuberculosis were estimated worldwide in 2011.[9] Although relatively rare, a resurgence of tuberculosis in pregnancy has occurred as a result of the start of the HIV epidemic, the increase in drug-resistant tuberculosis, changes in socio-economic conditions, and increased migration. Data from selected populations show a prevalence of tuberculosis in pregnant women ranging from 0.06–0.53% in HIV-uninfected women to 1–11% in HIV-infected women.[10]

Potential risk factors for developing tuberculosis in pregnancy include HIV infection, prior close contact with a case of contagious tuberculosis, and a past history of tuberculosis. A high index of suspicion for tuberculosis in pregnancy is also needed for individuals emigrating from endemic regions to the developed world. Current WHO guidelines for tuberculosis infection-screening differ between tuberculosis low- and high-burden countries. In areas of low prevalence, screening, which includes a tuberculin skin test (TST) or interferon-gamma-release assay (IGRA), is recommended for high-risk individuals only, followed by treatment of infected individuals after tuberculosis disease has been excluded.[11] In high-burden settings, routine screening is not

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Table 13.1. Definitions for maternal-infant tuberculosis used in this chapter

Neonate or newborn	First 28 days of life
Prematurity	Born at less than 37 weeks' gestational age
Low birth weight	Birth weight of less than 2500 grams
Infant	Less than 12 months
Congenital tuberculosis	Newborn presenting with tuberculosis disease at birth or shortly thereafter. Tuberculosis infection took place *in utero* or during birth
Postnatal tuberculosis	Infection occurs post-partum and disease presents shortly after birth
Perinatal tuberculosis	Combined congenital and postnatal tuberculosis
Perinatal period	Period around birth (5 months before and 1 month after)
Close contact	Someone sharing an enclosed space with the index case for extended daily periods*
Tuberculosis-exposed newborn	A newborn in close contact with someone with infectious tuberculosis, normally the mother or another caregiver
Tuberculosis screening	A systematic process to establish the diagnosis or its exclusion in someone with clinical signs and symptoms suggestive of tuberculosis disease
Tuberculosis infection	No symptoms or signs of tuberculosis, but the person is infected with tubercle bacilli, following exposure
Tuberculosis disease	Illness that occurs in someone infected with *M. tuberculosis*, characterized by clinical symptoms and signs, with or without laboratory or radiological evidence*
Treatment for tuberculosis infection	Treatment offered to contacts who are at risk of developing tuberculosis disease, following exposure to an infectious person, in order to reduce that risk*
Tuberculosis treatment	A 2-month intensive phase with 3 or 4 antituberculosis drugs, followed by a 4-month continuation phase with 2 drugs

*Adapted from the WHO's *Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children.* 2nd ed. Geneva: WHO; 2014.

recommended, with the exception of HIV-infected individuals and children below 5 years of age with a known contact.[11] Divergent results have been reported for the TST, with earlier studies suggesting diminished tuberculin sensitivity in pregnancy,[12] while more recent studies showed no difference.[13,14] In an Indian study performed in pregnant women, an IGRA resulted in more positive results than the TST; of the 401 women included, 150 (37%) had a positive IGRA, compared to 59 (14%) positive TST results ($p < 0.005$) among a background prevalence of tuberculosis infection of 35–40% in the population.[15] A potential decrease in the sensitivity of both TST and the IGRAs during pregnancy is postulated to be caused by increasing levels of progesterone, favoring a Th2-type immune response, and suppressing the cell-mediated Th1 immune response, which must be intact for both the TST and the IGRA assays to function properly.[16]

Increased awareness for diagnosing tuberculosis in pregnancy is required during the ante-, intra-, and post-partum period, as symptoms are often vague and nonspecific. The recommended tuberculosis symptom-screening tool (cough, fever, night sweats, and weight loss) does not perform well in pregnancy as poor weight gain is an unreliable predictor for the disease in pregnant women. Confounding matters further is the fact that additional symptoms of

tuberculosis, like tiredness and fatigue, frequently occur with pregnancy, potentially leading to delayed diagnosis. The tuberculosis symptom-screening tool has high specificity of between 84–90.9%, but its sensitivity is very poor, ranging from 28–54.5%, as shown by two recent studies reporting on tuberculosis screening in HIV-infected pregnant women.[17,18] Despite this low reported sensitivity, the tuberculosis symptom-screening tool is currently the best one available when deciding upon further evaluation for tuberculosis in a pregnant woman. Noteworthy is the finding from a recent large United Kingdom cohort that women in the early post-partum period are twice as likely to develop tuberculosis as non-pregnant women.[19] Health care providers caring for mothers need to have a high index of suspicion for tuberculosis in the puerperal period, a period of extremely high risk for transmission of *M. tuberculosis* to the newborn. If any tuberculosis-related symptoms are present in a pregnant or post-partum woman, a thorough history, a clinical examination, with or without shielded chest radiology, and other special investigations to exclude tuberculosis disease should be conducted.

The clinical presentation of tuberculosis in pregnancy varies widely. Women can be asymptomatic, or develop typical pulmonary tuberculosis (PTB), but they may also present with more severe forms of tuberculosis, including disseminated disease. PTB is the most common form of disease, but extrapulmonary tuberculosis (EPTB) occurs in 5–10% of pregnant women with tuberculosis.[20] However, EPTB has become more frequent since the start the HIV epidemic, presenting more commonly in immune-compromised individuals. The type of perinatal tuberculosis in the fetus or newborn—congenital or postnatal—depends mainly on the type of tuberculosis in the mother (Figure 13.2). *In utero* and at-birth transmission (congenital) are more likely in pregnant women with primary tuberculosis, presenting with pleural effusion, disseminated disease (miliary tuberculosis or meningitis), or other EPTB that has a bacillemic phase; postnatal transmission is more likely in post-partum women with typical cavitating PTB.[21,22] Regardless of the mode or time of transmission, the approach to a tuberculosis-exposed newborn is the same.

Tuberculosis-Exposed Newborn

A tuberculosis-exposed newborn is a neonate who has been in direct contact with a tuberculosis source case, most often the mother, who may pose an infectious risk irrespective of the type of tuberculosis or sputum acid-fast smear or mycobacterial culture results. Previous studies have shown a 60–80% risk of transmission to infants from a close acid-fast sputum smear-positive contact, and 30–40% from an acid-fast sputum smear-negative contact.[23] More pronounced adverse perinatal outcomes have been reported when the mother has advanced pulmonary lesions and when tuberculosis is either treated late in pregnancy or incompletely treated.[24] In South Africa, a high-burden setting, the Southern African Society for Paediatric Infectious Diseases (SASPID) defined a potentially infectious mother as someone who has received less than two months of effective treatment for tuberculosis disease at the time of delivery, or whose sputum smear has not yet become negative or is unknown at the time of birth.[25]

Guidelines regarding the management of the tuberculosis-exposed newborn vary widely across different countries, with little evidence to support current practices.[26] The approach to a tuberculosis-exposed newborn depends largely on clinical circumstances and available resources. We therefore propose a strategy used within a tuberculosis high-burden setting, based on clinical experience
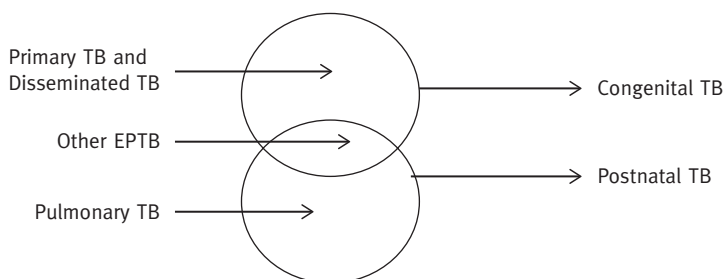

FIGURE 13.2   Types of maternal tuberculosis associated with perinatal tuberculosis.

and expert opinion, realizing that this may not suit all settings, and cases may need to be individualized. In this strategy, two issues are key to ensuring appropriate management: (1) Establish whether the newborn is well or unwell; (2) determine whether the possible source case is infectious. The risk for disease progression in a newborn may vary following infection with *M. tuberculosis* (see Figure 13.3).

An unwell newborn is defined as a tuberculosis-exposed newborn with symptoms and signs suggestive of tuberculosis disease, which may include respiratory distress, hepatosplenomegaly, or fever. Any unwell tuberculosis-exposed newborn, regardless of the mother's infectious status, needs urgent newborn care, as well as evaluation for tuberculosis disease. After stabilization of the newborn, testing should be conducted in the maternal-infant pair, including a tuberculosis symptom-screening tool given to the mother, chest radiography for the mother, and a clinical examination and tuberculosis-directed special investigations for the newborn. *If tuberculosis in the newborn is suspected or present, appropriate treatment should be initiated immediately*, as delay in treatment will worsen prognosis. Once tuberculosis has been excluded in the newborn and mother, and the underlying condition treated in the newborn, regular follow-up visits should ensue to monitor the well-being of the infant.

In the case of a well tuberculosis-exposed newborn whose mother does not have infectious tuberculosis disease or who has received more than two months of appropriate tuberculosis treatment and is responding well, a more conservative approach may be advised. In this scenario, no treatment is indicated for the infant while the child remains asymptomatic, but a BCG vaccine should be given where this is standard practice. Routine management and regular follow-up of the newborn is paramount in this setting. The infant should be evaluated for symptoms and signs of tuberculosis at each well-child visit, and promptly investigated for tuberculosis if indicated.

In the case of a mother with potentially contagious tuberculosis, the approach to a tuberculosis-exposed newborn at risk for *M. tuberculosis* infection and disease progression becomes more challenging, and the strategy in Figure 13.4 is proposed.

"Unwell" (symptoms and signs suggestive of tuberculosis) and "well and high-risk" tuberculosis-exposed newborns who are diagnosed with confirmed or probable tuberculosis disease should receive at least six months of treatment with three or four antituberculosis drugs in the intensive phase, and two drugs during the continuation phase. Other facets of care are important, including regular follow-up, weight checks, and drug-dose adjustments according to weight gain. A "well and high-risk" tuberculosis-exposed newborn who has had tuberculosis excluded, and a "well and low-risk" exposed newborn should receive six to nine months of isoniazid for possible tuberculosis infection. Monthly follow-up visits should be conducted for the duration of treatment, and isoniazid dosage adjusted according to weight gain. At each visit, screening for symptoms and signs suggestive of tuberculosis should be performed, and an infant who develops these should be evaluated for tuberculosis disease. Some guidelines recommend performing a TST toward the end of treatment—if the result is negative, a single dose of a BCG vaccine is given when it is standard practice, or where the risk of exposure to additional cases of tuberculosis is high.[27] BCG vaccine protects against the more severe types of disseminated tuberculosis, miliary and meningitis disease, but is contraindicated in HIV-infected newborns. Careful follow-up of the tuberculosis-exposed newborn should continue for a period of at least two years.

Where the health system makes use of "Road to Health" cards, it is essential that the diagnosis and steps taken for treatment and management be briefly noted.
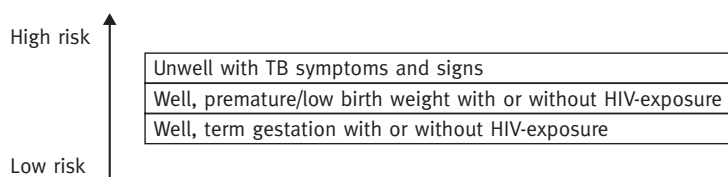

FIGURE 13.3   Risk for a tuberculosis-exposed newborn to develop tuberculosis disease.
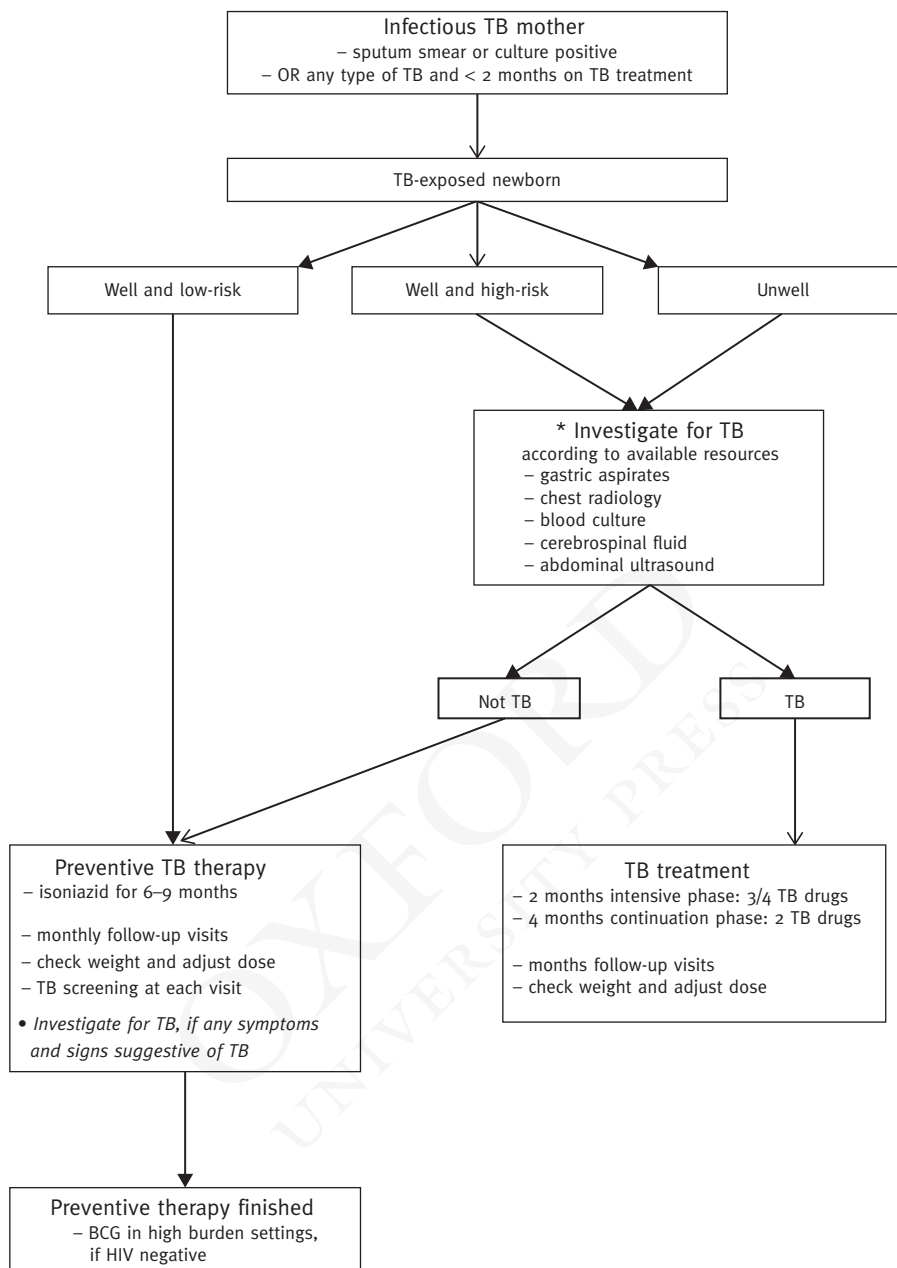
```
┌─────────────────────────────────────────────────────┐
│              Infectious TB mother                   │
│        – sputum smear or culture positive           │
│  – OR any type of TB and < 2 months on TB treatment │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
            ┌─────────────────────────────┐
            │      TB-exposed newborn     │
            └─────────────────────────────┘
        ┌─────────────┬─────────────┬─────────────┐
        ▼             ▼             ▼
┌───────────────┐ ┌───────────────┐ ┌───────────────┐
│ Well and      │ │ Well and      │ │    Unwell     │
│ low-risk      │ │ high-risk     │ │               │
└───────────────┘ └───────────────┘ └───────────────┘
```

* Investigate for TB
according to available resources
– gastric aspirates
– chest radiology
– blood culture
– cerebrospinal fluid
– abdominal ultrasound

Not TB   TB

Preventive TB therapy
– isoniazid for 6–9 months

– monthly follow-up visits
– check weight and adjust dose
– TB screening at each visit

• *Investigate for TB, if any symptoms
and signs suggestive of TB*

TB treatment
– 2 months intensive phase: 3/4 TB drugs
– 4 months continuation phase: 2 TB drugs

– months follow-up visits
– check weight and adjust dose

Preventive therapy finished
– BCG in high burden settings,
if HIV negative

FIGURE 13.4   Approach to a tuberculosis-exposed newborn from a potentially infectious mother.

PERINATAL AND INFANT TUBERCULOSIS

Tuberculosis disease in the young is not a single entity; it represents a continuum of disease, with many overlapping symptoms and signs. The term "perinatal tuberculosis" encompasses both congenital and postnatal disease (Figure 13.5), where the exact time point and mode of infection with *M. tuberculosis* is difficult to determine, and the clinical and radiological presentations overlap. Newborns can also be infected with *M. tuberculosis* later in infancy, via droplet spread from an infectious contact. "Infant tuberculosis" refers to children diagnosed in the first year of life. Early diagnosis of tuberculosis may be difficult, with neonates and infants
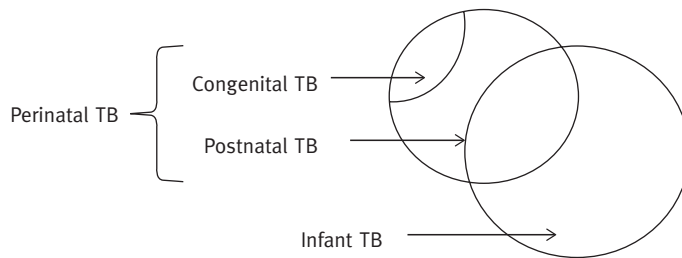
FIGURE 13.5   Tuberculosis disease terminology in neonates and infants.

often being asymptomatic, and symptoms and signs only becoming apparent in the late neonatal period and early infancy. TST and IGRA are insensitive for neonates and infants and are more often negative in these children than in older children with tuberculosis infection or disease.[28] In the following section, important differences between the earlier (mainly congenital) and later (mainly postnatal and infant) presentations of tuberculosis are highlighted.

Early Presentation of Tuberculosis in Infancy

True congenital tuberculosis is rare, with fewer than 300 cases reported in the literature prior to 1994.[22] Since the recognition of HIV infection, and with more women of childbearing age developing tuberculosis, an increase in vertical transmission of *M. tuberculosis* has been observed. In Durban, South Africa, prior to the routine use of ART in HIV-pregnant women, a 16% vertical transmission rate of *M. tuberculosis* was documented in tuberculosis-exposed newborns, from both HIV-infected and HIV-uninfected pregnant women.[29] Maternal tuberculosis in HIV-infected women is also a risk factor for increased vertical HIV transmission.[30] The revised Cantwell's criteria from 1994 define true congenital tuberculosis as disease diagnosed in any infant with a tuberculous lesion *and* one or more of the following: (i) the lesion being present in the first week of life; (ii) a primary hepatic complex or caseating hepatic granuloma; (iii) infection of the placenta or endometrial tuberculosis in the mother; or (iv) exclusion of the possibility of postnatal transmission by excluding tuberculosis in other contacts.[22]

Symptoms and signs of congenital tuberculosis may be present at birth, but they often occur in the first weeks of life, and mainly involve the lung and liver. Combined data from 75 individual congenital

tuberculosis cases demonstrated a median age of presentation at 2–3 weeks and reported the following symptoms and signs: respiratory distress (including tachypnea), hepatomegaly, splenomegaly, fever (low-grade), prematurity, and low birthweight occurred in more than 40% of cases; cough (acute or chronic), poor feeding, failure to thrive, abdominal distention (including ascites) in 25–40% cases; and irritability, peripheral lymphadenopathy, and sepsis syndrome in 10–25% of cases. Less than 10% of these cases presented with tuberculous meningitis, obstructive jaundice, skin lesions, otorrhea or mastoiditis, wheeze or stridor, apnea or cyanosis attacks, facial nerve palsy, or shock.[21] Chest radiography was available for 53 of the 75 infants, with miliary disease (30%), bronchopneumonia (32%), and lobar opacification (34%) being the most common radiological presentations.

A mycobacterial blood culture should be performed in suspected disseminated tuberculosis cases. Placental histology and culture and post-partum endometrial biopsy may be of value in confirming tuberculosis in the mother and congenital infection of the neonate. However, finding evidence of *M. tuberculosis* in the placenta does not confirm congenital infection, nor does its absence rule out infection of the neonate. Abdominal ultrasound should be performed in all suspected cases of congenital tuberculosis, as hypodense lesions may be observed in both the liver and spleen of the infant. In a review of 170 congenital tuberculosis cases from the literature, caseating hepatic granulomas were found in 15 infants subjected to liver biopsy, and a primary hepatic complex was found in two infants, probably pathognomic of congenital infection.[31]

Late Presentation of Tuberculosis in Infancy

PTB, also referred to as "intrathoracic tuberculosis" that includes hilar and mediastinal

lymphadenopathy, is present in up to 90% of infants with tuberculosis: EPTB occurs in 15–30% of cases.[8,21] In a case series of 47 infants, the most common symptoms and signs for infant tuberculosis included cough (79%), fever (64%), poor feeding (43%), localized rales or wheezing (38%), and decreased breath sounds.[8] These clinical features relate to the pathophysiology of PTB disease in the infant, which involves marked hilar and mediastinal lymphadenopathy. Enlarged glands can easily compress the airways of infants, which are of small caliber. Partial compression of the large airways may present with stridor, whereas compression of the smaller airways leads to signs of air trapping, including wheezing and focal decreased breath sounds. Progressive obstruction may cause a "ball-valve" effect, which can lead to hyperinflation of parts of the lungs or even an entire lung. Complete obstruction can result in collapse of certain sections of the lung. Enlarged lymph nodes are also known to erode into the bronchus, spreading caseous material to the lungs, which in turn may create segmental parenchymal lesions. A parenchymal lesion in the lung can also enlarge and cause widespread opacification in a segment or lobe of the lung, presenting with symptoms and signs similar to pneumonia. In the case series of 47 infants, 44 had parenchymal disease, and one infant had hilar adenopathy only. The majority of infants (86%) had radiologically confirmed segmental lesions associated with hilar adenopathy.[8] A similar report showed radiological evidence of air-trapping (56%), lobar or segmental opacification (52%), lymphadenopathy (52%), and large airway compression (48%) in 27 younger infants with PTB.[32] Pleural effusions do not occur commonly in infants,[21] although slight thickening of the pleura near a primary focus is not uncommon.

Gastric aspirates for acid-fast smear, GeneXpert MTB/RIF, and culture are routinely recommended when investigating young infants for tuberculosis disease. Sputum induction may also be successfully conducted in older infants. Although tuberculosis disease in children is paucibacillary in nature, studies have reported that more than 70% of clinically suspected cases in infants can be confirmed by culture.[8,21,33] This higher microbiological yield may be partially explained by the often-late diagnosis in the young, as well as the infants' immature immune system, which allow for uncontrolled multiplication of the tubercle bacilli.[21] In a study performed in a tuberculosis-endemic region, peripheral lymphadenopathy, mainly cervical, occurred less frequently

in infants than older children.[34] Prior to the universal use of ART in HIV-infected infants, axillary lymphadenopathy was a frequent adverse event in HIV-infected infants receiving BCG vaccination at birth. Tuberculous meningitis is unusual in infants less than three months of age, probably because of the time needed (several weeks or months) for the spread of *M. tuberculosis* infection from the primary focus to the brain and the establishment of a "Rich focus" close to the meninges.[35]

ANTITUBERCULOSIS DRUGS IN PREGNANCY AND INFANTS

This section highlights specific drug issues and matters related to pregnancy, neonates, and infants. The focus is on first-line drugs, as limited data are available for second-line drugs within these vulnerable populations.

First-line Antituberculosis Drugs in Pregnancy

First-line antituberculosis drugs—isoniazid (INH), rifampicin (RMP), ethambutol (EMB), and pyrazinamide (PZA)—are used in the treatment of drug-susceptible tuberculosis. They are safe and widely used in pregnancy, with no evidence associating them with increased human fetal malformations.[36] Streptomycin and other injectable drugs, however, are contraindicated in pregnancy because of the potential risk for fetal ototoxicity.[37]

INH as a single drug is recommended for the treatment of tuberculosis infection, both in low-burden settings and for HIV-infected individuals in high-burden settings. Due to the potential risk of INH hepatotoxicity, health care workers are hesitant to administer this drug in pregnancy or the immediate post-partum period, and many defer treatment until after delivery of the baby. Preexisting liver disease and the use of other hepatotoxic drugs may predispose to liver injury in pregnant women; therefore, baseline transaminases should be performed prior to starting treatment with INH. Regular monitoring of transaminases should be conducted in pregnant women taking INH, and the development of symptoms that may be referrable to the liver or an increase of serum transaminases of 3–5 times more than the upper limit of normal in an asymptomatic pregnant woman should prompt immediate cessation of any potentially hepatotoxic drug, including

INH.[38,39] A large randomized international trial is underway with the aim of establishing the safety of antepartum-initiated INH versus deferred or post-partum initiated INH among HIV-infected women in tuberculosis high-burden settings. Results from this study will guide future use of INH during pregnancy and the post-partum period. Pyridoxine supplementation is indicated in most patients taking INH, including pregnant women, to prevent the development of peripheral neuropathy.

Recommended first-line tuberculosis treatment regimens for pregnant women differ between countries, with many recommending the standard adult regimen (two-month intensive phase of INH, RMP, EMB, and PZA, followed by four months of INH and RMP).[40] In the United States, PZA is currently not recommended for use in pregnancy because of lack of specific teratogenicity studies in animals, and an alternative regimen of INH, RMP, and EMB for two months, followed by seven months of INH and RMP, is recommended. RMP induces the cytochrome P450 microsomal hepatic enzymes, which play an important role in the metabolism of several drugs, and can lead to lower drug-exposures of certain concomitant drugs. Because bleeding tendencies have been observed with RMP, giving prophylactic vitamin K to the newborn of a mother being treated for tuberculosis is of paramount importance to prevent hemorrhagic disease of the newborn. All of the first-line drugs cross the placenta, and minimal amounts are excreted into breast milk. Breastfeeding is recommended and should be encouraged for mothers being treated for tuberculosis who are no longer infectious.[40]

Second-line Antituberculosis Drugs in Pregnancy

Globally, the proportion of new cases with multidrug-resistant tuberculosis (MDR-TB), resistant to INH and RMP, was 3.5% in 2013.[1] Limited data are available for the use of second-line antituberculosis drugs in pregnancy, and it is largely unknown to what extent they cross the placenta or are excreted in the breast milk. Most second-line drugs have a greater incidence of adverse effects in the mother and/or an increased risk to the fetus in pregnancy.[36] Injectable drugs can cause neurosensory hearing loss in the fetus. The safety of the fluoroquinolones in pregnancy has not been established, and there are no data in this regard for newer drugs such as linezolid. However, untreated or poorly treated tuberculosis in the pregnant woman is associated with many poor fetal outcomes, including prematurity and stillbirth. The benefits of treating tuberculosis disease in pregnancy usually outweigh the risks of the specific drugs for the fetus, but the advice of a specialist in tuberculosis should be obtained when managing drug-resistant tuberculosis in pregnant women.

Antituberculosis Drugs in Infants with Tuberculosis Disease

INH, RMP, and PZA, with or without EMB, remain the cornerstones of first-line tuberculosis drug regimens in children of all ages (Table 13.2). The recommended standard regimen for PTB and peripheral lymphadenitis in the young consists of six months of treatment; three or four drugs (INH, RMP, PZA, and EMB) for two months (intensive phase), followed by two drugs (INH and RMP) for the following four months.[27] Ethionamide (ETH), a second-line drug, is sometimes preferred to EMB in the very young because of good CNS penetration in tuberculous meningitis, and due to the difficulty in monitoring for ocular toxicity that is caused rarely by EMB in this age group. However, in a literature review of EMB in children, the drug was stopped due to fears about possible ocular toxicity in only two of 3,811 patients.[41,42] A dose of 15–20 mg/kg/day is recommended for ETH use.[43]

In 2009, the WHO recommended higher doses of all first-line drugs for children than previously

Table 13.2. Revised WHO-recommended tuberculosis drug-dosing guidelines for children[27]

FIRST-LINE ANTITUBERCULOSIS DRUGS	DAILY DOSE
INH	10 mg/kg (range 7–15 mg/kg)
RMP	15 mg/kg (range 10–20 mg/kg)
PZA	35 mg/kg (range 30–40 mg/kg)
EMB	20 mg/kg (range 15–25 mg/kg)

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recommended: INH 10 mg versus 5 mg/kg/day, RMP 15 mg versus 10 mg/kg/day, PZA 35 mg versus 25 mg/kg/day, and EMB 20 mg versus 15 mg/kg/day.[44] These recommendations were based on evidence from pharmacokinetic (PK) studies involving mainly older children, which showed that higher milligram-per-kilogram dosing is necessary to achieve target drug concentrations that correlate with efficacy in adults. The effect of these higher doses on young infants, with immature organ systems and who are more prone to drug injury, remains largely unknown. Limited PK and safety data are available for neonates and infants for first-line drugs, with none available for long-term use of second-line drugs. Fortunately, adverse reactions to first-line drugs are seen less frequently in infants and children than in adults.[45] A transient increase in transaminases may occur with the hepatotoxic drugs, but clinically significant hepatitis is rare and documented in only 0.1% of children.[46] As older children generally tolerate first-line drugs well, routine laboratory monitoring of safety data is not standard of care in the otherwise healthy older child receiving first-line drugs.

However, caution about hepatotoxicity may be advised in neonates and infants with immature organ systems, and many experts recommend biochemical monitoring in this age group. Young infants have unique developmental and physiological changes that may influence the absorption, metabolism, and excretion of specific drugs. For INH, both *N-acetyltransferase 2* (*NAT2*) genotyping and enzyme maturation determine INH serum concentration. The rate of INH elimination shows genetic polymorphism, with individuals classified as homozygous fast (FF), heterozygous fast (FS), or homozygous slow (SS) acetylators, depending on their ability to eliminate this drug. Despite genetic differences influencing the rate of elimination of INH, evidence suggests that *NAT2* expression phenotypically matures with age as enzyme maturation develops, with faster acetylators metabolizing INH more rapidly with increasing age. The exact time of enzyme maturation remains unclear, with full maturity of the enzymatic pathways responsible for INH metabolism only reached at an estimated 2–4 years of age.[47] Genetically determined fast acetylators may therefore behave like slow acetylators in the very young with reduced clearance and relatively higher serum concentrations of INH. In a study conducted in 20 low-birthweight infants receiving 10 mg/kg/day of INH (dosed at the lower end of the WHO-recommended dosing guideline), all 20 LBW

infants achieved at least adult INH target values, and some were much higher, cautioning against the use of high-dose INH in the neonate or infant.[48] Some of the infants were monitored with periodic measurement of alanine transaminases (ALT); most results were normal, but one asymptomatic infant had a three times elevated value of ALT, which normalized at six months of age with continued treatment. The high serum concentrations of INH achieved in this group require further investigation, and careful safety monitoring is imperative in the very young.

Rifampicin is a strong inducer of cytochrome CYP3A4, and since large numbers of medications are CYP3A4 substrates, RMP use leads to reduced concentrations and, in some cases, reduced effectiveness of other drugs. In HIV high-prevalence settings, potential interactions may occur with specific ART drugs, leading to decreased serum levels of these drugs in neonates and infants. The protease inhibitor lopinavir/ritonavir (LPV/r) and the non-nucleoside reverse transcriptase inhibitor nevirapine (NVP), used in prevention of mother to child transmission, have been documented to have lower serum concentrations in the presence of RMP co-administration.[49] Overlapping toxicity of antituberculosis drugs and other concomitant drugs may increase the risk of adverse events. RMP is also known for its large inter-individual variation in serum concentrations, with some evidence suggesting lower RMP exposures in the young.[50] Whether higher doses of RMP should be considered when treating disseminated tuberculosis disease (high bacillary load), HIV co-infection (drug–drug interactions), and also within a background of rising INH resistance, remains uncertain. Genotyping may influence RMP exposures, with recent data indicating that a single-nucleotide polymorphism (SNP) in *SLCO1B1* is associated with rifampicin plasma concentrations.

The treatment of neonatal and infant tuberculosis remains a challenge due to the paucity of PK and safety data on the use of antituberculosis drugs in this age group. Ongoing PK studies will inform future guidelines for the optimal dosing of first- and second-line drugs in the young.

INFECTION CONTROL

General Principles for Maternal-Infant Pairs

Mothers with tuberculosis disease and their newborns should preferably *not* be separated from

each other. The mother with undiagnosed PTB or recently started PTB treatment poses the highest infectious risk to other people, including her own baby. Transmission of *M. tuberculosis* depends the degree and duration of exposure to the infectious individual. Patients with acid-fast sputum smear-positive PTB are more likely to transmit the organism than sputum smear-negative, culture-positive patients, although both carry an infectious risk. Breastfeeding should be encouraged, especially within resource-constrained settings, where it may be essential for infant survival. The recent WHO guidelines recommend breastfeeding, irrespective of the tuberculosis status of the mother,[27] while the American Academy of Pediatrics recommends that women with tuberculosis who have been treated appropriately for two weeks or more and who are not contagious may breastfeed.[51] It is evident that the mother should start appropriate treatment as soon as the diagnosis of tuberculosis disease is made, which will decrease her infectious risk. The mother should wear a protective mask during breastfeeding, and treatment for the newborn considered, depending on the likely contagiousness of the mother. The risk of transmission through breast milk is negligible, and only small amounts of antituberculosis drugs are excreted in breast milk. Management of drug-resistant tuberculosis cases should be discussed with a specialist familiar with treating these cases.

Isolation of potentially infectious tuberculosis cases should be facilitated within a health care facility, for both mother and newborn, to offer protection to fellow patients and other health care providers. Good ventilation strategies within the isolation facility will reduce transmission risk, with negative-pressure ventilation the preferred option. PTB patients initiated on treatment can safely leave isolation once the following criteria are met: at least two consecutive sputum acid-fast smear microscopy samples negative for mycobacteria; evidence of clinical improvement; and adherence to an adequate treatment regimen for two weeks or more.[52]

Undiagnosed PTB may potentially occur in any patient or health care provider, and caution is advised, especially in resource-constrained congregate settings where mothers share facilities; for example, during "kangaroo mother care" practices (skin–skin contact and nursing of premature babies). Heyns et al.[53] reported four infants that were infected and developed tuberculosis disease following exposure to a different mother with undiagnosed tuberculosis, illustrating that nosocomial transmission of *M. tuberculosis* may occur within a kangaroo mother care unit. In tuberculosis high-burden settings, symptom screening is advised for all rooming-in mothers, and a high index of suspicion for tuberculosis is essential, including for health care providers.

In the unfortunate event of a new drug-susceptible PTB diagnosis within a neonatal care setting, immediate steps should to be taken to assist the index case and protect all exposed individuals. If babies are exposed to tuberculosis in the neonatal care setting, appropriate testing and treatment should always be carried out. The index case should be started on appropriate treatment as soon as possible, and remain home until noninfectious. Health care providers are known to be at higher risk for tuberculosis disease in high-burden settings. In a recent study from South Africa, 133 primary health care facilities were reviewed, showing an incidence ratio of acid-fast smear-positive tuberculosis in primary health care workers of more than double that of the general population.[54] In low-burden settings, asymptomatic exposed individuals may be offered TST or IGRA assays, and if results are positive and conversion happened recently, treatment of tuberculosis infection is recommended. In high-burden settings, adults exposed to the index case usually will be followed without treatment. After a detailed history and clinical examination, regular follow-up should be conducted to identify suggestive symptoms and signs for tuberculosis disease. However, it could be argued that health care providers who care for neonates and infants and have been infected recently with *M. tuberculosis* should always be offered treatment to ensure that transmission to the young patients does not occur. Treatment of tuberculosis infection is recommended for HIV-infected individuals in all settings.

Optimal management of tuberculosis-exposed maternal-infant pairs requires integration of maternal and infant health services, which relies on good communication between those caring for the pregnant mother and those responsible for the newborn.

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CHAPTER 2.2

PHARMACOLOGICAL CONSIDERATIONS OF FIRST-LINE ANTITUBERCULOSIS DRUGS IN INFANTS

Changes in body composition, organ function, and enzyme maturation early in life all affect the absorption, distribution, metabolism and elimination of drugs (56, 57). These dynamic developmental changes influencing drug disposition occur mainly in infancy, resulting in a potential need for age- and weight-dependent dose adjustments. Cognizance should be taken of developmental changes over time, especially when long-term antituberculosis drug treatment is prescribed, to optimally guide effective and safe drug therapy in neonates and infants. Adverse effects of first-line antituberculosis drugs generally occur less frequently in children than in adults (64-67). As children generally tolerate these drugs well, routine laboratory monitoring of safety parameters is not standard of care in the otherwise healthy child receiving first-line antituberculosis drugs in resource-limited settings. However, caution may be appropriate in the very young with immature organ systems, i.e. liver and kidney, which may be more prone to drug injury. Because of the reduced drug clearance in neonates and infants, blood concentrations are relatively higher, potentially leading to adverse events. To advance our understanding of first-line antituberculosis drugs, I summarized the mode of action for isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol (EMB), the currently proposed target pharmacokinetic parameters and the main safety considerations below. Additional considerations of drug disposition and developmental pharmacology, the role of *N-acetyltransferase 2 (NAT2)* genotyping in INH metabolism, formulation considerations and potential drug-drug interactions to ensure dose optimization in infants are also addressed.

The use of isoniazid, rifampicin, pyrazinamide and ethambutol in drug-susceptible tuberculosis

Isoniazid

INH is the most bactericidal antituberculosis agent in use, with a high potency and a wide therapeutic margin (68). INH is a prodrug that needs to be converted to its active

form by the bacterial enzyme KatG, a catalase peroxidase. Following activation, INH inhibits the biosynthesis of mycolic acids in the mycobacterial cell wall. INH has little protein binding, is subject to first pass metabolism and is metabolised in the liver and intestine. INH has good early bactericidal activity (69), and prevents resistance in companion drugs in the intensive phase of treatment. The most important pathway for INH metabolism is dependent on trimodal *NAT2* genotype, which influences the clearance rate of INH in the body. Faster acetylators acetylate INH more rapidly than slow acetylators, and may require higher drug doses to achieve optimal pharmacokinetic target concentrations. The time to maximum concentration (C_{max}) for INH is reached within 1-3 hours irrespective of age, and the desirable pharmacokinetic target is a 2 hour plasma concentration of between 3 and 5 µg/ml based on adult studies (70). The main adverse events for INH, both of which are rare in children, are neurologic and hepatic toxicity (71). Children are less susceptible to developing peripheral neuropathy from INH (72, 73), which can be prevented and counteracted by administering pyridoxine supplementation. The more serious adverse event, hepatotoxicity resulting in liver failure, has occasionally been reported in children receiving INH at a dose of 10 mg/kg or less (74-76). Asymptomatic transient transaminase elevation occurs more frequently and has been reported in 5-10% of children on INH, with the highest rates documented in adolescents (77-79). In a recent study of 277 children receiving IPT, 113 (41%) had elevated transaminase levels, however, INH had to be discontinued in only four children (80). Special care should be taken when prescribing INH in the young, as a recent literature review reported an increased risk of hepatotoxicity with slow acetylator status, an increased INH dose, and when administered in combination with RMP (81).

Rifampicin

RMP is a bactericidal drug, of high potency and its sterilising activity on *M. tuberculosis* is by far the most important aspect of its function (82, 83). It acts on all populations of bacilli, including dormant bacilli, allowing for a shortening of duration of treatment (from 9-12 months previously to current "short-course" 6 months therapy). RMP inhibits DNA-dependent RNA polymerase of bacteria by binding its ß-unit and thereby preventing the translation of proteins (84). RMP is mainly metabolized by the liver, and induces its own metabolism leading to a progressively shortened half-life within the first 14 days of treatment (85). All rifamycins induce

hepatic and intestinal cytochrome CYP3A4, and since large numbers of medications are CYP3A4 substrates, the use of rifamycins lead to reduced concentrations of these medications, and thus, reduced efficacy. RMP formulations are known for their considerable intra- and inter-individual variability (85-88), which may partly be explained by differences in RMP bioavailability (89, 90). In a study including 20 children, only 50% +/- 22% of a freshly prepared oral RMP suspension was absorbed compared to an intravenous dose (91). Although the cause for this variability remains unclear, the following factors may impact on the bioavailability of RMP: raw material characteristics, changes in the crystalline nature of the RMP, excipients, degradation in the gastro-intestinal tract, and inherent variability in absorption and metabolism (92). RMP is the only hydrophobic drug of the first-line antituberculosis drugs, characterized by low solubility, easily adsorbed by pharmaceutical excipients, and showing a pH-dependent solubility that affects absorption (93). All of these factors highlight the need for stringent quality assurance of antituberculosis drugs and formulations in clinical care and research.

RMP's peak concentration occurs approximately 2 hours after dosing and a serum C_{max} of $\geq$ 8 µg/ml has been proposed as a pharmacodynamic surrogate for RMP when treating *M. tuberculosis* (94). However, few adult and paediatric patients reach the recommended minimum RMP 2-hour concentration of 8 µg/ml (95, 96), with youngest children (0-2 years) at high risk of "sub-therapeutic" RMP concentrations (97-99). This proposed RMP target concentration of $\geq$ 8 µg/ml was derived from studies conducted in healthy adult volunteers and adults with TB disease (70, 96), in which pharmacokinetic and pharmacodynamic parameters may differ from children with TB disease. Dose recommendations for RMP in adults, at 8-12 mg/kg (a maximum of 600 mg/kg), which leads to an "adequate" RMP pharmacokinetic target concentration of 8 µg/ml, were largely chosen because of the high cost of RMP production at the time, and not because of maximal efficacy (100). This "adequate" RMP pharmacokinetic target concentration of 8 µg/ml is controversial, with adult studies with low RMP exposures associated with treatment failure and the emergence of drug resistance (101). A recent study from India also showed an association between a lower C_{max} of RMP in HIV-infected and uninfected children, and death and treatment failures (102); higher RMP dosing strategies are currently being investigated in adults. The PanACEA consortium performed a study on higher-dose

RMP given as part of a multidrug TB treatment regimen in drug-susceptible TB for 12 weeks in South African and Tanzanian TB patients. Higher doses of RMP showed a disproportionate plasma exposure increase with dose, (20 mg/kg resulted in a C_{max} of 21.6 µg/ml and AUC of 113 h. µg/ml, and 35 mg/kg resulted in a C_{max} of 35.2 µg/ml and AUC of 235 h. µg/ml, respectively), and higher doses were safe and well tolerated over a 12-week period. Faster culture conversion in liquid media was obtained when RMP was dosed at 35 mg/kg as compared to the control regimen of RMP at 10 mg/kg/day (medians 62 and 49 days, adjusted hazard ratio 1.78 [95%CI 1.22 - 2.58], p=0.003), suggesting an increased efficacy in the higher dosing group (55). How these RMP optimization studies translate into clinical practice and paediatric care, especially with regards to shortening of tuberculosis regimens, and achieving higher concentrations within cerebrospinal fluid with tuberculosis meningitis, still need to be determined.

RMP is generally well tolerated by both adults and children with few major adverse events documented. Rash (0.8%), fever (0.5%), and nausea and vomiting (1.5%) have been reported (103), but severe hepatotoxicity is rare. Transient elevated transaminases do occur, and in a study of adult TB patients on 600 mg of RMP, mild hepatotoxicity occurred in about half of adult patients, but no one developed severe hepatotoxicity (104). Limited adverse event data are available in children using RMP alone. One small study performed in 25 children, reported no adverse events (79), and in a larger adolescent study (n=157), 41 (26%) mild adverse events occurred, however RMP use was discontinued in only one child, due to raised liver enzymes (105). In a literature review on antituberculosis drug-induced hepatotoxicity among children receiving multi-drug therapy for TB disease, liver enzymes were abnormal in 380/3855 children (9.9%) and jaundice occurred in 75 children (0.83%) (81). It showed that pre-existing liver injury, as well as concomitant INH and/or PZA use (both of which are hepatotoxic drugs) might predispose to hepatotoxicity (81, 106). RMP hypersensitivity syndrome, an immunologic phenomenon characterized by a flu-like syndrome, and in severe cases, with hypotension, renal failure, and thrombocytopenia, occurs rarely and is almost exclusively seen with intermittent dosing of RMP (107), but has also been reported with prolonged use (103). Although RMP has shown a relatively safe drug profile in children, alanine aminotransferase monitoring is advised in neonates and infants on multiple antituberculosis hepatotoxic

drugs. Safety of RMP should be carefully monitored, especially when considering higher dosing strategies.

Pyrazinamide

PZA is of low bactericidal activity but has important sterilising action following 2-3 months of treatment, especially when combined with RMP. It acts on slow-growing bacteria and is most useful in the initial phase of antituberculosis treatment. PZA is a nicotinamide analogue, a pro-drug that is converted by pyrazinamidase to the active form pyrazinoic acid in the bacterial cytoplasm (108). Absorption is generally good and maximum serum levels are achieved 1 hour after oral ingestion (87). Children achieve C_{max} values of approximately 40 μg/ml when PZA is administered at a dose of 25 mg/kg, comparable to those of adults at similar weight-based doses (109). The half-life of 10 hours is long, making it suitable for once daily dosing. Effective PZA serum levels are not well defined, but current recommendations suggest that plasma concentrations of 20-60 μg/ml 1-2 hours post-dose, serve as a suitable pharmacokinetic target to achieve optimal effect (70). Limited evidence from a pharmacokinetic study in children < 2 years of age showed that comparable adult target concentrations were achieved at revised WHO-recommended doses (60).

Despite PZA being the most hepatotoxic of the first-line antituberculosis drugs (110, 111), hepatic failure and death attributed to PZA seldom occur (112). Evidence on dose dependent liver injury is conflicting (113), but a recent literature review suggested that PZA induced hepatotoxicity is idiosyncratic rather than dose-dependent, and supports safe dosing with recommended doses of 30-40 mg/kg (114). Elevated transaminases occur infrequently with PZA use in children, and severe hepatotoxicity is very rare, mainly reported with use of multi-drug therapy for TB disease (115-118). Asymptomatic hyperuricaemia is frequently reported in adults (119), but rarely in children (120, 121). Symptoms of gout in children were mostly mild and elevated hyperuricaemia values returned to normal post PZA treatment (115, 116, 120).

Ethambutol

EMB has a bacteriostatic function and is of low potency at the doses currently demanded by fear of toxicity (122); it does not contribute to the sterilisation of TB

lesions (123). Combining EMB with other companion antituberculosis drugs may aid in the prevention of drug resistance (122). EMB is thought to act on the mycobacterial cell wall, with slow and incomplete absorption occurs in adults and children (124) and the time to maximum serum concentration (T_{max}) is between 2-4 hours. Tissue distribution of EMB is good, with the exception of the central nervous system. EMB drug concentrations are found to be lower in children than adults when using similar body weight doses (124-127), although none of these studies were performed in children less than 1 year of age. Effective therapeutic concentrations are defined as a $C_{max} \geq 2$ µg/ml and the majority of children should obtain this desirable serum level when EMB is administered at a dose of 20 mg/kg/day (122) This dose is now recommended for use in children by the WHO (58). The excretion of this drug is predominantly renal and impairment of kidney function is a relative contraindication for EMB use. The most concerning adverse effect from EMB is the dose-dependent ocular toxicity (122). Signs of toxicity include loss of visual acuity and colour vision or reduction in visual fields (71). This is of concern in young children owing to the difficulty in assessing ocular function and particular caution is warranted. However, in a literature review performed by Donald et al., EMB dosed at 15-30 mg/kg was stopped due to concerns about possible ocular toxicity in only 2 of 3811 children (128, 129). In a recent study, 3 of 11 children < 5 years of age developed reversible visual impairment secondary to 23, 25 and 27 mg/kg/day of EMB (130). More data on EMB optic neuritis is required in children, particular in the very young.

Drug disposition and developmental pharmacology

To achieve optimal pharmacokinetic target concentrations for first-line antituberculosis drugs, good drug bioavailability and drug stability is essential, as INH, RMP, PZA and EMB are all routinely administered orally. In neonates, small gastrointestinal tract surface area influences absorption of drugs. Slow gastric emptying and low intestinal motility also contribute to slower drug absorption, and thus a longer time to achieve maximal drug concentrations (56). The stability of some drugs are influenced by pH, with an increased absorption of acid-labile drugs observed in neonates with an elevated intra-gastric pH (131). In addition, evidence suggests that the maturation of drug-metabolizing enzymes and activity of efflux transporters in young children may alter the bioavailability of drugs (56) impacting directly on pharmacokinetic target concentrations achieved. Larger total body water

and extracellular compartments in neonates and young infants all influence drug distribution. Clearance increases rapidly with age in children, and the non-linear relationship that exists between body weight and drug clearance leads to a higher dose per kilogram body weight necessary to achieve similar pharmacokinetic concentrations (132). This may differ in neonates and infants with immature organ function and clearance processes, which cautions against routine use of high dose first-line antituberculosis drugs in this age group. Very limited pharmacokinetic data of first-line antituberculosis drugs is however available in the youngest age groups where drug exposures are the most difficult to predict.

Role of *NAT2*-genotyping in the metabolism of isoniazid

Pharmacogenetic factors have been shown to predict drug exposure in children receiving INH (59, 60, 133). The rate of INH elimination shows genetic polymorphism with individuals classified as homozygous fast (FF), heterozygous fast (FS) or homozygous slow (SS) acetylators depending on their ability to eliminate this drug (134). *NAT2* genotyping and enzyme maturation in the young child may also impact on INH serum concentration in infants. Despite genetic differences influencing the rate of elimination of INH, evidence suggests that *NAT2* expression phenotypically matures with age as enzyme maturation develops, with faster acetylators metabolizing INH more rapidly with increasing age. The exact time of enzyme maturation remains unclear, with full maturity of the enzymatic pathways responsible for INH metabolism reached at an estimated 2-4 years of age (133, 135). Knowledge of the existing genotype of the individual together with the phenotypical behaviour will assist in better defining the time point where enzymes fully mature. Changes in the maturity of liver enzymes and the pharmacogenetic determinant(s) of acetylating capacity may therefore all influence the dose of drug needed to achieve adequate blood levels and antimycobacterial action. Little is known about the role of enzyme maturation on INH target drug concentrations in infants.

Impact of antituberculosis drug formulations on drug exposure

Not many antituberculosis formulations are suitable for neonates and infants. This age group cannot swallow whole tablets, and the effect on bioavailability when crushing these tablets is poorly defined. Liquid suspensions also typically have unknown bioavailability, a short shelf life and may require refrigeration, which can be

problematic and impractical to use in resource-limited settings. Antituberculosis drugs are not always palatable and pill burden and long treatment durations may be challenging in children. Paediatric dispersible fixed-dosed combination (FDC) antituberculosis drugs, adapted for the revised 2009 WHO recommended drug doses (58), were only launched in December 2015 by the TB Alliance. Only one FDC drug is available to date (Macleods Pharmaceuticals Ltd.; Mumbai, India), which is not yet licensed in South Africa. Limited pharmacokinetic studies of these new FDCs have been completed in children (136, 137), and none specifically in infants.

Use of relevant concomitant medications in infants

The impact of cART on antituberculosis drugs in infants, given as part of PMTCT, or as HIV treatment has not yet been well characterized. Lower serum antituberculosis drug concentrations have been shown in HIV-infected adults with TB (127, 138), which may be attributed to malabsorption caused by drug-drug interactions. Co-administration of ART and other drugs given either as prevention or treatment to HIV-exposed infants may be potentially harmful to infants. Potential interactions may occur with specific ART leading to decreased serum levels of these drugs in infants. The protease inhibitor (PI), lopinavir/ritonavir (LPV/r) and the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine have both been documented to have lower serum drug concentrations in the presence of RMP co-administration (139). Overlapping toxicity and adverse events encountered with use of antituberculosis drugs and ART are common, including skin rashes, gastrointestinal intolerance, hepatotoxicity and peripheral neuropathy (139). An increased risk of adverse events has been reported in a Canadian study performed in adult TB patients, with HIV-infected individuals reported to be 3.8 times more likely to develop an adverse event, compared to their HIV-uninfected counterparts (140). In adult studies, TB patients with advanced HIV infection commonly experienced peripheral neuropathy, other neurological complications, rashes and gastro-intestinal events (141).

Hepatotoxicity is an adverse effect of many antituberculosis and antiretroviral drugs, with two adult studies reporting between 6-18% severe hepatotoxicity in TB patients in the cART era (141, 142). Very limited reports of adverse events on both antituberculosis and antiretroviral drugs are available for children, and more safety

data should be generated. This is especially true for infants in high HIV prevalence settings, where ART is widely used in the context of PMTCT.

Conclusions

Rigorously designed pharmacokinetic studies are important to generate representative data on the pharmacokinetic properties and safety of first-line antituberculosis drugs in children, including infants. Non-compartmental analysis will be used in the studies described in chapters 6 and 7 due to ease of interpretation and clinical relevance. The prevention and treatment of TB in infants remains a challenge, complicated by paucity of pharmacokinetic data on the use of first-line antituberculosis drugs at the currently recommended WHO doses. Data generated by the studies in chapters 6 and 7 will establish whether comparable adult target levels can safely be achieved by using these recommended dosages for first-line antituberculosis drugs in infants. INH exposures in newborns will be evaluated in the presence of maternal HIV infection, a major consideration in settings with a high burden of TB and HIV. In addition *NAT2* genotyping will be described for all infants receiving INH. Potential interactions between antituberculosis and ART drugs are explored and safety data are systematically reported.

CHAPTER 3

TUBERCULOSIS EXPOSURE AMONG NEWBORNS IN A HIGH BURDEN TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS SETTING

Infants (<12 months) born to women with tuberculosis (TB) are at high risk of TB exposure, infection and disease. In high prevalence human immunodeficiency virus (HIV) settings, TB disproportionately affects women of childbearing age (2, 4). In Cape Town, South Africa, the peak TB notification rates exceeded 1400 per 100 000 population among young adults (age 25-45 years) in some areas in 2009 (14), with a 16.9% HIV prevalence amongst women attending public antenatal clinics (15). The burden of TB during pregnancy and related perinatal outcome has not been reported in this setting and remains largely unknown.

The primary aim of this study was to describe the indications and current practices for routine TB screening in neonates (< 28 days of life) at Tygerberg Hospital (TBH). Secondary aims included determining clinical characteristics of TB-exposed neonates and their mothers, treatment decisions in neonates, and infant outcomes prior to hospital discharge. I conducted a retrospective folder review of neonates screened for TB in 2009. Potentially eligible neonates were comprehensively identified using a three-pronged surveillance strategy: 1) laboratory data for mycobacteriological information, 2) clinical records of TB-exposed neonates, as identified by attending clinicians; and 3) pharmacy records of neonates discharged on TB medications.

Results

A key finding of this study was that a large number of neonates (n=70) were routinely screened for TB at our hospital during a one-year period. The majority of these neonates were screened for TB because of confirmed (41; 59%) or suspected (9; 13%) maternal TB. Another 5 (7%) neonates were investigated because of a household TB contact, and 15 (21%) due to severe neonatal illness compatible with TB, but without any known TB exposure. Infants born to women with TB were often premature (< 37 weeks gestational age) and of low birth weight (LBW: < 2500 grams). Of the 38 women treated for TB, of whom 25 (66%) were HIV-infected, five women had disseminated TB and a quarter of the women with TB presented with pleural effusions

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during pregnancy. Prospective data from pregnant and postpartum women with TB at the same facility are presented in chapter 4. Three women died postpartum with confirmed TB: 1 woman from pneumonia without any TB treatment being initiated, 1 woman from pneumonia and drug-resistant TB, and one woman from post-partum haemorrhage two months into TB treatment. Vertical transmission of TB was bacteriologically confirmed in 3/50 (6%) neonates born to women with confirmed or suspected TB. HIV infection status was poorly documented in HIV-exposed infants. Thirty-six (72%) neonates born to 50 women with suspected and confirmed TB were initiated on isoniazid preventive therapy (IPT). Only a third of these infants were traceable 12 months later, and only 5/12 traceable infants completed six months of IPT, as per standard recommended WHO and South African National TB programme (SANTP) guidelines (39, 42).

Limitations of this study were the use of routine retrospective hospital data and the possibility that not all neonates screened for TB may have been studied, despite using a three-pronged surveillance strategy. Notwithstanding these limitations this is the first study conducted in a TB and HIV endemic setting to investigate TB exposure and indications for TB screening in neonates. A large number of neonates were screened for TB due to suspected or confirmed maternal TB, and two-thirds of all neonates screened for TB (n=70) were born to HIV-infected women. A large proportion of neonates were started on IPT suggesting delayed TB diagnosis in pregnancy (with ongoing positive sputum status and risk of transmission of *M. tuberculosis* to the infant). TB treatment outcome data for maternal-infant pairs affected by TB, with or without HIV, were particularly limited. This study highlighted the need to perform a prospective study in pregnant women with TB and their neonates, to more comprehensively describe maternal TB and maternal and infants outcomes in a high burden setting.

By collecting better quality data on the spectrum of TB disease during pregnancy, including outcome data, a better understanding of the TB disease burden in pregnant women could be developed and strategies designed to minimise adverse outcomes for both mother and neonate. This study also highlighted major operational gaps in IPT delivery to at-risk infants exposed to TB. By identifying barriers to completion of antituberculosis treatment (preventive and curative) in TB-exposed neonates,

strategies to achieve better control of maternal-infant TB can be designed. These research gaps will be addressed in chapters 4 and 5, respectively (study 2 of this thesis), where maternal-infant TB pairs were followed prospectively.

High tuberculosis exposure among neonates in a high tuberculosis and human immunodeficiency virus burden setting

A. Bekker,* K. Du Preez,† H. S. Schaaf,*† M. F. Cotton,* A. C. Hesseling†

*Department of Paediatrics and Child Health, and †Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Science, Stellenbosch University, Cape Town, South Africa

SUMMARY

BACKGROUND: Maternal and neonatal tuberculosis (TB) are under-recognised, particularly in settings with a high burden of human immunodeficiency virus (HIV) infection.

DESIGN AND SETTING: Retrospective audit of neonates routinely screened for TB in a South African hospital during 2009. Surveillance sources reviewed included routine clinical, laboratory and pharmacy records.

RESULTS: Among 70 neonates (60% HIV-exposed) screened for TB, the median gestational age was 35.5 weeks (IQR 33–38), and the median birth weight was 2000 g (IQR 1530–2484). The neonates were grouped according to a history of documented TB exposure: maternal TB in 41/70 (59%), suspected maternal TB in 9/70 (13%), other documented household TB exposure in 5/70 (7%), and no known TB exposure 15/70 (21%). Of the 50 neonates exposed to confirmed or suspected maternal TB, 36 (72%) were initiated on TB chemoprophylaxis, 5 (10%) received TB treatment and 9 (18%) received no intervention. Eight (8/50, 16%) were diagnosed with TB, all of whom were born to mothers with suspected or proven TB.

CONCLUSIONS: Maternal TB, primarily among HIV-infected women, was the main indication for TB screening of neonates. Routine TB screening of pregnant women and TB care in mothers and infants should be improved in settings with a high burden of TB and HIV.

KEY WORDS: newborns; low birth weight; TB treatment; IPT; outcome

IN 2009, the World Health Organization (WHO) reported that South Africa had more than 20% of the global human immunodeficiency virus (HIV) co-infected tuberculosis (TB) cases,[1] with 60% of all TB cases being HIV-infected.[2] Women in their reproductive prime (age 15–29 years) are disproportionately affected by the dual TB-HIV epidemic.[3] In a study performed in post-partum HIV-infected South African women prior to the roll-out of combination antiretroviral therapy (cART), 11% of HIV-infected women with a positive tuberculin skin test had undiagnosed TB.[4]

TB and HIV are associated with increased maternal and infant mortality.[5–7] Women with TB alone are twice as likely to have premature and low birth weight (LBW, <2500 g) infants than women without TB.[8] A study from Tanzania found that TB contributed to 17% of prevalent LBW among infants, and HIV-infected women were twice as likely to give birth to LBW infants than non-HIV-infected women.[9] Maternal TB in HIV-infected women is also a risk factor for vertical HIV transmission.[10,11]

TB-HIV co-infection contributes significantly to infant mortality. A study from Pune, India, reported an almost four-fold increase in infant mortality with maternal HIV-associated TB. Women with incident TB and their infants had respectively an adjusted 2.2- and 3.4-fold increased probability of death compared to woman-infant pairs without TB.[10] Infants born to HIV-infected women with TB are at high risk of developing disease, including disseminated and complicated TB. In the absence of appropriate chemoprophylaxis, up to 50% of infants will develop TB, 30% of whom will have progressive pulmonary or disseminated disease, following exposure and infection.[12] Data regarding the presentation and outcome of TB-exposed neonates are limited, particularly in settings with a high burden of TB and HIV.

Our aim was to describe the spectrum and outcome of routine neonatal TB screening at a tertiary referral hospital in a setting with a high burden of TB and HIV. We describe indications for TB screening, sources of TB exposure, and the clinical presentation, management and outcome of mother-infant pairs.

Correspondence to: A Bekker, Division of Neonatology, Department of Pediatrics and Child Health, Faculty of Health Science, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa. Tel: (+27) 219 389 219. Fax: (+27) 219 389 138. e-mail: adrie@sun.ac.za

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METHODS

Design and setting

This was a retrospective, descriptive study of neonates routinely screened for TB at Tygerberg Hospital (TBH), a tertiary referral hospital in Cape Town, Western Cape Province, South Africa. TBH manages up to 6000 high-risk deliveries per year, serving approximately 30% of all provincial referrals. Neonatal wards have 112 beds, including 8 neonatal intensive care beds and 33 high-care beds. In this resource-limited health care setting, there were 4470 admissions to the neonatal wards in 2009; more than 20% of all neonates delivered were LBW. During the study period, no standard maternal or neonatal TB screening protocol was in place.

During 2009, the peak TB notification rate in Cape Town was among young adults (age 25–45 years), exceeding 1400 per 100 000.[13] Childhood TB (age 0–14 years) contributed 17.3% of the total provincial disease burden, with a notification rate of 620/100 000 in 2008 (unpublished data, Western Cape Department of Health). The provincial maternal HIV prevalence for this period was 16.9% among public antenatal clinic attendees.[14] Pregnant woman with a CD4 count $< 250 \times 10^6$/l qualified for cART, consisting of zidovudine (ZDV), lamivudine and nevirapine (NVP); for women with higher CD4 counts, prevention of maternal-to-child transmission (PMTCT) therapy included antenatal ZDV and a single dose of NVP at the onset of labour.

Sources and type of data

Based on the limited documentation of TB screening practice among newborns in TBH, we reviewed and included several sources of routine surveillance data to comprehensively identify neonates screened for TB. These included 1) laboratory data: all samples routinely collected from neonates (age <28 days) submitted to the routine hospital National Health Laboratory Service (NHLS) for mycobacterial investigation during the study period; 2) clinical records: all neonates with TB exposure or suspected TB disease as documented by attending clinicians in the neonatal wards; and 3) pharmacy records: the routine hospital pharmacy database was reviewed and all records of neonates discharged on any TB medications were retrieved.

Thereafter, neonatal and maternal hospital folders, chest radiographs (CXR; where available) and additional laboratory records were reviewed to collect available clinical and demographic data on mother-infant pairs. A standard data collection tool was designed to extract folder data. The following neonatal data were collected: estimated gestational age, birth weight, HIV exposure (maternal HIV infection status), clinical characteristics, including radiological investigations, TB exposure status and source and type of exposure, laboratory investigations, including mycobacterial investigation (culture, smear and drug susceptibility testing), placental investigations, treatments, including TB preventive therapy and treatment and outcome at discharge from hospital. Maternal data included age, gravidity, parity, HIV status, CD4 count and cART, CXR, TB treatment history, information regarding current TB investigations including bacteriology, and the observed spectrum of TB disease.

Case definitions

A case of neonatal TB was defined as clinical presentation compatible with disease in conjunction with isolation of *Mycobacterium tuberculosis* from gastric/tracheal aspirate, or clinically diagnosed TB; in the latter, a decision was made to treat together with infectious disease specialists, based on the presence of maternal TB with radiological or other clinical evidence in the neonate suggestive of TB in the absence of culture confirmation in the neonate.

A case of maternal TB was classified as a mother already on anti-tuberculosis treatment or started on anti-tuberculosis treatment during the labour admission, after either bacteriological confirmation or clinical findings compatible with TB (suggestive symptoms and CXR). If there was a strong clinical suspicion of TB during hospitalisation, but inadequate proof to make the diagnosis of TB, women were classified as having suspected maternal TB. Women found to have a subsequent positive *M. tuberculosis* culture were re-classified as having maternal TB following review of the available data.

Neonates received chemoprophylaxis according to standard WHO and South African National TB Programme (SANTP) guidelines.[15] All neonates in close contact with a TB case were eligible for chemoprophylaxis, consisting of daily isoniazid (INH) therapy (10 mg/kg/day) for 6 months. Anti-tuberculosis treatment in neonates comprised a 2-month intensive phase of daily INH (10 mg/kg), rifampicin (RMP; 10 mg/kg), pyrazinamide (25 mg/kg) ± ethionamide (ETH; 20 mg/kg), followed by a 4-month continuation phase of INH and RMP at the same dose. ETH was the drug of choice in cases with extensive disease (instead of ethambutol) due to the difficulty in evaluating visual acuity in neonates.

Maternal and neonatal mortality during hospitalisation was documented. In January 2011, all surviving mothers/care givers, excluding infant deaths during hospitalisation, were contacted by telephone to establish outcome. A standard questionnaire was administered to all contactable participants. Three attempts were made before participants were classified as lost to follow-up.

Approval for the study was obtained from the Health Research Ethics Committee of Stellenbosch University and TBH (N09/12/354). Waiver of informed consent was granted.

Statistical analysis

Due to the descriptive nature of the study and the limited sample size, our analysis was not hypothesis driven. This study was not designed or powered to investigate the association between HIV infection and TB exposure in neonates. Our analysis was therefore mainly descriptive. Categorical variables were reported as actual numbers and proportions, and continuous variables as medians and interquartile ranges (IQRs). We ascertained the number of neonates screened for TB, and described the clinical characteristics of mothers and neonates.

RESULTS

During the period under review, 74/4470 (2%) neonatal admissions were screened for TB, of which four were excluded due to inappropriate investigation (clinical condition not deemed to be TB-related). The Figure illustrates the three surveillance sources used to identify the study sample, the contribution of each source and the reported indications for TB screening among neonates. Only 10 patients were identified using all three surveillance strategies. The majority of the neonates were screened for TB due to a history of maternal TB (41/70, 59%) or suspected maternal TB (9/70, 13%). Of the remaining 20 neonates, 5/70 (7%) were investigated because of another household TB source case and 15/70 (21%) due to severe neonatal illness without known TB exposure. No information on maternal TB was documented for these 20 neonates, and only one of these qualified for TB prophylaxis based on local guidelines.

The cohort of 70 neonates included four sets of twins. The median gestational age was 35.5 weeks (IQR 33–38), median birth weight was 2000 g (IQR
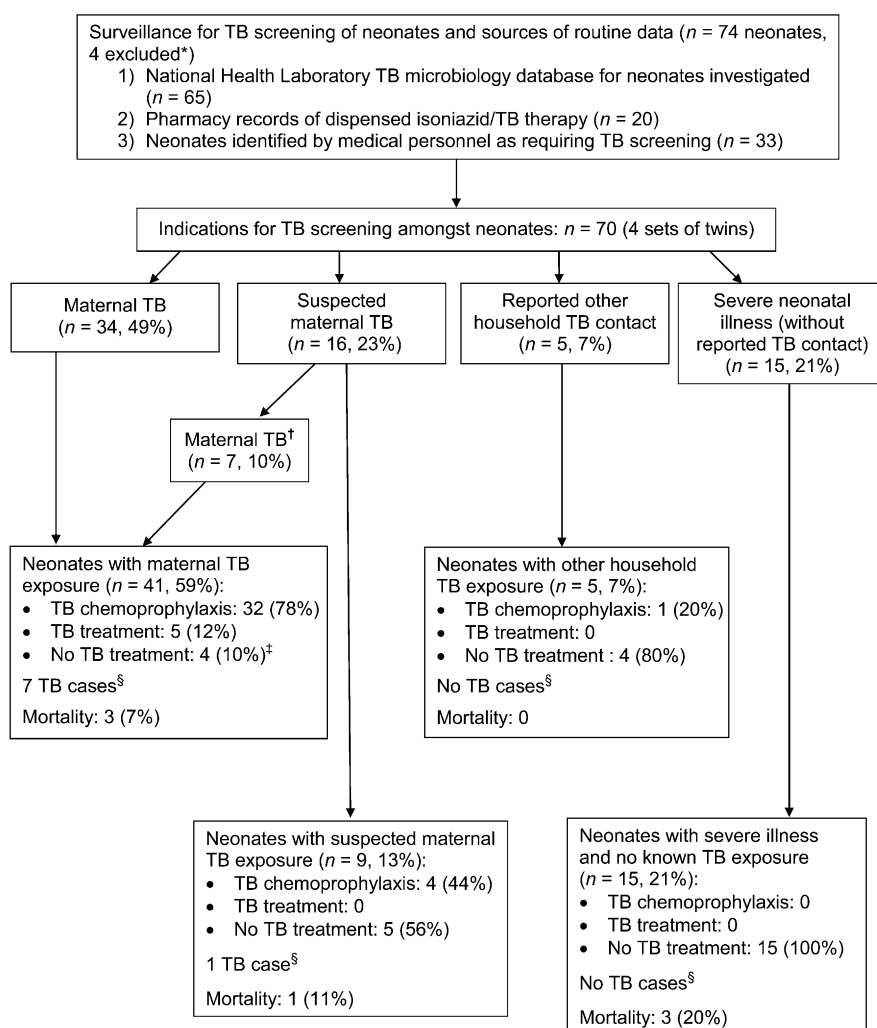

Figure   Flow diagram of TB screening and TB exposure in neonates born during 2009 at Tygerberg Hospital. *Excluded due to inappropriate investigations for TB: swabs from eyes (*n* = 3) and joints (*n* = 1). †Mother started on anti-tuberculosis treatment during delivery admission to hospital or positive *M. tuberculosis* culture that was taken during delivery admission to hospital. ‡Anti-tuberculosis treatment was not initiated in 4 neonates: 1 died due to nosocomial sepsis, 1 died due to congenital abnormalities, 1 was thought to have a low infection risk (mother in last month of anti-tuberculosis treatment), and 1 was discharged into the care of the aunt following the death of the mother. §TB case = neonate treated for TB. TB = tuberculosis.

Table 1 Clinical characteristics of neonates exposed to mothers treated for TB (*n* = 41)*

Neonates	*n* (%)
Gestation, weeks, median [IQR]	36 [33–38]
Birth weight, g, median [IQR]	1950 [1537–2283]
Male sex	16 (39)
HIV-exposed	27 (66)
Chest radiography	
Non-specific changes	24 (58)
Hyaline membrane disease	4 (10)
Transient tachypnoea of newborn	2 (5)
Pneumonia	4 (10)
Acute respiratory distress syndrome	1 (2)
Not performed	6 (15)
TB bacteriology (neonate)	
Smear-positive	0
Culture-positive	2 (5)†
Anti-tuberculosis treatment initiated	
Anti-tuberculosis treatment	5 (12)
TB prophylaxis	32 (78)
No treatment‡	4 (10)
Possible maternal TB transmission	7§
Mortality at discharge from hospital	3 (7)

*Includes three sets of twins.
†Another neonate was *M. tuberculosis*-positive, but the mother was only suspected to have TB and was not treated for TB.
‡Anti-tuberculosis treatment was not initiated in 4 neonates: 1 died from nosocomial sepsis, 1 from congenital abnormalities, 1 was thought to have a low infection risk (mother in her last month of anti-tuberculosis treatment) and 1 was discharged into the care of the aunt due to the death of the mother.
§One additional neonate with possible maternal TB transmission was identified from the group of mothers with suspected TB (eight neonates in total were considered to be neonatal TB cases).
TB = tuberculosis; IQR = interquartile range; HIV = human immunodeficiency virus.

Table 2 Clinical characteristics of mothers treated for TB (*n* = 38)*

	n (%)
Age, years, median [IQR]	27 [23–31]
HIV-infected	25 (66)
Absolute CD4 count (HIV-infected women), median [IQR]	138 [76–250]
Time of TB diagnosis	
Ante partum	21 (55)
Intra/post partum	17 (45)
Confirmed vs. probable maternal TB†	
Confirmed	22 (58)
Probable	16 (42)
CXR	
Pleural effusion	9 (24)
Cavities	2 (5)
Lymphadenopathy	2 (5)
Bronchopneumonia	3 (8)
Miliary TB	3 (8)
Non-specific changes	7 (18)
Not performed	12 (32)
TB disease classification‡	
PTB only	22 (58)
EPTB only	7 (18)
Both PTB and EPTB	9 (24)
All disseminated TB§	5 (13)
Bacteriology	
Smear-positive only¶	1 (3)
Culture-positive only	10 (26)
Smear- and culture-positive	11 (29)
Negative smear and culture	8 (21)
No evidence of bacteriological investigation	8 (21)
Mortality at discharge from hospital	3 (8)

*Includes three sets of twins.
†Confirmed maternal TB = bacteriological confirmation by smear or culture for *M. tuberculosis*; probable maternal TB = clinical diagnoses of TB and treatment initiation without available laboratory proof of bacteriological confirmation.
‡PTB = sputum AFB-positive, *M. tuberculosis* on sputum culture, started based on suggestive CXR findings; EPTB = TB involving abdominal and superficial skin lymph nodes.
§Disseminated TB in mothers: 3 miliary, 1 blood culture and 1 placenta Ziehl-Nielsen-positive.
¶Culture not performed.
TB = tuberculosis; IQR = interquartile range; HIV = human immunodeficiency virus; CXR = chest radiography; PTB = pulmonary TB; EPTB = extra-pulmonary TB; AFB = acid-fast bacilli.

1530–2484), and sex was equally distributed. The majority (60%) of the neonates were HIV-exposed, and the median CD4 count of the HIV-infected mothers was 176×10^6/l (IQR 90–328).

Data summarised in Table 1 include CXR findings, bacteriology and treatment decisions for all 41 neonates with proven maternal TB exposure. Neonates born to the 50 mothers with confirmed or suspected maternal TB yielded eight (16%) cases of neonatal TB, of which six were born to HIV-infected mothers. Of the 50 neonates exposed to confirmed or suspected maternal TB, 36 (72%) were initiated on chemoprophylaxis, 5 (10%) received anti-tuberculosis treatment and 9 (18%) received no TB chemotherapy.

Clinical characteristics of the 38 women with TB are documented in Table 2. The spectrum of maternal TB was classified as pulmonary TB, extra-pulmonary TB or both.[2] A large proportion of all women with TB (17/38, 45%) were diagnosed with TB during the intrapartum period or shortly postpartum, with three infants developing confirmed or presumed TB. A high proportion of HIV-infected women with TB had pleural effusions (8/25, 32%) or disseminated TB (5/25, 20%).

Clinical data on the cases of confirmed and presumed neonatal TB are shown in Table 3, while data on maternal and neonatal mortality are summarised in Table 4. Overall neonatal mortality during hospital admission was 7/70 (10%); maternal mortality was 3/66 (5%). All maternal deaths were associated with TB, while TB might have contributed to one neonatal death. Telephonic follow-up to establish outcome in the cohort of 70 neonates could only be obtained in 29/70 (41%) infants. Of the 2/5 traceable neonates who were initiated on anti-tuberculosis treatment, both were reported to be well and to have completed 6 months of TB treatment. Of the 13/37 (35%) traceable neonates who were started on TB chemoprophylaxis, one was subsequently started on anti-tuberculosis treatment; only 5/12 patients (42%) completed 6 months of INH.

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Table 3 Characteristics of neonates treated for confirmed or presumed TB ($n = 8$)

Birth weight g	Gestational age weeks	Sex	HIV exposed	Maternal TB type	Clinical manifestations	CXR	Bacteriological evidence of TB	Anti-TB treatment	Outcome at hospital discharge	Final outcome
1320	32	F	No	PTB (smear-positive)	Prematurity, RDS	Lobar opacification	None	Yes	Alive	Lost to follow-up
1620*	35	M	Yes, PCR-negative	Disseminated TB (miliary)	Prematurity, pneumonia	Normal	None	Yes	Alive	Lost to follow-up
1200*	35	F	Yes, PCR-negative	Disseminated TB (miliary)	Prematurity, pneumonia	Normal	None	Yes	Alive	Lost to follow-up
1700	37	M	Yes, PCR-negative	Disseminated TB (blood culture) and PTB (smear-positive)	IUGR, pneumonia	Normal	None	Yes	Alive	Completed 6 months of treatment; alive at 6 months of age
800	27	F	Yes, PCR-negative	Disseminated TB (placenta)	Prematurity, RDS	Normal	None	Yes	Alive	Completed 6 months of treatment; alive at 9 months of age
2480	36	M	Yes, PCR not performed	Pleural effusion	Congenital abnormality, Pierre Robin syndrome	Bilateral alveolar opacification	Tracheal aspirate (positive culture for *M. tuberculosis*)	No	Died	Died <24 h following birth
2141	40	F	Yes, PCR not performed	Pleural effusion (second episode of TB)	IUGR, RDS	Bilateral alveolar opacification	Gastric aspirate (positive culture for *M. tuberculosis*)	IPT†	Alive	Alive at 5 months of age (telephonic follow-up)
1091	27	F	No	Investigated twice for TB, unconfirmed TB	Prematurity, RDS	Bilateral alveolar opacification	Tracheal aspirate (positive culture for *M. tuberculosis*)	No	Died	Died: prematurity and *Acinetobacter baumanni* septicaemia

* Twins.
† IPT started initially, mother contacted at time of culture result but did not return for follow-up visit.
TB = tuberculosis; HIV = human immunodeficiency virus; CXR = chest radiography; F = female; PTB = pulmonary TB; RDS = respiratory distress syndrome; M = male; PCR = polymerase chain reaction for HIV; IUGR = intra-uterine growth restriction; IPT = isoniazid preventive therapy.

DISCUSSION

This is the first study investigating neonatal TB exposure in the Western Cape Province, South Africa, a setting with high burdens of TB and HIV. The majority of neonates in this hospital setting were routinely investigated for TB due to maternal or suspected maternal TB. In addition, two thirds of the women with TB were also HIV-infected, indicating the high burden of co-infection in this setting. Almost half of all the women treated for TB were diagnosed only during the intra- and postpartum period, illustrating the lack of appropriate routine antenatal TB screening, the importance of in-hospital screening and the potential risk of nosocomial transmission in hospital settings such as ours.

Of the 50 neonates screened for TB due to confirmed or suspected maternal TB, a high proportion (8/50, 16%) were diagnosed with TB; 3/50 (6%) had confirmed TB (assumed maternal source of infection) and 5/50 (10%) suspected transmitted TB. Those treated for presumed congenital TB (one set of twins) were judged to be at particularly high risk for TB infection, although no confirmatory bacteriological evidence was available. The mothers of all infants had confirmed TB, and three were HIV-infected. The neonates were premature, had LBW and clinical signs of pneumonia and respiratory distress syndrome. A single additional South African study from KwaZulu-Natal (1997–1999)[16] had previously reported a 16% incidence of vertical TB transmission in a setting with 30% maternal HIV prevalence prior to the availability of cART. Our data highlight the risk of congenital TB, particularly in the context of a high burden of maternal HIV.

The presentation of congenital TB may be varied and difficult to diagnose. Mortality is typically high, at 22–50%.[17,18] It is therefore imperative to maintain a high index of suspicion for TB among pregnant or postpartum women and their infants in settings with a high TB burden. TB screening during the antenatal and postnatal period is currently not adequately implemented in high-burden settings, for example in PMTCT programmes. Our results confirm that almost half of the women with TB were only diagnosed when admitted to hospital in the intra- and postpartum period. Mothers were frequently only investigated for TB when they had suggestive symptoms after hospitalisation for the delivery of their babies, by which time there was substantial risk of morbidity to both mother and infant. Furthermore, two thirds of the women with confirmed TB were also HIV-infected, with a tendency towards paucibacillary disease, indicating the need for rigorous screening using other tools in addition to sputum smear microscopy.

In our study, the infants were mostly LBW and premature, consistent with studies from India, where infants born to mothers with TB had significantly

Table 4   Data on neonatal and maternal in-hospital mortality

HIV-exposed	Maternal TB status	Bacteriological evidence of TB in neonate	Treated for TB	Age at death	Attributed cause of death
Neonatal deaths (*n* = 7/70, 10%)					
Yes (PCR not performed)	Suspected	None	No	7 days	Prematurity and *Klebsiella pneumoniae* septicaemia
Yes (PCR not performed)	Confirmed (pleural effusion)	*M. tuberculosis*-positive	No*	1 day	Congenital abnormalities (Pierre Robin syndrome)
Yes (PCR-positive)	Confirmed	None	Yes	6 months	Stage 4 HIV and septicaemia
No	Suspected	*M. tuberculosis*-positive	No*	16 days	*Acinetobacter baumanni* septicaemia
Yes (PCR-negative)	Not TB	None	No	51 days	Congenital brain abnormality
Yes (PCR not performed)	Not TB	None	No	16 days	Respiratory syncytial virus pneumonia
No	Not TB	None	No	24 days	*Group B streptococcus* infection
Maternal deaths (*n* = 3/66, 5%)					
Negative	Yes, confirmed	*M. tuberculosis*-positive	None	10 days	Pneumonia
Positive	Yes, confirmed	*M. tuberculosis*-positive (INH- and RMP-resistant)	None	2 days	Miliary TB (multilobar pneumonia requiring ventilation)
Negative	Yes, confirmed	*M. tuberculosis*-positive	2 months	1 day	Post-partum haemorrhage

*Infant died before gastric aspirate result became available.
HIV = human immunodeficiency virus; TB = tuberculosis; PCR = polymerase chain reaction for HIV; INH = isoniazid; RMP = rifampicin.

lower weight and a two-fold increased risk of prematurity. These effects were more prevalent when anti-tuberculosis treatment was commenced late in pregnancy, where poor maternal drug adherence was present and when the mother was sputum smear-positive for *M. tuberculosis*.[8] These data further emphasise the importance of earlier maternal TB case detection. The high maternal mortality (5%) in women with TB is of concern; the delayed TB diagnosis in the majority of these women may be a contributing factor.

Women with TB were mostly young and HIV-infected. The TB disease spectrum among mothers varied. Noteworthy observations were that pleural effusions occurred in nearly a quarter and disseminated TB in 5/25 (20%) HIV-infected mothers. It is possible that the risk of transmitting *M. tuberculosis* in utero is higher in pregnant mothers with recent *M. tuberculosis* infection or primary TB, e.g., those presenting with pleural effusion or with disseminated TB (miliary TB or tuberculous meningitis), which has a bacillaemic phase.[17,19] In support of this hypothesis, of the eight neonatal TB cases (one set of twins), two mothers had tuberculous pleural effusions and three had disseminated TB disease. In a review of 75 cases of congenital TB with information on maternal TB available in 65 (43 with CXR results), 12/43 (28%) had pleural effusions, 8/43 (19%) had miliary TB and 6/43 (14%) central nervous system disease.[20]

Gupta et al. recently documented that infants born to HIV-infected mothers with TB had a 2.5-fold (95% confidence interval [CI] 1.05–6.02, *P* = 0.04) increased odds of being HIV-infected compared to infants born to non-HIV-infected mothers, adjusting for maternal and infant factors.[10] Despite the incom-

plete data on infant HIV polymerase chain reaction (PCR) testing (available for only 19/41 HIV-exposed infants in the present study), the number of HIV-infected infants was high compared to routine reported provincial HIV vertical transmission rates (3–5%). We found that 5/19 (26%) infants tested for HIV were PCR-positive: 3 from the confirmed maternal TB group and 2 from mothers with suspected TB. However, these results should be interpreted with caution: selection bias might have occurred, as more severely ill infants were potentially more likely to be tested for HIV.

Although the attrition for neonates screened for TB was high, an alarmingly small number of infants were referred for TB chemoprophylaxis, and 5/12 (42%) completed 6 months' chemoprophylaxis. This highlights the remaining operational challenges in linking hospital community care and improving adherence to INH preventive therapy in this highly vulnerable age group.

This study has several limitations, including the use of routine retrospective hospital data and the possible incomplete documentation of neonates screened for TB despite using a multisource surveillance strategy. As no existing maternal TB screening protocol was in place, the entry point for our study was infants screened for TB and not infants eligible for TB screening, leading to the potential omission of some infants. Data routinely recorded in folders were of varying quality, and outcome data were particularly limited, including those on completion of chemoprophylaxis and anti-tuberculosis treatment in this mobile and impoverished urban study population.

In a setting with high TB and HIV burdens, both routine antenatal TB and HIV screening are imperative.

Screening for TB diagnosis and prevention clearly lags behind that of HIV within PMTCT programmes. Where several HIV-infected mothers received cART, few were screened antenatally for TB. Early detection of both diseases will aid in the appropriate and earlier treatment of the mother and infant, reducing adverse outcomes, including neonatal TB, prematurity and LBW. Our data indicate the need for prospective studies and interventions for improved maternal and infant TB screening and management in high-burden settings.

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_____ R É S U M É

CONTEXTE : La tuberculose (TB) maternelle et néonatale n'est pas suffisamment reconnue dans les contextes où le fardeau du virus de l'immunodéficience humaine (VIH) est élevé.

SCHÉMA ET DISPOSITION : Il s'agit d'un audit rétrospectif des nouveau-nés dépistés en routine pour TB dans un hôpital d'Afrique du Sud pendant l'année 2009. Comme sources de surveillance, on a utilisé les dossiers de routine en matière de clinique, de laboratoire et de pharmacie.

RÉSULTATS : On a dépisté 70 nouveau-nés pour TB (60% exposés au VIH). L'âge gestationnel médian a été de 35,5 semaines (IQR 33–38) et le poids médian à la naissance de 2000 g (IQR 1.530–2.484). Les nouveaunés ont été regroupés en fonction de leurs antécédents documentés d'exposition à la TB : TB maternelle chez 41/70 (59%) ; suspicion de TB maternelle chez 9/70

(13%) ; autres expositions documentées au niveau du ménage chez 5/70 (7%) ; et aucune exposition connue à la TB chez 15/70 (21%). Sur les 50 nouveau-nés exposés à une TB maternelle confirmée ou suspectée, 36 (72%) ont été mis sous chimioprophylaxie de la TB, 5 (10%) ont reçu un traitement de la TB et chez 9 (18%), il n'y a eu aucune intervention. Le diagnostic de TB a été porté chez 8/50 nouveau-nés (16%), tous nés de mères atteintes d'une TB suspectée ou démontrée.

CONCLUSIONS : La TB maternelle, principalement chez les femmes infectées par le VIH, a constitué l'indication principale du dépistage chez les nouveau-nés. Un dépistage de routine de la TB chez les femmes enceintes et les soins de TB chez les mères et chez les nourrissons devraient être améliorés dans les contextes où le fardeau de TB et de VIH est élevé.

_____ R E S U M E N

MARCO DE REFERENCIA: La tuberculosis (TB) materna y la TB neonatal suelen pasar desapercibidas, sobre todo en los entornos con una alta carga de morbilidad por la infección causada por el virus de la inmunodeficiencia humana (VIH).

MÉTODO: Evaluar la detección sistemática de la TB en los recién nacidos en un hospital en Sudáfrica en el 2009. Las fuentes de datos de vigilancia analizadas fueron los registros corrientes clínicos, de laboratorio y de farmacia del hospital.

RESULTADOS: Se practicó el cribado para TB a 70 recién nacidos (60% expuestos al VIH). La mediana de la edad gestacional fue 35,5 semanas (IQR 33 a 38) con una mediana del peso al nacer de 2000 g (IQR 1530 a 2484). Los recién nacidos se categorizaron en función del antecedente de exposición a la TB de la siguiente manera: TB materna en 41/70 (59%), presunción clí

nica de TB materna en 9 (13%), otra exposición directa documentada en 5 (7%) y sin exposición a la TB en 15/70 recién nacidos (21%). De los 50 neonatos expuestos a una TB materna presunta o confirmada, se inició la quimioprofilaxis tuberculosa en 36 (72%), 5 (10%) recibieron tratamiento antituberculoso y en 9 (18%) no se aplicó ninguna intervención. Se estableció el diagnóstico de TB en ocho recién nacidos (8/50, 16%), cuyas madres presentaban ya sea una presunción clínica o un diagnóstico confirmado de la enfermedad.

CONCLUSIÓN: La TB materna, sobre todo en las mujeres con infección por el VIH, constituye la principal indicación de detección de la TB en el recién nacido. Es importante mejorar la detección sistemática de la TB en las mujeres embarazadas y la atención de la TB de las madres y los lactantes en los entornos con una alta carga de morbilidad por TB e infección por el VIH.

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CHAPTER 4

CLINICAL DISEASE PRESENTATION AND TREATMENT OUTCOMES OF PREGNANT WOMEN WITH TB AT A REFERRAL HOSPITAL

It became evident that exposure to *M. tuberculosis* was common and a serious clinical concern among neonates routinely admitted to a large provincial referral hospital in the Western Cape Province, Tygerberg Hospital (TBH) (143). Maternal TB was the main indication for completing TB screening in neonates, of which the majority was born to HIV-infected women. Isoniazid preventive therapy (IPT) was indicated in a large proportion of neonates because mothers were judged to pose an infectious risk of TB to their newborn infants (143). This delayed maternal TB diagnosis, together with the high number of poorly characterized and very ill pregnant women with TB who were often co-infected with HIV, were points of concern. No standard recording and reporting of TB during pregnancy was routinely required by the national TB programme during the time this research was conducted, and the burden of TB in pregnant and postpartum women at a facility such as TBH was therefore unknown. TB during pregnancy is associated with high morbidity and mortality in both mothers and infants (20, 23, 28, 29). It was therefore thought to be important to describe the epidemiology and clinical presentation of maternal-infant TB at TBH, and collect data on infant outcomes and maternal TB treatment outcomes.

A prospective cohort study was conducted among pregnant and postpartum women routinely admitted to the obstetric services at TBH during 2011. HIV-infected and uninfected women, identified to be on TB treatment were consecutively enrolled from January 2011 through December 2011. All pregnant women on TB treatment and those initiated on TB treatment by their routine clinicians were eligible for inclusion. During the study period, 8471 women were admitted to the TBH obstetric services, where 5864 high-risk deliveries were managed during 2011. Low birth weight (LBW; <2500 g) infants constituted 2179 (37%) of all live births during this period (144). Baseline maternal and infant data were extracted from hospital records, and maternal TB treatment outcomes were assessed at TB treatment completion. Paper-based TB

treatment clinic registers and the national electronic TB registry (ETR.net) were used to determine maternal TB treatment outcomes. Standard World Health Organization (WHO) TB treatment outcome definitions were applied to classify maternal TB disease outcome: cured, treatment completed, treatment failure, loss to follow-up and death (145). Cured or treatment completed were considered as favourable TB treatment outcomes, while treatment failure, loss to follow-up or death were classified as unfavourable. Analysis was mainly descriptive; in addition, a multivariable regression model was used to identify predictors associated with unfavourable maternal TB treatment outcome.

Results from this study showed that a large number (n=74) of pregnant and postpartum women with TB were admitted to routine obstetric services at TBH during a single year. Of the 74 women with TB, 35 (47%) women were diagnosed only after hospital admission for labour, and 22 (30%) reported a previous TB treatment episode. Fifty-three (72%) of 74 women were HIV-infected and 31/53 (58%) HIV-infected women had a CD_4 count of < 200 cells/mm^3. Only HIV-infected women had manifestations of severe extrapulmonary (EPTB), including TB meningitis, TB spine, TB pericarditis, abdominal TB and bacteremia. Despite free, accessible antiretroviral therapy (ART) in the public sector, only 34/53 (64%) HIV-infected pregnant women had received any form of prevention of mother-to-child transmission (PMTCT) therapy or combination ART (cART). All five maternal deaths were amongst HIV-infected women. The four stillbirths and 6 newborn deaths all occurred amongst infants born to HIV-infected women. Similar high rates of maternal and newborn mortality have previously been observed in pregnant and postpartum women with TB and HIV in earlier studies from India and KwaZulu-Natal, South Africa, where access to cART was not yet widely available (20, 23).

Maternal TB treatment outcomes were poor in general, with 41/74 (55%) women only having favourable TB treatment outcomes. There was a high proportion of premature (49/75; 65%) and LBW infants (44/75; 59%) born to women with TB (2 sets of twins; 1 foetus died *in utero*). Women with unfavourable TB outcomes were 3.83 times more likely to deliver a LBW infant (95% confidence interval 1.40 – 10.53, p=0.009), suggesting additional challenges for infant care in women with LBW infants beyond their own personal healthcare.

In conclusion, this study found a large number of women with TB routinely admitted and treated for TB at a large provincial referral hospital during 2011. The diagnosis of maternal TB was delayed with almost half of women initiated on treatment after hospital admission for labour. Maternal HIV infection was associated with high maternal and newborn mortality. Maternal TB treatment outcomes were poor. Health system strengthening is required to increase routine antenatal TB screening in settings with high burden of TB and HIV. Early TB diagnosis and treatment of pregnant women with TB, and PMTCT or cART in HIV co-infected women, will likely improve both maternal health and perinatal outcomes. Careful clinical management and follow-up is required for pregnant women with TB to ensure better treatment outcomes.

Tuberculosis Disease during Pregnancy and Treatment Outcomes in HIV-Infected and Uninfected Women at a Referral Hospital in Cape Town

Adrie Bekker[1]*, Hendrik S. Schaaf[1], Heather R. Draper[1], Magdalena Kriel[2], Anneke C. Hesseling[1]

1 Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa, **2** DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/Medical Research Council (MRC) Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

* adrie@sun.ac.za

Abstract

Background

Tuberculosis during pregnancy and treatment outcomes are poorly defined in high preva-lence tuberculosis and HIV settings.

Methods

A prospective cohort study of pregnant and postpartum women identified to be routinely on antituberculosis treatment was conducted at Tygerberg Hospital, Cape Town, South Africa, from January 2011 through December 2011. Maternal tuberculosis disease spectrum and tuberculosis-exposed newborns were characterized by maternal HIV status. Maternal tuberculosis treatment outcomes were documented and a multivariable regression model identified predictors of unfavourable tuberculosis treatment outcomes. Infant outcomes were also described.

Results

Seventy-four women with tuberculosis, 53 (72%) HIV-infected, were consecutively enrolled; 35 (47%) were diagnosed at delivery or postpartum and 22 (30%) of women reported previous antituberculosis treatment. HIV-infected women were 5.67 times more likely to have extrapulmonary tuberculosis (95% CI 1.18–27.25, p = 0.03). All 5 maternal deaths were amongst HIV-infected women. Birth outcomes were available for 75 newborns (2 sets of twins, missing data for 1 stillbirth). Of the 75 newborns, 49 (65%) were premature and 44 (59%) were low birth weight (LBW; <2500 grams). All 6 infants who died and the 4 stillbirths were born to HIV-infected women. Unfavourable tuberculosis treatment outcomes

were documented in 33/74 (45%) women. Unfavourable maternal tuberculosis outcome
was associated with delivery of LBW infants (OR 3.83; 95% CI 1.40–10.53, p = 0.009).

Conclusions

A large number of pregnant women with tuberculosis presented at a provincial referral hospital. All maternal and infant deaths occurred in HIV-infected women and their newborns.
Maternal tuberculosis treatment outcomes were poor.

Introduction

Tuberculosis (TB) during pregnancy and maternal TB treatment outcomes are poorly defined
in settings with high burden of TB and human immunodeficiency virus (HIV). TB and HIV
infection are both associated with increased morbidity and mortality in pregnant women and
their infants [1–4]. The World Health Organization (WHO) estimated that there were 9.6 million new TB cases globally in 2014, of which 1.2 million (12%) were HIV-infected, 74% from
the African region [5]. Of these incident TB cases, 3.2 million occurred in women, with 480,
000 estimated TB deaths [5].

It is unclear how many of these women were pregnant, as most countries do not report the
pregnancy status of female TB cases. Women of reproductive age are disproportionately
affected by TB in high HIV prevalence settings [6–7]. HIV-infected women are at increased
risk of TB with a prevalence of TB in pregnant women ranging from 1–11%, compared to
0.06–0.53% in HIV-uninfected women [8]. Immunological changes during pregnancy may
predispose to the susceptibility of new infection and the activation of latent TB infection [8].
Whether this increased risk of TB in HIV-infected women is further increased by being pregnant, remains unknown. In South Africa, where 61% of all reported TB cases were HIV-
infected in 2014 [5], non-pregnancy related infections (mainly deaths in HIV-infected women
complicated by TB and pneumonia) are the single most common cause of maternal mortality,
accounting for more than 35% of all maternal deaths [9]. TB in pregnant women also adversely
impacts on perinatal and infant outcomes. A two-fold risk of delivering premature (<37 weeks
gestational age) and low birth weight (LBW; <2500 grams) infants, and a six-fold increase in
perinatal deaths have been reported in women with TB [10]. Limited data are available for TB
treatment outcomes in pregnant women with TB, despite both pregnancy and TB linked to
poor outcomes [11].

The aim of this study was to describe the clinical presentation of TB in HIV-infected and
HIV-uninfected pregnant women and their perinatal outcomes at a large provincial referral
hospital in Cape Town. Maternal TB treatment outcomes were assessed at the end of antituberculosis treatment. Predictors of unfavourable TB treatment outcomes in pregnant women were
identified and reported.

Methods

Study design and setting

We conducted a prospective cohort study in HIV-infected and HIV-uninfected pregnant
women with TB routinely admitted to the obstetric services at Tygerberg Hospital (TBH),
Cape Town, South Africa, from January 2011 through December 2011. TBH is a large secondary and tertiary provincial referral hospital in the Western Cape Province serving

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approximately one-third of the provincial population. During 2011, 8471 women were admitted to the obstetric services at TBH, which managed 5864 high-risk deliveries. Obstetric service admissions included women with antepartum (prior to delivery) complications, women in labour (intrapartum), and women with postpartum complications (until 6 weeks post delivery). LBW infants constituted 2179 (37%) of all live births during this period [12]. In 2009, Cape Town had a mid-year population of 3,443,010 (3,241,508 HIV-uninfected and 201,502 HIV-infected individuals), and 29,478 newly notified TB cases were recorded in the electronic TB registry (ETR.net) [13]. Pregnant and postpartum women identified to be on routine antituberculosis treatment at TBH were consecutively enrolled and baseline information was obtained from mothers and their newborns.

Pregnant women with tuberculosis

Antenatal TB screening was not routinely recommended or implemented by the South African National TB programme (NTP) during the study period, despite the fact that all HIV-infected persons were recommended to have had routine TB screening [14]. TB screening in pregnant women at TBH consisted of identifying any of the following symptoms: presence of chronic cough (>2 weeks), night sweats, fever, and loss of weight or failure to gain weight during pregnancy. If any of these were present, investigations were performed, including sputum/other samples for microscopy for acid-fast bacilli (AFB) and mycobacterial culture, and shielded chest radiography in the case of suspected pulmonary TB (PTB) [14]. For women with suspected extrapulmonary TB (EPTB), directed special investigations were performed as clinically indicated. The type of TB was classified according to the anatomical site of disease using standard WHO definitions: PTB only involved the lung parenchyma or the tracheobronchial tree; EPTB involved organs other than the lungs, i.e. pleura, lymph nodes, pericardium, bones, meninges or positive blood cultures for *Mycobacterium tuberculosis*; both PTB and EPTB involved a PTB case complicated by pleural effusion [15]. For the purpose of the study, severe EPTB manifestations were defined as EPTB not isolated to only peripheral lymph nodes or pleural effusions, i.e. TB meningitis, *M. tuberculosis* positive on blood culture, abdominal TB, TB spine and TB pericarditis. Standard WHO TB case definitions were applied: both a bacteriologically confirmed TB case (a biological specimen that is positive by smear, microscopy, culture or Xpert MTB/RIF) or a clinically diagnosis of TB (no bacteriological confirmation but diagnosed as TB disease with a decision to treat by a routine clinician) were classified as maternal TB cases [15]. The standard antituberculosis treatment regimen for drug-susceptible TB, also in pregnant women, included a 2-month intensive phase of isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol, followed by a 4-month continuation phase of INH and RMP [14]. Antituberculosis treatment was individualised based on WHO treatment guidance. Drug-resistant antituberculosis treatment regimens consisted of at least four effective drugs and the total antituberculosis treatment duration was maintained for a minimum of 18 months [16]. No injectable agents were used in pregnant women with drug-resistant TB due to the risk of foetal toxicity [14].

TB-exposed newborns

If any mother with TB was judged to pose a risk of transmitting *M. tuberculosis* to her newborn (i.e. mother on antituberculosis treatment for < 2 months or not yet sputum smear or culture converted), infants were investigated for TB disease. Infant screening included clinical examination, gastric aspirates/other sample for culture for *M. tuberculosis*, chest radiography and other imaging/investigations as clinically indicated. Antituberculosis treatment was started in infants if TB was suspected or confirmed. In disease-free infants, where the mother might still

be infectious, 10 mg/kg/day of isoniazid preventive therapy (IPT) for six months was given in infants born to drug-susceptible TB mothers, while infants born to mothers with drug-resistant TB received tailored preventive therapy regimen according to the mother's drug susceptibility test result. Infants born to mothers with TB who were no longer judged to be infectious did not receive antituberculosis treatment, but were closely followed as part of routine care. Infant TB outcomes were available for TB-exposed infants at 6 months of age in a subset of infants enrolled on a complementary study of IPT delivery [17].

HIV infection in women and newborns

Maternal HIV testing included two confirmatory enzyme-linked immunosorbent assays (ELISA) for HIV antibodies, routinely performed in all pregnant women with an unknown HIV status upon hospital admission. The prevention of mother-to-child HIV transmission (PMTCT) policy at the time included lifelong combination antiretroviral therapy (cART) for all HIV-infected pregnant women with a CD_4 count $\leq$350 cells/mm^3 and limited ART from 14 weeks onwards during pregnancy for HIV-infected women with CD_4 count >350 cells/mm^3 [18]. HIV-infected pregnant women on cART received the following drugs, tenofovir (TDF), lamivudine (3TC)/emtracitabine (FTC) plus nevirapine (NVP). Limited ART regimens included antepartum (just before delivery) zidovudine (AZT), intrapartum (during delivery) NVP and AZT, and a single dose of TDF and FTC postpartum. Daily NVP for 6 weeks was administered as newborn preventive therapy for HIV, or daily NVP was given until discontinuation of breastfeeding for mothers not on cART. HIV polymerase chain reaction (PCR) testing was routinely performed at 6 weeks of age in HIV-exposed infants and earlier if clinically indicated. All HIV-infected infants were fast-tracked for cART initiation [19]. HIV PCR results for infants were extracted from the provincial laboratory database 6 months post-delivery.

Maternal TB treatment outcomes

Maternal TB treatment outcomes were assessed at the end of antituberculosis treatment, which was routinely administered by TB services. Facility-based TB treatment clinic treatment registers (from local clinics) were reviewed and verified against the national electronic TB registry. Standard WHO TB treatment outcome definitions were applied to classify maternal TB disease outcomes: cured, treatment completed, treatment failure, lost to follow-up or death [15]. Cured or treatment completion was considered favourable TB treatment outcomes, while treatment failure, lost to follow-up (LTFU) or death were classified as unfavourable TB treatment outcomes. Maternal mortality was defined using the International Classification of Diseases revised version 10, which included late maternal deaths between 42 days and one-year post abortion, miscarriage or delivery [20].

Data collection

Written informed consent was obtained from eligible women with TB. Baseline information was extracted from maternal and infant hospital folders using a standard data collection tool. Maternal data included age, ethnicity (black versus mixed race), haemoglobin at admission, previous antituberculosis treatment, the presence of patient-reported TB symptoms (cough of any duration, night sweats, perceived weight loss, chest pain or shortness of breath, fever, tiredness or malaise), the timing of TB diagnosis (ante- versus intra- or postpartum), type of TB (PTB, EPTB or both), TB treatment regimen, and microbiology results including AFB smear-microscopy, culture, and drug susceptibility testing where available. All chest radiographs were read by two independent readers, using the modified Timika radiology score [21], which estimates the extent of active PTB seen on a posteroanterior chest radiograph according to

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percentage of lung involvement and the presence/absence of cavities [22]. In HIV-infected women CD_4 count during pregnancy and ART delivery were documented. Perinatal outcomes included gestational age, birth weight, birth type (singleton versus twin status), and death (still-birth defined as death of a foetus after 28 weeks gestation). All antituberculosis treatment decisions in newborns and deaths prior to hospital discharge were recorded. Maternal TB treatment outcomes were assessed at antituberculosis treatment completion.

Data analysis

Maternal TB and infant characteristics, including infant outcomes, were evaluated by maternal HIV status using summary statistics, odds ratios (OR) and 95% confidence intervals (CI). Bivariate associations between categorical variables were evaluated using the Chi-square or Fisher's exact tests and continuous variables by t-test or Wilcoxon sign-rank-sum test for normally and non-normally distributed data, respectively. A p-value <0.05 was used to determine statistical significance. In addition, a multivariable regression model was used to identify whether any covariates were associated with unfavourable maternal TB treatment outcome. Any covariate with a univariable logistic regression p-value <0.1 was included in the final model. Maternal HIV status was considered clinically relevant and was included in the final model regardless of its univariable p-value.

The study was approved by the Stellenbosch University Health Research Ethics Committee (N10/08/279), and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (944/10), Paris, France.

Results

Characteristics of pregnancy-associated TB

Seventy-four pregnant and postpartum women were identified to be on antituberculosis treatment at TBH (Fig 1). Sixty-one (82%) of 74 women delivered at TBH and 13/74 (18%) women were transferred in post-delivery. Of the 74 women with TB, 39 (53%) were started on antituberculosis treatment prior to the delivery (3.6 months median duration of TB treatment) and 35 (47%) women were initiated on TB treatment upon hospital admission for delivery or within 6 weeks post-delivery. On admission to the obstetric services, symptoms suggestive of TB were documented in 31/74 (42%) women: 28/31 (90%) reported cough of any duration, 11/31 (35%) night sweats, 11/31 (35%) perceived weight loss, 11/31 (35%) chest pain or shortness of breath, 6/31 (19%) reported fever and 2/31 (6%) complained of tiredness and malaise.

Table 1 describes the maternal TB characteristics by HIV status. Fifty-three (72%) of 74 women on antituberculosis treatment were HIV-infected (median CD_4 count 155 cells/mm³; range 11–565 cells/mm³); 29/53 (55%) were on cART at time of delivery (median time on cART 2 months; IQR: 1–5 months), 5/53 (9%) on PMTCT, 10/53 (19%) received no ART, and 9/53 (17%) had no documentation of ART. Age, ethnicity and EPTB only were associated with maternal HIV status. All severe EPTB manifestations occurred in HIV-infected women. Six (14%) of 42 women in whom TB was bacteriologically confirmed had drug-resistant TB; 4 were HIV-infected and 4 overall had unfavourable treatment outcome (Table 2).

Maternal TB treatment outcomes

Forty-one (55%) of 74 women with TB had favourable TB treatment outcomes and 33 (45%) women had unfavourable treatment outcomes (Fig 1). Of the 26/74 (35%) women who were LTFU, 13 were LTFU before entering the clinic TB registry and 13 women were classified as LTFU at the time of TB treatment completion. HIV status was not associated with
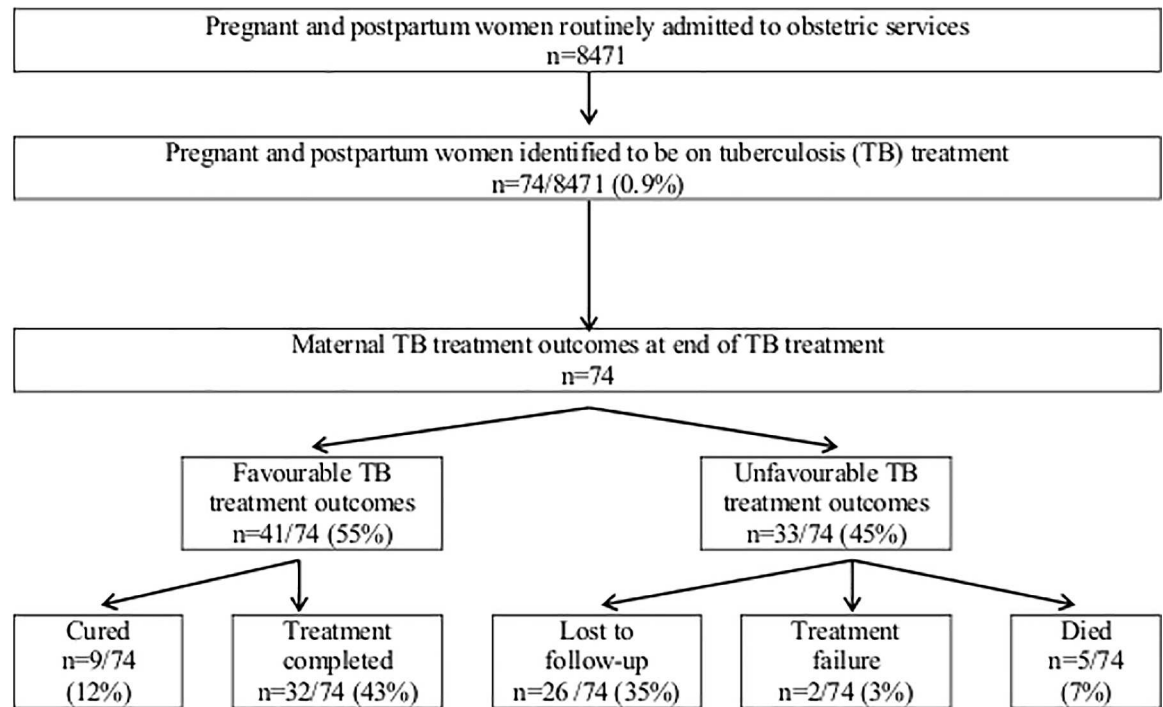
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Fig 1. Pregnant and postpartum women on tuberculosis treatment and their outcomes (n = 74).

unfavourable maternal TB treatment outcome (OR 1.1, 95% CI 0.4–3.1, p = 0.85), but all 5 women who died were HIV co-infected. Deaths amongst pregnant women are described in Table 3. All five maternal deaths (3 during hospital delivery admission and 2 after discharge from hospital) were classified as TB-related by routine attending clinicians, but no autopsies were performed.

Characteristics and outcomes of TB-exposed infants

Table 4 summarizes the characteristics and hospital outcomes of infants by maternal HIV status (n = 76; 2 sets of twins). Gestational age and birth weight was unknown for 1 stillbirth.

Forty-nine of 75 (65%) infants were premature and 44/75 (59%) infants were LBW. No differences in the gestational age and birth weight were observed between HIV-exposed and HIV-unexposed infants. All ten deaths (4 stillbirths, and 6 newborn deaths) were in infants born to HIV-infected women; the causes of infant deaths are listed in Table 4. Treatment decisions were made for all 72 live-born TB-exposed infants (4 stillbirths); 11 (15%) were given no anti-tuberculosis treatment by routine attending clinicians (3 newborn deaths, 8 born to women judged not to be infectious); 57 (79%) were initiated on IPT (all born to women judged to pose significant *M.tuberculosis* transmission risk), and 4 (6%) were started on antituberculosis treatment (3 completed 6 months of antituberculosis treatment and 1 died). *M.tuberculosis* was confirmed in 2/72 (3%) TB-exposed infants; both infants had congenital TB and both were born to HIV-infected women.

Of the 57 infants initiated on IPT, outcome data were available in 39 (68%) infants at 6 months following hospital discharge: 24/39 (62%) completed IPT, 13/39 (33%) did not complete IPT, and 2/39 (5%) died (17). HIV PCR tests were performed in 45 (82%) of 55 HIV-exposed newborns; HIV PCR testing was not done in 10 infants (4 stillbirths, 4 newborn deaths, and in 2 newborns who were LTFU). Of the 45 newborns who had HIV testing, 42

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Table 1. Maternal tuberculosis characteristics by HIV status (n = 74).

	All pregnant women	Maternal HIV status		OR (95% CI)	p-value
		HIV-infected (n = 53)	HIV-uninfected (n = 21)		
Age in years, mean (SD)	29.8 (6)	31.1 (6)	26.7 (6)	1.16 (1.04–1.30)	0.006
Black ethnicity (%) [1]	48 (65)	42 (80)	6 (29)	9.55 (3.00–30.34)	<0.001
Haemoglobin in g/dL, mean (SD) [n = 70]	9.8 (1.7)	9.6 (1.8)	10.1 (1.5)	0.86 (0.64–1.18)	0.352
Previous TB treatment (%)	22 (30)	16 (30)	6 (29)	1.08 (0.35–3.29)	0.891
Timing of TB diagnosis					
Antepartum (%)	39 (53)	27 (51)	12 (57)	-Ref-	
Intra- or postpartum (%)	35 (47)	26 (49)	9 (43)	1.28 (0.46–3.55)	0.630
TB case definitions					
Bacteriologically confirmed (%)	50 (68)	33 (62)	17 (80)	0.39 (0.11–1.32)	0.129
Clinically diagnosed (%)	24 (32)	20 (38)	4 (20)	Ref	
TB disease type					
PTB only (%)	47 (63)	30 (56)	17 (80)	Ref	
EPTB only (%)	22 (30)	20 (38)	2 (10)	5.67 (1.18–27.25)	0.030
Both PTB and EPTB (%)	5 (7)	3 (6)	2 (10)	0.85 (0.13–5.60)	0.866
EPTB disease manifestation [n = 27] [2]					
Severe EPTB					
TB meningitis (%)	8 (29)	8 (15)	0 (0)		
Blood culture positive for *M. tuberculosis* (%)	1 (4)	1 (2)	0 (0)		
Abdominal TB (%)	1 (4)	1 (2)	0 (0)		
TB spine (%)	1 (4)	1 (2)	0 (0)		
TB pericarditis (%)	1 (4)	1 (2)	0 (0)		
Non severe EPTB (%)					
Peripheral lymph nodes (%)	2 (7)	2 (4)	0 (0)		
Pleural effusions (%)	13 (48)	9 (17)	4 (19)		
Microbiological results					
Smear positive (%) [n = 73] [3]	28 (38)	18 (34)	10 (50)	0.44 (0.16–1.24)	0.121
Culture positive (%)	42 (57)	28 (53)	14 (67)	0.56 (0.19–1.61)	0.282
Chest radiographic features [n = 52] [4]	52 (70)	39 (74)	13 (62)		
Percentage affected lungs, median (IQR)	14 (8–32)	14 (8–33)	14 (8–28)	0.99 (0.96–1.02)	0.382
Presence of cavities (%)	9 (17)	6 (15)	3 (23)	0.61 (0.13–2.87)	0.528
Final score, median (IQR)	15 (8–38)	19 (8–38)	14 (8–28)	0.99 (0.97–1.01)	0.346
TB treatment regimen					
First-line TB treatment (%)	68 (92)	49 (93)	19 (91)	1.29 (0.22–7.63)	0.779
Second-line TB treatment (%)	6 (8)	4 (7)	2 (9)		
Maternal mortality (%)	5 (7)	5 (9)	0 (0.0)	—	0.313

OR, odds ratio; SD, standard deviation; TB, tuberculosis; Ref, reference; PTB, pulmonary TB; EPTB, extrapulmonary TB; IQR, interquartile range.

[1] Women were of black ethnicity or mixed race

[2] *M. tuberculosis* was cultured in 15/27 (56%) EPTB women; on cerebrospinal fluid in 3, on bone biospy in 1, on pericardial fluid in 1, on blood culture in 1, on lymph node tissue in 3 (1 abdominal TB and 2 peripheral lymphadenopathy), and on pleural fluid in 6.

[3] Xpert MTB/RIF was positive and culture positive for *M. tuberculosis* in the sputum of 1 women. The positive Xpert MTB/RIF result was excluded from the smear positive analysis.

[4] Chest radiographic features were only available for 52 pregnant women with any TB disease type.

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(93%) had a negative test result and 3 (7%) were HIV PCR positive. All 3 HIV-infected infants were initiated on cART and were alive and well at six months of age.

Table 2. Characteristics and outcomes of pregnant women with confirmed drug-resistant tuberculosis (n = 6).

Maternal characteristics			Outcomes	
TB presentation	TB treatment regimen	HIV features	Perinatal and infant [1]	Maternal TB
• Pulmonary TB[2] • *M. tb* (INH and RMP resistant) cultured from sputum within 13 d • 38 y, • Mixed race	• 3 m of OFX,ETO,PZA,EMB,TRD prior to delivery; • 9 m interruption post- delivery, and presented again with *M.tb* sputum (INH, RMP, ETH and OFX resistant)	• CD$_4$ 24 cells/mm^3 • Previously diagnosed with HIV infection, 3 m prior to delivery (TDF, 3TC, NVP)	• Male, • 1940 g, • 38 w GA • Alive at 9 m and completed 6 m of high dose INH, in place of safety. • PCR HIV negative	Unfavourable (LTF)
• Pulmonary TB[2] • *M. tb* (INH and RMP resistant) cultured from sputum within 22 d • 28 y, • African	• 8 m of OFX, ETO, PZA,EMB, TRD and 5 m of TB treatment interruption prior to delivery. • Mom was re-initiated on TB treatment post-delivery—adhered for 3 m, and then re-located	• CD$_4$ 410 cells/mm^3 • Previously diagnosed with HIV infection, but defaulted ART 5 m prior to delivery PMTCT at delivery	• Male, • 3100 g, • 40 w GA • Alive at 3 m and completed 3 m of high dose INH, then LTF. • PCR HIV negative	Unfavourable (LTF)
• TB meningitis and miliary TB • *M. tb* (INH and RMP resistant) cultured from CSF within 18 d, • 29 y, • Mixed race	• 11 days of INH,RMP,PZA,EMB • *M.tb* culture results only became available after the mother had died	• CD$_4$ 87 cells/mm^3 • Previously diagnosed with HIV infection, but defaulted ART 3 m prior to delivery	• Foetus died *in utero*	Unfavourable (died)
• Pulmonary TB [2] • *M. tb* (INH and RMP resistant) cultured from sputum within 22 d • 30 y, • African	• 18 m of OFX,ETO,PZA,EMB, TRD • 14 m prior to delivery, and 4 m post-delivery	• CD$_4$ 154 cells/mm^3 • Previously diagnosed with HIV infection 14 m prior to delivery (ZDV, 3TC, EFV)	• Female, • 2690 g, • 40 w GA • Alive at discharge from hospital. No TB medication indicated • PCR HIV negative	Favourable (cured)
• Pulmonary TB • *M. tb* (INH and RMP resistant) cultured from sputum within 22 d • 38 y, • Mixed race	• 24 m of OFX,ETO,PZA,EMB, TRD • 9 m prior to delivery, and 15 m post-delivery	• HIV uninfected	• Male, • 2920 g, • 38 w GA • Alive at discharged from hospital. • No TB medication indicated	Favourable (cured)
Pulmonary TB • s*M. tb* (INH, RMP, ETH, OFX and AMK resistant) cultured from sputum within 24 d • 30 y, • African	• 1 m of OFX,ETO,PZA,EMB,TRD • Prior to delivery, followed by 8 m of extensively drug-resistant TB treatment [3]	• HIV uninfected	• Female, • 3625 g, • 40 w GA • Alive at 1 month and completed 1 month of high dose INH—then LTF (re-located with grandmother)	Unfavourable (LTF)

TB, tuberculosis; *M. tb*, *Mycobacterium tuberculosis*; INH, isoniazid; RMP, rifampicin; d, days; y, years; m, months; OFX, ofloxacin; ETO, ethionamide; PZA, pyrazinamide; EMB, ethambutol; TRD, terizidone; TDF, tenofovir; 3TC, lamivudine; NVP, nevirapine, g, grams; ZDV, zidovudine; EFV, efavirenz; w, weeks; GA, gestational age; ART, antiretroviral therapy; PCR, polymerase chain reaction; PMTCT, Prevention of mother-to-child-HIV transmission; CSF, cerebrospinal fluid

[1] No fetal abnormalities were detected at birth

[2] Previously treated for drug-susceptible TB

[3] Mother was subsequently discharged from TB hospital and lost to follow-up

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Predictors of unfavourable maternal TB treatment outcomes

In univariable logistic regression, delivery of a LBW infant was associated with unfavourable maternal TB treatment outcome (p = 0.009). Maternal HIV status, maternal age, haemoglobin upon hospital admission, the presence of any EPTB, bacteriologically confirmed tuberculosis, and intra- and postpartum TB diagnosis were not associated with an unfavourable treatment outcome (Table 5). In multivariable regression, adjusting for maternal HIV infection, women delivering LBW infants were 3.83 times more likely to have an unfavourable TB treatment

Table 3. Deaths amongst pregnant women with tuberculosis and infant outcomes (n = 5).

Maternal characteristics			Outcomes	
TB presentation	TB treatment regimen duration prior to delivery	HIV features at delivery	Perinatal and infant	Maternal death
• Pulmonary TB • Respiratory failure (Sputum negative for *M. tb*) • 31 y and African • Hb 11.4 g/dL	• 1 w • RMP,INH,PZA and EMB	• *Pneumocystis jiroveci* pneumonia • CD$_4$ 16 cells/mm^3. • Newly diagnosed with HIV • No prior cART	• Male, 1290 g, 29 weeks GA • Alive at 3 m and on TB preventive therapy • PCR HIV negative	• 2 d after delivery
• Abdominal TB • *M. tb* (INH and RMP susceptible) cultured from lymph node within 6 d. • Adenosine deaminase of 185 IU/L on ascitic fluid • 24 y and African • Hb 9.8 g/dL	• 1 d • RMP,INH,PZA and EMB	• Kaposi sarcoma • CD$_4$ 50 cells/mm^3 • Diagnosed with HIV 2 m prior to delivery • cART initiated at time of HIVdiagnosis	• Female, 2030 g, 35 weeks GA • *M. tb* cultured from gastric aspirate (x2). Alive at 6 m and completed TB treatment • PCR HIV negative	• 96 d after delivery
• Bacteraemia • *M. tb* (INH and RMP susceptible) cultured from blood within 28 d • 27 y and African • Hb 7.8 g/dL	• Less than 1 m • RMP,INH,PZA and EMB	• CD$_4$ 102 cells/mm^3 • Newly diagnosed with HIV • cART initiated < 1 m prior to delivery	• Male, 980 g, 30 weeks GA • Died on day of birth—cause of death: prematurity and hyaline membrane disease • PCR HIV negative	• On day of delivery
• TB meningitis and miliary TB • *M. tb* (INH and RMP resistant) cultured from CSF within 18 d • 29 y and of mixed race • Hb 8.2 g/dL	• 11 d • RMP,INH,PZA and EMB	• CD$_4$ 87 cells/mm^3 • Newly diagnosed with HIV • No prior cART	• Foetus died *in utero*	• 11 d after admission
• TB meningitis • Tuberculoma on CT scan • 27 y and of mixed race • Hb 7.3 g/dL	• 1 d • RMP,INH,PZA and EMB	• CD4 37 cells/mm^3 • Newly HIV diagnosed • No prior cART	• Male, 1260 g, 30 weeks GA • Alive at 6 m and completed TB treatment • PCR HIV negative	• 54 d after delivery

M. tb, *M. tuberculosis*; y, years; RMP, rifampicin; INH, isoniaizd; PZA, pyrazinamide; EMB, ethambutol; cART, combination antiretroviral therapy; g, grams; w, weeks; GA, gestational age; PCR, polymerase chain reaction; d, days; Hb, haemoglobin; m, months; CSF, cerebrospinal fluid; CT, computerized tomography

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outcome (95% CI 1.40–10.53, p = 0.009), compared to women delivering infants weighing > 2500 grams.

Discussion

This study represents one of the largest prospective cohorts of HIV-infected and uninfected pregnant and postpartum women with TB, including reporting of maternal TB treatment outcomes. TBH is a provincial referral hospital that manages high-risk and complicated deliveries and serves communities with high burdens of TB and HIV. Of the 74 women with TB, nearly half were only diagnosed at delivery or in the postpartum period, and almost a third of women reported prior tuberculosis treatment. More than two-thirds of women on antituberculosis treatment were HIV-infected, the majority were severely immune suppressed, and many presented with severe manifestations of EPTB. All deaths occurred in HIV-infected women. All stillbirths and newborns who died were born to HIV-infected women. Maternal TB treatment outcomes were poor with unfavourable TB treatment outcomes in 33/74 (45%) of women. Prematurity and LBW status were common amongst infants. LBW deliveries were associated with unfavourable maternal TB treatment outcomes.

The challenges of diagnosing TB during pregnancy may lead to under-recognition of TB in pregnant women. Early TB symptoms are often non-specific [23], and overlap with pregnancy

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Table 4. Characteristics and outcome of TB-exposed infants at hospital discharge, by infant HIV exposure status (N = 76).

	All infants	Infant HIV Status		OR (95% CI)	p-value
		HIV-exposed (n = 55)	HIV-unexposed (n = 21)		
Gestational age in weeks, median (IQR) [n = 75] [1]	36 (31–38)	36 (31–38)	37 (33–38)	0.99 (0.87–1.13)	0.851
Premature (%) [n = 75] [1]	49 (65)	38 (70)	11 (52)	2.16 (0.77–6.09)	0.146
Birth weight in grams, median (IQR) [n = 75] [1]	2120 (1386–2920)	2080 (1350–2930)	2230 (1900–2885)	1.0 (1.00–1.00)	0.563
Low birth weight, <2500 grams (%) [n = 75] [1]	44 (59)	32 (59)	12 (57)	1.09 (0.39–3.03)	0.867
Birth type					
Singletons (%)	72 (95)	51 (93)	21 (100)	—	0.571
Number of twin babies (%)	4 (5)	4 (7)	0 (0)		
TB treatment decision in live born infants [n = 72]					
No TB treatment (%)	11 (15)	7 (14)	4 (19)	Ref	
TB preventive therapy (%)	57 (79)	41 (80)	16 (76)	1.46 (0.38–5.69)	0.582
TB treatment (%) [2]	4 (6)	3 (6)	1 (5)	1.71 (0.13–22.51)	0.682
Deaths	10 (13)	10 (18)	0 (0.0)	—	0.054
Stillbirths (%)	4 (5)	4 (7)	0 (0.0)		
Newborn deaths (%) [3]	6 (8)	6 (11)	0 (0.0)		

OR, odds ratio; CI, confidence interval; IQR, interquartile range; Ref, reference; AFB, acid fast bacilli

[1] Unknown gestational age and birth weight for one fetus who was still in utero when mother died.

[2] *M. tuberculosis* cultured from 2 gastric aspirates in 1 infant, 1 symptomatic ventilated infant had AFB on tracheal aspirate, 1 infant had miliary TB, and 1 infant had suggestive chest radiography.

[3] Respiratory distress syndrome in 2, necrotising enterocolitis in 1, presumed nosocomial sepsis in 1, vein of Galen malformation in 1, and duodenal web in 1.

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symptoms [11]. Poor performance of TB symptom-screening tools has been noted in two recent studies in HIV-infected pregnant women, reporting a sensitivity of 28% and 54%, respectively [24–25]. A third of women from our cohort reported previous TB treatment, highlighting the possible importance of documentation of previous treatment as a risk factor for maternal TB. Consideration of previous TB may therefore be useful as an additional part of current WHO TB screening algorithms in pregnant women. Furthermore, TB diagnosis in pregnant women was often delayed. Almost half of the women in our cohort were only diagnosed with TB upon hospital admission for labour and up to 6 weeks post-delivery. Delayed TB diagnosis can be explained both by the difficulty in diagnosing TB during pregnancy and the increased risk of TB in the post-partum period. A large epidemiological study from the United Kingdom that was conducted in 192,801 women (a total of 264,136 pregnancies) recently found that early postpartum women were twice as likely to develop TB as non-pregnant women [26]. Delayed TB diagnosis in 47% of our cohort likely contributed to the

Table 5. Predictors of unfavourable maternal tuberculosis treatment outcome (n = 74).

	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Maternal HIV infection	1.10 (0.40–3.06)	0,850	1.06 (0.36–3.10)	0.921
Low birth weight infant (<2500g)	3.83 (1.40–10.53)	0,009	3.83 (1.40–10.53)	0.009
Any extrapulmonary TB	1.59 (0.61–4.12)	0,342		
Maternal age	0.99 (0.92–1.07)	0,837		
Intra- and postpartum TB diagnosis	1.69 (0.67–4.27)	0,264		
Haemoglobin prior to delivery	0.87 (0.66–1.15)	0,319		
Bacteriologically confirmed TB	1.54 (0.57–4.16)	0,396		

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pronounced maternal morbidity and mortality. Untreated maternal TB carries a significant risk of TB exposure to infants and risk of TB in infants, and led to IPT being initiated in three-quarters of TB-exposed infants.

More than two-thirds (72%) of women with TB were HIV-infected, which is comparable to the 2009 national data with an HIV prevalence of 61% in all notified TB cases [5]. Despite wide access to maternal ART, only 64% ART uptake was documented in pregnant women with TB in our study. Only HIV-infected women had severe EPTB manifestations, including TB meningitis, TB spine, TB pericarditis, abdominal TB and bacteraemia. Increased EPTB manifestations in HIV-infected pregnant women have also been reported in studies of TB during pregnancy (1, 3). Whether this increased risk for EPTB is purely because of immunosuppression from HIV co-infection [27–28], and whether the immunological changes from pregnancy co-contribute, remain uncertain. All five maternal deaths occurred in HIV-infected women and were attributed to TB. All the women who died were newly diagnosed with HIV upon hospital admission, were severely immune suppressed, and had received a very short period of ART, if any, prior to delivery. They all had a short antituberculosis treatment duration (between 1 day and 1 month), making immune reconstitution inflammatory syndrome (IRIS) unlikely. Drug-resistant TB was confirmed in 6/42 (14%) women in whom *M.tuberculosis* was bacteriologically confirmed. We describe the drug-resistant TB from our cohort due to the lack of published data in this subgroup of TB patients. Noteworthy is that none of these women routinely received antituberculosis injectable agents during pregnancy, and that unfavourable maternal TB treatment outcomes were documented in four of these women.

Two-thirds of infants born to pregnant women with TB were premature and had LBW, exceeding the already high burden of LBW infant deliveries of 37% at our provincial referral hospital during the study period [12]. An increased risk of prematurity and LBW infant deliveries have previously been described in pregnant women with TB [10, 29]. Previous literature has also reported a high mortality in infants born to mothers affected by TB and HIV [4], all stillbirths and newborn deaths in our cohort were born to HIV-infected mothers. Congenital TB was only confirmed in 2/72 (3%) newborns, both in infants born to HIV-infected women, a lower figure than the 16% reported by Pillay et al., between 1997–1999, at a referral hospital in Durban, South Africa, prior to any ART use in HIV-infected pregnant women [1].

TB treatment completion or cure was only achieved in 41 (55%) women. These poor treatment outcomes are of concern and should be seen in the context of an academic hospital setting with more ill women referred for complications during pregnancy or birth. Almost a fifth of women with TB were lost to follow-up before being documented to continue treatment at the local TB clinic services (initial loss to follow-up), emphasizing the need to improve linking of clinical services between hospital and community-based TB clinics. Maternal HIV infection was not a predictor for an unfavourable TB treatment outcome; however, LBW deliveries were associated with unfavourable maternal TB treatment outcomes. It is possible that women with LBW infants may have had additional challenges to attend personal healthcare, given the challenges of also caring for these LBW infants.

This study has several limitations and does not represent the general population of pregnant women with TB in the study setting. Selection bias might have occurred as only women with complicated pregnancies are referred to TBH for specialised care. Despite this limitation, this relatively large cohort study of seventy-four women adds to the current limited knowledge base of TB disease during pregnancy and maternal TB treatment outcomes in a setting with high HIV prevalence. Another limitation was that the exact time of HIV diagnosis was not systematically recorded in all HIV-infected women. However, all women were tested for HIV prior to hospital discharge and all ART provision was recorded. TB and HIV treatment were provided as part of routine national programmatic services and antituberculosis treatment adherence

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was not monitored in this study. Follow-up was conducted by routine TB services, and only maternal TB treatment outcome data were collected by the study team. Our definition of unfavourable maternal TB treatment outcomes included women who were LTFU, as recommended by WHO. Although we cannot confirm that these women did not complete antituberculosis treatment elsewhere or whether they died, the use of two different TB registry documents (paper based and electronic) could not trace them.

In settings with high burden of TB and HIV, successful implementation of PMTCT programmes in HIV-infected women, with earlier initiation of ART, is critical to reduce maternal and infant morbidity and mortality. In addition, preventing TB deaths among HIV-infected women requires intensified scale-up of TB prevention, diagnosis and treatment interventions within maternal health and TB programmes. Basic antenatal TB screening should routinely be included in high burden TB/HIV settings, and the high risk of TB in the postpartum period should also be considered in TB screening guidelines [26]. Ascertaining previous TB treatment episodes may improve the sensitivity of the current TB symptom-screening tool. The use of improved molecular diagnostics, including Xpert MTB/RIF, which was not routinely available at the time of the study, may reduce diagnostic delays and result in more rapid initiation of antituberculosis treatment, also in pregnant women. A high degree of clinical awareness is essential to diagnose TB in pregnancy and the postpartum period. Improved recording and reporting of maternal TB and treatment outcomes during pregnancy will contribute to better estimates of disease burden, and inform much needed guidelines to improve TB case finding and outcomes amongst women and their infants.

Supporting Information

S1 Dataset.
(XLSX)

Author Contributions

Conceptualization: AB ACH HSS.

Formal analysis: AB HRD.

Investigation: AB MK.

Methodology: AB HRD.

Writing – original draft: AB.

Writing – review & editing: AB HSS HRD MK ACH.

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CHAPTER 5

COMPLETION OF PREVENTIVE ANTITUBERCULOSIS TREATMENT AMONG TB-EXPOSED NEWBORNS IN A RESOURCE-LIMITED SETTING

Isoniazid preventive therapy (IPT) uptake is typically low but variable in resource constrained settings worldwide, ranging from 5-50% (44, 46-49). This poor IPT uptake in high burden countries has been observed despite the current World Health Organization (WHO) guidelines recommending contact investigation and daily isoniazid (INH) preventive treatment for 6 months in children < 5 years exposed to *Mycobacterium tuberculosis* (41). Adherence to, and completion of unsupervised IPT in children less than 5 years of age has been very poor in Cape Town, South Africa (44, 51, 146). Between 2003-2005, a study collecting data from two Cape Town suburbs, reported completion of unsupervised IPT in 36/180 (20%) children with a known TB index case (50). In 2010, a large retrospective record review performed in 14 primary health care facilities in Cape Town, identified 525 child contacts from 1179 infectious adult TB case-records. Of these 525 children, 141 (27%) children were initiated on IPT, and less than 14% (19/141) completed 6 months of routine programmatic delivery of IPT (147). IPT uptake and completion are hampered by many operational challenges in resource-limited settings (43, 44, 146). It is therefore essential to study IPT in TB-exposed newborns, who are at high risk of TB disease progression and who develop severe forms of TB disease (32).

Chapter 3 (study 1) showed poor traceability and low IPT completion rates in a retrospective study (audit) conducted amongst TB-exposed neonates who were routinely initiated on preventive therapy at a Tygerberg hospital (TBH). A decentralized model of TB care was adopted by South Africa, where most patients are investigated, diagnosed and treated at their local TB clinic. Newborns initiated on IPT at referral hospital level (typically specialist care) have the potential for not "linking" to TB care at community-based TB clinics. Down-referral from hospital to the community-based TB clinic is thought to be a possible barrier to IPT completion (148). We therefore designed and implemented study 2, with the aim of prospectively investigating health system, maternal and socio-economic determinants of IPT completion among TB-exposed newborns.

A prospective cohort study was conducted at TBH, Cape Town, South Africa, from January 2011 to June 2012. In this high burden TB and HIV setting, TBH manages up to 6000 high-risk deliveries per annum and has a neonatal service that consists of 136 beds (144). Following routine investigation for TB, newborns initiated on antituberculosis treatment (IPT or TB treatment) were followed to 6 months of age by the study team. For the purpose of this study, antituberculosis treatment was defined as either 6 months of IPT *or* 6 months of antituberculosis treatment for disease. The tested study hypothesis was, that in the presence of certain determinants including health system-, maternal- and socio-economic factors, TB-exposed newborns would be less likely to complete antituberculosis treatment than TB-exposed newborns in whom these determinants were absent. The primary determinant (risk factor) was evidence of an appropriate TB treatment referral, i.e. evidence of a standard TB clinic referral letter *or* proof of a hospital pharmacy prescription for antituberculosis treatment from the hospital to the local community clinic/other health facility, which was used as a proxy for health system function. Covariates included maternal age, HIV status, knowledge of neonatal TB, type of caregiver (more than one), completion of maternal TB treatment, possession of a child support grant, and monthly household income (table 1 of article).

The primary outcome was completion of antituberculosis treatment at 6 months. Cox proportional hazard models were used to calculate the unadjusted and adjusted hazard ratios for the association of an appropriate TB treatment referral on completion of antituberculosis treatment. Continuation of treatment was verified telephonically at 1 month, and study visits were completed at 3 and 6 months. Newborns with incomplete follow-up were censored at the last documented study visit attended. Given the high risk of TB disease progression in TB-exposed newborns in the absence of preventive therapy, treatment was reinstituted (additional study intervention) when there was no evidence of treatment initiation or if treatment had been interrupted (discontinuation of antituberculosis treatment for > 1 month) in the routine healthcare system. For analysis purposes, newborns who successfully completed treatment with the aid of additional study interventions for adherence, described above, were excluded from the 'completion' TB treatment group.

Results

Of the 56 TB-exposed newborns included in the study (49 with a maternal TB source case, and 7 with another infectious household TB source case), 44 (79%) newborns were followed to 6 months. Twelve patients left the study: 1 died, 4 withdrew and 7 were lost to follow-up. Of the 44 newborns followed to 6 months, 29 (66%) completed antituberculosis treatment (26 completed a course of IPT and 3 newborns completed TB treatment for disease). Another eight newborns only completed IPT after an additional study intervention. For analysis purposes, these eight newborns were included in the 'non-completion' TB treatment group. The documentation of an appropriate TB treatment referral was the main determinant predicting completion of antituberculosis treatment. In the presence of maternal HIV this association was strengthened, which may be indicative of more frequent health care service attendance in HIV-infected women for their own and their infants's care.

In summary, a simple hospital-based strategy, including an appropriate TB treatment referral and linkage to care from hospital to local TB clinic substantially improved completion of IPT in TB-exposed newborns. Training of health care professionals on appropriate IPT referral of newborns from hospital to TB clinic is important. Health systems strengthening for models of maternal-infant TB care is required in high-burden settings to improve IPT completion amongst infants. Further simple linkage to care programmes from hospital to community TB clinics is being investigated for TB patients in this setting to improve linkage and retention in TB care. During 2012, 239/282 (85%) children identified at TBH with TB disease were appropriately referred for TB care at community level, and accurately reported in routine provincial TB surveillance data (Du Preez et al, work in progress).

Determinants of tuberculosis treatment completion among newborns in a high-burden setting

A. Bekker, A. L. Slogrove, H. S. Schaaf, K. Du Preez, A. C. Hesseling

Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

SUMMARY

BACKGROUND: Newborns exposed to *Mycobacterium tuberculosis* are at high risk of progression to tuberculosis (TB) disease.

DESIGN AND SETTING: A prospective cohort study conducted in Cape Town, South Africa, from January 2011 to June 2012. TB-exposed newborns requiring isoniazid preventive therapy (IPT) or anti-tuberculosis treatment were followed to 6 months of age. Appropriate tuberculosis treatment referral, maternal and socio-economic determinants were evaluated. The primary outcome, completion of treatment (6 months IPT, 3 months IPT with a negative tuberculin skin test, or 6 months' treatment for disease) was measured at 6 months. Data were collected from folders and care giver interviews. Cox regression was used to determine hazard ratios (HR) for non-completion of treatment.

RESULTS: Fifty-six (63% human immunodeficiency virus [HIV] exposed) TB-exposed newborns were included; median gestational age and mean birth weight were respectively 36 weeks and 2242 g. Of the 56 newborns, 44 (79%) were followed to 6 months; 29/44 (66%) completed anti-tuberculosis treatment without study team intervention. Appropriate treatment referral was associated with a lower hazard of non-completion of treatment (unadjusted HR 0.34, 95%CI 0.12–0.93). This relationship was maintained in multivariable adjustment for maternal HIV status and type of care giver (adjusted HR 0.26, 95%CI 0.09–0.77).

CONCLUSIONS: Appropriate anti-tuberculosis treatment referral improves completion of treatment in infants.

KEY WORDS: IPT; exposure; HIV; maternal

IN SETTINGS such as South Africa, with a high burden of tuberculosis (TB) and human immunodeficiency virus (HIV), young adults aged 20–39 years, including women of reproductive age, are disproportionally represented among TB cases.[1] In 2011, 65% of all TB cases in South Africa were reported to be HIV-infected,[2] contributing to increased mortality among both women and their offspring, especially in the presence of maternal TB.[3,4] Surviving TB-exposed newborns are at high risk of tuberculous infection and disease progression; up to 50% of *Mycobacterium tuberculosis*-infected infants aged <1 year will develop TB, with 30% of these developing progressive pulmonary or disseminated disease in the absence of appropriate anti-tuberculosis preventive therapy.[5]

Despite post-exposure isoniazid preventive therapy (IPT), which reduces the risk of progression to TB disease by 60–65% over 5 years,[6,7] and the World Health Organization recommendations to administer IPT in the presence of *M. tuberculosis* exposure/infection in children aged <5 years,[8] programmatic delivery of IPT is typically poor in high-burden settings. In the Western Cape, South Africa, many operational challenges to IPT delivery have been reported, including a lack of IPT management tools.[9,10] A low completion rate of IPT among young child contacts has been reported in this setting, with figures ranging from 20% to 30% for 6 months of unsupervised daily isoniazid monotherapy.[11,12] In a study from Tygerberg Children's Hospital, Cape Town, only 5 of 37 TB-exposed newborns initiated on IPT were known to have completed 6 months of IPT.[13] Given the high risk of progression to TB disease in this paediatric group, the improved delivery of IPT is clearly a priority.

The aim of the present study was to investigate health system, maternal and socio-economic predictors of TB treatment completion among TB-exposed newborns.

METHODS

Study setting and design

A prospective cohort study was conducted at Tygerberg Children's Hospital (TCH), Cape Town, South

Correspondence to: Adrie Bekker, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505 South Africa. Tel: (+27) 21 938 9198. Fax: (+27) 21 938 9138. e-mail: adrie@sun.ac.za

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Africa, from January 2011 to June 2012. TCH is a tertiary referral hospital in the Western Cape Province that manages up to 6000 high-risk deliveries per annum, with a neonatal service consisting of 124 beds. In this high-burden setting, the peak TB notification rate among young adults exceeded 1400 per 100 000 population;[14] maternal HIV prevalence was 16.9% among public antenatal clinic attendees in 2009.[15]

TB-exposed newborns requiring treatment were followed to 6 months of age. A TB-exposed newborn was defined as a neonate (<28 days) in close contact with an infectious TB source case (maternal TB or other TB household case who was sputum smear- or culture-positive). If the TB source case posed a significant risk of infection (on anti-tuberculosis treatment for <2 months or had not yet sputum converted), the routine clinical care team initiated IPT in all newborns, unless they had symptoms or signs suggestive of TB.[16] Standard of care included deferral of bacille Calmette-Guérin (BCG) vaccine until IPT completion. Screening for TB disease was at the clinician's discretion and included clinical examination, respiratory samples for mycobacterial culture and chest radiographs. For the purpose of this study, anti-tuberculosis treatment was defined as either IPT or a full course of treatment for disease.

IPT consisted of isoniazid (INH) administered at 10 mg/kg/day, once daily, 7 days a week, for 6 months (standard regimen) or INH for 3 months followed by a negative tuberculin skin test (TST), as recommended by the local programme.[16]

Anti-tuberculosis treatment consisted of a 2-month intensive phase, given once daily, 7 days a week, of INH (10–15 mg/kg), rifampicin (RMP; 15 mg/kg), pyrazinamide (35 mg/kg) with or without ethionamide (15–20 mg/kg), followed by a 4-month continuation phase of INH and RMP (same dose).

The recommended standard of care for newborns initiated on anti-tuberculosis treatment included the routine completion of a provincial TB clinic referral letter and a 1-month prescription of anti-tuberculosis treatment following hospital discharge. Newborns receiving treatment for TB disease are routinely notified to the National TB Programme; however, there is no routine notification process for IPT. TB-exposed newborns on anti-tuberculosis treatment are routinely referred for treatment continuation to their local TB clinic following discharge, given the decentralised (clinic-based) model of TB care in this setting. TB clinics typically dispense IPT once monthly, and anti-tuberculosis treatment once weekly. Directly observed treatment is not provided for IPT.

Data collection

All TB-exposed newborns routinely initiated on therapy at TCH from January 2011 to January 2012 were followed to 6 months of age. The primary entry point for recruitment was all mothers routinely identified as having been on anti-tuberculosis treatment,

either antepartum (before delivery), intrapartum (at the time of delivery) or postpartum (shortly following delivery, but within the same hospitalisation period). Written informed consent was obtained from the care giver (mother/legal guardian). If the newborn died before hospital discharge, or if no legal guardian was yet appointed to provide informed consent (e.g., when the mother was too ill or had died), they were excluded. Surveillance was performed from Monday to Friday to identify all potentially eligible participants. Hospital folders from the weekend were reviewed on the following Monday to ensure identification of all maternal TB admissions and discharges.

Potential determinants of anti-tuberculosis treatment completion were collected at baseline. Data from maternal and infant hospital folders were extracted using a standard data collection tool, including information on maternal, infant and socio-economic factors. Using structured interviews, the mother's knowledge of neonatal TB was ascertained by asking the following three questions: Was she aware of any TB risk to her newborn? Did she know that preventive therapy could be given to her newborn? Did she know if the duration of anti-tuberculosis treatment for her newborn constituted days, weeks or months? Maternal knowledge of neonatal TB was classified according to the number of correct answers. If all three were answered correctly, knowledge was categorised as 'good'; if one or more questions were answered correctly, knowledge was categorised as 'some'; if all questions were answered incorrectly, knowledge was categorised as 'poor'. The study team later provided the correct answers to the questions. Post-hospital discharge, the infants' hospital folders were checked for an appropriate anti-tuberculosis treatment referral, defined as evidence of a standard TB clinic referral letter or proof of a hospital pharmacy prescription for anti-tuberculosis treatment. An appropriate treatment referral was used as a proxy for health system function.

All participants were contacted by telephone within the first week after hospital discharge to confirm TB treatment status in the newborn. If the care giver reported not receiving treatment, they were recalled by the study team and treatment was initiated. If a care giver could not be reached by telephone, a home visit was conducted. Continuation of treatment was again verified at 1 month, by telephoning the care giver and the TB clinic. If either reported no anti-tuberculosis treatment for the newborn, the study team recalled them and re-initiated treatment. Further study visits with the study team were scheduled at 3 and 6 months, and care givers were asked to bring the prescribed medication with them to the visit. Due to the high risk of progression to TB disease in infants, treatment was reinstituted when there was no evidence of treatment initiation or if treatment interruption was documented. Treatment interruption was defined as the discontinuation of

anti-tuberculosis medication for >1 month. Interventions constituted a recall study visit, where treatment was initiated or re-instituted. The primary outcome, the completion of anti-tuberculosis treatment (defined as 6 months of IPT, 3 months of IPT followed by a negative TST or 6 months of anti-tuberculosis treatment in the case of suspected or confirmed disease) was measured at 6 months of age. Non-completion of treatment was defined as the failure of any of these criteria. Treatment outcomes for the infectious TB source case were also collected.

Data analysis

The primary study hypothesis was that infants with an appropriate treatment referral (primary determinant) would be less likely than those with an inappropriate referral not to complete anti-tuberculosis treatment (primary outcome). Descriptive statistics are presented as frequencies for categorical variables, mean and standard deviation (SD) for normally distributed continuous variables or median with interquartile range (IQR) if not normally distributed. Bivariable associations between categorical variables were evaluated using the χ^2 or Fisher's exact tests and continuous variables by Student's *t*-test or Wilcoxon sign-rank-sum test, as appropriate. All hypothesis tests were two-sided, with an α value of 0.05. Cox proportional hazards models were used to calculate the unadjusted and adjusted hazard ratios (HRs) for the primary association; the degree of precision around the HR point estimates were represented by 95% confidence intervals (CIs).

Infants with incomplete follow-up were censored at the last study visit attended. The timing of occurrence of the event, non-completion of treatment, was set as the first study visit at which discontinuation of treatment was identified. Variables considered a priori as potential confounders included maternal age, knowledge and HIV status, duration of infant hospitalisation, and maternal socio-economic status. Variables were retained in the multivariable model if they shifted the co-efficient of the point estimate for the primary determinant by ⩾10%. The Schoenfeld test was used to confirm that models met the proportional hazards assumption. Statistical analysis was conducted using R version 2.15.1, with the survival package (R Foundation for Statistical Computing, Vienna, Austria).

Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (N10/08/279), Cape Town, South Africa, and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (44/10), Paris, France.

RESULTS

Inclusion pathways and outcomes of anti-tuberculosis treatment for the study cohort (*n* = 56) are illustrated in the Figure. Of 73 women (51 HIV-infected) identified as being on anti-tuberculosis treatment (clinical data to be reported elsewhere), 49 (67%) newborns were included. In addition, another seven newborns initiated on IPT due to the presence of a non-maternal infectious household TB source case identified during the infant's hospital admission were included, resulting in 56 TB-exposed newborns initiated on IPT. Nineteen newborns had inadvertently received BCG, 30 had not and in 7 there was no documentation of BCG. All 37 non-vaccinated infants were referred for vaccination upon completion of IPT. The median gestational age was 36 weeks (IQR 36–39); the mean birth weight was 2242 g (SD 815). Of the total cohort, 35 (63%) infants were born to HIV-infected women; 2 (6%) infants were HIV-infected. Both HIV-infected infants were initiated on combination antiretroviral therapy (cART) by 3 months of age.

Of the 56 TB-exposed newborns on IPT, 45 (80%) were screened for TB; 43 had gastric/tracheal aspirates and all 45 had a chest radiograph. All 56 were originally initiated on IPT. However, 3 (5%) were later transferred to a full course of anti-tuberculosis treatment due to suspected or confirmed TB disease: 1 infant developed suspected miliary TB and was started on anti-tuberculosis treatment at 2 months of age (the mother had drug-susceptible TB, and the infant had been hospitalised since birth); in 1 asymptomatic infant, *M. tuberculosis* was cultured from two gastric aspirates taken at birth and treatment was initiated at 2 weeks of age; and in a third newborn who was on ventilation for respiratory insufficiency, a tracheal aspirate was smear-positive for acid-fast bacilli. All three completed 6 months of anti-tuberculosis treatment.

Of 56 TB-exposed newborns, 44 (79%) had known TB treatment outcomes at 6 months and 29/44 (66%) completed their anti-tuberculosis treatment; 20 completed 6 months of IPT (70%), 6 completed 3 months of IPT followed by a negative TST (20%) and 3 completed 6 months of TB treatment (10%). The presence of INH tablets was checked at follow-up visits. Of the 44 patients, 15 (34%) failed to complete treatment, with 8 additional patients successfully completing treatment only with a study intervention. For analysis purposes, these 8 were included into the 'non-completion' TB treatment group, as they required additional treatment support. Reasons for non-completion included: discharge from hospital without IPT (*n* = 5), incorrect stoppage of IPT by health facilities (*n* = 6) and care giver's failure to attend TB clinics for IPT (*n* = 4).

Table 1 describes the characteristics of and treatment outcomes for TB-exposed newborns. In the 44 infants with a known outcome, 22/29 (76%) with an appropriate treatment referral completed anti-tuberculosis treatment vs. 7/15 (47%) who failed to complete (*P* = 0.05), measured at 6 months. Of the 27/44 (61%) HIV-infected mothers with known
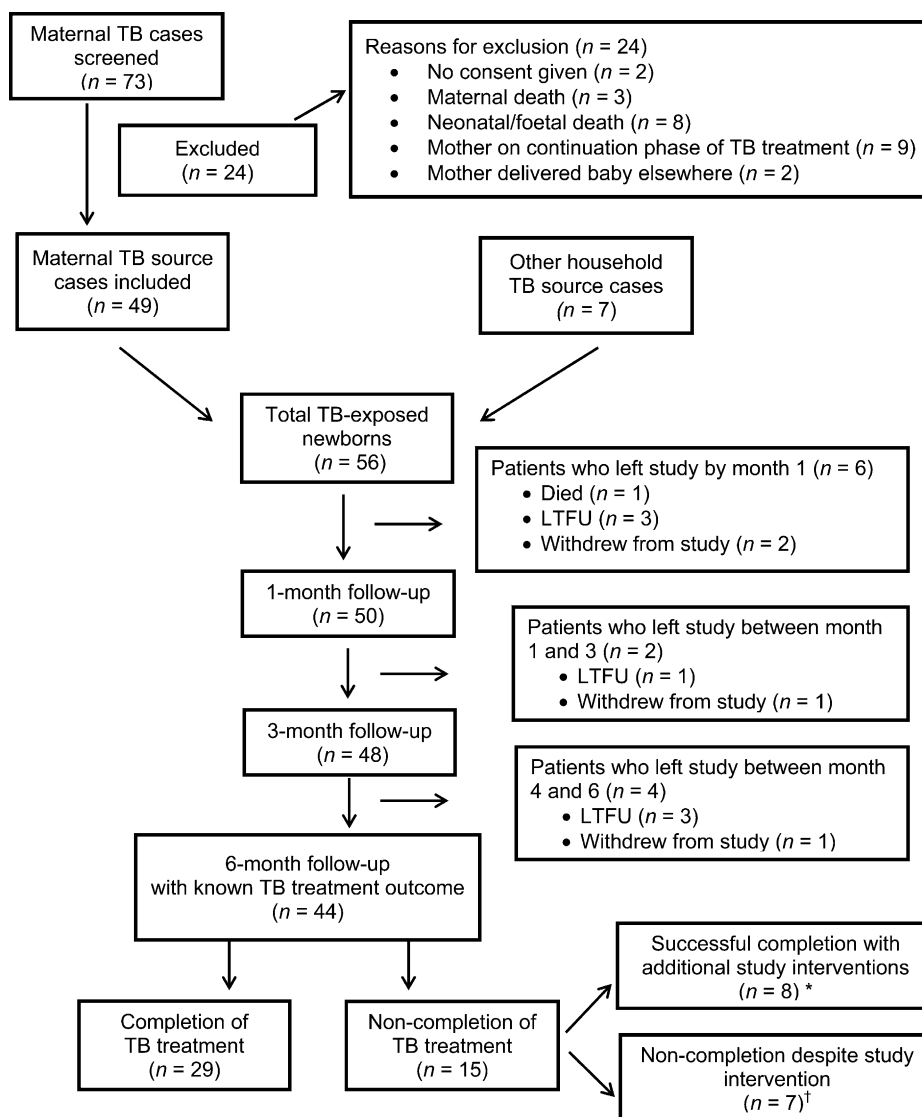
Figure Inclusion pathways and treatment outcomes in TB-exposed infants ($n = 56$). *5 patients were discharged from hospital without IPT, in 2 the health facility stopped IPT incorrectly, in 1 the care giver did not attend the TB clinic. †4 patients had their IPT stopped incorrectly by the health facility; 3 care givers refused to attend the TB clinic. TB = tuberculosis; LTFU = lost to follow-up.

infant TB treatment outcomes, 20/27 (74%) infants completed TB treatment, compared to 9/17 (53%) born to non-HIV-infected mothers. The mother was the primary care giver in 15/29 (52%) cases where the infant's treatment was completed. Eight mothers had 'good' knowledge of TB. Seven of these underwent appropriate TB referral and all infants completed treatment. Treatment was successfully completed in 31/44 (71%) maternal and other TB source cases; of these, 21/31 (68%) infants also completed treatment.

Table 2 presents the HR for non-completion of anti-tuberculosis treatment ($n = 56$). Appropriate treatment referral was associated with a 66% lower hazard of non-completion of treatment (unadjusted HR 0.34, 95%CI 0.12–0.93). Maternal HIV infection also had a protective effect, while the mother being the primary and only care giver was associated

with an increased hazard for non-completion of treatment, although both CIs crossed one. When included in a multivariable model adjusted for maternal HIV status and type of care giver, the association between appropriate referral and non-completion of treatment was marginally strengthened (adjusted HR 0.26, 95%CI 0.09–0.77). The addition of maternal knowledge and source case TB treatment episode outcome, factors also possibly associated with the outcome of interest, did not substantially alter the relationship in this model.

DISCUSSION

To our knowledge, this is the first study specifically investigating the determinants of anti-tuberculosis treatment completion in newborns. Appropriate and

Table 1   Characteristics of newborn infants without and those with a known TB treatment outcome (*n* = 56)

Characteristic	Total cohort (N = 56) n (%)	Unknown treatment status (n = 12) n (%)	Known completion of TB treatment* (n = 29) n (%)	Known non-completion of TB treatment* (n = 15) n (%)	P value†
Health system					
Appropriate TB treatment referral	38 (68)	9 (75)	22 (76)	7 (47)	0.05
Maternal					
Age, years, mean ± SD	29 ± 6	29 ± 6	29 ± 6	29 ± 6	0.87
Knowledge of neonatal TB					0.24
Poor	31 (55)	7 (58)	16 (55)	8 (53)	
Some	16 (29)	4 (33)	6 (21)	6 (40)	
Good	9 (16)	1 (8)	7 (24)	1 (7)	
HIV-infected	35 (63)	8 (67)	20 (69)	7 (47)	0.15
Infant					
Female	32 (57)	9 (75)	15 (52)	8 (53)	0.92
Gestational age, median [IQR]	36 [33–39]	37 [33–40]	36 [33–38]	35 [32–39]	0.59
Birth weight, g, mean ± SD	2242 ± 815	2486 ± 815	2170 ± 744	2186 ± 932	0.95
Duration of hospitalisation, days, median [IQR]	6 [3–28]	6 [3–13]	7 [3–29]	4 [3–16]	0.49
TB source case (maternal and other)					
Relationship with TB source case					
Mother	49 (88)	12 (100)	25 (86)	12 (80)	0.67
Close household contact	7 (12)	0	4 (14)	3 (20)	
Time of TB diagnosis					
Antepartum	34 (64)	9 (75)	16 (55)	9 (60)	0.76
Intra-/postpartum	22 (36)	3 (25)	13 (45)	6 (40)	
TB treatment episode					
First time	32 (57)	9 (75)	13 (45)	10 (67)	0.21
Second time	24 (43)	3 (25)	16 (55)	5 (33)	
TB treatment outcome					
Completed 6 months	36 (64)	5 (42)	21 (72)	10 (67)	0.74
Defaulted/LTFU/died	20 (36)	7 (58)	8 (28)	5 (33)‡	
Socio-economic					
Mother the primary care giver	35 (63)	9 (75)	15 (52)	11 (73)	0.17
Additional care giver	21 (37)	3 (25)	14 (48)	4 (27)	
Walking distance from clinic	45 (80)	10 (83)	23 (79)	12 (80)	1.0
Type of housing					
Brick house	32 (57)	6 (50)	18 (62)	8 (53)	0.58
Shack/yard house/flat	24 (43)	6 (50)	11 (38)	7 (47)	
Household monthly income, ZAR					
<2500	38 (69)	8 (67)	18 (62)	12 (80)	0.31
≥2500	18 (31)	4 (33)	11 (38)	3 (20)	
Receiving child care support	28 (50)	5 (42)	15 (52)	8 (53)	0.91

*Included isoniazid preventive therapy and treatment for TB disease.
†Calculated for comparison between known completion of TB treatment (*n* = 29) and non-completion of TB treatment (*n* = 15).
‡2 maternal TB source cases died after their newborns were discharged from hospital; 3 maternal TB source cases were LTFU.
TB = tuberculosis; SD = standard deviation; HIV = human immunodeficiency virus; IQR = interquartile range; LTFU = lost to follow-up; ZAR = South African Rand.

Table 2   Hazard ratios for non-completion of treatment in TB-exposed infants (*N* = 56)

	Non-completion of TB treatment	
	Unadjusted HR (95%CI)	Adjusted HR (95%CI)*
TB treatment referral type		
Appropriate	0.34 (0.12–0.93)	0.26 (0.09–0.77)
Inappropriate	Reference	Reference
Maternal HIV status		
HIV-infected	0.44 (0.16–1.21)	0.30 (0.10–0.92)
Non-HIV-infected	Reference	Reference
Care giver type		
Mother only	2.03 (0.65–6.41)	3.10 (0.89–10.82)
Other care giver and/or mother	Reference	Reference

*Cox proportional hazards model for the hazard of not completing TB treatment by referral type, adjusted for maternal HIV status and care giver type.
TB = tuberculosis; HR = hazard ratio; CI = confidence interval; HIV = human immunodeficiency virus.

correct down-referral from hospital to community-based clinics was strongly associated with improved treatment completion. We further document high IPT completion rates in young children compared to those reported in the literature under routine programmatic conditions.[9–12] These completion rates were achieved with the aid of a dedicated TB care team, consisting of a nurse and doctor who provided IPT support and ensured regular follow-up. Two thirds of the cohort completed treatment, while a large proportion (84%) finally completed with the support of a simple intervention, which included an additional recall visit and re-institution of anti-tuberculosis treatment. Although additional resources were required, a dedicated TB care team substantially improved the delivery of treatment in this high-risk group of TB-exposed newborns. Given the extreme vulnerability of TB-affected

pregnant women and their infants, additional health care resources are justified in this context, given that two thirds of women with TB were also HIV-co-infected and severely ill, and that the majority of infants were premature and had low birth weight (<2500 g). It is important to note that in the majority of infants where treatment was discontinued, this was primarily due to health system factors.

The strongest predictor for completion of anti-tuberculosis treatment was appropriate treatment referral. The presence of a hospital TB referral letter addressed to the TB clinic or proof of IPT dispensed by the hospital on discharge contributed substantially to the completion of treatment. Linking of hospital and community TB care is therefore of paramount importance to ensure completion of treatment in infants. Strengthening of health services in the context of busy, resource-poor hospitals and clinics can be achieved using a dedicated care team. Strategies could be simplified to be delivered by community care workers with the involvement of the mothers. Appropriate treatment referral and the assistance of an additional care giver were associated with better completion of infant anti-tuberculosis treatment in the presence of maternal HIV infection. One possible explanation may be that HIV-infected mothers, supported by other care givers, attend health care services more frequently for their own and their infant's care, including prevention of mother-to-child-transmission of HIV (PMTCT) and HIV care, which both have strong models of treatment and adherence in this setting, and may have led to improved TB adherence in their infants. Given the high prevalence of maternal HIV in settings such as ours, models of TB care should consider linking into existing maternal and infant health programmes, including PMTCT programmes.

Limitations to our study included the modest sample size. However, this is a relatively large sample for the very specific paediatric group targeted, i.e., TB-exposed newborns. The primary outcome was unknown for a substantial proportion of infants; however, this group did not differ markedly regarding baseline characteristics compared to the rest of the cohort, and is unlikely to have biased results. Failure to retain study participants was mainly due to loss to follow-up. Half of the lost participants were reported by neighbours to have re-located back to their original provinces, a common occurrence where patients temporarily transfer to other provinces for social and other reasons.

In conclusion, appropriate treatment referral in newborn infants improves completion of IPT and anti-tuberculosis treatment. Targeted interventions conducted by a dedicated care team further improve continuity of care. Health systems should be strengthened to improve TB care in this vulnerable paediatric subgroup.

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RÉSUMÉ

CONTEXTE : Les nouveau-nés exposés à *Mycobacterium tuberculosis* ont un risqué élevé de progression vers la maladie tuberculeuse.

SCHÉMA : Une étude de cohorte prospective a été conduite au Cap, en Afrique du Sud, de janvier 2011 à juin 2012. Des nouveau-nés exposés à la tuberculose (TB) ayant bénéficié soit d'une chimioprophylaxie par isoniazide ou d'un traitement anti-tuberculeux ont été suivis jusqu'à l'âge de 6 mois. L'étude a évalué le caractère approprié de la référence thérapeutique et les déterminants maternels et socioéconomiques. Le résultat initial, l'achèvement du traitement (6 mois d'IPT, 3 mois d'IPT en cas de cutiréaction négative ou 6 mois de traitement pour la TB maladie) ont été mesurés à 6 mois. Les données ont été recueillies à partir des dossiers et des entretiens avec les soignants. La méthode de régression de Cox a permis de déterminer le ratio de risque (HR) de non achèvement du traitement.

RÉSULTATS : Cinquante-six nouveau-nés exposés à la TB, dont 63% exposés également au virus de l'immunodéficience humaine (VIH), ont été inclus ; l'âge gestationnel médian était de 36 semaines et le poids de naissance moyen de 2242 g. Sur les 56 nouveau-nés, 44 (79%) ont été suivis pendant 6 mois ; 29/44 (66%) ont achevé leur traitement sans intervention de l'équipe d'étude. Une référence appropriée était associée à un moindre risque de non-achèvement du traitement anti-tuberculeux (HR non ajusté 0,34 ; IC95% 0,12–0,93). Cette relation s'est maintenue en ajustement multivarié selon le statut maternel VIH et le type de soignant (HR ajusté 0,26 ; IC95% 0,09–0,77).

CONCLUSIONS : Une référence appropriée pour le traitement antituberculeux améliore l'achèvement du traitement chez les nourrissons.

RESUMEN

MARCO DE REFERENCIA: Los recién nacidos expuestos a *Mycobacterium tuberculosis* presentan un alto riesgo de progresión hacia la enfermedad tuberculosa.

MÉTODO: En un estudio prospectivo de cohortes llevado a cabo en la Ciudad del Cabo en Suráfrica de enero del 2011 a junio del 2012, se practicó un seguimiento hasta los 6 meses de edad a los recién nacidos expuestos a la tuberculosis (TB) que precisaron tratamiento preventivo con isoniazida (IPT) o tratamiento antituberculoso. Se evaluaron la remisión apropiada a fin de recibir el tratamiento antituberculoso y los factores determinantes de compleción del tratamiento de carácter socioeconómico y dependientes de la madre. El criterio primario de valoración se midió a los 6 meses y consistió en la compleción del tratamiento (IPT durante 6 meses, tratamiento preventivo durante 3 meses con una prueba tuberculínica negativa o 6 meses de tratamiento por TB activa). Se recogieron datos a partir de los registros y las entrevistas a los cuidadores. Se aplicó un análisis de regresión de Cox con el fin de determinar el cociente de riesgos instantáneos (HR) de no completar el tratamiento.

RESULTADOS: Se incluyeron en el estudio 56 recién nacidos expuestos a la TB (63% expuestos al virus de la inmunodeficiencia humana [VIH]); la mediana de la edad gestacional fue 36 semanas y el promedio del peso al nacer fue 2242 g. De los 56 recién nacidos, se siguieron 44 durante 6 meses (79%); 29 de los 44 completaron el tratamiento antituberculoso sin intervención del equipo del estudio (66%). Una remisión apropiada con el fin de recibir el tratamiento se asoció con un menor riesgo de no completar el tratamiento contra la TB (HR non ajustado 0,34; IC95% de 0,12 a 0,93). Esta correlación se mantuvo tras la corrección de las variables según la situación de la madre frente al VIH y el tipo de cuidador (HR ajustado 0,26; IC95% de 0,09 a 0,77).

CONCLUSIÓN: Una remisión apropiada encaminada a la administración del tratamiento antituberculoso mejora el índice de compleción del tratamiento de los lactantes menores.

CHAPTER 6

PHARMACOKINETICS OF ISONIAZID IN LOW BIRTH WEIGHT AND PREMATURE INFANTS

Chapter 3 to 5 addressed the high burden of TB-exposed newborns at a referral hospital in Cape Town, South Africa (143, 149, 150). TB-exposed newborns, who are at high risk of TB disease progression (32), urgently need optimal and safe isoniazid (INH) dosing. We have shown that there are considerable numbers of premature (< 37 weeks gestational age) and low birth weight (LBW; < 2500 grams) infants requiring post-exposure INH preventive therapy (IPT) in high burden TB and HIV settings (143, 149). Despite implementation of health system strengthening strategies to link infants to TB care, research presented in chapter 5 showed suboptimal IPT completion (150). Determining the appropriate INH dose in TB-exposed newborns was the next knowledge gap to be addressed in the prevention and treatment of perinatal and infant TB.

To our knowledge, only one INH pharmacokinetic study has been performed in newborns, who received the drug transplacentally; women were given 300 mg INH intramuscularly on the day of delivery (52). Only two newborns were studied and both had prolonged INH half-lives of 7.8 and 19.8 hours, respectively (52). These limited pharmacokinetic data suggest a slow elimination rate of INH and cautions against high INH dosing in newborns. The 2009 revised World Health Organization (WHO) paediatric dosing guidelines recommended an increase in the INH dose from 5 mg/kg (4-6 mg/kg) to 10 mg/kg (7-15 mg/kg) (58). The impact of this higher INH dosing in newborns has yet to be studied. The aim of study 3 was therefore to determine whether the current WHO-recommended INH dose of 10 mg/kg achieves adequate target drug concentrations in TB-exposed premature and LBW infants, compared to the proposed adult INH target pharmacokinetic concentrations. Examining the influence of the *N-acetyltransferase (NAT2)* genotype on INH pharmacokinetics was a secondary aim of this study.

An intensive sampling pharmacokinetic study was conducted at Tygerberg Hospital, between May 2011 and May 2012. LBW infants born to HIV-infected and uninfected

women, who were routinely initiated on INH for preventive or curative treatment, were consecutively recruited. Clinical characteristics of infants, and alanine aminotransferase (ALT) values taken as part of routine care were collected. On the day of pharmacokinetic sampling, infants were given an exact dose of 10 mg/kg of INH. INH in powder form, obtained from Fluka Chemie AG (Buchs, Switzerland), was accurately weighed (to the nearest 0.1 mg) to administer a dose of 10 mg/kg according to the naked newborn's weight (weighed by the study nurse on the day prior to study drug administration). Phlebotomy was performed at 2, 3, 4, and 5 hours post dosing. The high-performance liquid chromatographic (HPLC) method was used for the pharmacokinetic assays, and DNA was extracted from the remaining blood cells for *NAT2* genotyping. Non-compartmental analysis (NCA) was used to generate pharmacokinetic parameters, which were compared by *NAT2* genotype, birth weight, weight at the time of pharmacokinetic sampling, gestational age, age at sampling, gender, HIV exposure, and feeding type (breastfeeding versus formula).

Twenty LBW infants were recruited (14 [70%] male, 16 [80%] HIV-exposed) for this study, of whom 17 (85%) were premature. Of the 16 HIV-exposed infants, 15 infants were uninfected and 1 HIV-infected infant was on combination antiretroviral therapy. Infants had a median birth weight of 1575 grams (interquartile range: 1190 – 2035 grams) and a median gestational age of 35 weeks (interquartile range: 34 – 38 weeks). The summary pharmacokinetic parameters found a median maximum serum concentration (C_{max}) of 5.63 μg/ml, a time to C_{max} (T_{max}) of 2 hours, an area under the concentration-time curve 2-5 hours (AUC_{2-5}) of 13.56 μg.h/ml, and a half-life ($t_{½}$) of 4.69 hours. Nineteen (95%) infants achieved drug concentrations (range 2.9 – 10.7 μg/ml) above the current proposed adult pharmacokinetic target concentration of 3 μg/ml at 2 hours (70). All 20 infants achieved target drug concentrations > 1.5 μg/ml at 3 hours post dose (151), comparable to the adult pharmacokinetic target concentration. The median C_{max} of very low birth weight (VLBW; < 1500 grams) infants was significantly higher (6.58 μg/ml versus 4.99 μg/ml; p-value < 0.028) than the median C_{max} observed in infants weighing between 1500 – 2500 grams, suggesting reduced elimination in VLBW infants. Although a higher median C_{max} of 5.66 μg/ml in 17 premature infants was observed compared to a median C_{max} of 4.11 μg/ml in the 3 term infants (p=0.1530), the numbers were too small to show significance. The trimodal *NAT2* acetylation pattern was already apparent at this very

early stage of development, with pharmacokinetic parameters significantly different for all three acetylator groups (table 3, figure 2 in article). Slow acetylators had a decreased elimination rate compared to intermediate and fast acetylators. No other covariates studied showed any differences for INH pharmacokinetic parameters.

Hepatotoxicity is an uncommon but serious adverse event that may occur in patients receiving INH for preventive or curative treatment (81). ALT values obtained during routine care by attending clinicians were systematically collected for 19 infants at baseline, 14 infants at 3-months and 11 infants at the 6-months follow-up. Based on the standard Division of AIDS (DAIDS) grading table for adverse events, two 3-month values were abnormal; 1 was mildly elevated (<2.5 times elevated; DAIDS grade 1) and one moderately elevated (<5 times elevated; DAIDS grade 2) (152). The mildly elevated value was not repeated, and the moderately elevated ALT normalized at six months. Limited safety data collected among LBW infants in this study were reassuring.

In summary, this is the first INH pharmacokinetic study conducted in LBW and premature infants. INH drug concentrations achieved in LBW infants were comparable to adult proposed target drug concentrations. Peak concentrations were relatively high in LBW infants dosed at 10 mg/kg, the lower end of the recommended dosing guideline (58). Higher peak INH concentrations and exposures were observed in the smaller infants, and in the genetically determined slow acetylators. These high INH concentrations and reduced elimination observed in LBW infants, cautions against exceeding a dose of 10 mg/kg of INH in this population.

Pharmacokinetics of Isoniazid in Low-Birth-Weight and Premature Infants

A. Bekker,[a,b] H. S. Schaaf,[a,b] H. I. Seifart,[c] H. R. Draper,[b] C. J. Werely,[d] M. F. Cotton,[a] A. C. Hesseling[b]

Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa[a]; Desmond TB Tutu Centre, Department of Paediatrics and Child Health, Stellenbosch University, Tygerberg, South Africa[b]; Division for Clinical Pharmacology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa[c]; MRC Centre of Molecular & Cellular Biology and DST/NRF Centre of Excellence for Biomedical TB Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa[d]

Isoniazid (INH) is recommended for use as posttuberculosis exposure preventive therapy in children. However, no pharmacokinetic data are available for INH treatment in low-birth-weight (LBW) infants, who undergo substantial developmental and physiological changes. Our objectives in this study were to determine the pharmacokinetic parameters of INH at a dose of 10 mg/kg of body weight/day and to define its pharmacokinetics relative to the arylamine N-acetyltransferase-2 ($NAT2$) genotype. An intensive prospective pharmacokinetic sampling study was conducted at Tygerberg Children's Hospital, South Africa, in which we measured INH blood plasma concentrations at 2, 3, 4 and 5 h postdose. Twenty LBW infants (14 male, 16 exposed to HIV) were studied. The median birth weight was 1,575 g (interquartile range, 1,190 to 2,035 g) and the median gestational age was 35 weeks (interquartile range, 34 to 38 weeks). The $NAT2$ acetylation statuses of the infants were homozygous slow (SS) (5 infants), heterozygous intermediate (FS) (11 infants), and homozygous fast (FF) (4 infants). Using a noncompartmental analysis approach, the median maximum drug concentration in blood serum (C_{max}) was 5.63 µg/ml, the time after drug administration to reach C_{max} T_{max}) was 2 h, the area under the concentration-time curve from 2 to 5 h ($AUC_{2–5}$) was 13.56 µg · h/ml, the half-life ($t_{1/2}$) was 4.69 h, and the elimination constant rate (k_{el}) was 0.15 h^{-1}. The alanine aminotransferase levels were normal, apart from 2 isolated values at two and three times above the normal levels. Only the three-times-elevated value was repeated at 6 months and normalized. All LBW infants achieved target INH blood plasma concentrations comparable to the adult values. Reduced elimination was observed in smaller and younger infants and in slow acetylators, cautioning against higher doses. The safety data, although limited, were reassuring. More data, however, are required for newborn infants.

The HIV pandemic has been associated with a dramatic increase in tuberculosis (TB) rates among pregnant women (1). Maternal TB, regardless of HIV status, is associated with a high incidence of low birth weight (LBW) (defined as <2,500 g) and prematurity (a gestational age of <38 weeks) in infants (2–4). In South Africa, with its high burden of both TB and HIV, recent advances in neonatal care have improved infant survival, resulting in a considerable group of small and premature TB-exposed newborns requiring preventive therapy against TB. Isoniazid (INH) is the most widely recommended agent for preventing TB disease in children (5). Following *Mycobacterium tuberculosis* infection, up to 50% of infants (<12 months of age) will progress to have TB disease in the absence of INH preventive therapy (IPT) (6), while postexposure IPT reduces the risk of TB disease by 60 to 65% (7). There are limited pharmacokinetic data on the use of INH in young children and none in newborns or LBW infants requiring IPT due to maternal TB. INH is primarily metabolized through acetylation in the liver and intestines, and the acetylation rate is genetically determined (8). Developmental and physiological changes in the volume of distribution, maturity of liver enzymes, and the role of acetylation capacity may influence INH dosage requirements in this vulnerable population (9, 10). The aim of this study was to determine the pharmacokinetics of routinely administered INH at 10 mg/kg of body weight/day in TB-exposed LBW infants. The World Health Organization (WHO) recommends a dosage range of 10 to 15 mg/kg daily. We also describe the influence of the N-acetyltransferase 2 ($NAT2$) genotype on INH pharmacokinetics.

MATERIALS AND METHODS

Design and setting. An intensive sampling pharmacokinetic study was conducted at Tygerberg Children's Hospital (TCH), Cape Town, South Africa, between May 2011 and May 2012. TCH is a tertiary referral hospital for 50,000 deliveries per annum. Here, the peak TB notification rate among young adults was >1,400 per 100,000 population in 2009 (11), and the provincial maternal HIV prevalence was 16.9% among public antenatal clinic attendees in 2009 (12). TCH manages an average of 6,000 high-risk complicated deliveries per year, 24% of which are LBW infants. The neonatal service has 136 neonatal beds and includes intensive- and high-care facilities.

Study procedures and drug administration. LBW infants born to HIV-infected and HIV-uninfected women were consecutively recruited if routinely receiving daily INH either for 6 months of IPT (10 mg/kg/day) or as in TB treatment, as per the local guidelines (13). The treatment for TB disease consisted of a 2-month intensive phase comprising daily INH, rifampin (RMP), and pyrazinamide (PZA), with or without ethionamide (ETH), followed by a 4-month continuation phase of INH and RMP alone. Multidrug-resistant TB (MDR-TB) preventive therapy (consisting of daily INH, ETH, and ofloxacin) was administered in consultation with a pediatric infectious diseases specialist. The eligibility criteria included

TABLE 1 Clinical characteristics of low-birth-weight infants at sampling ($n = 20$)[a]

Clinical characteristics	Values
Sex (male) (no. [%])	14 (70)
Ethnicity (black) (no. [%])	12 (60)[b]
HIV exposed (no. [%])	16 (80)
Exclusive breastfeeding (no. [%])	9 (45)
Formula feeding (no. [%])	11 (55)
Birth wt (median [IQR]) (g)	1,575 (1,190–2,035)
>1,500 and <2,500 (no. [%])	10 (50)
<1,500 (no. [%])	10 (50)
Gestational age (median [IQR]) (wk)	35 (34–38)
Term (≥38 wk) (no. [%])	3 (15)
Premature (<38 wk) (no. [%])	17 (85)
Wt on day of PK sampling (median [IQR]) (g)	1,874 (1,361–2,120)
Age at time of PK sampling (median [IQR]) (days)	14 (9–31)
Concomitant medications (no. [%])[c]	
Nevirapine[d]	15 (75)
Theophylline	8 (40)
Combination of anti-TB drugs (RMP, PZA, ETH, ofloxacin)	4 (20)[e]
cART (abacavir, lopinavir/ritonavir, lamivudine) and cotrimoxazole	1 (5)[f]
Hydrochlorothiazide and spironolactone	1 (5)[g]

[a] PK, pharmacokinetic; RMP, rifampin; PZA, pyrazinamide; ETH, ethionamide; cART, combination antiretroviral therapy.
[b] Seven infants of mixed race, one white infant.
[c] Some infants were on a different combination of the listed concomitant medications.
[d] Two infants on nevirapine (NVP) and isoniazid (INH) had a raised alanine aminotransferase (ALT) level at 3 months.
[e] Two infants on RMP and PZA; 1 infant on RMP, PZA, and ETH; 1 infant on ofloxacin and ETH.
[f] One infant with HIV infection was treated with cART and cotrimoxazole on day 59 of life.
[g] Used to control mild cardiac failure in infant with a ventricular septal defect.

TABLE 2 Summary of pharmacokinetic parameters in low-birth-weight infants ($n = 20$)

Pharmacokinetic parameters[a]	Median (IQR)[b]
C_{max} (μg/ml)	5.63 (4.86–7.53)
T_{max} (h)	2 (2–2)
AUC_{2-5} (μg · h/ml)	13.56 (11.75–19.10)
k_{el} (h^{-1})[c]	0.15 (0.10–0.23)
$t_{1/2}$ (h)[c]	4.69 (3.08–7.60)

[a] C_{max}, maximum drug concentration; T_{max}, time to C_{max}; AUC_{2-5}, area under the concentration-time curve; k_{el}, first-order elimination rate constant; $t_{1/2}$, half-life.
[b] IQR, interquartile range.
[c] k_{el} and $t_{1/2}$ not calculated for 1 patient due to continuous high plasma drug concentrations.

rately weighed to administer a dose of 10 mg/kg according to the weight of a newborn when naked (weighed by the study nurse on the day prior to study drug administration). The INH powder was dissolved in 1 to 2 ml of sterile water, administered through a nasogastric tube, and flushed with 1 ml of water.

Pharmacokinetic sampling and laboratory analysis. On the day of pharmacokinetic assessment, the INH dose, time of administration, and data obtained by phlebotomy were documented precisely. Four arterial blood specimens of 0.5 ml each were taken at 2, 3, 4, and 5 h postdose and collected in EDTA-coated tubes. The specimens were kept on ice and delivered to the laboratory within 30 min of collection. After centrifugation, the separation plasma fragment was assayed using the high-performance liquid chromatographic (HPLC) method described previously (16). The remaining blood cells were used to extract DNA for *NAT2* genotyping. Genomic DNA (gDNA) was prepared with a simple salting-out procedure for extracting DNA from human nucleated cells (17). The gDNA was analyzed for the *NAT2*5, *NAT2*6, *NAT2*7, *NAT2*12, *NAT2*13, and *NAT2*14 alleles via a PCR-based strategy (18). Separate PCR aliquots were restricted with the MspI, FokI, KpnI, TaqI, DdeI, and BamHI restriction enzymes to delineate the polymorphisms at nucleotide positions 191, 282, 481, 590, 803, and 857, respectively. According to Vatsis nomenclature, the wild-type fast allele (F) was assigned as *NAT2*4, *NAT2*12, or *NAT2*13 (19). These alleles confer normal enzyme activity on the NAT2 protein, while the mutant slow alleles (S), classified as *NAT2*5, *NAT2*6, *NAT2*7, and *NAT2*14 in humans, confer decreased enzyme activity on the *NAT2* protein. Accordingly, the study participants were classified as homozygous fast (FF), heterozygous intermediate (FS), or homozygous slow (SS) acetylators, depending on the allele combination observed.

Pharmacokinetic parameters and statistical analysis. The following pharmacokinetic parameters were generated using fixed sampling times for each patient through noncompartmental analysis (NCA): C_{max} (the maximum drug concentration observed), T_{max} (the time after drug administration to reach C_{max}), the AUC_{2-5} (area under the time-concentration curve from 2 to 5 h), $t_{1/2}$ (the plasma half-life), and the k_{el} (elimination constant). The AUC was calculated according to the linear trapezoidal rule. The pharmacokinetic parameters were summarized using medians and interquartile ranges (IQR), except for T_{max}, which was summarized using means and standard deviations (SD). The pharmacokinetic parameters for the infants were compared by *NAT2* genotype, birth weight, weight at the time of pharmacokinetic sampling, gestational age, age at sampling, gender, HIV exposure status, and feeding type. All covariates were analyzed dichotomously, except for *NAT2*, which was analyzed categorically into three levels: FF (fast), FS (intermediate), and SS (slow) acetylator types. Since the sample sizes were small, the dichotomous covariates were analyzed using the Wilcoxon rank-sum test. *NAT2* was analyzed using the Kruskal-Wallis test, and if statistically significant, the trend test was used to assess for trends across ordered *NAT2* groups. Dichotomous confounders, weight at pharmacokinetic sampling, and feeding type were assessed using Fisher's exact test. Any P value of <0.05

weight of ≥1.2 kg on the day of pharmacokinetic sampling, being clinically stable in room air and tolerating oral preparations, and written informed consent from the mother/legal guardian. The maternal HIV infection status was routinely determined by an enzyme-linked immunosorbent assay, preceded by informed consent and appropriate pre- and posttest counseling. All HIV-exposed newborns (those born to HIV-infected mothers) received nevirapine (NVP) for 6 weeks postpartum or until cessation of breastfeeding, according to national prevention of mother-to-child transmission (PMTCT) guidelines (14). Exclusive breastfeeding was encouraged.

Data on the following characteristics were collected for the infants: gender, ethnicity, birth weight, gestational age, weight and age at pharmacokinetic sampling, feeding type (breastfeeding versus formula), HIV exposure, and concomitant medications. The gestational age was determined by the date of the mother's last menstrual period, early ultrasound findings, and/or a Ballard score performed by a single experienced neonatologist. Data on alanine aminotransferase (ALT), a proxy for drug-related hepatotoxicity, were collected at baseline (at pharmacokinetic sampling) and at 3- and 6-month follow-up visits. The Division of Microbiology and Infectious diseases (DMID) tables were used to grade potential hepatotoxicity (15). The infants required regular feeding (every 2 to 3 h), as per routine care. INH in powder form, obtained from Fluka Chemie AG (Buchs, Switzerland), was used on the pharmacokinetic sampling days. The INH powder was accu-
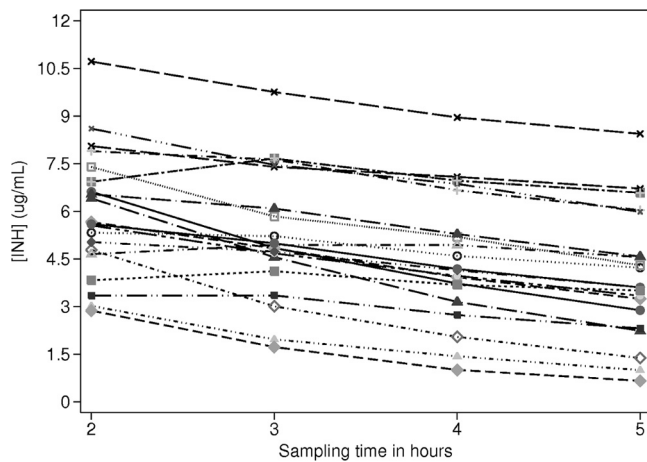
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FIG 1 Individual isoniazid drug concentrations in low-birth-weight infants ($n = 20$). Symbols show the progressions for individual infant subjects.

was considered statistically significant. All data were analyzed using Stata 12.1 special edition software (StataCorp, College Station, TX, USA).

Study approval was obtained from the Health Research Ethics Committee at Stellenbosch University (approval no. N10/07/232).

RESULTS

Twenty infants, 16 (80%) of whom were HIV exposed, were enrolled; 17 received IPT and 3 received treatment for TB. Ten infants (50%) had a birth weight of <1,500 g, and 17 (85%) were premature (Table 1). On the day of pharmacokinetic sampling, the median weight was 1,874 g (interquartile range, 1,361 to 2,120 g). Eight (40%) infants received theophylline for apnea of prematurity, and 15 (75%) of the 16 HIV-exposed infants received NVP for PMTCT (one HIV-infected infant received abacavir, lopinavir/ritonavir, and lamivudine).

The pharmacokinetic measures are shown in Table 2. The C_{max}, k_{el}, and consequently the $t_{1/2}$ could not be accurately determined for one infant in whom INH plasma concentrations remained high (2-h value, 4.6 µg/ml; 5-h value, 4.9 µg/ml). The $t_{1/2}$ ranged from 1.45 to 14.25 h. Figure 1 illustrates the INH plasma concentrations at 2, 3, 4, and 5 h postdose ($n = 20$). Nineteen infants achieved plasma concentrations above the reference of 3 µg/ml at 2 h (20), with a range of 2.9 to 10.7 µg/ml, while all 20 infants achieved target drug plasma concentrations of >1.5 µg/ml at 3 h postdose (21).

The pharmacokinetic parameters were compared by demo-

TABLE 3 Effect of clinical and other characteristics on isoniazid pharmacokinetic parameters in low-birth-weight infants ($n = 20$)

Subject characteristics	C_{max} parameter[a]			AUC$_{2-5}$ parameter[b]			$t_{1/2}$ parameter[c]		
	No. of infants	Median (IQR) value (µg/ml)	P	No. of infants	Median (IQR) value (µg · h/ml)	P	No. of infants	Median (IQR) value (h)	P
NAT2 genotype									
SS (slow)	5	6.54 (5.60–8.05)		5	16.93 (14.59–21.87)		5	6.56 (4.86–9.57)	
FS (intermediate)	11	6.41 (5.03–7.66)		11	13.21 (12.03–21.26)		11	4.52 (3.48–6.27)	
FF (fast)	4	3.90 (2.95–4.86)	0.0235	4	6.78 (4.96–11.31)	0.0199	3	1.78 (1.45–2.04)	0.0134
Birth wt									
Very low (<1,500 g)	10	6.58 (5.60–8.05)		10	15.35 (13.21–21.62)		10	4.56 (3.74–6.27)	
Low (≥1,500 to <2,500 g)	10	4.99 (4.11–6.41)	0.0284	10	12.61 (8.14–14.59)	0.0696	9	4.52 (1.95–6.56)	0.4142
Wt at PK time (g)									
<1,750	9	7.66 (5.60–8.05)		9	21.26 (13.21–21.62)		9	5.93 (4.12–9.15)	
≥1,750	11	5.03 (4.11–6.54)	0.0304	11	13.19 (8.14–14.59)	0.0441	10	3.60 (1.95–5.09)	0.0864
Gestational age									
<38 wk	17	5.66 (5.03–7.66)		17	14.48 (13.19–21.26)		16	4.69 (3.61–6.42)	
≥38 wk	3	4.11 (3.02–6.41)	0.1530	3	11.46 (5.41–12.03)	0.0502	3	2.04 (1.95–8.64)	0.4338
Corrected gestational age at PK time									
<36 wk	11	5.66 (4.94–8.05)		11	14.48 (13.09–21.62)		10	4.39 (3.74–6.27)	
≥36 wk	9	5.33 (4.11–6.54)	0.1837	9	13.19 (11.46–14.59)	0.1837	9	4.86 (2.04–6.56)	0.6242
Gender									
Female	6	5.14 (3.02–8.05)		6	14.54 (5.41–21.62)		5	6.27 (2.04–6.56)	
Male	14	6.04 (5.03–7.40)	0.4579	14	13.27 (12.03–16.93)	0.8690	14	4.39 (3.48–5.93)	0.7812
HIV status									
Exposed	16	5.45 (4.45–6.97)		16	13.49 (10.19–16.91)		15	4.52 (2.04–6.27)	
Negative	4	7.34 (6.14–9.39)	0.0472	4	17.60 (13.27–25.08)	0.1306	4	6.53 (3.08–11.91)	0.4237
Feeding type									
Breastfed	9	7.66 (5.66–8.05)		9	21.26 (13.33–21.62)		9	5.93 (4.12–9.15)	
Formula fed	11	4.94 (3.35–6.41)	0.0027	11	12.03 (8.14–14.59)	0.0135	10	4.13 (1.95–5.09)	0.1208

[a] C_{max}, maximum drug concentration; IQR, interquartile range.
[b] AUC$_{2-5}$, area under the concentration-time curve.
[c] $t_{1/2}$, half-life. The $t_{1/2}$ not calculated for 1 patient due to continuous high drug plasma concentrations.
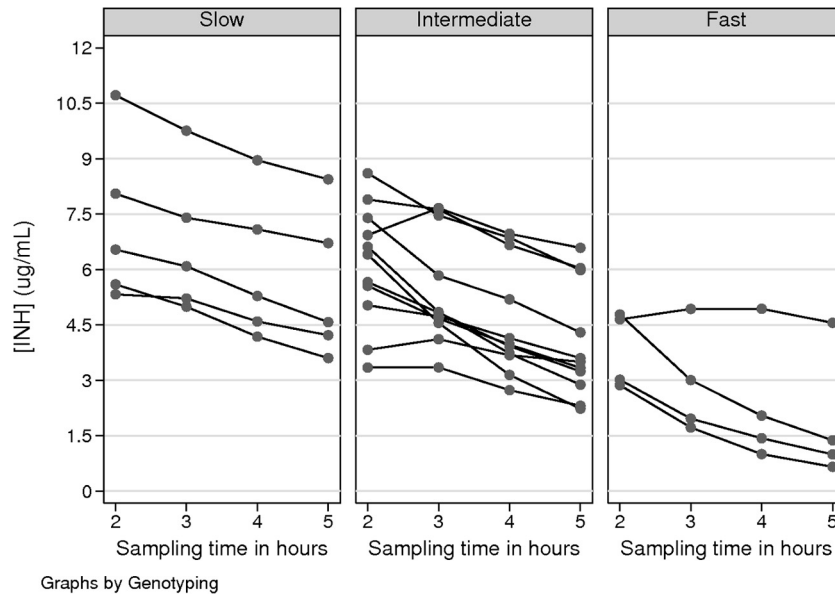
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FIG 2 Isoniazid concentrations in relation to *NAT2* genotyping by acetylator type. Each line represents a different infant subject (*n* = 20).

graphic and clinical covariates (Table 3). The distribution of acetylation status was as follows: 5 were slow, 11 were intermediate, and 4 were fast acetylators. Statistically significant differences in the C_{max}, AUC_{2-5}, $t_{1/2}$, and k_{el} were shown (P = 0.024, 0.020, 0.013, and 0.013, respectively) between slow, intermediate, and fast acetylators. Slow acetylators had a higher median C_{max} (6.5 μg/ml), larger AUC_{2-5} (16.93 μg · h/ml), and longer $t_{1/2}$ (6.56 h) than those of intermediate and fast acetylators, in decreasing order. Figure 2 illustrates INH concentrations in relation to the *NAT2* genotype.

Infants weighing <1,750 g on the day of pharmacokinetic sampling had a higher C_{max} and AUC_{2-5} than those of heavier infants (7.66 μg/ml versus 5.03 μg/ml [P = 0.030] and 21.26 μg · h/ml

versus 13.19 μg · h/ml [P = 0.044], respectively). The data in Fig. 3 suggest increased INH absorption and reduced clearance in smaller infants. Similar findings were observed in infants with younger gestational age; however, these differences were not statistically significant. Feeding also affected the C_{max} and AUC_{2-5} values: exclusively breastfed infants had a higher C_{max} and AUC_{2-5} than did formula-fed infants (7.66 μg/ml versus 4.94 μg/ml [P = 0.003] and 21.26 μg · h/ml versus 12.03 μg · h/ml [P = 0.014], respectively). Since weight at the time of pharmacokinetic sampling was associated with feeding status (P = 0.001), feeding and weight were likely confounded, with smaller babies more likely to receive breast milk. Gender did not influence the pharmacokinetic parameters.
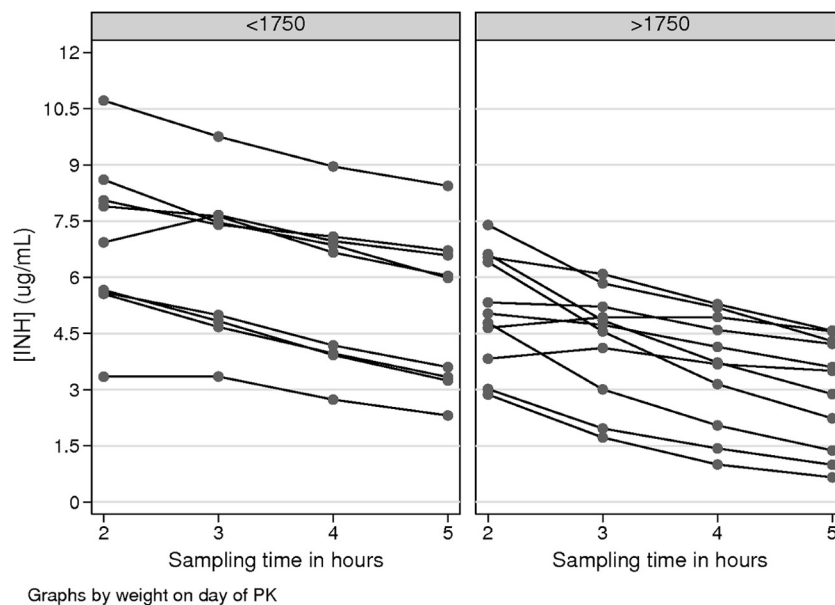

FIG 3 Isoniazid concentrations relative to current weight in low-birth-weight infants. Each line represents a different infant subject (*n* = 20).

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ALT levels were determined in 19 (95%) infants at baseline, in 14 (70%) at 3 months, and in 11 (55%) at 6 months. Two 3-month values were abnormal; 1 was mildly elevated (<2.5 times elevated [DMID grade 1]) and one moderately elevated (<5 times elevated [DMID grade 2]). The mildly elevated value was not repeated, and the moderately elevated ALT value normalized at 6 months. Both raised ALT values occurred in HIV-exposed infants receiving IPT and NVP.

DISCUSSION

This is the first study describing the pharmacokinetics of INH and correlating with *NAT2* genotypes in LBW and premature infants. The INH plasma concentrations in LBW infants, administered at a dose of 10 mg/kg, compared well to the published target adult values (20–22). An increased although variable half-life was observed in LBW infants, cautioning against higher-INH-dosing strategies. Markedly reduced clearance was present in smaller infants and in slow acetylators. The most important pathway for INH metabolism in humans is dependent on the trimodal *NAT2* acetylation (18) already apparent in this young age group.

Optimal and safe INH dosing for TB prevention and treatment are especially relevant for LBW infants. We administered INH at the lower end of the WHO recommended dosage of 10 to 15 mg/kg (23). The desirable pharmacokinetic targets for children include either a 2-h blood plasma concentration of 3 to 5 μg/ml (20) or a 3-h value of >1.5 μg/ml (21), both correlating with good clinical response in adults. We found good absorption of INH in all infants, and the adult pharmacokinetic target values were achieved. All but one infant achieved a 2-h drug plasma concentration of ≥3 μg/ml; the 2-h value for that infant was 2.9 μg/ml. All infants achieved a 3-h value of ≥1.5 μg/ml. The effect of routine feeding every 2 to 3 h for LBW infants did not influence the C_{max}. However, food intake is known to decrease the bioavailability of INH (24), and we were unable to evaluate pharmacokinetics in the absence of feeding. Concomitant medicines frequently used in LBW infants included theophylline and NVP, neither of which should impact the pharmacokinetic parameters of INH (25, 26).

In our study, we observed an increased but variable half-life (1.45 to 14.25 h) using a dose of 10 mg/kg of INH, in line with an earlier study of two neonates in whom the half-lives were 7.8 and 19.8 h, respectively (27). This is not surprising, since most drugs in newborns have a prolonged elimination half-life in general. This finding, however, cautions against using a high dose of INH in small infants. Furthermore, a study describing the INH pharmacokinetics according to phenotype, performed in 34 children (two infants < 1 month of age), showed a definite decrease of half-life with increasing age, suggesting slower elimination of INH in young children (28). Although desirable INH pharmacokinetic targets are essential for efficacy in infants, caution should be applied when dosing at the higher range to prevent potential toxicity.

In this study, markedly reduced INH clearance was noted in the smaller and younger LBW infants. INH has a significant first-pass metabolism, with the rate of drug metabolism depending largely on the maturation of hepatic enzymes. Impaired elimination in the smaller and younger LBW infants is therefore probably due to immature hepatic enzymes. The development of drug-metabolizing enzymes varies widely between neonates and may be prolonged in premature infants (29). Pharmacokinetic studies show that the grade of maturation of enzymes is the most impor-

tant factor in determining the rate of metabolism of a drug, with most liver enzymes maturing after the first year of life (30, 31). The effect of reduced INH elimination was even more pronounced in breastfeeding infants. However, this was confounded by the fact that smaller infants were more likely to receive breast milk. Antituberculosis drugs, including INH, are secreted in the breast milk of women on TB treatment, with levels ranging between 0.05 and 28% (32). The levels of antituberculosis drugs in breast milk are inadequate to prevent or treat infants but may increase the exposure to these medications. Therefore, Tran et al. recommended dosing at the lower end of the therapeutic range (i.e., 10 mg/kg/day of INH) in order to decrease the risk of potential toxicity (32).

Age and acetylator status influence INH pharmacokinetics in children (28, 33). INH is acetylated to acetylisoniazid by a hepatic and intestinal enzyme, *N*-acetyltransferase 2, which is coded for genetically. In our study, trimodal clearance of INH as a function of the *NAT2* genotype was already apparent, even in this young age group. Our results showed that all compared parameters were significantly different for all three acetylator groups, with slow acetylators having decreased elimination compared to intermediate and fast acetylators. Recent data from an IPT trial conducted in children 3 to 24 months of age illustrated not only the difference for each genotype group but also immature *NAT2* activity, particularly in fast acetylators, with the acquisition of activity to adult values occurring over the first 2 years of life (34). This is in keeping with the maturation of genetically determined *NAT2* activity over time. The impact of enzyme maturity on the INH dosing for LBW infants requires further study.

The transient elevation of blood serum transaminases occurs commonly with INH, but clinically manifested hepatotoxicity is rare. No formal relationship has been demonstrated between INH plasma concentrations and hepatotoxicity. Previous observations indicate that hepatotoxicity may be dose related (35, 36). In our study, only two ALT results were slightly raised at month 3, with the thrice-elevated value returning to normal at month 6. The limited safety data collected were reassuring, and no jaundice was observed in any infant. A limitation of the study was the few pharmacokinetic sampling points, mainly because of the small blood volume available in LBW infants. An earlier time point may have assisted with determining the C_{max} more accurately, as previous pharmacokinetic studies in children indicate that the T_{max} occurs any time between 1 and 2 h (28, 37).

In conclusion, a sound understanding of the pharmacokinetic properties of currently used antituberculosis drugs is essential for optimal use in newborns and infants. LBW infants receiving 10 mg/kg of INH had desirable blood drug concentrations, which were comparable to the adult target values. However, a prolonged half-life and reduced elimination of INH were noted in smaller and younger infants, especially in those genetically determined to be slow acetylators. Therefore, we caution against exceeding a dosage of 10 mg/kg in this population. Although no serious adverse effects were observed, more data on safety are needed. Also, more research is needed on the appropriate dosing requirements for TB drugs in newborns and infants.

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CHAPTER 7

PHARMACOKINETICS OF RIFAMPICIN, ISONIAZID, PYRAZINAMIDE, AND ETHAMBUTOL IN INFANTS

To ensure optimal curative treatment in infants (<12 months of age), a sound understanding is required of the pharmacokinetic properties of rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB), as drug disposition is influenced by developmental and physiological changes early in life (56). Age-dependent elimination of first-line antituberculosis drugs has been previously demonstrated, with substantially lower exposures documented in younger children for RMP (97-99), INH (97, 98, 153), PZA (97, 127), and EMB compared to adults (154). However, few pharmacokinetic studies have been conducted in children less than two years of age with TB (60, 97), and none specifically in infants. In addition, the World Health Organization (WHO) revised their first-line antituberculosis drug dosing guidelines for children in 2009, by recommending considerably higher doses of RMP (10-20 versus 8-12 mg/kg/day), INH (7-15 versus 4-6 mg/kg/day), PZA (30-40 versus 20-30 mg/kg/day), and EMB (15-25 versus 15-20 mg/kg/day) (39, 58). Study 4 was designed to test whether these substantially higher recommended mg/kg doses of first-line antituberculosis drugs, achieve adequate serum drug concentrations in infants, compared to current adult pharmacokinetic target concentrations.

An observational cohort study with intensive pharmacokinetic sampling (single day) was conducted among HIV-infected and HIV-uninfected infants routinely initiated on first-line antituberculosis treatment in Cape Town, South Africa, from March 2014 through March 2015. Participants were recruited from Tygerberg Hospital, Khayelitsha District Hospital and Brooklyn Chest Hospital. Routine South African National TB Programme (SANTP) antituberculosis drugs were given for the duration of TB treatment, and consisted of a fixed dose combination (FDC) tablet of RMP and INH (60:60) mg (Sandoz, South Africa), and a 500 mg PZA tablet (Sandoz, South Africa), with or without a 400 mg EMB tablet (Sandoz, South Africa). On the pharmacokinetic sampling day *only*, routine antituberculosis drugs were replaced by regulatory approved single-drug formulations. This strategy allowed for more accurate weight-banded drug dosing in our young study population, and more closely

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reflected the revised WHO recommended dosing guidelines. The new FDC tablets (1.5:1 ratio of RMP and INH), supporting the revised WHO dosing recommendations, were not yet available at the time the study was implemented, and the routinely used SANTP fixed-dose combination (FDC) tablets (1:1 ratio of RMP and INH) were not suitable for accurate dosing. A limitation of the study was that we did not procure all regulatory approved single-drug formulations prior to study implementation, although this provided us with RMP pharmacokinetic data for two RMP formulations. The manufacturer temporarily halted production of the RMP suspension (RMP formulation 1) halfway through the study; it was then substituted by a second RMP suspension (RMP formulation 2), manufactured in South Africa and registered by the Medicines Control Council of South Africa.

Pharmacokinetic blood samples were obtained pre-dose (0 hours) and at 1, 2, 4, 6 and 8 hours following the administration of the single-drug formulations. Blood samples for alanine aminotransferase (ALT) levels were collected on the day of pharmacokinetic sampling, and again at TB treatment completion (6 months after TB treatment initiation). Antituberculosis drug concentrations were determined at the Pharmacology Laboratory at the University of Cape Town using validated liquid chromatography mass spectrometry methods (136). Pharmacokinetic parameters were calculated using non-compartmental analysis (NCA). The C_{max}, AUC_{0-8} and $t_{½}$ for RMP, INH, PZA and EMB were compared by the clinical covariates of age at time of pharmacokinetic study evaluation (0-6 months versus 7-12 months), nutritional status (WAZ < -2 versus WAZ $\geq$ -2), prematurity (premature, < 37 weeks versus term), HIV status, ethnicity (black versus mixed race), gender and, for RMP pharmacokinetic parameters only, by RMP formulation and RMP dose (10-15 mg/kg versus 15-20 mg/kg). Multivariable regression models were generated to determine if any clinical covariates were associated with INH, RMP or PZA C_{max} or AUC_{0-8}.

Results

Thirty-nine infants on antituberculosis treatment were studied; 15 (38%) were premature (<37 weeks gestation) at birth, and 5 (13%) were HIV-infected and on antiretroviral therapy. The mean corrected age and weight was 6.6 months (standard deviation 3.3 months) and 6.45 kilograms (standard deviation 1.67 kilograms), respectively.

Table 1. Dosing ranges and pharmacokinetic parameters in infants on routine first-line antituberculosis drugs (n=39) (94)

First-line antituberculosis drugs	WHO recommended dose (mg/kg)	Mean (range) actual study dose (mg/kg)	Mean (range) C_{max} of study (μg/ml)	Adult PK target Concentrations (μg/ml)	C_{max} of study compared to adult PK target concentrations
Rifampicin	15 (10-20)	15 (10-21)	2.9 (0.6-7.9)	8-24	lower
Formulation 1 (n=14)		13 (10-18)	4.1 (0.7-7.9)		lower
Formulation 2 (n=25)		17 (10-21)	2.2 (0.6-6.9)		lower
Isoniazid (n=39)	10 (7-15)	13 (10-15)	7.9 (4 – 11.3)	3-6	similar
Pyrazinamide (n=39)	35 (30-40)	33 (28-38)	41.9 (26.3 – 68.4)	20-50	similar
Ethambutol (n=16)	20 (15-25)	20 (15-24)	1.3 (0.2 – 2)	2-6	lower

C_{max}=maximum serum concentration; PK=pharmacokinetics; WHO=World Health Organization

Very low RMP exposures were observed for both RMP formulations studied; none of the 39 infants achieved the minimum proposed target adult RMP peak concentration (C_{max}) of ≥ 8 μg/ml (94). Despite RMP formulation-1 being dosed at the lower range, the RMP C_{max} and AUC_{0-8} for RMP formulation-1 were significantly higher compared to RMP formulation-2 (C_{max}: 4.1 versus 2.2 μg/ml, p=0.007; AUC_{0-8}: 16.8 versus 9.5 μg.h/ml; p=0.021). In multivariable regression, adjusting for the effects of age and weight, RMP formulation was associated with RMP C_{max} and exposure. When stratified by RMP formulation, RMP dose did not have an effect. The bioavailability of these two RMP formulations was subsequently tested and is addressed elsewhere (89). For INH, all 39 infants achieved the proposed target adult C_{max} of ≥ 3 μg/ml (94). For PZA, all achieved the adult minimum target C_{max} of ≥ 20 μg/ml (94); 31/39 (80%) achieved a C_{max} ≥ 35 μg/ml for PZA, which was previously correlated with better TB treatment outcomes in adults (155). In this Botswana study, patients with a C_{max} of < 35 μg/ml were more than 3 times as likely to have poor TB treatment outcomes, compared to patients with a C_{max} of ≥ 35 μg/ml, after adjustment for HIV-infection and CD4 cell count (adjusted risk ratio, 3.38; 95% confidence interval, 1.84-6.22) (155). Multivariable analyses for PZA showed that HIV-infected infants had a lower C_{max} and AUC_{0-8} controlling for age and weight. This is similar to a Malawian study conducted in 27 children (mean age 5.7 years), where 18/27 (67 %) HIV-

infected children on PZA had a lower C_{max} than the PZA C_{max} observed in HIV-uninfected children (127). For EMB dosed at 20 mg/kg, only 1/16 (6%) infant achieved the minimum recommended adult C_{max} target value of ≥ 2 μg/ml (94). At doses exceeding 20 mg/kg ocular toxicity may occur (122). This cautions against higher EMB dosing, especially in infants where visual acuity and colour vision testing is challenging. No other important associations were found for the key pharmacokinetic parameters of first-line antituberculosis drugs including now effect of age, weight, weight-for-age Z-score, prematurity, HIV-status, ethnicity, gender or TB treatment outcome.

No study infants developed symptomatic hepatotoxicity. Alanine aminotransferase (ALT) values were performed at baseline and at treatment completion, 5/73 (7%) alanine aminotransferase (ALT) values were elevated in 5 infants. ALT values were graded by using the Division of AIDS (DAIDS) adverse event tables. Two Grade 1 adverse events (elevated 2-fold), and one Grade 3 event (elevated 7-fold) occurred on the day of pharmacokinetic visit; one Grade 1 event and one Grade 2 event (elevated 3-fold) occurred following 6 months of treatment. All resolved spontaneously at follow-up measurement. Final TB treatment outcomes were favourable (cure or treatment completion) in 37/39 (94%) infants while on routine programmatic FDC tablets (1:1 relation of RMP and INH), as per SANTP guidelines (156). TB treatment was reinitiated in four of them and six-months of TB treatment was subsequently completed. In two other infants, the treatment outcomes were unknown (both relocated to a different province after 3 months of treatment).

This is the first study reporting pharmacokinetic parameters for the four first-line antituberculosis drugs in infants, using the 2009 revised WHO dosing guidelines. INH, PZA and EMB concentrations were comparable to previous paediatric studies when administered at the revised higher WHO recommended doses, with INH and PZA reaching recommended adult target concentrations. However, RMP exposures were low regardless of the formulation used when administered at a dosing range of between 10-20 mg/kg. The effect of these low RMP exposures on TB treatment outcomes in infants with immature immunity is unclear. As routine programmatic antituberculosis FDC tablets were given for the duration of TB treatment in our cohort, we could not evaluate the relation between the low antituberculosis drug-

exposures for the single drug formulations and favourable TB treatment outcomes. Low RMP exposures described in paediatric pharmacokinetic studies (97-99, 157), supports RMP dose optimization in children. High RMP doses have become especially relevant in the context of treatment shortening regimens and when treating children with severe forms of TB. Future planned research by our group will investigate higher RMP doses in children, including safety, in line with adult studies (55). *N-acetyltransferase 2 (NAT2)* genotyping and enzyme maturation in the young child impacts on INH serum concentrations (158). *NAT2* genotyping was determined for all infants on INH in this study. We are currently in the process of analyzing the *NAT2* genotyping data in relation to the INH pharmacokinetic parameters, with the aim to determine the effect of acetylator status on INH exposures. Population pharmacokinetic modeling will be applied to a cohort of 96 children <2 years of age with both *NAT2* genotyping and INH pharmacokinetic measurements available; data from study 3 and 4 will be included. Data generated from this proposed study will clarify the role of enzyme maturation on target INH drug concentrations in young children.

Pharmacokinetics of Rifampin, Isoniazid, Pyrazinamide, and Ethambutol in Infants Dosed According to Revised WHO-Recommended Treatment Guidelines

A. Bekker,[a] H. S. Schaaf,[a] H. R. Draper,[a] L. van der Laan,[a] S. Murray,[b] L. Wiesner,[c] P. R. Donald,[a] H. M. McIlleron,[c] A. C. Hesseling[a]

Desmond Tutu TB Center, Department of Pediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa[a]; Department of Clinical Research, Global Alliance for TB Drug Development, New York, New York, USA[b]; Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa[c]

There are limited pharmacokinetic data for use of the first-line antituberculosis drugs during infancy (<12 months of age), when drug disposition may differ. Intensive pharmacokinetic sampling was performed in infants routinely receiving antituberculosis treatment, including rifampin, isoniazid, pyrazinamide, and ethambutol, using World Health Organization-recommended doses. Regulatory-approved single-drug formulations, including two rifampin suspensions, were used on the sampling day. Assays were conducted using liquid chromatography-mass spectrometry; pharmacokinetic parameters were generated using noncompartmental analysis. Thirty-nine infants were studied; 14 (36%) had culture-confirmed tuberculosis. Fifteen (38%) were premature (<37 weeks gestation); 5 (13%) were HIV infected. The mean corrected age and weight were 6.6 months and 6.45 kg, respectively. The mean maximum plasma concentrations (C_{max}) for rifampin, isoniazid, pyrazinamide, and ethambutol were 2.9, 7.9, 41.9, and 1.3 μg/ml, respectively (current recommended adult target concentrations: 8 to 24, 3 to 6, 20 to 50, and 2 to 6 μg/ml, respectively), and the mean areas under the concentration-time curves from 0 to 8 h (AUC_{0-8}) were 12.1, 24.7, 239.4, and 5.1 μg · h/ml, respectively. After adjusting for age and weight, rifampin exposures for the two formulations used differed in C_{max} (geometric mean ratio [GMR], 2.55; 95% confidence interval [CI], 1.47 to 4.41; $P = 0.001$) and AUC_{0-8} (GMR, 2.52; 95% CI, 1.34 to 4.73; $P = 0.005$). HIV status was associated with lower pyrazinamide C_{max} (GMR, 0.85; 95% CI, 0.75 to 0.96; $P = 0.013$) and AUC_{0-8} (GMR, 0.79; 95% CI, 0.69 to 0.90; $P < 0.001$) values. No other important differences were observed due to age, weight, prematurity, ethnicity, or gender. In summary, isoniazid and pyrazinamide concentrations in infants compared well with proposed adult target concentrations; ethambutol concentrations were lower but similar to previously reported pediatric studies. The low rifampin exposures require further investigation. (This study has been registered at ClinicalTrials.gov under registration no. NCT01637558.)

Infants (age <12 months) in settings with high-burden tuberculosis (TB) and HIV are at high risk of *Mycobacterium tuberculosis* exposure, infection, disease, and mortality, emphasizing the need for rigorous evidence from pharmacokinetic studies to guide optimal antituberculosis treatment in this vulnerable population. Up to 50% of infants exposed to and infected with *M. tuberculosis* will develop TB disease in the absence of preventive therapy, with up to 30% of these progressing to severe pulmonary or disseminated disease (1). A 4-fold increase in mortality has been reported among infants with maternal HIV-associated TB in India (2), while 24% mortality was observed in South African infants <3 months of age with culture-confirmed TB (3).

In 2010, the World Health Organization (WHO) revised pediatric TB dosing guidelines by recommending considerably higher doses of first-line antituberculosis drugs in children (rifampin [RMP], 10 to 20 versus 8 to 12 mg/kg of body weight/day; isoniazid [INH], 10 to 15 versus 4 to 6 mg/kg/day; pyrazinamide [PZA], 30 to 40 versus 20 to 30 mg/kg/day; and ethambutol [EMB], 15 to 25 versus 15 to 20 mg/kg/day) (4, 5). These guidelines were based on evidence from pharmacokinetic studies involving mainly older children, which showed that higher mg/kg body weight dosing was necessary to achieve equivalent adult target concentrations (6–10). The pharmacokinetic and safety profiles of antituberculosis drugs in infants at these higher doses remain largely unknown. Infants undergo developmental and physiological changes that may influence drug disposition and susceptibility to toxicity (11,

12). Enzyme maturation, efflux transporter activity, increased drug clearance for body weight, and formulations and their preparations are all important factors determining drug exposure in young children (12). Few pharmacokinetic studies have been completed in children <2 years of age with TB (13, 14), and none were specifically in infants. A study in Indian children found significantly lower maximum plasma concentrations (C_{max}) and areas under the concentration-time curves (AUC) for RMP, INH, and PZA in children <3 years of age compared to older children (ages 3.1 to 12.0 years) when the drugs were given thrice weekly, highlighting the need for age-dependent dosing considerations

(14). The aim of our study was to determine key pharmacokinetic parameters for RMP, INH, PZA, and EMB at the revised WHO-recommended doses in infants routinely treated for TB.

MATERIALS AND METHODS

Study design and setting. An observational cohort study with intensive pharmacokinetic sampling (single day) was conducted among HIV-infected and -uninfected infants routinely initiating first-line antituberculosis treatment in Cape Town, South Africa, from March 2014 through March 2015. Participants were recruited from Tygerberg Children's Hospital, Khayelitsha District Hospital, and Brooklyn Chest Hospital.

Study population and antituberculosis drugs. HIV-infected and -uninfected infants were eligible after at least 2 weeks of intensive phase first-line antituberculosis treatment, allowing for RMP autoinduction (15). Infants were consecutively recruited after parental informed consent was obtained. HIV testing was routinely performed. TB treatment consisted of a standard 2-month intensive phase of RMP, INH, and PZA, with or without EMB, given once daily, 7 days a week, followed by a 4-month continuation phase of INH and RMP at the same doses (16). Weight-banded doses were consistent with the WHO interim guidelines for treatment of TB in young children using the available dispersible fixed-dose combinations for children. EMB was added at clinician discretion (typically in the case of more severe TB disease), as per national guidelines (16). Routine South African national TB program antituberculosis drugs, consisting of a fixed-dose combination (FDC) tablet of RMP and INH (60:60 mg) (Sandoz, South Africa) and a 500-mg PZA tablet (Sandoz, South Africa) with or without a 400-mg EMB tablet (Sandoz, South Africa), were given for the duration of TB treatment. Nevirapine (NVP) as part of prevention of mother-to-child transmission and combination antiretroviral therapy (cART) consisting of abacavir, lamivudine, and lopinavir/ritonavir were given to HIV-exposed and -infected infants, respectively (17).

Pharmacokinetic sampling was deferred in critically ill infants, those with a weight of <1.8 kg, and those with a hemoglobin level of <8 g/dl. On the pharmacokinetic sampling day only, routine antituberculosis drugs were replaced by single-drug formulations. This allowed for more accurate dosing in the very young study population, more closely reflecting the revised WHO-recommended dosing guidelines, since the routinely used FDC tablets (1:1 relation of RMP and INH) were not suitable for accurate dosing and the new FDC tablets (1.5:1 relation of RMP and INH) supporting the revised WHO dosing recommendations were not yet available at the time of the study. INH, PZA, and EMB were available in tablets of 50, 150, and 100 mg, respectively, and RMP was a granulate for suspension (100 mg/5 ml; Eremfat, referred to here as RMP formulation 1). The RMP, INH, and EMB formulations were manufactured by Riemser Arzneimittel, Germany, were registered with a stringent regulatory authority, and appeared on the global fund list of approved medicines. Pyrazinamide (Svizera Laboratories, Pty Ltd., the Netherlands) was manufactured in a WHO-certified facility complying with good manufacturing practices. In June 2014, Riemser temporarily halted the manufacturing of Eremfat, necessitating its substitution with a second RMP suspension, R-cin (100 mg/5 ml; manufactured by Aspen Pharmacare, South Africa, and registered by the Medicines Control Council of South Africa; referred to here as RMP formulation 2).

Sample size calculations. The aim of the study was to enroll approximately 40 infants, based on prior knowledge of RMP variability, reported RMP exposures in adults and children, and the feasibility of enrolling this substantial number of infants with TB over a 12- to 18-month accrual period.

Pharmacokinetic investigation. Infants were fasted for 2 h prior to TB drug dosing to facilitate improved absorption (18, 19). A nasogastric tube (NGT) was inserted to ensure accurate dosing, and the pH of gastric aspirates was measured using an instacheck 0-13 pHydrion pH test kit (Micro Essential Laboratory, USA). An indwelling peripheral venous catheter was inserted, and a predose (0-h) blood sample was obtained for

drug concentration determination and for albumin and alanine aminotransferase (ALT) levels. The RMP suspension, followed by INH and PZA with or without EMB tablets crushed and mixed with water, was given via NGT, which was then flushed with 5 ml of water. Five more samples of 0.6 ml each at 1, 2, 4, 6, and 8 h postdose were collected in EDTA-coated tubes and centrifuged to separate the plasma before freezing at −80°C within 30 min of sampling. Drug concentrations were determined at the pharmacology laboratory at the University of Cape Town using validated liquid chromatography-mass spectrometry methods. The methods were validated over the concentration ranges of 0.0977 to 26.0 μg/ml for isoniazid, 0.117 to 30.0 μg/ml for rifampin, 0.200 to 80.0 μg/ml for pyrazinamide, and 0.0844 to 5.46 μg/ml for ethambutol (20). All samples that were below the level of quantification (BLQ) were set to half of the lower limit of quantification (LLOQ). ALT measures were repeated at the end of 6 months of TB treatment.

Pharmacokinetic parameters and statistical analysis. Pharmacokinetic parameters were calculated using noncompartmental analysis (NCA). Stata 12.1 SE software (StataCorp 2011, College Station, TX, USA) was used for analyses. C_{max} and time to C_{max} (T_{max}) were recorded directly from the concentration-time data. The AUC_{0-8} was calculated using the linear trapezoidal rule. The elimination half-life ($t_{1/2}$) was denoted as $\ln2/K_{el}$, where K_{el} (elimination rate constant) was the negative slope of the log-linear regression of three final data points of the concentration-time curve. The C_{max}, AUC_{0-8}, and $t_{1/2}$ for RMP, INH, PZA, and EMB were compared by the clinical covariates of age at time of pharmacokinetic study evaluation (0 to 6 versus 7 to 12 months), nutritional status (Weight-for-age Z-score [WAZ] < −2 versus ≥ −2), prematurity (premature, <37 weeks, versus term), HIV status, ethnicity (black versus mixed race), and gender as well as, for RMP pharmacokinetic parameters only, by RMP formulation and RMP dose (10 to 15 versus 15 to 20 mg/kg). All comparisons were generated using t tests. For preterm infants, ages corrected for gestational age were calculated. Age was analyzed with and without corrected ages; no differences were found, so unadjusted age at the time of pharmacokinetic sampling was used. WAZs were calculated using the United Kingdom WHO preterm growth chart, in which preterm infant (<37 weeks) WAZs were adjusted for gestational age (21). Clearance was calculated for all first-line antituberculosis drugs using dose and AUC_{0-8} for age comparisons. Adverse events were classified using the division of AIDS (DAIDS) tables. TB treatment outcomes were defined using standard international criteria (22): cured or treatment completion was considered favorable, while treatment failure, death, and loss to follow-up, including treatment interruption of >2 months, were classified as unfavorable outcomes. TB treatment outcome was compared by the C_{max} and AUC_{0-8} for the first-line antituberculosis drugs.

Multivariable regression models were generated to determine if any clinical covariates were associated with the INH, RMP, or PZA C_{max} or AUC_{0-8}. In total, six models were generated using the INH, RMP, and PZA log-transformed C_{max} and AUC_{0-8} values as dependent variables, each analyzed separately. The geometric mean ratios (GMR) were reported along with 95% confidence intervals (CI) and P values. The clinical covariates considered for inclusion into the models were age at time of pharmacokinetic study (continuous), weight on pharmacokinetic day (continuous), HIV status, prematurity (premature versus term), ethnicity, nutritional status (WAZ < −2 versus WAZ ≥ −2), and gender. Covariates with a P value of <0.10 and factors known to affect drug disposition (age and weight) were included in each multivariable model. To determine if RMP dose was associated with C_{max} or AUC_{0-8}, a subanalysis was done using multivariable linear regression stratified by RMP formulation, where dose was analyzed, categorically controlling for continuous age and weight. The resulting regression coefficients (βs) for dose, along with the corresponding 95% CI and P values, are reported.

Regulatory approval. The study (NCT01637558) was approved by Medicines Control Council of South Africa, by the research ethics committees of Stellenbosch University (N13/03/031), and by the University of Cape Town (180/2011).

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RESULTS

Patient characteristics at pharmacokinetic assessment. Table 1 displays the characteristics of the 39 infants, of whom 15 (39%) were documented as premature. At pharmacokinetic sampling, the mean age, corrected for gestational age, and weight were 6.6 months and 6.5 kg, respectively. *M. tuberculosis* infection was confirmed in 14 (36%) infants. Twenty-two (56%) infants were born to HIV-infected mothers; 5 infants seroconverted shortly after birth. All 5 HIV-infected infants were established on cART at the time of pharmacokinetic assessment. All infants received RMP, PZA, and INH; 16 (41%) also received EMB. Ethionamide was given in 7 (18%) infants ($n = 2$ with TB meningitis, $n = 1$ with miliary TB, and $n = 4$ due to clinician preference). The dosing ranges and summary statistics for the pharmacokinetic parameters for RMP (i.e., formulation 1 and formulation 2), INH, PZA, and EMB are displayed in Table 2. No difference was noted for clearance of the first-line antituberculosis drugs by age (data not shown).

Rifampin. None of the 39 infants achieved the target adult peak RMP concentration of ≥ 8 μg/ml. Table 3 provides the RMP pharmacokinetic parameter comparisons for C_{max}, AUC_{0-8}, and $t_{1/2}$ by clinical covariates; the comparison for RMP C_{max}, AUC_{0-8}, and $t_{1/2}$ by RMP formulation and RMP dose are also displayed. On the pharmacokinetic sampling day, 14 of 39 (36%) infants received RMP formulation 1 (median dose, 12.1 mg/kg) and 25 of 39 (64%) infants received RMP formulation 2 (median dose, 18.6 mg/kg; difference between the median doses of RMP formulations was $P = 0.025$). Despite the lower dose range of RMP formulation 1, the RMP C_{max} and AUC_{0-8} for RMP formulation 1 were higher than those of RMP formulation 2 (C_{max}: 4.1 versus 2.2 μg/ml, $P = 0.007$; AUC_{0-8}: 16.8 versus 9.5 μg · h/ml; $P = 0.021$). While this comparison was not adjusted for dose, it is worthwhile to note that the lower-dosed formulation resulted in higher C_{max} and AUC_{0-8}. When the analysis of formulation was adjusted for dose, the differences in C_{max} and AUC_{0-8} remained, and the formulation effect was increased in the same direction (results not shown). In multivariable analysis stratified on formulation, RMP dose (10 to 15 versus 15 to 20 mg/kg) was not associated with either C_{max} or AUC_{0-8} after adjusting for age and weight (C_{max} for subset of RMP formulation 1: $\beta_{dose} = 1.33$, 95% CI, -0.43 to 3.09, $P = 0.132$; C_{max} for subset of RMP formulation 2: $\beta_{dose} = 2.02$, 95% CI, -1.30 to 5.34, $P = 0.206$; AUC_{0-8} for subset of RMP formulation 1: $\beta_{dose} = 5.93$, 95% CI, -2.17 to 14.03, $P = 0.143$; AUC_{0-8} for subset of RMP formulation 2: $\beta_{dose} = 9.81$, 95% CI, -3.68 to 23.31, $P = 0.136$). Figure 1 illustrates RMP concentrations over time by formulation, including suggested adult target values (23, 24). In multivariable regression adjusting for the effects of age and weight, RMP formulation was associated with RMP peak concentrations and exposure. For C_{max}, the GMR for the RMP formulation 1 to the RMP formulation 2 was 2.55 (95% CI, 1.47 to 4.41; $P = 0.001$); for AUC_{0-8}, the GMR for the same comparison was 2.52 (95% CI, 1.34 to 4.73; $P = 0.005$).

Isoniazid. All 39 infants achieved the target adult peak INH concentration of ≥ 3 μg/ml. Table S1 in the supplemental material provides the INH pharmacokinetic parameter comparisons for C_{max}, AUC_{0-8}, and $t_{1/2}$. Infants with a lower WAZ (≤ -2.0) had a shorter $t_{1/2}$ (1.77 versus 2.2 h; $P = 0.046$). Figure 2 shows the INH concentration over time for infant participants, including re-

TABLE 1 Characteristics of infants with tuberculosis at the time of pharmacokinetic sampling ($n = 39^a$)

Characteristic	Value
No. (%) male	26 (67)
No. (%) black ethnicity	29 (74)
Mean (SD) corrected age, mo	6.6 (3.3)
Mean (SD) weight, kg	6.45 (1.67)
Mean (SD) WAZ[b]	−1.62 (1.53)
Mean (SD) WLZ[c]	−0.41 (1.26)
No. (%) with premature birth (<37 wk gestational age)	15 (38)
Median (IQR[d]) birth weight, kg	2.70 (2.33–3.08)
Mean (SD) hemoglobin, g/dl	10.9 (1.6)
Mean (SD) albumin, g/liter	42 (3)
No. (%) with gastric pH ≤3 ($n = 11$)	11 (100)
Maternal HIV status	
No. (%) with HIV-infected mother	22 (56)
Median maternal log viral load ($n = 15$)	2.8
No. (%) with maternal cART/PMTCT[e] ($n = 21$)	15 (71)
Infant HIV status	
No. (%) HIV infected ($n = 39$)	5 (13)[f]
Median (IQR) infant CD4 percentage	35.4 (26.9–38.5)
Median (IQR) infant log viral load	6.33 (6.14–6.7)
Median (IQR) cART in weeks before PK study	8 (5–8)
Infant TB characteristics	
No. (%) with culture and/or Xpert MTB[g]/rifampin confirmed	14 (36)[h]
Median (IQR) duration of TB treatment in weeks at PK	7 (5–9)
No. (%) with BCG[i] vaccination history/scar	32 (82)
No. (%) with positive tuberculin skin test ($n = 16$)	7 (44)
No. (%) with documented TB source case ($n = 28$)	28 (72)
No. (%) with mother as source case	15 (54)
No. (%) with other household TB source case	8 (29)
No. (%) with nonhousehold TB source case	5 (17)
No. (%) with smear/culture and/or Xpert positive source case	18 (64)
No. (%) with type of TB[j]	
PTB	36 (92)
EPTB ($n = 1$ TBM)	1 (3)
PTB and EPTB ($n = 1$ miliary TB; $n = 1$ TBM)	2 (5)
Mean (range) routine antituberculosis drug dose, mg/kg	
Rifampin ($n = 38$)	13.8 (9.0–19.7)
Isoniazid ($n = 38$)	13.8 (9.0–19.7)
Pyrazinamide ($n = 39$)	31.5 (18.7–44.6)
Ethambutol ($n = 16$)	19.9 (13.3–29.0)
No. (%) with concomitant medications[k]	
ABC, 3TC, LPV/r[l]	5 (12)
Prednisone	1 (3)
Diuretics and spironolactone	1 (3)

[a] Total number of infants evaluated was 39, unless otherwise noted in table.
[b] WAZ, weight for age Z-score.
[c] WLZ, weight for length Z-score.
[d] IQR, interquartile range.
[e] cART, combination antiretroviral therapy; PMTCT, prevention of mother-to-child transmission.
[f] Three of these mothers received no PMTCT or cART before delivery.
[g] Xpert: Gene Xpert for MTB/rifampin.
[h] Culture and Xpert positive for 10 infants ($n = 3$, only culture; $n = 1$, only Gene Xpert).
[i] BCG, bacillus Calmette-Guérin.
[j] Types of TB: PTB, pulmonary TB; EPTB, extrapulmonary TB.
[k] Nevirapine therapy was completed for HIV-exposed infants; steroid therapy, for severe airway compression; diuretics, for ventral septal defect.
[l] ABC, abacavir; 3TC, lamivudine; LPV/r, lopinavir and ritonavir.

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TABLE 2 Summary statistics for infant pharmacokinetic parameters of rifampin, isoniazid, pyrazinamide, and ethambutol ($n = 39^a$)

First-line antituberculosis drug	Recommended dose (range) (mg/kg)	Mean (range) actual dose (mg/kg)	Mean (range) C_{max} (µg)	Mean (IQR[b]) T_{max} (h)	Mean (range) AUC_{0-8} (µg · h/ml)	Mean (range) $t_{1/2}$ (h)[c]	Mean (range) CL/F^d (liter/h/kg)
Rifampin	15 (10–20)	15.37 (10.13–20.51)	2.90 (0.59–7.96)	2.0 (2.0–2.0)	12.12 (1.78–33.01)	2.05 (1.06–4.06)	2.57 (0.37–16.68)
Formulation 1 ($n = 14$)		12.91 (10.13–18.18)	4.13 (0.65–7.96)	2.0 (1.0–2.0)	16.77 (1.59–33.01)	2.07 (1.11–4.06)	1.55 (0.37–7.07)
Formulation 2 ($n = 25$)		16.75 (10.13–20.51)	2.22 (0.59–6.94)	2.0 (2.0–2.0)	9.52 (1.78–32.29)	2.04 (1.06–3.93)	3.14 (0.58–16.68)
Isoniazid	10 (10–15)	12.80 (10.31–15.38)	7.92 (3.97–11.30)	1.0 (1.0–1.0)	24.68 (11.56–50.18)	2.00 (1.00–3.92)	0.57 (0.26–1.00)
Pyrazinamide	35 (30–40)	33.32 (28.48–38.46)	41.9 (26.3–68.4)	1.0 (1.0–2.0)	239.4 (147.1–450.0)	8.01 (4.40–20.63)	0.15 (0.08–0.20)
Ethambutol ($n = 16$)	20 (15–25)	20.18 (15.38–24.10)	1.26 (0.24–2.01)	2.0 (1.5–3.0)	5.09 (1. 24–8.87)	3.59 (1.61–6.94)	4.69 (2.13–12.45)

[a] Total number of infants evaluated was 39 unless otherwise noted.
[b] IQR, interquartile range.
[c] $t_{1/2}$, half-life of the drug. Due to late peaks, $t_{1/2}$ was not calculated in the following infant samples: $n = 4$ rifampin (of $n = 36$); $n = 1$ isoniazid (of $n = 38$); $n = 1$ pyrazinamide (of $n = 38$); and $n = 1$ ethambutol (of $n = 15$).
[d] CL, clearance; F, fraction absorbed.

ported adult target values (23, 24). No significant associations were found in log-transformed multivariable regression.

Pyrazinamide. All 39 infants achieved adult peak PZA concentrations of ≥20 µg/ml, and 31 of 39 (80%) achieved a C_{max} of ≥35 µg/ml. Table S2 in the supplemental material provides the PZA pharmacokinetic parameters comparisons for C_{max}, AUC_{0-8}, and $t_{1/2}$. Younger infants had a longer $t_{1/2}$ (8.92 versus 7.01 h; $P = 0.047$), while malnourished infants, versus those with normal nu-

TABLE 3 Rifampin pharmacokinetic parameters by age, nutritional status, prematurity, HIV status, ethnicity, gender, formulation, and dose ($n = 39$)

Variable	Parameter data for:								
	C_{max} (µg/ml)			AUC_{0-8} (µg · h/ml)			$t_{1/2}$ (h)[a]		
	No. evaluated	Mean (SD) value	P	No. evaluated	Mean (SD) value	P	No. evaluated	Mean (SD) value	P
Age at PK sampling, mo									
0–6	20	2.89 (2.16)		20	12.78 (9.72)		17	2.17 (0.70)	
7–12	19	2.92 (2.24)	0.975	19	11.43 (9.63)	0.664	19	1.95 (0.88)	0.425
Nutritional status on day of PK									
≥−2.0	21	2.65 (1.94)		21	10.60 (8.20)		20	2.02 (0.78)	
<−2.0	18	3.21 (2.44)	0.429	18	13.90 (10.93)	0.289	16	2.08 (0.84)	0.830
Prematurity									
Term (≥38 wk)	24	2.73 (1.90)		24	11.46 (8.65)		23	1.99 (0.84)	
Premature (<37 wk)	15	3.18 (2.60)	0.538	15	13.18 (11.12)	0.593	13	2.17 (0.73)	0.520
HIV status									
HIV infected	5	3.67 (3.31)		5	16.46 (15.00)		5	2.27 (1.14)	
HIV uninfected	34	2.79 (2.00)	0.404	34	11.48 (8.66)	0.283	31	2.02 (0.75)	0.522
Ethnicity									
African	29	3.00 (2.37)		29	12.04 (10.20)		28	1.88 (0.64)	
Mixed race	10	2.63 (1.52)	0.644	10	12.37 (7.94)	0.926	8	2.67 (1.04)	0.011
Gender									
Female	13	3.27 (2.39)		13	13.00 (10.01)		13	1.75 (0.57)	
Male	26	2.72 (2.08)	0.468	26	11.68 (9.52)	0.692	23	2.22 (0.87)	0.088
Rifampin formulation									
Formulation 1	14	4.13 (2.35)		14	16.77 (10.50)		14	2.07 (0.84)	
Formulation 2	25	2.22 (1.77)	0.007	25	9.52 (8.11)	0.021	22	2.04 (0.79)	0.918
Rifampin dose, mg/kg									
10–15	17	2.74 (2.34)		17	10.86 (10.01)		17	1.91 (0.71)	
15–20	22	3.03 (2.09)	0.691	22	13.10 (9.34)	0.447	19	2.17 (0.87)	0.340

[a] $t_{1/2}$, half-life of the drug.
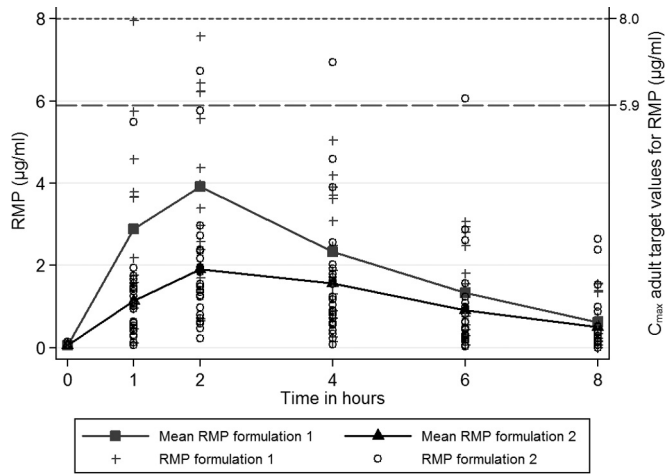
FIG 1 Mean plasma rifampin (RMP) concentrations (µg/ml) after the intake of a mean dose of 12.9 mg/kg for RMP formulation 1 ($n = 14$) and of a mean dose of 16.7 mg/kg for RMP formulation 2 ($n = 25$). C_{max} adult target values, 8 to 24 µg/ml when RMP is administered at 600 mg daily in American adults (23); C_{max}, 5.9 µg/ml when RMP is administered at 10.9 mg/kg in South African adults (24).

FIG 3 Mean plasma pyrazinamide (PZA) concentrations (µg/ml) after the intake of a mean dose of 33.3 mg/kg of PZA ($n = 39$). Adult target C_{max} values, 20 to 50 µg/ml when PZA is administered at 25 mg/kg/day in American adults (23); C_{max}, 52.7 µg/ml when INH is administered at 35.7 mg/kg in South African adults (24).

trition, had a higher AUC_{0-8} (268.09 versus 214.72 µg · h/ml; $P = 0.018$). Figure 3 shows the PZA concentration over time, including reported adult target values (23, 24). Multivariable analyses from a log-transformed regression model for PZA showed that HIV-infected infants had a lower C_{max} (GMR, 0.85; 95% CI, 0.75 to 0.96; $P = 0.013$) and a lower AUC_{0-8} (GMR, 0.79; 95% CI, 0.69 to 0.90; $P = 0.001$), controlling for age and weight. No other significant associations were observed.

Ethambutol. Only one of 16 infants (6%) had a $C_{max} >2$ µg/ml, which is the recommended adult target value (Fig. 4). Table S3 in the supplemental material displays the EMB pharmacokinetic parameter comparisons for C_{max}, AUC_{0-8}, and $t_{1/2}$. HIV-infected infants had a lower C_{max} (0.4 versus 1.4 µg/ml; $P = 0.004$) and a

lower AUC_{0-8} (2.0 versus 5.5 µg · h/ml; $P = 0.008$) than HIV-uninfected infants.

Safety. The median ALT at the time of sampling was 21 U/liter (interquartile range [IQR], 15 to 35 U/liter; $n = 37$) and at treatment completion was 22 U/liter (IQR, 18 to 30 U/liter; $n = 36$). In total, 5 of 73 (7%) ALT values in 5 separate infants were elevated. Two grade 1 adverse events (elevated 2-fold) and one grade 3 event (elevated 7-fold) occurred on the pharmacokinetic visit; one grade 1 event and one grade 2 event (elevated 3-fold) occurred following 6 months of treatment. All resolved spontaneously at a follow-up measurement. These safety data are relevant only to the routine antituberculosis drugs, as study drugs were administered only on the PK sampling day.

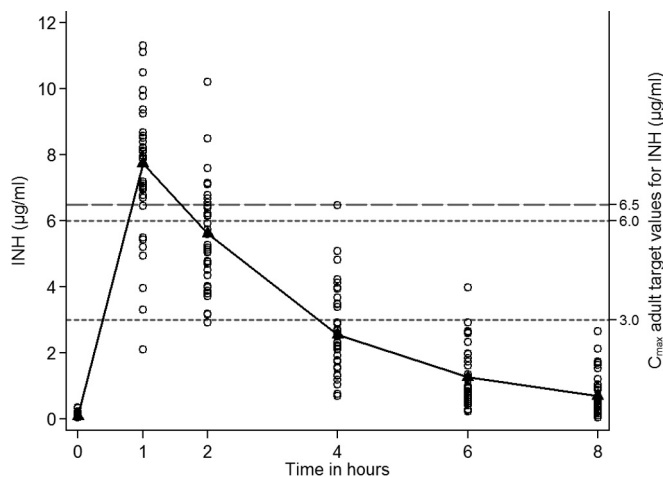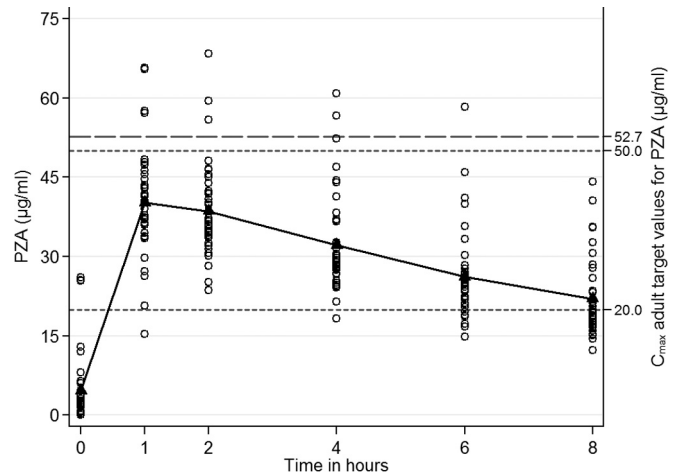**TB treatment outcome.** Thirty-three of the infants (85%) had favorable TB treatment outcomes; cure occurred in 4, and treat-
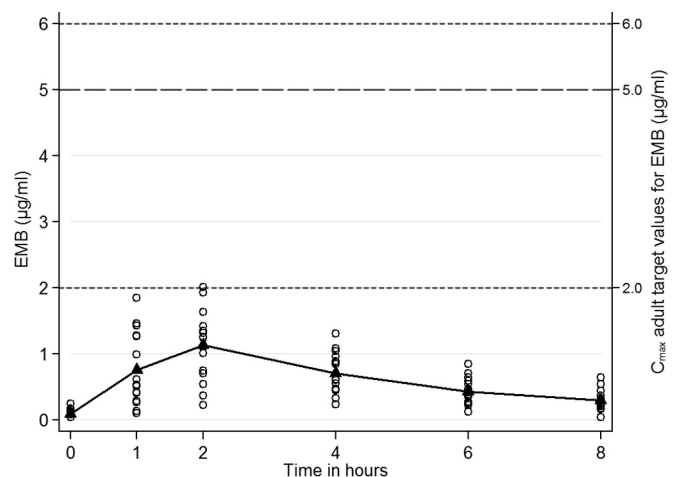

FIG 2 Mean plasma isoniazid (INH) concentrations (µg/ml) after the intake of a mean dose of 12.8 mg/kg of INH ($n = 39$). Adult target C_{max} values, 3 to 6 µg/ml when INH is administered at 300 mg daily in American adults (23); C_{max}, 6.5 µg/ml when INH is administered at 6.5 mg/kg in South African adults (24).

FIG 4 Mean plasma ethambutol (EMB) concentrations (µg/ml) after the intake of a mean dose of 20.2 mg/kg of EMB ($n = 16$). Adult target C_{max} values, 2 to 6 µg/ml administered at 25 mg/kg/day in American adults (23); C_{max}, 5 µg/ml when EMB is administered at 24.5 mg/kg in South African adults (24).

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ment completion was documented in 29. Of six infants, all with poor social circumstances, initially classified as having unfavorable treatment outcomes, 4 infants ($n = 1$ HIV-infected and $n = 3$ HIV-exposed) initially classified as lost to follow-up were successfully reinitiated on TB treatment ($n = 1$ each at month 3, 4, 5, and 6) and completed 6 months of TB treatment. In the other 2 infants, the treatment outcome was unknown (both relocated to a different province after 3 months of treatment). C_{max} and AUC_{0-8} for RMP, INH, PZA, and EMB were not associated with TB treatment outcome.

DISCUSSION

This is the first study reporting pharmacokinetic parameters for the four first-line antituberculosis drugs, using the 2010 revised WHO dosing guidelines, specifically in infants. Encouragingly, infants demonstrated INH and PZA concentrations similar to those reported in adult studies; EMB concentrations were low but comparable to other pediatric pharmacokinetic studies using the same dose. However, very low RMP exposures were observed for both RMP formulations studied. RMP formulation 2, which was dosed at a higher dosing range, had lower RMP C_{max} and AUC_{0-8} than RMP formulation 1. The RMP dose was not associated with RMP C_{max} and AUC_{0-8} when stratified by formulation; therefore, the formulation probably contributed more than the dose toward the observed difference in RMP pharmacokinetic parameters. PZA and EMB exposures were lower in the few HIV-infected infants studied. No other important associations were found for the key pharmacokinetic parameters of first-line antituberculosis drugs by age, weight, weight-for-age Z-score, prematurity, HIV status, ethnicity, gender, or TB treatment outcome. No infants developed symptomatic hepatotoxicity, and at least 85% of infants had a favorable TB treatment outcome.

A markedly low RMP mean C_{max} of 2.9 μg/ml was observed in infants, even when dosed at 12 to 20 mg/kg/day. Desirable RMP 2-hour postdose concentrations of between 8 and 24 μg/ml have been suggested in adult healthy volunteers and in TB patients receiving a dose of 9 to 12 mg/kg (23); however, few adult and pediatric patients reach the often-recommended minimum RMP 2-hour concentration of 8 μg/ml (24, 25). In our study, RMP exposures were low using both RMP suspensions, and no infant achieved a 2-hour value of >8 μg/ml. Infants on RMP formulation 1 achieved significantly higher exposures than those on RMP formulation 2, despite the lower-mg/kg administered dose (Fig. 1). RMP dose itself was not associated with RMP C_{max}, or AUC_{0-8}; however, these findings and conclusions must be interpreted with caution. Further analysis showed that the formulation mainly contributed toward observed differences in pharmacokinetic parameters, emphasizing the importance of bioavailability of drug formulations and the use of high-quality formulations. High-performance liquid chromatography (HPLC) testing revealed similar RMP concentrations for both formulations, and, in a laboratory-based study, aliquots of the RMP formulation 1 used in the study that were left to stand for 30 min at room temperature in 3 nasogastric tubes had RMP concentrations similar to a control aliquot of the suspension (26). McIlleron et al. (26) also reported no impact of NGT drug delivery on the effect of RMP formulation on the AUC in the large DATiC (dosing antiretroviral- and tuberculosis drugs in children) study, where children were given an RMP suspension either by NGT or *per os*. The peak RMP concentrations of 4.0 and 2.2 μg/ml for the two different RMP formulations,

respectively, were similar to those reported in other pediatric studies, documenting low peak concentrations of 2.8, 2.9, 3.5, 3.8, and 3.9 μg/ml following an oral administration of 10 to 20 mg/kg (27–31). Higher median RMP peak concentrations of 10 and 12 μg/ml were achieved when dosed at 10 and 15 mg/kg ($P = 0.008$), respectively, in Indian children (32). RMP mean peak concentrations of 6.4 and 11.7 μg/ml, when dosed at 10 and 15 mg/kg ($P = 0.005$), respectively, were observed in 11 South African children younger than 2 years of age using a different RMP formulation. The authors noted that children with high drug concentrations at the lower RMP dose did not necessarily have comparatively high drug concentrations following the higher dose (13). RMP formulations are known for considerable intra- and interindividual variability, which may partly be explained by differences in RMP bioavailability. In a study with 20 children, only 50% ± 22% of a freshly prepared oral suspension was absorbed compared to an intravenous dose (33). Although the cause for this variable RMP bioavailability remains unclear, the following factors have been proposed: raw material characteristics, changes in the crystalline habit of the RMP, excipients, manufacturing and/or process variables, degradation in the gastrointestinal tract, and inherent variability in absorption and metabolism (34). RMP is the only hydrophobic drug of the first-line antituberculosis drugs, is characterized by low solubility, is easily adsorbed by common pharmaceutical excipients, and also shows a pH-dependent solubility affecting its absorption (35). All of these factors highlight the need for stringent quality assurance of antituberculosis drugs in clinical care and research. A limitation of this study was that all of the study drugs were not purchased prior to the start of the study. The effect of gastric pH on RMP bioavailability remains unclear. A low gastric pH, as found in 11 study infants tested, was associated with low RMP exposures; Khalil et al. described reduced oral absorption of RMP when combined with antacids (36).There are limited data available on the ontogeny of the expression of p-glycoprotein, known to influence RMP absorption, with developmental differences in efflux transporter activity markedly altering the bioavailability of drugs (37). The specific relevance of p-glycoprotein in the dosing of young children with RMP requires further investigation in young children.

All infants had 2-hour plasma INH concentrations of >3 μg/ml following a mean dose of 12.8 mg/kg, which is above the suggested adult lower limit target range of 3 to 6 μg/ml (23). Infant INH concentrations also compared well with other pediatric pharmacokinetic studies with similar 2-hour concentrations reported of 4.5, 5.6, and 3.9 to 8.6, following 10 to 15 mg/kg (7, 27, 38). The effect of acetylator status on INH exposures is not reported here (future work).

PZA is probably the best absorbed among the first-line antituberculosis drugs, and infants achieved a high mean C_{max} of 41.9 μg/ml at 1 h, following a dose of 33.3 mg/kg. A proposed PZA adult target range of 20 to 60 μg/ml has been suggested (23), but a study from Botswana showed that a C_{max} of less than 35 μg/ml was associated with a poor outcome in adult pulmonary TB patients (39). Our results were similar to other pediatric studies reporting peak concentrations of 34.6 to 47.8 μg/ml when dosed at 30 to 40 mg/kg (8, 13, 28, 32).

The mean EMB C_{max} of 1.3 μg/ml at 2 h following a mean dose of 20.2 mg/kg was low, with a proposed adult therapeutic 2-hour peak concentration of 2 μg/ml (23). Other pediatric studies reported similarly low concentrations of between 0.78 and 2.1

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μg/ml when dosed at 10 to 20 mg/kg (27, 28, 32, 40, 41). Increasing EMB dosage in children has been reported to result in higher peak concentrations (42, 43); however, dose-dependent ocular toxicity is a limiting factor in dosing increases (44) especially in young children, in whom clinical evaluation is more challenging. In a recent study, 3 of 11 children <5 years of age developed reversible visual impairment secondary to 23, 25, and 27 mg/kg/day of EMB (45). Routine eye examinations are not common in resource-constrained settings; therefore, caution should be advised with increased EMB dosage. Ethionamide is sometimes preferentially given to infants due to clinical concerns of optic neuritis. EMB is the least efficacious of the first-line antituberculosis drugs and is used mainly to protect companion drugs against resistance. The clinical relevance of these findings in infants, who typically have paucibacillary TB and in whom the theoretical risk of acquision of drug resistance is low, needs further evaluation.

Age-dependent elimination of first-line antituberculosis drugs has been shown, with lower exposures documented in younger children for RMP (10, 14, 40), INH (14, 40, 46), PZA (8, 14), and EMB (42). These findings have mainly been attributed to the more rapid clearance of drugs and to a relatively larger liver-to-body size ratio. In our study, however, where all children were <1 year of age, age did not influence clearance. To our knowledge, this is the first study specifically performed in infants on antituberculosis drugs that also incorporated postconceptual age, and we found no remaining influence of prematurity. There is no clear evidence regarding the potential association between low TB drug exposures and concomitant cART (8, 13, 27, 47). In our study, we observed lower PZA and EMB exposures in HIV-infected infants; however, we studied only 5 HIV-coinfected infants. The higher PZA AUC_{0-8} observed in malnourished infants, compared to those with normal nutrition, was confounded by the fact that malnourished infants were dosed at the higher end of the dosing range.

Hepatotoxicity is the main adverse event associated with RMP, INH, and PZA. All infants were asymptomatic for symptoms and signs of hepatotoxicity, but ALT values were raised in 5 (7%) infants while on routine antituberculosis drugs. However, ALT values returned to normal within 3 months without intervention. Few pediatric studies have reported on hepatotoxicity at the revised WHO dosing recommendations. Hiruy et al. documented an ALT range of 9 to 71 U/liter in 31 children younger than 10 years at the revised higher doses (27). Two (3.1%) of 64 children developed hepatitis on the previously recommended lower doses, and 3 (4.8%) of 63 children did so on the revised higher doses in a study conducted in Indian children younger than 15 years of age (32).

The majority of infants had favorable TB treatment outcomes, despite the observed low RMP exposures for both formulations and the extensive disease in most. Higher RMP dosing strategies are currently being investigated in early bactericidal activity studies in adults; the short-term safety results have been reassuring. In adult RMP dose-escalation studies of up to 35 mg/kg/day for 2 weeks, higher doses showed a disproportionate plasma exposure increase with dose and a greater fall in bacterial load (48). Multiple factors influence the relationship between drug concentrations and TB treatment outcome, including *M. tuberculosis* genotype, gene polymorphisms, extent of TB disease, bacillary burden, and immune status, including HIV infection status (46). A limitation of our study is that the study drug was administered only on the pharmacokinetic day, while routine drugs were used for the 6-month treatment; this limits the interpretation of drug concentrations on TB treatment outcome.

NCA was used due to its timeliness and also for ease of interpretation to a broad clinical audience. Multivariable analysis was used to determine if pharmacokinetic parameters were associated with clinical covariates applicable to a clinical audience. In the absence of pediatric pharmacodynamic data, the optimal required target concentrations remain unclear and are typically extrapolated from adult studies. Furthermore, direct comparison of pharmacokinetic studies is complicated by variations in dose administered, type of formulation, single drugs versus fixed-dose combinations, daily versus intermittent therapy, and different sampling schedules, all of which influence plasma drug concentrations. In our study, INH, PZA, and EMB concentrations were comparable to other pediatric studies when dosed at the revised higher WHO recommendations, with INH and PZA reaching recommended adult target concentrations. However, RMP exposures were low regardless of the formulation used when administered at a dosing range of 10 to 20 mg/kg, highlighting the importance of using high-quality formulations and of bioavailability studies for RMP formulations in children and potentially arguing in favor of using dispersible tablets instead of liquid formulations in young children. In addition, the low RMP exposures argue for RMP dose-optimization studies in children. Reported TB treatment outcomes are generally good in children with drug-susceptible TB, independent of drug concentrations achieved, but this may be different for infants with immature immunity, for HIV-infected children, and in those with extensive and disseminated TB disease. Therefore, the low RMP exposures may become increasingly relevant in children who receive treatment-shortening regimens and in children with severe forms of TB; both populations are currently under evaluation.

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CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

The interwoven epidemics of tuberculosis (TB) and human immunodeficiency virus (HIV) have led to a global resurgence of TB, especially in sub-Saharan Africa (1). In settings with high prevalence of HIV, women of childbearing age (20-39 years) are disproportionately affected by TB (2, 4). Infants (<12 months of age) born to women with TB are therefore also at high risk of *Mycobacterium tuberculosis* exposure (33, 34) early in life. Up to 50% of infants infected with *M. tuberculosis* will develop TB disease in the absence of preventive chemotherapy and bacillus Calmette-Guérin (BCG) vaccination (32, 34). Poor completion of isoniazid preventive therapy (IPT) in children (44, 46, 47) contributes towards increased risk of TB and TB-related morbidity. Addressing barriers to IPT delivery in newborns may lead to improved IPT initiation and completion in resource-constrained settings.

Rigorous pharmacokinetic data regarding the use of first-line antituberculosis drugs in neonates (<28 days of age) and infants, where developmental changes may substantially influence drug disposition (56), is essential to inform the effective and safe dosing of antituberculosis drugs in young children. Prior to the pharmacokinetic study described in chapter 6, no pharmacokinetic data were available to guide the use of isoniazid (INH), a key first-line antituberculosis drug, in neonates, and there was very limited pharmacokinetic data regarding the use of first-line antituberculosis drugs in infants. The aim of this dissertation was therefore to comprehensively investigate strategies to prevent and treat perinatal and infant TB in the context of maternal HIV infection.

The first study (chapter 3) demonstrated that a high number of neonates were investigated for TB at a large provincial referral hospital, Tygerberg Hospital (TBH) in Cape Town, South Africa. Despite the high background prevalence of TB and HIV in this setting, no routine data had previously been collected on maternal-infant TB in this setting. Despite the retrospective nature of this study, valuable information was obtained by documenting high TB exposure among neonates. Seventy neonates (60%

HIV-exposed) were investigated for TB during a single year. Maternal TB was the primary indication for TB screening among infants. Nearly three-quarters of neonates screened for TB were initiated on IPT due to concerns regarding maternal infectiousness, suggesting delayed TB diagnosis in pregnant women. Data on the completion of IPT was limited, although overall the IPT completion was poor. Telephonic follow-up at one-year post hospital discharge traced only a third of neonates confirmed to have initiated IPT. Of the 12 traceable infants, only 5 had completed 6 months of IPT. This study highlighted the critical gaps in knowledge and implementation for maternal and infant TB control interventions in high prevalence TB and HIV-endemic settings, informing the design and implementation of a prospective cohort study (study 2; chapters 4 and 5). This next prospective study aimed to generate more rigorous clinical and epidemiological data on maternal TB disease and maternal and infant outcomes, collected prospectively. Identifying barriers to IPT completion in TB-exposed neonates was also prioritized as an important research gap to be addressed.

The second study (chapter 4) therefore prospectively enrolled pregnant and postpartum women on TB treatment at TBH during 2011. Of 74 women routinely treated for TB, 35 (47%) women were diagnosed with TB only upon hospital admission for labour *or* within 6 weeks of delivery. This delayed TB diagnosis in pregnant women illustrated the lack of appropriate antenatal TB screening and the frequency of delayed presentation and diagnosis of TB in the postpartum period. Furthermore, 22 (30%) of 74 women reported a previous TB treatment episode, highlighting the importance of previous TB episodes as a risk factor for maternal TB. Consideration of previous TB may therefore be useful as an additional part of current World Health Organization (WHO) TB-screening algorithms in pregnant women, given the poor performance of the algorithm in HIV-infected pregnant women documented in two recent studies with reported sensitivities of 28% and 54%, respectively (9, 10). In addition, improved molecular diagnostic tools, including Xpert MTB/RIF (Cepheid), which was not available at the time of the study, may potentially decrease diagnostic delays and result in more rapid initiation of TB treatment for pregnant women. Xpert MTB/RIF would also allow for the identification of potential drug-resistant TB, which was present in 6/42 (14%) women with bacteriologically-confirmed TB in our study. Maternal HIV infection was associated with high maternal

and newborn mortality in this study. All five TB-related maternal deaths occurred in HIV-infected women, and the four stillbirths and six newborn deaths were all from HIV-infected women. A large proportion 53/74 (72%) of these women were HIV co-infected, many with severe immune suppression. Only 34/53 (64%) HIV-infected women received any form of prevention of mother-to-child transmission (PMTCT) therapy or combination antiretroviral therapy (cART) in the context of widespread access to ART in the public sector. Not surprisingly, women presented with severe extrapulmonary TB (EPTB) manifestations, i.e. TB meningitis, TB spine, TB pericarditis, abdominal TB and TB-associated bacteraemia, experiencing high morbidity. Earlier initiation of ART in HIV-infected pregnant women could reduce the high morbidity and mortality presently observed in HIV co-infected women and their infants.

Maternal TB treatment outcomes in this cohort of pregnant women, who mostly had high-risk pregnancies, were poor. TB cure and treatment completion (favourable TB treatment outcomes) was achieved in only 41/74 (55%) of pregnant women treated for TB. Intensified scale-up of TB prevention, diagnosis and treatment interventions in PMTCT and TB programmes will lead to earlier TB diagnosis during pregnancy and better completion and outcomes of maternal TB treatment. Both TB and HIV impact adversely on perinatal outcomes and have been associated with premature and low birth weight (<2500g; LBW) deliveries (29, 159). This study also showed a high risk of delivering a LBW infant in the presence of maternal TB (59%), against an already high background of 37% LBW deliveries at our referral hospital during the study period (144). Earlier treatment of both TB and HIV will likely lead to healthier pregnant women who are more likely to carry to term, reducing the risk of preterm labour and LBW infants. In a pregnant woman with TB, giving birth to a LBW infant was associated with increased risk of unfavourable maternal TB treatment outcomes (OR 3.83, 95% CI 1.40-10.53, p=0.009). It is possible that women with LBW infants would have had additional challenges to attend TB clinics for their own treatment, given the challenges of caring for these LBW infants. Two (3%) of 72 live born infants had bacteriologically-confirmed TB (2 sets of twins), and completed 6 months of antituberculosis treatment. Three (7%) of 45 HIV-exposed infants had HIV-infection diagnosed on routine postnatal testing and were initiated on cART. This vertical transmission rate for HIV was higher than national transmission rate of 2.8%

in 2011 (160), however this cohort consisted of a selected group of high-risk pregnancies. Important clinical epidemiological data were generated by prospectively characterizing TB during pregnancy and systematically documenting perinatal, infant and maternal TB treatment outcomes. Data generated from this study contributed towards current understanding of maternal and infant TB in the context of maternal HIV, and may assist with improved strategies to prevent perinatal and infant TB.

Of note was the large proportion of TB-exposed newborns who required IPT because of a maternal infectious TB source case. Unsupervised IPT completion rates in children below 30% were reported from two studies in Cape Town (50, 51). Very few of the newborns initiated on IPT at TBH in the first study of this dissertation were traceable and known to have completed IPT. This led me to conduct a prospective study to identify barriers to completion of IPT in TB-exposed newborns. A cohort of 56 newborns who had been routinely initiated on IPT at TBH in 2011 was enrolled. Of the 56 TB-exposed newborns included, 49 had a maternal TB source case and 7 had another infectious household TB-source case. The aim of this study was to identify health system, socio-economic and maternal predictors of IPT completion among TB-exposed newborns. A dedicated hospital-based TB care team followed 44/56 newborns to 6 months; 12 patients left the study (1 died, 4 withdrew and 7 were loss to follow-up). Twenty-nine (66%) of the 44 TB-exposed newborns who were followed to 6 months completed IPT without study team intervention. Linkage to care, which included an appropriate TB treatment referral resulted in improved IPT completion, after adjusting for maternal HIV status and type of caregiver. In conclusion, a simple intervention, including hospital-community linkage of care and appropriate TB referral from hospital to local TB clinic, resulted in improved IPT completion in TB-exposed newborns. Further operational research is required in South Africa and other high-burden settings, to identify health system strengthening strategies to improve the down-referral of newborns initiated on IPT from hospital to community-based TB clinics.

Antituberculosis drug doses in newborns and infants

Infants undergo substantial physiological changes influencing drug disposition during the first year of life (56, 57). For this reason, achieving safe and effective dosing of first-line antituberculosis drugs in neonates and infants is an important consideration

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for the chemoprevention and treatment of perinatal and infant TB. Although children with drug-susceptible TB often have paucibacillary TB disease and good treatment outcomes, infants are more prone to develop progressive pulmonary and disseminated TB disease, highlighting the importance of optimal antituberculosis drug concentrations in infants to ensure good outcomes. Substantial uncertainty remains around the optimal target concentrations and exposures for several of the first-line antituberculosis drugs. Additional studies are needed to establish the appropriate dose in children, in order to inform paediatric dosing to achieve current adult target levels. Recent studies have shown that higher mg/kg body weight doses of antituberculosis drugs are necessary in children to achieve comparable adult antituberculosis drug target concentrations. This evidence led to the WHO revising its paediatric antituberculosis dosing guidelines for first-line antituberculosis drugs in 2009, recommending higher doses in children (58). Pharmacokinetic evidence for these higher doses is lacking in premature and LBW infants, with only sparse pharmacokinetic data available for infants overall. These higher doses should be thoroughly investigated in neonates and infants, where reduced clearance of drugs may lead to high exposures and possible toxicity, and too low a dose may not be successful in achieving cure.

In the third study, an intensive pharmacokinetic sampling study was conducted in 20 LBW infants receiving INH at a dose of 10 mg/kg/day, prescribed in the course of routine care for the prevention or treatment of TB disease. To our knowledge, this was the first INH pharmacokinetic study conducted in LBW infants. The median 2-hour post dose INH concentration in LBW infants was 5.63 μg/ml, ranging from 2.9 – 10.7 μg/ml, which is similar or higher than the recommended adult minimum 2-hour post dose concentration of 3 μg/ml (94). Very low birth weight (VLBW; < 1500 grams) infants had significantly higher INH exposures, compared to infants weighing between 1500 – 2500 grams, suggesting reduced INH elimination in the smaller infants. All 20 LBW infants phenotypically behaved as slow acetylators, reflected by the high-observed INH concentrations. Interestingly, the trimodal N-acetyltransferase 2 (NAT2) acetylation pattern was already apparent at this very early stage of development. Genotypically slow acetylators had a decreased elimination rate compared to genotypically intermediate- and fast acetylators. NAT2 enzyme maturation differences have never been documented this early in life. Zhu and

colleagues illustrated a different NAT2 enzyme maturation profile present at 3 months of age for each of the three acetylation groups in another study, with no change in the apparent clearance of the slow acetylator group over the course of 24 months (133). Further research on the developmental expression of the *NAT2* gene in the neonatal period and infancy is needed to understand the impact of enzyme maturation on clearance, and ultimately inform dosing of INH in infancy. The limited safety data collected during our study was reassuring; however, caution should be expressed against exceeding an INH dose of 10 mg/kg/day in this very young paediatric population, given the INH exposures achieved using the lower range of the current WHO dosage recommendations.

The aim of the fourth study was to determine whether the revised higher 2009 WHO-recommended doses in children for RMP, INH, PZA and EMB, achieved adequate drug concentrations in infants compared to current adult pharmacokinetic target concentrations for these drugs. An observational cohort study with intensive pharmacokinetic sampling was conducted among HIV-infected and HIV-uninfected infants who were routinely started on first-line antituberculosis treatment, from March 2014 through March 2015 in Cape Town, South Africa. On the pharmacokinetic sampling day *only*, routine antituberculosis drugs were replaced by regulatory approved single-drug formulations to more closely reflect the revised WHO recommended doses. Thirty-nine infants on RMP, INH and PZA were studied; 14 on RMP formulation-1 and 25 on RMP formulation-2. Sixteen infants were also on EMB and pharmacokinetic samples were obtained for all first-line antituberculosis drugs. Very low RMP exposures were observed for both RMP formulations studied, when dosed at 10-20 mg/kg. Not one of the 39 infants achieved the minimum proposed target adult peak concentration (C_{max}) of ≥ 8 μg/ml (94). Few adult and paediatric patients reach this often-recommended minimum RMP 2-hour target of 8 μg/ml, when dosed at 8-12 mg/kg (95, 96). This fixed RMP dose of 600 mg in adults (8-12 mg/kg in children) was originally recommended on the basis of cost rather than on efficacy or safety (55, 161). Higher RMP dosing strategies are currently being investigated in adults in the context of dose-escalation and efficacy trials. RMP given at 35 mg/kg/day for two weeks in adults, showed a disproportionate plasma exposure increase with dose, a greater fall in bacterial load, and provided reassuring short-term safety results (55). There is a need to conduct RMP dose-escalation studies in

children. Low RMP exposures may become increasingly relevant in the context of treatment shortening regimens in children and in children with severe forms of TB, e.g. TB meningitis. For INH, all 39 infants achieved the proposed target adult C_{max} of ≥ 3 μg/ml (94), and *NAT2* genotyping analysis is pending for this cohort. For PZA, all infants achieved the adult minimum target C_{max} of ≥ 20 μg/ml (94). PZA differs from other first-line antituberculosis drugs regarding the need of a higher mg/kg dose in children than in adults to achieve the same C_{max}. In a literature review, Donald et al., demonstrated little difference between the C_{max} achieved in children and adults following various mg/kg body weight doses (162). For EMB, dosed at 20 mg/kg, only one of sixteen infants achieved a $C_{max} \geq 2$ μg/ml, which is the recommended adult C_{max} target value (94). Due to EMB dose-related ocular toxicity (122) and difficulty of testing vision in infants, caution should be advised against higher dosing of EMB in infants at this stage. No other important associations were found for the key pharmacokinetic parameters of first-line antituberculosis drugs including effect of age, weight, weight-for-age Z-score, prematurity, HIV-status, ethnicity, gender or TB treatment outcome. There were no clinically significant safety concerns and no indication of liver toxicity.

Summary of pharmacokinetic studies

Data from the pharmacokinetic studies forming part of this dissertation showed relatively high INH C_{max} in LBW infants dosed at 10 mg/kg/day, compared to adult proposed INH target C_{max}. Both the smaller infants, and the genotypically slow acetylators, had higher INH exposures and longer half-lives, suggesting reduced elimination of INH. This study showed that the INH dose in premature and LBW infants should probably not exceed 10 mg/kg/day. The final study was conducted in infants treated for TB disease at the revised higher mg/kg body weight dose recommended by WHO for first-line antituberculosis drugs (58). Similar INH and PZA target C_{max} were achieved in infants, compared to adults. However, low target C_{max} in infants were observed for RMP and EMB, compared to adults. RMP is difficult to dose, with considerable intra- and interindividual variability, partly explained by differences in RMP bioavailability (89, 92). In addition, uncertainty exists around the historical target RMP C_{max} of 8 μg/ml in adults, chosen mainly due to cost implications rather than for efficacy and safety reasons (55). Recent RMP dose-escalation studies in adults show promise with regards to better efficacy, and

low toxicity when administering RMP at higher doses over short dosing periods. Although EMB peak concentrations in children were low, the dose dependent adverse effect of optic neuritis currently prevents the recommendation of substantially higher doses at this stage. More pharmacokinetic data of first-line antituberculosis drugs are needed in a larger numbers of neonates and infants of different genetic backgrounds, with and without HIV co-infection and with different drug doses, to determine optimal dosing in this age group. Furthermore, there is a need for better defining target pharmacokinetic concentrations of first-line antituberculosis drugs in neonates and infants to guide dose finding. Higher doses of RMP in infants should be considered. A pharmacokinetic/pharmacodynamic model-based approach, using pharmacokinetic data from children and adults, including outcome data from adult TB meningitis trials, showed that RMP doses of at least 30 mg/kg/day are needed to attain proposed target RMP exposures (163).

Future directions

Several factors should be considered in the prevention and treatment of perinatal and infant TB. Important considerations for reducing the burden of TB in infants are the prevention, early diagnosis and optimal treatment of TB disease in pregnant women. Untreated TB in pregnancy poses a significant risk to the mother and her newborn and is associated with high morbidity and mortality (20, 28, 143, 149). The WHO recommends all four first-line antituberculosis drugs (RMP, INH, PZA and EMB) for use in pregnancy (164), which are widely regarded as safe and do not appear to have harmful effects on the foetus. Although the US Centers for Disease Control and Prevention (CDC) does not recommend the routine use of PZA during pregnancy, CDC supports the inclusion of PZA for HIV-infected pregnant women, as it may outweigh the undetermined potential risk to the foetus (165). Isolated cases of drug-induced hepatitis on antituberculosis drugs have been reported in pregnancy (166), but this low risk of fatal liver damage is also present in non-pregnant women (35, 167). Uncertainty exists as to whether the risk of drug-induced hepatitis is increased during pregnancy.

Ongoing and future research

The International Maternal, Pediatric, Adolescent AIDS Clinical Trials (IMPAACT) network have recently completed enrolment for the P1078 trial, including several

South African sites, to evaluate the safety of antepartum versus postpartum INH in HIV-infected pregnant women (n=950). Safety data generated from this trial will inform much needed guidance on the use of INH in HIV-infected pregnant women. Limited pharmacokinetic data of first- and second-line antituberculosis drugs are available in pregnant women with TB to guide the optimal dosing of both first- and second-line antituberculosis drugs during pregnancy (168). The IMPAACT P1026 study is currently investigating pharmacokinetic properties of first and second-line antituberculosis drugs in pregnant women with and without HIV co-infection. Evidence from this study will hopefully guide safe and effective dosing in pregnant and postpartum women with TB. Early initiation of ART in HIV-infected pregnant women also reduces TB disease progression, and the risk of TB and HIV in newborn infants. Integration of maternal HIV and TB services is essential to improve early case detection.

The incidence of TB during pregnancy remains unknown and there are poor data to inform global estimates of TB burden during pregnancy. The development and implementation of a TB pregnancy registry at national and international level to record and report cases of TB in pregnant women, may assist in better estimating the true TB incidence and treatment outcomes during pregnancy. As a first step towards establishing better data on TB in pregnant women, a TB pregnancy registry protocol has been developed to establish the first global registry of pregnancy-associated TB (Chair: Bekker; beyond the scope of this PhD). The proposed project would pool data on pregnant women enrolled in completed, ongoing, and planned trials of HIV and TB treatment, generating much needed data on TB during pregnancy and outcomes of women and infants affected by TB.

Robust and representative pharmacokinetic data of antituberculosis drugs in LBW infants are needed to inform the appropriate use of the first-line antituberculosis drugs. No data are available for second-line antituberculosis drugs in this population. The ongoing multi-center IMPAACT P1106 study (protocol vice-chair: Bekker) aims to describe the pharmacokinetic and safety characteristics of ART and first-line antituberculosis medicines in LBW infants. Mathematical modeling (population pharmacokinetic models) will be done to optimally characterize the pharmacokinetics of these drugs in LBW infants, and inform dosing guidelines. Future research should

further study the association between INH concentrations and *NAT2* genotyping in young children. The role of enzyme maturation in this population should be explored, as this may potentially influence dosing recommendations. Low RMP concentrations observed in the infant pharmacokinetic study (study 4) were of concern. A phase I/II open label multi-cohort dose escalation study to evaluate the pharmacokinetics and safety of increased doses of RMP in HIV-negative children with TB disease will start recruitment in the last quarter of 2016. Findings from this study will potentially inform future dosing for TB treatment shortening trials in children and provide guidance on optimal treatment of severe forms of TB disease in infants, including TB meningitis.

In summary, pregnant women and TB-exposed infants face a considerable burden of TB disease, especially in the context of maternal HIV infection. Pregnant women and infants should be included in observational and interventional trials of TB prevention and treatment. Concurrently, health systems should be strengthened and antenatal TB screening, diagnosis and treatment improved. Health care workers practicing in high burden TB and HIV settings should have a high index of suspicion to investigate newborns for TB. Pharmacokinetic data generated from pregnant women and infants on existing and new antituberculosis drugs will optimize preventive and curative TB treatment, especially in the context of maternal HIV infection, where outcomes are currently poor for both women and their infants. Health systems should be strengthened to improve the care of pregnant women with TB and their infants and new findings from clinical research should inform both policy development and implementation to improve TB care in these vulnerable populations.

APPENDICES

OTHER CONTRIBUTING WORKS

1. Schaaf HS, Collins A, **Bekker A**, Davies PD. Tuberculosis at extremes of age. Respirology 2010;15:747-763.

This review describes the tuberculosis epidemiology, clinical presentation and public health aspects and outcomes in infants (<1 year) and the elderly (>65 years). Mortality is increase at the extremes of age where both the young and the elderly are reliant on others for adherence of treatment. Public health measures should be improved to effectively prevent and treat TB in both groups.

2. McIlleron H, Hundt H, Smythe W, **Bekker A**, Winckler J, van der Laan L, Smith P, Zar HJ, Hesseling AC, Maartens G, Wiesner L, van Rie A. Bioavailability of two licensed paediatric rifampicin suspensions: implications for quality control programmes. Int J Tuberc Lung Dis. 2016 Jul;20(7):915-9.

The rifampicin formulation effect of two formulations was determined on the pharmacokinetics of rifampicin in 146 children (median age 1.4 years). The 2-hour peak concentrations differed substantially for the two formulations (the median bioavailability of one formulation was only 25% of the other). The quality of rifampicin suspension should be ensured when treating children with tuberculosis.

INVITED REVIEW SERIES: TUBERCULOSIS
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Tuberculosis at extremes of age

H. Simon Schaaf,[1] Andrea Collins,[2] Adrie Bekker[1] and Peter D.O. Davies[2]

[1]Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University and Tygerberg Children's Hospital, Tygerberg, South Africa, and [2]Tuberculosis Research and Resources Unit, Liverpool Heart and Chest Hospital, Liverpool, UK

ABSTRACT

Although tuberculosis (TB) has its highest burden among young adults, especially since the advent of HIV infection, two other groups with low immunity, the very young (<1 year) with immature immunity and the elderly (>65 years) with waning immunity, are vulnerable groups not to be forgotten. This review describes the epidemiology, clinical aspects, public health aspects and outcome of TB in patients at the extremes of age. The epidemiology differs therein that TB in infants occurs in developing countries with high incidences of TB and HIV, while TB in the elderly occurs in developed countries with ageing populations. The clinical presentation may be non-specific, history of contact with TB is often not known and TB is often not considered at these age extremes, and when the diagnosis is considered, disease progression may already be advanced. Anti-TB treatment regimens are the same as in other age groups, but drug dosages may need adjustment according to weight, renal function, liver function and other potentially complicating factors. Adverse events are more difficult to observe and both the young and the elderly are reliant on others for adherence to treatment. Mortality at both age extremes is higher than in the general TB population. For all the above reasons, public health measures to: prevent transmission of infection; identify those infected and providing preventive therapy; high index of suspicion in order to make an early diagnosis; and timely initiation of treatment are important in both the very young and the elderly.

Key words: tuberculosis, congenital, infant, perinatal, elderly.

The Authors: Professor Peter Davies qualified at Oxford and St Tomas's Hospital in 1973. He was appointed a consultant respiratory physician to Fazakerly Hospital (Now Aintree University Hospital) and the Cardiothoracic Centre Trusts, Liverpool in 1988. In 1990 he set up the Tuberculosis Research and Resources Unit (TBRRU), which he is Director of and from where he has conducted research into many epidemiological aspects of tuberculosis in Liverpool and other parts of the world. He is the editor of Clinical Tuberculosis, now in its fourth edition and the only international standard reference book on clinical aspects of tuberculosis published outside the USA. In 2004 he was appointed Honorary Professor to Liverpool University. Professor Hendrik Simon Schaaf is a paediatrician (sub-speciality infectious diseases) at Tygerberg Children's Hospital and the Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa. He has a special interest in childhood tuberculosis and has done much research and published widely in this field. Dr Andrea Collins is a respiratory registrar currently at the Liverpool Heart and Chest Hospital in the north of England, UK. Her specialist interests include tuberculosis, pleural disease and bronchiectasis. Dr Adrie Bekker is a paediatrician (sub-speciality neonatology) at Tygerberg Children's Hospital and the Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa. She has a special interest in neonatal infectious diseases and is currently furthering her career in this field.

Correspondence: Peter D.O. Davies, Tuberculosis Research and Resources Unit, Liverpool Heart and Chest Hospital, Liverpool L14 3PE, UK. Email: peter.davies@lhch.nhs.uk

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INTRODUCTION

Tuberculosis (TB) is one of the world's most serious diseases. According to the World Health Organization (WHO), one-third of the world's population are infected with TB. Each year, there are over nine million cases of TB and almost 1.8 million TB-related deaths worldwide. Furthermore, TB is one of the leading causes of death in HIV-infected people.[1] More than 90% of global TB cases and deaths occur in the developing world. In the USA, 10–15 million people are estimated to be TB-infected, with 14 093 active cases of TB in 2005.[1]

Apart from severe immune compromise, such as seen with HIV infection, immune immaturity related to young age (mainly children infected before <2 years of age) is associated with a greatly increased risk to develop active disease following infection with *Mycobacterium tuberculosis*. Infants (i.e. children <12 months of age) experience the highest risk; 50% develop TB after infection in the absence of preventive measures and up to 30% develop progressive pulmonary or disseminated (miliary) TB.[2] HIV infection increases this risk even further; a recent study in the Western Cape province, an area with a high TB incidence of 1037/100 000 population in 2006, demonstrated a relative risk of 24 (95% CI: 17–34) for culture-confirmed TB in HIV-infected infants

compared with HIV-uninfected infants.[3] Reasons postulated for this increase were immunosuppression, more exposure to adult TB source cases because of increased susceptibility to TB in adults caring for these infants and the likelihood that Bacille Calmette-Guérin (BCG) vaccination is less effective in HIV-infected children.[3] It is possible that not only HIV-infected but also HIV-exposed uninfected infants are at higher risk of TB, because of increased exposure to TB from immunodeficient household members and potential immunosuppression in these infants.[4,5]

On the other end of the spectrum, life expectancy continues to increase in the developed world; therefore, TB in the elderly is likely to be a continuing, if not an increasing problem. In many developing countries, especially in Africa, life expectancy is decreasing mainly because of HIV infection in young adults, leading to a likely decline in number of elderly people, but with the general population having increased exposure to TB, it is likely that worldwide TB in the elderly may become an increasing problem.

Although HIV infection is relatively uncommon in the elderly and therefore not a major risk factor in this age group, immunity in the elderly is often waning, and because of high infection rates with *M. tuberculosis* at young age even in currently developed countries, the elderly remain at high risk for TB disease mainly due to reactivation.

In both extremes of age, the symptoms at presentation are often uncharacteristic at best, and often vague. Alertness and awareness of TB as a possible diagnosis in these age groups is paramount. Adverse events to anti-TB treatment are more common in the elderly and more difficult to identify in the very young; therefore, more careful monitoring is needed. Identifying patients at risk in both age groups is challenging, because mothers or other household members are often not yet diagnosed with TB or unaware of the risk to the infant if an adult has TB, while contact tracing of patients living in residential or nursing homes can be very problematic and resource intense. The mortality at both extremes of age is therefore, not surprisingly, high.

It is vital that all those involved in the care of very young and elderly patients consider TB as a differential diagnosis and be aware of the pitfalls and difficulties in diagnosis and treatment, especially as it is potentially a curable disease, unlike many conditions that either an infant or elderly patients present with.

METHODS

Search strategies

Tuberculosis in the very young—for this review we refer to TB in infants. However, TB in infants is a diverse group. Pubmed was searched under the terms 'congenital tuberculosis (or TB)', 'perinatal tuberculosis', 'infant tuberculosis' and 'connatal tuberculosis' and available articles or abstracts with sufficient information about epidemiology and clinical aspects were used for this overview. Additional articles were

Table 1 Symptoms and signs in congenital TB: combined data from 75 individual cases of congenital TB, median age 2–3 weeks with range 1–112 days of age (collected from 38 case series and reports)[6–43]

Symptoms and signs	Occurrence
Respiratory distress including tachypnoea	Common (i.e. >40%)
Hepatomegaly, splenomegaly	
Fever (usually low grade)	
Prematurity/low birth weight	
Cough, may be acute or chronic	Frequent (i.e. 25–40%)
Poor feeding	
Failure to thrive	
Abdominal distension (including ascites)	
Irritability	Infrequent (i.e. 10–25%)
Peripheral lymphadenopathy	
Sepsis syndrome	
Skin papular/pustular or ulcerative lesions	Rare (i.e. <10%)
TB meningitis	
Jaundice (obstructive)	
Otorrhoea/mastoiditis	
Wheeze or stridor	
Apnoea or cyanosis attacks	
Facial nerve palsy	
Shock	

TB, tuberculosis.

found by using references from articles identified by these searches. We have used our own experience with surveillance of TB in children in the Western Cape of South Africa (population 4.8 million), an area with a TB incidence of >1000/100 000 population in 2008 and a HIV prevalence in 2008 of approximately 15% in pregnant women attending public health antenatal clinics, to demonstrate some points.

From the available literature we have reviewed 75 cases of congenital TB for this study, a group of patients that is often referred to in this article and some findings are summarized in Tables 1 and 2.[6–43]

Tuberculosis in the elderly: Pubmed was searched under the terms 'elderly tuberculosis (or TB)' and 'Old age TB'. A Google search using the same words was also performed. Additional articles from the references of these obtained articles were also used particularly for epidemiology data.

The authors of this review have published extensively on TB; therefore, the authors' own work and experiences have also been incorporated into this article.

Definitions

Infant TB is all TB diagnosed in the first year of life. However, it is not a single entity, as TB in infants can be congenitally acquired or they can be infected

Table 2 Comparison of chest radiograph features in infants with culture-confirmed congenital TB versus those <3 months of age with mainly postnatal TB

Radiographical feature	Congenital TB[6–43] $n = 53$ (%)	TB in infants[44] (<3 months of age) $n = 27$ (%)
Lymphadenopathy (hilar/paratracheal)	4 (8)	14 (52)
Lobar/segmental opacification (unilateral or bilateral)	18 (34)	14 (52)
Airtrapping	NA	15 (56)
Large airway compression	NA	13 (48)
Bronchopneumonia (bilateral)	17 (32)	5 (19)
Miliary TB	16 (30)	7 (26)
Ghon focus	NA	2 (7)
Cavities or cystic lesions	4 (8)	NA
Lobar collapse	NA	4 (15)
Pleural effusion	1 (2)	2 (7)
Normal chest radiograph	4 (8)	1 (4)

NA, not applicable; TB, tuberculosis.

shortly after birth (usually within the first few weeks after birth and often referred to as postnatal TB) or later in infancy. Congenital TB occurs when *M. tuberculosis* is transmitted from the mother to the fetus *in utero* or during birth. Postnatal TB (transmission of infection within first weeks after birth) is acquired after birth from the mother or another source case. Some authors prefer to combine the entities of congenital and postnatal TB as perinatal TB, as it is often difficult to distinguish at what point the infant was infected.[45,46]

Tuberculosis in the elderly: for this review the term 'elderly' is used to define those persons in the population over 65 years old. Because of the lack of clear definition, many referenced papers have different age cut-offs, such as age ≥60, ≥75 or ≥80 years. Where not mentioned the age referred to should be assumed to be ≥65 years old.

TUBERCULOSIS IN THE VERY YOUNG

Epidemiology

Despite the high risk of developing TB after infection, not many studies address TB in infants. In a survey of culture-confirmed TB cases in children less than 13 years of age from March 2003 through February 2005 at the two major tertiary paediatric referral hospitals in the Western Cape province of South Africa, 156 of the 596 (26.2%) culture-confirmed childhood TB cases were less than 12 months of age at diagnosis,[47] similar to a study in 1985 in the same province with 26% of culture-confirmed children <12 months of age.[48] Another study from the Western Cape (same referral hospitals) confirms that infants are exposed to TB early in infancy with 10% of infants 3–4 months of age already exposed to a known adult TB source case usually someone in the same household or regular close contact.[49] Adults are presenting with TB at younger age not only in high TB burden countries but also in developed countries with low TB burdens,

which may impact on infant TB.[45,50,51] In high TB burden countries, such as South Africa, TB among pregnant women is strongly associated with HIV infection, as more younger women at child-bearing age develop TB when HIV-infected.[52]

Congenital TB, however, is often reported to be a rare disease, but this is challenged by some authors.[6] Many continue to quote a figure of 300 reported cases in the English literature,[7,8,45] but these authors do not take into account many reports published after the 300 cases mentioned in Cantwell's report in 1994[9] or the fact that many cases are either not reported or the diagnosis is missed.[53] Culture-confirmed childhood (<13 years of age) TB surveillance data from March 2003 through February 2009 at Tygerberg Children's Hospital, one of the two main referral hospitals in the Western Cape province, found 72 of 905 cases (8%) were <3 months of age at diagnosis and of these at least 12 (1.3%) had congenital TB (H.S. Schaaf, unpubl. data, 2010). Pillay *et al.*[10] reported that 16 babies born to 107 mothers (15%) with TB in pregnancy developed TB in the first 3 weeks of life, 12 in the first week, which most likely represent congenital TB.

Perinatal tuberculosis

It is often difficult to clinically distinguish between true congenital TB, transmitted *in utero* by haematogenous spread via the umbilical vein or ingestion/aspiration of infected amniotic fluid during birth, and postnatal transmission that occurs by inhalation of bacilli spread by the airborne route from a mother or other close source case with infectious pulmonary TB. The term perinatal TB is now often preferred. The revised criteria from Cantwell[9] help to distinguish true congenital TB from postnatal transmission. According to these criteria, any infant with a TB lesion and one or more of the following most likely has congenital TB: (i) present within the first week of life; (ii) a primary hepatic complex or caseating hepatic granuloma; (iii)

TB infection of the placenta or endometrial TB in the mother; or (iv) exclusion of the possibility of postnatal transmission by excluding TB in other contacts. Newborns are these days rarely removed from an infectious mother and often the mother's TB is diagnosed only after the infant is diagnosed with TB. Pregnant mothers with recent TB infection or primary TB, presenting with pleural effusion, disseminated TB (miliary TB or tuberculous meningitis) or other extrapulmonary TB, which has a bacillaemic phase, are more likely to transmit *in utero* than mothers with typical cavitating adult-type disease, who in turn are more likely to transmit postnatally.[9,11] In the review of 75 congenital TB cases (see Methods) we found that information on maternal TB was available in 65; 43 had chest radiograph results. Of these 43, 14 (33%) had a normal chest radiograph with confirmed extrapulmonary TB, 12 (28%) had pleural effusions of whom six also had other pulmonary infiltrates (only one culture-positive), eight (19%) had miliary TB and 10 (23%) had only pulmonary TB, most of which was culture-negative TB.[6–43] Other types of extrapulmonary TB identified in these mothers were endometrial TB confirmed in 22 of 25 tested, central nervous system TB in six, osteoarticular and abdominal TB in three each.

Close contact with an infectious pulmonary TB source case poses a transmission risk of 30–80% in infants, with risk between 60% and 80% for sputum acid-fast bacilli (AFB) smear-positive and 30–40% for smear-negative source cases.[54] Mothers, nursing staff, visitors or even other neonates may have undiagnosed pulmonary TB. Heyns *et al.* described four infants that were infected and developed disease after being exposed to a mother with undiagnosed pulmonary TB in a kangaroo mother care unit (i.e. where mothers nurse their premature babies in a hospital setting).[55] Although infection in a neonatal unit is not common, mainly because of relatively short duration of exposure in healthy newborns, transmission may occur.[56] A single case of neonatal transmission via contaminated respiratory equipment has been described, confirmed by DNA fingerprinting of the organism.[57]

The clinical presentation of congenital TB in the review of 75 cases is summarized in Table 1.[6–43] Although postnatal TB may clinically be indistinguishable from congenital TB, the presence of a hepatic granuloma is fairly pathognomic of congenital TB.[9] The clinical and radiological picture may vary in perinatal TB.[44] Postnatal TB often presents with cough, tachypnoea, wheeze and stridor, while congenital TB cases present with acute respiratory distress and/or hepatomegaly and abdominal signs; other presenting symptoms and signs, such as poor weight gain, are similar.[44,51] The radiological features described in 53 of the 75 congenital TB cases are compared with features of culture-confirmed TB in infants less than 3 months in Table 2.

Infant tuberculosis

Although perinatal TB forms part of the larger infant TB group, this section highlights a few points in the larger infant group compared with TB in children >1 year of age. Few articles on TB in infancy, addressing the peculiarities of TB in infancy, have been published in the last 20 years.[44,51] Pulmonary TB (or intrathoracic TB as many paediatricians prefer to refer to it because hilar and/or mediastinal lymphadenopathy is included, while in adults it is mostly classified as extrapulmonary TB) is present in >90% and extrapulmonary TB occurs in 15–30% of infant cases.[51]

Common and less common types of tuberculosis in infancy

Hilar and paratracheal lymphadenopathy (with or without primary lung parenchymal lesions) is a common presentation, often causing complications, such as large airway compression with distal lung pathology. The thymic shadow may obscure hilar adenopathy on the anteroposterior view; a lateral chest radiograph may identify hilar adenopathy.[58] The anteroposterior chest radiograph should be carefully examined for compression of the large airways, which may be present without obviously visible lymphadenopathy. Bronchoscopy and CT may be of value if available.[59] If response to anti-TB treatment and steroids is inadequate, thoracotomy with lymph node enucleation (removing the liquefied inner part of the nodes) may be indicated to restore airway patency.[60]

Cavitating TB, a condition more likely to be associated with adult-type TB, also occurs in infants.[47,61] The pathogenesis of lung cavities may be different in infants compared with adults, and can be placed in two categories. First, poor containment of primary infection with enlargement of the primary (Ghon) focus with caseous liquefaction.[62,63] This then ruptures into an airway causing endobronchial spread of TB resulting in bronchopneumonic opacification and eventually widespread cystic cavities. Second, lymphobronchial TB caused by lymph node obstruction of large airways with distal caseating pneumonia resulting in bulging fissures (expansile pneumonia) and thick-walled cavities.[64] Cavities are present in 63% of children with TB expansile pneumonia.[59] Progressive, uncontained pulmonary TB in infants may lead to lung destruction and death.

Miliary TB is common in all infants, but tuberculous meningitis occurs more frequently in older infants (>3 months).[44,51] This is because TB meningitis is usually preceded by infection in the lungs a few weeks to months earlier.[65] Disseminated TB disease (miliary TB and tuberculous meningitis) is responsible for most of the TB-related mortality seen in infants.

Peripheral lymphadenopathy, mainly cervical, is the most common form of extrapulmonary TB in older children (>1 year), but is uncommon in infants.[66] However, axillary lymphadenopathy has become a frequent adverse event in especially HIV-infected infants who received BCG vaccination at birth.[67]

Other types of extrapulmonary TB, such as osteoarticular TB and abdominal TB, are rare in older infants (abdominal TB common in congenital TB).[47] Tuberculous otitis media, caused by haematogenous or direct spread to the ear, is uncommon but seen more

frequently in infants. It should be considered in any chronic suppurative otitis media.[68] HIV infection has lead to an increase in tuberculous otitis media.[69]

Complications of Bacille Calmette-Guérin vaccination

In countries or regions with high TB burden, BCG is given to infants at birth to prevent TB and it is currently the most widely used vaccine worldwide. The vaccine is mainly effective in preventing miliary TB and tuberculous meningitis.[70] BCG adverse events, ranging from a local vaccination site abscess, ipsilateral axillary lymphadenopathy to disseminated BCG disease (i.e. *M. bovis* BCG spreading to other organs, e.g. the lungs), almost always present during infancy. These adverse events are more common in HIV-infected than in HIV-uninfected infants. The clinical picture of BCG adverse events may be indistinguishable from TB.[67] To further complicate matters *M. bovis* BCG is part of the group of organisms identified as *M. tuberculosis* complex, and specific PCR tests are needed to differentiate *M. bovis* BCG from *M. tuberculosis* var *hominis* (i.e. true TB).

Current WHO guidelines for the management of HIV-infected children recommend that all infants diagnosed with HIV should start antiretroviral therapy (ART) irrespective of their clinical or immunological stage.[71] This reduces death from HIV and most likely will prevent disseminated BCG disease, but could cause restoration of the immune response, which in some children may lead to an immune reconstitution inflammatory syndrome (IRIS; a paradoxical reaction of either unmasking of disease or worsening of symptoms or signs) to BCG at the injection site or draining lymph nodes. Although these BCG-IRIS abscesses may need aspiration of pus or drainage for relieve of symptoms, ART should be continued and no anti-TB treatment is indicated.[72,73]

Clinical aspects

Diagnosis

The recognition of TB in infants, whether it be congenital, postnatal or later transmission, may be difficult due to non-specific or even absent symptoms until disease progression occurs. A high index of suspicion is necessary to make the diagnosis. In infants, symptoms are often acute (days) rather than chronic (weeks), especially in those infants less than 3 months of age. Available diagnostic tests, such as the tuberculin skin test (TST) and the new interferon-gamma release assays (IGRA), are more often negative in infants with TB compared with older children and are therefore often not helpful in the diagnosis.[74,75] Infants with congenital TB may be symptomatic within the first week of life, but mostly present between 2 and 4 weeks of age. Infants tend to develop disease after infection much more rapidly than older children—within weeks rather than months.

Perinatal TB should be considered at least in the following situations: (i) pneumonia not responding to broad spectrum antibiotics, especially in TB endemic settings or if the mother/primary caregiver has TB; (ii) non-specific symptoms but mother (or other source case) diagnosed with TB; (iii) high lymphocyte count in cerebrospinal fluid with no identified pathogen; (iv) fever and hepatosplenomegaly; or (v) abdominal distension with ascites and hepatomegaly.[76]

As in other children, TB in infants is mostly diagnosed by a constellation of history of contact with an infectious source case, chronic (weeks) symptoms and signs, a positive TST and suggestive chest radiographical findings. Microbiological confirmation is often sought to confirm the diagnosis. Although children often have low mycobacterial yields of approximately 10–20% smear positivity and 40% culture positivity,[47,77] infants may have culture-confirmed TB in up to 70% of cases.[51] Of the 75 congenital TB cases reviewed in this report, 53 (71%) yielded specimens (e.g. gastric aspirates, endotracheal and bronchial aspirates, ear swabs and biopsy specimens of lymph nodes, liver and skin) smear-positive for acid-fast bacilli, which confirmed the diagnosis of TB in suspected cases. This high bacillary load with positive smears may be because of late recognition of disease or because of uncontrolled multiplication of bacilli in the absence of a well-developed immune system. It is, however, possible that this high yield of positive smears is due to publication bias, as mainly cases with confirmed TB are likely to be reported.

Broth-based automated mycobacterial culture methods, such as Mycobacterial Growth Indicator Tube (Becton Dickenson, Cockeysville, MD, USA), have significantly reduced time to culture detection from the traditional solid culture media. However, new rapid culture and drug susceptibility testing (DST) methods are necessary and methods, such as microscopic observation drug susceptibility assay, may prove to be useful in poorly resourced areas,[78] giving results for both culture and DST in 7–10 days. Confirmation of *M. tuberculosis* complex and DST for rifampicin and isoniazid in smear-positive cases can be done by line probe assay, such as the MTBDRplus assay (Hain Lifescience, Nehren, Germany), and yield results within as little as 2 days under field conditions.[79]

Abdominal ultrasound examination is valuable in cases with suspected congenital TB, the majority of true cases have hepatomegaly. Sonography can identify hypoechoeic foci in the liver and spleen, confirm ascites in neonates with abdominal distension and identify lymphadenopathy. Ultrasound-guided biopsy from hepatic lesions can confirm the diagnosis if AFB or granulomatous lesions are found.[12] Other imaging investigations for abdominal involvement do not yield much more information compared with ultrasound in congenital TB cases.

Treatment

Prompt treatment is essential to prevent severe morbidity and mortality in infants. Current WHO and

Table 3 New WHO dosage recommendations for first-line anti-tuberculosis drugs in children including infants

First-line drug	Dosage in mg/kg body weight (range)
Isoniazid	10 (10–15)
Rifampicin	15 (10–20)
Pyrazinamide	35 (30–40)
Ethambutol	20 (15–20)
Streptomycin	15 (12–18)

WHO, World Health Organization.

International Union against Tuberculosis and Lung Disease recommended dosages of first-line anti-TB drugs for children have been challenged lately because of several studies showing lower concentrations of serum drug levels in children compared with adults at the same mg/kg body weight dose for isoniazid, rifampicin, pyrazinamide and ethambutol.[80–84] New WHO recommended dosages for children including infants are summarized in Table 3. No studies have been carried out to determine anti-TB drug levels in premature or full-term neonates. Although neonates will most likely require higher doses, pharmacokinetic studies in this age group still need to be done.

Treatment regimens used to treat infants with congenital TB vary widely. A combination of isoniazid, rifampicin and pyrazinamide with or without a fourth drug (mainly an aminoglycoside or ethambutol) was used in the intensive phase of 2 months in most reported cases,[6–43] with a continuation phase of isoniazid and rifampicin varying from 4 to 10 months. Adverse events are seldom reported, but hepatotoxicity (isoniazid, rifampicin and pyrazinamide), hearing loss (aminoglycosides) and visual disturbance (ethambutol) should be considered. Hearing loss and visual testing are difficult in infants, and alternative fourth drug, such as ethionamide, should be considered, although with correct dosaging of ethambutol <1% of children develop visual problems and it is mostly reversible once drug is stopped.[84] In infants with miliary TB or TB meningitis some experts recommend ethionamide as the fourth drug because of better cerebrospinal fluid penetration compared with ethambutol and the aminoglycosides.[85]

Infants with TB may be co-infected with HIV. These TB/HIV co-infected infants require additional treatment to improve response to anti-TB treatment. As mentioned above, WHO recommends that all HIV-infected infants receive ART irrespective of clinical or immunological stage.[71] If not already on ART, anti-TB treatment should first be introduced followed by ART within 2–4 weeks to reduce morbidity and mortality. The delay in starting ART is to distinguish possible adverse events from anti-TB drugs from those of antiretroviral drugs that could be similar, and also to reduce the risk of TB-IRIS (i.e. is worsening of symptoms or signs due to restoration of immune response to the TB bacilli). All TB/HIV co-infected infants should receive co-trimoxazole prophylaxis against *Pneumocystis jiroveci* pneumonia. Pyridoxine levels

in TB/HIV co-infected children were shown to be consistently low in one study and some antiretroviral drugs could also cause peripheral neuropathy; therefore, these infants are also recommended to receive pyridoxine supplementation.[86]

Nearly 5% of all incident TB cases in 2007 worldwide were multidrug-resistant (MDR, i.e. resistant to isoniazid and rifampicin).[87] Infants in contact with adults with drug-resistant TB will most likely be infected with the same resistant strain.[88,89] If TB is diagnosed in these infants they should be treated according to the DST result of the adult source case and not wait for the infant's own culture and DST results.[85] If a positive culture of the infant is obtained, DST should still be done and treatment changed according to the infant's isolate DST if indicated.

Outcome

Mortality in congenital TB remains high at 22–50%.[9,90] Of the 75 cases with congenital TB reviewed in this study, 48 of 70 (69%) were alive at discharge or follow up and 22 of the 70 with known outcome (31%) died. In a study of 596 culture-confirmed TB cases in children, eight of 33 (24%) infants <3 months of age died, while only 6% deaths occurred in older children.[47] Vallejo *et al.* reported two out of 47 (4%) deaths in children 3–12 months of age in a developing country.[51] The outcome of isolated pulmonary TB is usually good if treatment is started early. In a study of 17 infants 2–5 months of age ventilated for respiratory failure because of TB all survived, although some with chronic lung disease.[60]

Public health aspects

Infants are mainly infected by household contact with an infectious adult TB case, frequently the mother or primary caregiver. Early identification and treatment of TB in pregnancy will improve outcome of both mother and infant and it will reduce the risk of perinatal TB.[45,46,50] However, diagnosis may be difficult due to non-specific symptoms, often ascribed to the pregnancy itself.[90] A high index of suspicion is required in TB endemic areas, where screening of all pregnant women, especially those who are HIV-infected, seems warranted.[53] A thorough history of recent TB contact, and screening for TB-associated symptoms would suffice in most cases. Shielded chest radiography and sputum microbiology for *M. tuberculosis* should be done if TB is suspected. Pregnant mothers may have extrapulmonary TB and be sputum smear and culture-negative. First-line anti-TB drugs with the exception of streptomycin are safe in pregnancy. Some second-line drugs, such as ethionamide, aminoglycosides and capreomycin used for the treatment of MDR-TB, have been associated with teratogenic effects or adverse events in newborns. Outcome of MDR-TB treatment in pregnancy for both mother and infant has been improved by starting treatment during pregnancy in the few cases described; therefore, risk and benefit for both mother and infant of

starting treatment during pregnancy or delaying full MDR-TB treatment until after birth in these cases should be weighed in each individual case.[50,91–93]

Infants born to mothers with TB diagnosed during pregnancy or soon after delivery should be screened for TB. If no respiratory symptoms, feeding problems or abdominal pathology is found and the infant is clinically well, chemoprophylaxis should be provided and BCG should be withheld until after completion of chemoprophylaxis. The recommended chemoprophylaxis regimen in drug-susceptible contacts is isoniazid for 6–9 months, but isoniazid plus rifampicin for 3 months is an acceptable alternative.[46,85] If BCG was withheld in a HIV-exposed infant at birth, BCG should not be administered later if the infant is confirmed HIV-infected.[94]

Breastfeeding is not contraindicated in drug-susceptible TB, and infants and mothers do not have to be separated provided mothers receive treatment and infants receive chemoprophylaxis. The decision about breastfeeding and separation of infants and mothers are more complex in MDR and extensive drug-resistant TB (i.e. resistance to isoniazid, rifampicin, the fluoroquinolones and a second-line injectable anti-TB agent). Some experts recommend separation of mother and infant in all MDR-TB cases. Standard first-line chemoprophylaxis has failed in these cases.[95] Separation is not always feasible, and chemoprophylaxis with two drugs to which the mother's *M. tuberculosis* isolate is susceptible (including a fluoroquinolone) has been used, together with continuing or starting the mother on MDR-TB treatment. Respiratory protection (mask for the mother) is advised and the infant should sleep in a separate room. Close follow up for 2 years is recommended.[96] All infants should be separated from mothers with extensive drug-resistant TB. Infants exposed to infectious TB cases after birth should receive chemoprophylaxis as above after excluding TB disease.

Bacille Calmette-Guérin reduces the risk of disseminated TB (miliary TB and TB meningitis) in infants and young children by approximately 75%.[97] It is the most widely used vaccine and is safe in immunocompetent infants. However, recent studies have shown a high incidence of disseminated BCG disease in HIV-infected infants and the benefit versus risk of BCG in these infants has been questioned.[70] WHO has recently recommended not giving BCG to HIV-infected infants; this would mean delaying BCG vaccination in all HIV-exposed infants until HIV status is confirmed.[98] This is not feasible in settings with both a high TB incidence and a high prevalence of HIV infection, as with a well-established prevention of mother-to-child-HIV-transmission programme, fewer than 5% of infants will be infected and in most high prevalence HIV areas, PCR screening for HIV infection in HIV-exposed infants only occur at 6 weeks—this could lead to many vulnerable HIV-exposed but uninfected infants who are at high risk of exposure to infectious TB cases not receiving BCG in timely manner or at all.[94] Early initiation of ART in infants has shown to reduce IRIS associated with TB in HIV-infected infants and will most likely also reduce both the risk of TB disease and disseminated BCG disease.[99,100]

TUBERCULOSIS IN THE ELDERLY

The ageing population

In the USA, the percentage of the population ≥75 years old in 1990 was 5.3% and increased to 5.9% in 2000.[101] In the UK, ≥65-year age group increased from 13% in 1991 to 16% in 2001, and is expected to rise to 23% by 2031. In Europe, a rise in the mean age of the population is expected from 34.9 years in 2004 to 44.4 years in 2024.[102] In India, an increase in life expectancy and the resultant increase in the older population (≥50 years old) has led to an increase in the number of TB cases in this age group.[103] On the other hand, life expectancy in sub-Saharan African countries has significantly declined due to HIV/AIDS and its related opportunistic infections, in particular TB.

Epidemiology

Within the elderly population in the UK, Europe and North America, it is the indigenous white populations in whom TB is principally an issue, with rates highest in this group.[104,105] This is because many of these people were alive when TB was much more prevalent and a great number of them would have been infected with the tubercle bacilli.[106] This was also before the time of BCG vaccination and effective anti-TB treatment. An increase in the rates of TB in the elderly has been noted in some economically developed countries.

In countries with a high prevalence of TB there is a contrasting picture, the disease still remains mostly a disease of the young in Africa,[105] but in the Indian subcontinent is increasingly common in the elderly.

Worldwide, despite the increasing availability of effective treatment, TB remains one of the leading causes of human illness and premature death.[107] According to the latest WHO report, the estimated global incidence of TB peaked in 2004 and is now slowly declining by about 1% per year.[108] The number of cases, however, continues to rise as a result of population growth. The most recent WHO estimates indicated that there were 9.4 million cases and 1.8 million deaths caused by TB in 2008.[108]

In 2008 in the UK, 8655 cases of TB were reported, a rate of 14.1 per 100 000 population. This represents an increase of 2.2% in the rate of disease (Table 4).[109] The majority of all TB cases continue to occur in the non-UK born (72%) and those aged 15–44 years (61%). Those ≥65 years old accounted for 14%. In Wales, Northern Ireland and North East of England, a greater proportion of cases were found in those ≥65 years compared with other areas of the UK.

In the USA, the annual Centers for Disease Control and Prevention report showed a total of 12 904 TB cases (rate of 4.2 per 100 000) in 2008. The TB rate in 2008 was the lowest recorded since national reporting began in 1953. The TB rate has been declining in the USA each year since 1992 (Fig. 1). TB rates are declining in all age groups (Fig. 2). However, the number of cases among non-USA born individuals, particularly

Table 4 Tuberculosis case reports and rates by age group and gender, UK, 2008 ([109] with permission from HPA data)

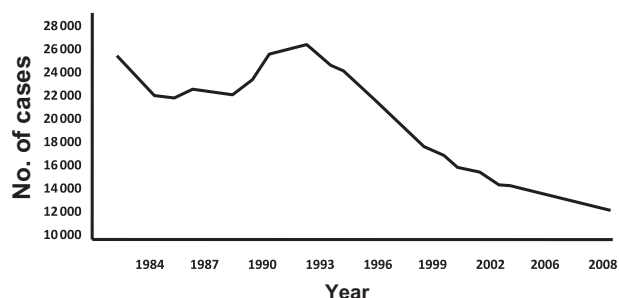| Age group (in years)	Number of male cases	Rate (per 100 000) male	Number of female cases	Rate (per 100 000) female	Total number of cases	Total rate (per 100 000)
0–14	229	4.2	261	5.0	495	4.6
15–44	2870	22.5	2377	19.0	5265	20.8
45–64	961	12.7	722	9.2	1690	11.0
65+	639	14.8	559	10.0	1204	12.1
Total	4700	15.6	3919	12.5	8655	14.1

Figure 1 Reported tuberculosis cases in USA, 1982–2008;[1] updated as of 20 May 2009.
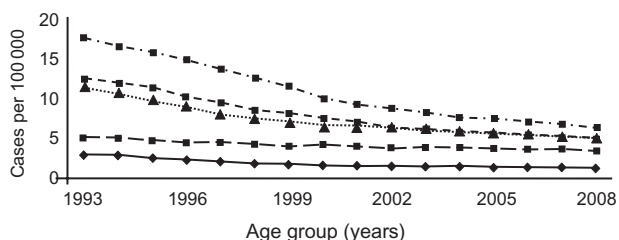

Figure 2 Tuberculosis case rates by age group in USA, 1993–2008;[1] updated as of 20 May 2009. (–◆–) <15 years, (–■–) 15–24 years, (··▲··) 25–44 years, (–■·) 45–64 years, (–■·) ≥65 years.

Figure 3 Reported tuberculosis cases by age group in USA, 2008.[1] (▨) <15 years, (▨) 15–24 years, (▨) 25–44 years, (▨) 45–64 years, (■) ≥65 years.

in those ≥45 years old, is steadily rising.[1] Around 20% of newly diagnosed cases of TB occur in those aged ≥65 years old (Fig. 3).

There is concern that previously infected elderly individuals will develop TB due to reactivation while others may be more likely to develop active TB if exposed to an index case, as immunity declines with increasing age.

Predisposing factors

It is well known that immunocompetency declines with increasing age. In TB, cell-mediated immunity plays a key role in controlling infection. As age advances the related decline in immunity increases the chance of reactivation of latent disease.

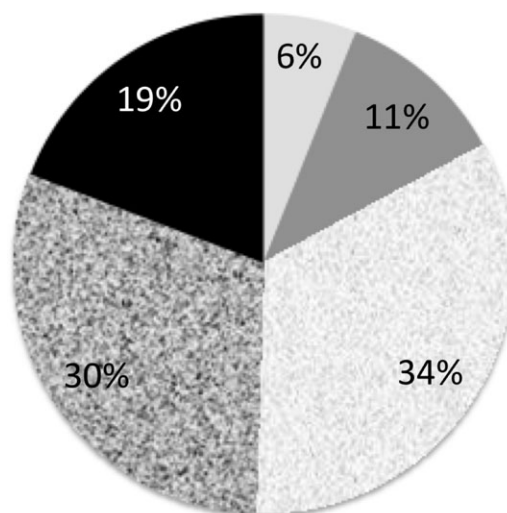Much of the research into the ageing immune system has been done in mice rather than humans.

Elderly mice have a strong innate immune system. Conflicting data exist. Some data suggest that the elderly may develop a lack of regulation in pro-inflammatory cytokines and enzymes that control the expression of reactive oxygen species and inflammatory mediators, and that cytokine expression, responsible for interferon-gamma (IFN-γ) transcription from CD4+ T cells, can be restored by dietary supplementation in mice. Mucosal immune responses were also noted to be reduced in ageing mice.[110] Dietary vitamin E supplementation restored both the humoral and mucosal immune responses to normal adult mice levels. This implies that much of the immune dysfunction is correctable.[110]

It is unlikely that the reduced T cell-mediated response is entirely responsible for the increased susceptibility of the elderly to TB. IL-2 and IFN-γ, both especially important in host defence against TB, are reduced in the elderly but are potentially reversible.[111] However, other studies on the humoral response in elderly TB patients showed no decline in response[112] and well preserved cytokine production in response to stimulation with *M. tuberculosis*.[113]

Vesosky and Turner[114] showed that in response to TB infection, although old mice had impaired generation of antigen-specific CD4+ T-cell immunity, they

had a transient enhanced resistance in the first few weeks; this resistance was associated with the presence of CD8+ T cells, and therefore IFN-γ, before younger mice.[114] Further research may allow researchers to enhance this effector function and improve the immune response in elderly patients to TB.

Comorbidities that are more common in the elderly, such as diabetes mellitus, chronic kidney disease, intercurrent illness, malnutrition, excess alcohol use, underlying malignancy and the use of immunosuppressant drugs (most commonly steroids) can clearly impair the cell-mediated immune system, increasing the risk of both reactivation and new infection progressing to disease, and decreasing the chance of cure in those diagnosed with active TB.

The mode of TB infection is always difficult to determine. However, over 90% of TB in the elderly is felt to be reactivation of dormant infection (endogenous) rather than new (exogenous) infection.[115] Various types of pulmonary TB are seen. New infection is less commonly seen in the elderly in developed countries.[115] If this does occur it is usually in care homes with an outbreak affecting several residents from one index case.[116]

Clinical aspects

Diagnosis

Diagnostic difficulties in the elderly are common in many diseases, not solely TB. Problems, such as poor memory, deafness, blindness/partial sight, impaired speech, poor short-term memory, all contribute, often making an accurate history difficult. Patient, family and doctor may often attribute symptoms to 'old age'. Comorbidities often further complicate matters, especially malignancy that may often coexist.[116–118]

Many papers have been published with regards to clinical and radiologic presentation of TB in varying ages. Unfortunately none of these studies is truly comprehensive or definitive. In many the sample size is small and cut-off age arbitrary. The term 'atypical' in terms of clinical presentation is best avoided as this may lead to the incorrect conclusion that the patient in fact has an environmental mycobacterial infection or a less typical site of TB infection.

A classical TB presentation is difficult to define. Non-specific symptoms with a lack of focal signs are more common in patients with reduced immune competency. Patients may present with a lack of respiratory symptoms and may be unable to expectorate sputum due to weakness. In a comparison between clinical features in the young and old (adults), the classical symptoms of productive cough, night sweats, fever, weight loss and haemoptysis were much less common in the older age group.[119] Confusion was common in the elderly. One study showed that biochemical and haematological abnormalities were more common in older patients, such as anaemia, deranged liver function tests, low sodium, potassium and albumin.[120] This may suggest silent extrapulmonary disease or be secondary to comorbidities.

Katz *et al.*[121] found no significant difference in presenting symptoms between younger adults and the elderly. Cavitatory lesions and haemoptysis were significantly less likely in the elderly but complaints of dyspnoea and lower lobe infiltrates were more common. Chan *et al.*[122] found that older patients had lower body weight, less haemoptysis but more non-specific complaints, but the differences were not significant. Past history of TB and comorbidities were more common but upper lobe infiltrates were less common in the older group. Older patients had more extensive disease but less cavity formation. Age-related mortality was significantly higher in the elderly.

A study from Brazil comparing 117 patients ≥60 years old and 464 patients aged 15–49 years showed dyspnoea ($P = 0.018$) and weight loss ($P = 0.047$) were more predominant in the older age group. In the younger group, haemoptysis ($P = 0.002$), chest pain ($P = 0.027$) and fever ($P = 0.006$) were more common.[123] A study from Korea of 326 patients agreed that haemoptysis and fever were more frequent in the ≤65-year age group, whereas weakness, dyspnoea, anorexia and mental change were more frequent in the elderly.[124]

Non-pulmonary TB, such as TB meningitis, bone and joint TB, genito-urinary and gastrointestinal TB are seen in all age groups [125].

The elderly patient may present with 'atypical' radiological features, such as middle or lower lobe (rather than upper lobe) infiltrates,[117,121,124] mass-like lesions or nodules appearing more like cancers, extensive bronchopneumonia without cavitation or non-resolving infiltrates. Lesions are frequently misdiagnosed as pneumonia or lung cancer in the elderly.[124] Some authorities suggest that any elderly person admitted to hospital for pneumonia should have at least one sputum culture for TB.[1] In another study the most common radiological abnormalities were infiltrates and cavitations.[126] Bilateral involvement was more common in the elderly patients ($P = 0.009$).[126] Some studies have reported that pleural effusion is more common in the elderly, but a recent study showed the highest incidence of pleural disease to be in men aged 30–59 years old rather than older patients.[127]

Miliary TB is the most common form of TB seen in the elderly. With miliary TB there are large concentrations of multiplying tubercle bacilli with no or minimal immune response. It often presents in a chronic, indolent form, possibly with prolonged low-grade fever without focal signs or symptoms, and as a protracted wasting illness rather than the classical presentation of high-grade intermittent fever in an acute or subacute pattern. On examination hepatosplenomegaly and anaemia may be present. A normal chest radiograph is compatible with miliary TB.[128] The majority of both undiagnosed and diagnosed fatal cases are seen in patients ≥60 years old.[129,130]

There does therefore not appear to be a consistent difference in the clinical presentation of TB in the elderly according to the current literature, except that the elderly do appear to more commonly have miliary

Table 5 Treatment completion and death before or during treatment by age group, UK, 2007 ([109] with permission from HPA data)

Age group (in years)	Number (%) completed treatment	Number died (%)	Total cases outcomes determined
0–14	418 (90.7)	2 (0.4)	461
15–44	4115 (83.7)	88 (1.8)	4914
45–64	1202 (80.0)	116 (7.7)	1502
65+	722 (65.3)	267 (24.1)	1106

disease and lower-zone infiltrates on chest radiography,[131] and haemoptysis is less common than in younger adult TB patients.

Tuberculin skin test sensitivity is known to wane with age and is therefore undoubtedly less helpful in older rather than younger age groups. TST studies show a steady decline in skin test positivity from 15% in the over 70s to 3% in the over 90s.[132] Extensive infection, advancing age and malnutrition leading to immunosuppression can all be associated with non-reactive TST despite the presence of active disease. The booster effect, an increasing TST reaction to repeated TST inoculation over a limited period (usually months), which may be wrongly interpreted as new infection, occurs at all ages but is more common in the elderly. Van den Brande and Demedts[133] suggest that the failing immune response to tuberculin can be restored progressively by repeated TST administration. Care should therefore be taken in interpreting any positive 'test–retest TST' as conversion, when in fact they are due to the booster response.

Kobashi *et al.* evaluated the usefulness of the QuantiFERON TB-2G (QFT-2G) test and the TST in patients with active TB disease stratified by age in 10-year increments in a Japanese population.[134] The positive rate on TST was 55% in patients aged 70–79 years and 33% in patients ≥80 years. However, the positive rate on QFT-2G testing was 79% and 75% respectively. The indeterminate result rate of QFT-2G increased with age. It was suggested that this may be due to comorbidities. They suggest that although the positive rate of QFT-2G was seen to decrease with age, it may be a useful supportive diagnostic method for active TB disease compared with the TST in elderly patients. QFT-2G could potentially be used in elderly patients in whom TB is suspected but where all other investigations are inconclusive or not practical because of the patients' general condition.

Diagnosis of miliary TB can be difficult and anti-TB treatment may have to be started without definitive evidence of TB in the critically ill elderly patient. Transbronchial biopsy may be useful in association with bronchial washings performed at bronchoscopy. Bone marrow with or without liver biopsies should be considered if miliary TB is suspected with a normal chest radiograph. The yield for bone marrow biopsy and liver biopsy is likely to be better if anaemia is present or liver function tests are abnormal respectively. The risks of invasive investigations are greater in the elderly due to increasing comorbidities. The risks of invasive procedures therefore need to be balanced against the risk of potentially missing the correct and curable condition of TB.

Treatment

The adherence to and tolerance of anti-TB treatment are the main issues, with the former being the main reason for treatment failure in all age groups. Adherence is a larger problem in the elderly due to factors, such as poor vision (unable to read labels and therefore use correct doses/frequency), poor mobility and low income (unable to collect prescription), poor memory (unable to remember to take drugs or at correct dose/time), increased risk of drug interactions and adverse events, and increased rates of depression/low mood (may lack determination to complete full 6-month treatment regimen).

These reasons may lead to a patient receiving inadequate treatment. Closer monitoring to ensure adherence is needed and the role of the TB nurse is therefore vital. In many countries treatment is therefore directly observed by health-care providers.

In the UK, treatment completion declines with age, but the proportion of cases reported as dying before completing treatment increases (Table 5). TB was known to have caused or contributed to 30% of the deaths among cases aged 65 years or older.[109]

An Indian study showed a completion rate under directly observed therapy, short-course (DOTS) of 82% in 50- to 65-year age group and 70% in ≥65-year age group and treatment default rates of 7.5% and 12.5% respectively.[103]

Adverse events

It is well known that, in general, adverse drug events occur more commonly with increasing age, due to age-related physiological changes and the coexistence of other diseases leading to polypharmacy. More careful/rigorous monitoring of adverse events is needed especially if there is known renal or liver dysfunction. Studies have shown that increasing age is a predictor of hepatotoxicity due to both isoniazid and rifampicin, manifesting as hepatitis and fatal fulminant hepatic necrosis.[135,136] Rifampicin in combination with isoniazid has an additive, although not synergistic effect of liver dysfunction. It has been suggested that the elderly are almost three times as likely to experience adverse events compared with younger

Table 6 Number and proportion of tuberculosis cases with drug resistance by age group, UK, 2008 ([109] with permission from HPA data)

Age group (in years)	Isoniazid-resistant (%)	Multi-drug-resistant (%)	Resistant to any first-line drug (%)	Total[†]
0–14	7.6	3.4	8.4	119
15–44	6.8	1.3	7.8	3204
45–65	5.4	0.9	6.2	883
65+	2.0	0.0	2.2	602

[†] Culture-confirmed cases with drug susceptibility test results for at least isoniazid and rifampicin.

patients.[136,137] Clinical monitoring for hepatotoxicity is most important. Some clinicians recommend monthly monitoring of serum transaminases, but this is not generally accepted. An additional problem is that the elderly may not recognize adverse events because they may put them down to age, general poor health or other medication.

The role of toxic metabolites in adverse events is not clear. Isoniazid metabolites (hydrazine and monoacetylhydrazine) are both hepatotoxic. One study showed the maximum concentration of hydrazine after the first dose was significantly higher in older patients (all patients were on four-drug regimen).[138] Combination therapy may predispose to adverse events. Metabolic induction by rifampicin may lead to increased levels of hepatotoxic isoniazid metabolites.[139] A Korean study involving 207 younger patients (<65 years old) and 119 elderly patients (≥65 years old) confirmed that the elderly had a higher frequency of adverse events compared with younger adults (18.5% vs 40.7%, $P < 0.05$).[124]

Non-pharmacological factors may also increase the risk of hepatotoxicity, such as liver involvement by extensive mycobacterial disease, underlying chronic liver disease and poor nutritional state (drug metabolism and toxicity is affected by dietary intake). The latter may partly explain the higher rates of hepatotoxicity in patients in India (3–22%) compared with the USA (2–3%).[140]

Clinicians should take the above factors into consideration to help avoid hepatotoxicity. Nutrition support should be offered where necessary and close clinical monitoring of all patients is essential. More pharmacokinetic and pharmacodynamic studies are needed to determine optimal dosages for elderly patients, taking into account these different factors, without compromising cure.

Ethambutol can potentially cause reduction in visual acuity, central scotomas and colour vision defects due to optic neuritis. The risks and benefits of ethambutol in anti-TB treatment regimens in the elderly with retinopathy or cataracts (in whom initial assessment of visual acuity may be difficult) should be carefully considered.

Streptomycin may cause oto- and nephrotoxicity more frequently in those with renal dysfunction, and these adverse events are generally irreversible. The use of streptomycin in the elderly should take risks and benefits of treatment into account.

Drug interactions

Drug interaction is more frequently a problem because elderly patients often receive polypharmacy. A variety of drugs may interact, notably, anticonvulsants, for example, phenytoin (isoniazid can elevate levels, whereas rifampicin can lower levels due to inhibition and induction of metabolism respectively—overall phenytoin levels are usually lower on first-line anti-TB therapy),[141] digoxin and steroids (rifampicin can reduce levels). Isoniazid, rifampicin and pyrazinamide are predominately metabolized by the liver.[142] Hepatic volume and blood flow decrease with age[143] and the rate of hepatic metabolism of certain drugs may decline.[144,145] There are only a few studies on the pharmacokinetics of anti-TB drugs, many of them assessing each drug in isolation rather than in combination.

In the elderly, malnutrition and chronic disease mean lower serum albumin levels.[146] Acidic drugs bind to albumin; therefore, free-drug concentrations may rise in those with lower serum albumin levels. The relationship between free-drug levels and toxicity is not known, but this may play a role in increasing adverse events in the elderly.[147]

Drug resistance

The incidence of drug resistance (39% vs 16%, $P < 0.05$) was significantly higher in the older patients in China.[148] Lung resection surgery can be used as an adjuvant to anti-TB therapy in patients with MDR-TB. A Japanese study suggests that pulmonary resection achieves a high cure rate with low morbidity and mortality. The oldest person in this study was 65 years, but the authors suggest that pulmonary resection should be considered in all age groups including the elderly.[149]

In the UK, isoniazid resistance, MDR-TB and resistance to any first-line drug decreased as age increased (Table 6). The majority of drug-resistant TB cases were in the age group 15 to 44 years old.[109]

Mortality

High mortality in the elderly is a common finding across the world. Patients with comorbidities tend to

have higher mortality rates whatever the disease process; and elderly people, in general, have higher rates of comorbidity and therefore mortality. The key to reducing mortality is earlier diagnosis. Worldwide mortality rates of up to 30–50% can be expected in those aged over 70 years, and especially those in their 80s.

A survey conducted in England and Wales in 1978–1979 showed that 12% of adult patients notified with TB died before completing treatment. Multivariate analysis showed there to be a significant association between age, extent of disease, positive smear result and mortality.[150] A later mortality survey (1974–1987) showed a 32% mortality in those aged 75 years and above, 4% in those aged 35–54 years, 1% in those aged 15–34 years but encouragingly a steady decline in the yearly mortality over that period.[151] A further study from 2001, showed a mortality rate of 27% by 2001 ≥75 years old, mortality in other age groups remained static.[152] A study from Birmingham in the UK, showed higher mortality rates in Caucasians than Asians (median population age of 66 years). Diagnostic delay was a contributory factor in many of these cases, with the authors concluding that TB is most likely to be missed in the elderly Caucasian population.[153] Extent of disease can also contribute to increased mortality with more elderly patients presenting with disseminated or miliary disease. A recent study from China suggested an overall case fatality rate of 5.5%. Of these deaths 50.5% were attributed to causes other than TB. Eighty-six per cent of the deaths were among patients ≥60 years old, with male gender, sputum smear positivity, comorbidities and advancing age being significant independent risk factors for death.[154]

Non-specific symptoms, such as mild weight loss, reduced appetite and breathlessness, can be attributed to age-related change or other diagnoses, such as COPD or pulmonary fibrosis. For these reasons the diagnosis may be considerably delayed, leading to more advanced disease at initial presentation, or an entirely missed diagnosis, made at post-mortem.[135,155]

One study showed that initial diagnosis of TB at first visit was less frequent in the elderly than in the younger adult group (38.6% vs 47.3%), although symptoms and signs at first visit were similar in each of the age groups. Elapsed time from the first visit to suspicion of TB and initiation of anti-TB treatment was frequently delayed in elderly patients (mean 22 days, SD 23 days vs 13 days, SD 20 days, $P < 0.05$).[148]

Reports from older studies have shown that a significant number of cases of TB in the elderly were diagnosed at post-mortem. With the declining rates of post-mortems in the UK and many other countries, these cases of TB may completely be missed. A Swiss post-mortem study in the elderly from 1989 found active TB in 3%.[107] A Ghanaian study showed that mortality was strongly related to age ($P < 0.001$).[156]

A UK study showed that elderly patients were over 20 times more likely to be diagnosed with TB at post-mortem than younger patients, and six times more likely to die from TB (130). Higher TB-related mortality (1.3% vs 11.1%, $P < 0.05$) was noted in the elderly in Korea.[124] A recent Indian study showed mortality rates

of 12.5% in the ≥65-year age group as compared with 5.5% in 50- to 65-year age group.[103]

Public health aspects

Prevention

Measures to prevent increasing cases of active TB in the elderly could include poverty reduction, provision of adequate health care, preventative therapy (for latent TB), good ventilation in aggregate settings, such as institutional care, avoidance of overcrowding, optimal nutrition and improvement of general health status.

Several studies report increased prevalence in the elderly living in institutional care (care homes, residential homes and nursing homes). This is thought to be due to both a predisposition to reactivation of latent TB as well as an increased risk of cross-infection from an index case within the care home environment.[157] A study in Caucasian residents of old age homes in South Africa showed rates of 798/100 000 population, compared with 16/100 000 in the general population.[158] A study from Hong Kong in 1993 showed rates of 1200–2600/100 000 in care homes compared with 100–400/100 000 in the general elderly population. Other studies have, however, shown no increased risk in the elderly in such settings.[132,159]

In the USA in 1984–1985 a study showed an incidence of 39.2/100 000 among nursing home residents, compared with 21.5/100 000 in those living in the community. The Centers for Disease Control and Prevention in the USA has established recommendations for surveillance, control and reporting of TB in long-term care facilities.[1] All new residents and employees have an initial TST and then annual screening. Chest radiography is performed on all those with a positive TST. As noted earlier TB case rates are highest in ≥65-year age group in the USA.

In 1989 the strategic plan for elimination of TB in the USA stated that 'control and prevention of TB among the elderly must be addressed aggressively to achieve the goal of eliminating TB in the United States by the year 2010'.[160] In the USA, more elderly persons live in nursing homes than in any other type of residential institution; approximately 5% of all elderly persons live in a nursing home. However, elderly persons represent 88% of the nation's approximately 1.7 million nursing home residents.[161] Such concentrations of elderly persons, many of whom have latent TB and some of whom are immunosuppressed, create high-risk situations for TB transmission.

The results of a recent prospective observational study in the USA (entry closed January 2009) are awaited. This study set out to compare the TST with IGRA (T-SPOT.*TB*, Oxford Immunotec, Oxford, UK) testing for the screening of patients ≥65 years old in USA nursing homes.

Screening

The usefulness of TST depends on the prevalence within and the age of a particular population.

Universal guidance on screening is not possible as this will vary depending on the prevalence within a country. Where the prevalence is high sputum examination and chest radiography remain the most cost-effective screening method. Screening using TST, IGRA, sputum smear and culture, and chest radiography may be considered. This screening policy was advocated in the USA in 1989.[160]

Methods of screening vary across Europe and screening of the elderly in long-term facilities only occurs in 16/50 (32%) of European countries.[162]

In general the adverse effects of treatment for latent TB in the elderly outweigh the potential benefits of disease prevention. If it is believed that the elderly have an increased susceptibility; surveillance and screening should be considered to be paramount if we are to eliminate TB worldwide.[1]

A study from Hong Kong showed that when screening an elderly population for TB, low BMI (<18.5 kg/m^2) was associated with the future development of active TB. If low BMI in an elderly individual can increase their susceptibility to developing TB, it should be taken into account in screening programmes[163] A study in 2006 showed that the rates of active and latent TB in old age homes in Hong Kong remain high. The average age in the study was 82 years. Patients were screened using interview, medical record review, two-stage TST and chest radiography with or without sputum microscopy as required. The estimated prevalence rate of active TB in the population was 669/100 000 and was notably significantly higher in men (1101 vs 530/100 000).[160]

The absolute number of older people in the developed world continues to increase; TB is likely to become an increasing problem in this age. It is likely that it will continue to be a problem in the developing world. A high index of suspicion is vital. Close therapeutic monitoring is necessary. Many advocacy agencies aim to increase the awareness of TB worldwide, hopefully leading to increased survival worldwide.

More vigorous clinical management and prevention strategies by both the TB control programme and other public health programmes are essential to improve TB treatment outcomes especially in developing countries.[164] Uncharacteristic clinical presentation may occur. Medical and psychosocial aspects of ageing, comorbidity and misperception of symptoms, may make the diagnosis of TB difficult in the elderly. Appropriate investigations and prompt institution of treatment will help to decrease morbidity and mortality in the elderly.

CONCLUSION

Tuberculosis in the very young and the elderly poses problems for the clinician, microbiologist and public health programme. Presentation may be varied and diagnosis therefore difficult. Both the young and the old human frame may be reluctant to divulge its secrets in the form of positive bacteriology. Treatment is not often straightforward and drug dosages will have to be adjusted according to weight, renal function, liver function and other potentially complicating factors. Both the very young and the elderly are subject to increased rates of infection leading to disease as immunity may be compromised. Doctors and other health professionals who are to manage patients at the extremes of life will require special skills and experience.

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Bioavailability of two licensed paediatric rifampicin suspensions: implications for quality control programmes

H. McIlleron,* H. Hundt,* W. Smythe,*† A. Bekker,‡ J. Winckler,§ L. van der Laan,‡ P. Smith,*
H. J. Zar,§¶ A. C. Hesseling,‡ G. Maartens,* L. Wiesner,* A. van Rie#**

*Division of Clinical Pharmacology, Department of Medicine, and †Clinical Research Centre, Health Sciences
Faculty, University of Cape Town, Cape Town; ‡Desmond Tutu TB Centre, Department of Paediatrics and Child
Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town; §Department of Paediatrics
and Child Health, and Medical Research Council Unit on Child and Adolescent Health, University of Cape Town,
Cape Town; ¶Red Cross War Memorial Children's Hospital, Cape Town, South Africa; #Department of Epidemiology,
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; **International Health Unit,
Epidemiology and Social Medicine, Faculty of Medicine, University of Antwerp, Antwerp, Belgium

S U M M A R Y

S E T T I N G : To assess the revised World Health Organization-recommended dose of 10–20 mg/kg rifampicin (RMP), we studied the steady state pharmacokinetics of RMP in South African children who received standard treatment for drug-susceptible tuberculosis (TB).

O B J E C T I V E : To determine the formulation effect on the pharmacokinetics of RMP.

D E S I G N : RMP plasma concentrations were characterised in 146 children (median age 1.4 years, range 0.2–10.2). The morning dose on the day of the pharmacokinetic evaluation was administered as one of two RMP single-drug oral suspensions.

R E S U L T S : While one formulation achieved 2 h concentrations in the range of those observed in adults (median 6.54 mg/l, interquartile range [IQR] 4.47–8.84), the other attained a median bioavailability of only 25% of this, with a median 2 h concentration of 1.59 mg/l (IQR 0.89–2.38).

C O N C L U S I O N : RMP is a key drug for the treatment of TB. It is critical that the quality of RMP suspensions used to treat childhood TB is ensured.

K E Y W O R D S : tuberculosis; first-line drug; quality assurance; children

FEW STUDIES have been conducted in children to support drug dosing for tuberculosis (TB) or to evaluate drug formulations.[1] Instead, paediatric doses have generally been extrapolated from those used in adults. The World Health Organization (WHO) reviewed the evidence on first-line anti-tuberculosis drug formulation and dosage in children and found that rifampicin (RMP) concentrations were low and varied widely between and within studies.[2,3] Although sampling processes and assay methods varied across studies, and forms of dosage, methods of administration and dosing schedules were not consistently described, experts concluded that the RMP dose in children needed to be increased to achieve concentrations comparable to those in adults. The WHO accordingly revised its dosing guidelines in 2010, recommending a 50% increase in the dose of RMP, from 10 (8–12) to 15 (10–20) mg/kg.[4] We evaluated the pharmacokinetics of RMP, isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) in children with TB at doses consistent with the revised WHO guidelines. Here we report findings regarding formulation effects on the pharmacokinetics of RMP.

STUDY POPULATION AND METHODS

Children aged <12 years diagnosed with drug-susceptible TB who were receiving RMP, INH and PZA (with or without EMB or ethionamide) in daily doses at Red Cross Children's Hospital, Tygerberg Hospital and Khayelitsha District Hospital, Cape Town, South Africa, were enrolled. The children were treated using fixed-dose combination (FDC) products available in the public health sector, with doses approximating the WHO's 2010 guidelines.[4] Children underwent pharmacokinetic evaluation after at least 2 weeks of anti-tuberculosis treatment. On the day of pharmacokinetic evaluation, single-drug formulations of registered products were administered by the study team, as FDC products providing doses in accordance with the revised WHO recommenda-

tions are not yet available. A granulate preparation of RMP for suspension (Eremfat®, RIEMSER Arzneimittel, Germany, registered for use in several European countries) was reconstituted to a concentration of 100 mg/5 ml and administered in accurately measured doses of 10–20 mg/kg using a syringe, or via a nasogastric tube in very young children. Due to an interruption in the supply of Eremfat, R-Cin® suspension (100 mg/5 ml; Aspen Pharmacare, Durban, South Africa, registered for use in South Africa) was used in subsequently enrolled children.

Serial 0.6 ml blood samples were drawn to determine the plasma pharmacokinetics of the antituberculosis drugs. A pre-dose sample and samples at 2 and 4 h after the dose were drawn in all 146 children. An additional sample was drawn at 8 h in 86 children; in 20 children additional samples were taken at 1, 6 and 10 h; and in 40 children an additional sample was drawn at 1, 6 and 8 h after the dose. The samples were centrifuged to separate the plasma within 0.5 h of sampling and stored immediately at −70°C until analysis.

The study (NCT01637558) protocol was approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, Cape Town, and the South African Medicines Control Council, Pretoria, South Africa.

RMP plasma concentrations were determined using a liquid chromatography–mass spectrometry assay validated according to Federal Drug Administration (FDA) and European Medicines Agency (EMA) guidelines.[4–6] The samples were processed with a protein precipitation extraction method using 20 µl plasma with 500 µl acetonitrile containing a stable isotope-labelled internal standard, RMP-D3. Five microliters of the supernatant were injected onto the high-performance liquid chromatography column. Chromatographic separation was achieved on a Discovery C18, 5 µm, 50 × 4.6 mm analytical column using acetonitrile, methanol and 0.1% formic acid in water (6:1:3, v/v/v) as the mobile phase, delivered at a constant flow rate of 400 µl/min. An AB Sciex API 3000 mass spectrometer (GenTech, Arcade, NY, USA) was operated at unit resolution in the multiple-reaction monitoring mode, monitoring the transition of the protonated molecular ions at m/z 823.4 to the product ions at m/z 791.4 for RMP and the protonated molecular ions at m/z 826.5 to the product ions at m/z 794.4 for the internal standard. Electrospray ionisation was used for ion production. The assay was validated over the concentration range of 0.117–30 µg/ml. The combined accuracy and precision statistics of the limit of quantification, low, medium and high quality controls (three validation batches, $n = 18$) were between 101% and 107%, and 2.7% and 3.7%, respectively.

A truncated area under the RMP concentration-time curve to 4 h after the dose (AUC_{0-4}) was computed as a measure of systemic RMP exposure using the concentrations from the sampling times common to all children (pre-dose, 2 and 4 h) in a non-compartmental analysis. Concentrations below the limit of quantification of the assay (respectively 95%, 0% and 2% for the pre-dose, 2 h and 4 h concentrations) were imputed a value of 0.06 mg/l. Differences between the groups of children receiving Eremfat and R-Cin RMP suspensions were compared using the Mann-Whitney U-test. Quantile regression was used as a robust approach to adjust for the effects of age, sex, dose per kg of body weight, human immunodeficiency virus (HIV) status and administration by nasogastric tube when evaluating the effect of formulation on AUC_{0-4}. Each of these variables was tested separately, and then added to the base model describing the effect of formulation on the AUC_{0-4}. Covariate effects with $P < 0.2$ were retained in the final model. Non-compartmental analysis and all statistical analyses were performed using Stata 13.1 (StataCorp LP, College Station, TX, USA).

The RMP content of the Eremfat and R-Cin formulations was compared using product batches used during the study. A fresh suspension of Eremfat was prepared as described in the product insert. The suspension was shaken well before 200 µl was added to 40 ml methanol. Similarly, 200 µl of the well-shaken R-Cin suspension from a freshly opened bottle was added to 40 ml methanol. The two suspensions were chromatographed with a gradient high-performance liquid chromatography-ultraviolet assay at a flow rate of 0.4 ml/min. Mobile phase A consisted of a mixture of 0.1% formic acid in water and acetonitrile (85:15, v/v), and mobile phase B consisted of 0.1% formic acid in acetonitrile. A mobile phase gradient was run from 100% A to 100% B over 3 min and remained at 100% B for another minute before returning to 100% over 0.5 min. Five microliters were injected onto a Phenomenex Max-RP 3 µ 50 × 2 mm column (Phenomenex, Torrance, CA, USA), and RMP was detected at a wavelength of 334 nm. A similar method was used to evaluate the effect of passage through a nasogastric tube on the RMP concentrations in a RMP suspension.

RESULTS

Among the 146 children included in the analysis, 92 received Eremfat and 54 received R-Cin formulations (Table 1). Children receiving R-Cin RMP suspension were younger (median 0.88 vs. 1.97 years, $P < 0.001$), and thus had lower body weight (median 8.37 vs. 11.48 kg, $P < 0.001$). They also received slightly higher doses of RMP in mg/kg (median 16.51 vs. 14.95). The children underwent pharmacokinetic sampling a median of 1.3 months (interquartile range [IQR] 1.1–1.6) after starting anti-tuberculosis treatment.

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Table 1 Characteristics of enrolled children, RMP dose per kg of body weight, and administration by nasogastric tube on the day of pharmacokinetic evaluation by RMP formulation

Formulation	Eremfat® RMP (n = 92) median [IQR]	R-Cin® RMP (n = 54) median [IQR]	P value
Age, years	1.97 [0.94–4.37]	0.88 [0.5–2.47]	<0.001
Weight, kg	11.48 [8.27–15.45]	8.37 [5.7–13.3]	<0.001
HIV-infected, n (%)*	5 (5)	6 (11)	0.217
Female, n (%)	43 (47)	17 (31)	0.070
Dose, mg/kg	14.95 [12.20–16.68]	16.51 [14.85–18.67]	0.002
Nasogastric tube, n (%)	14 (15)	26 (48)	<0.001

* At the time of pharmacokinetic sampling, four children had not started antiretroviral treatment, one child was receiving an efavirenz-based regimen and the remaining children were on lopinavir/ritonavir with two nucleoside reverse transcriptase inhibitors.
RMP = rifampicin; IQR = interquartile range; HIV = human immunodeficiency virus.

Plasma RMP concentrations by time after dose are shown in the Figure. The median 2 h concentration was 6.54 mg/l (IQR 4.47–8.84) in children receiving Eremfat, higher than the 1.59 mg/l (IQR 0.89–2.38) for those receiving R-Cin (Figure). The median RMP AUC_{0-4} was respectively 16.85 (IQR 11.80–23.24) and 4.19 (IQR 2.68–6.68) mg.h/l in the groups who received Eremfat and R-Cin RMP. When stratified by age, the RMP AUC_{0-4} remained consistently lower in children receiving R-Cin than in those who received Eremfat (Table 2, $P < 0.001$).

In univariate quantile regression, R-Cin was associated with a 12.81 mg.h/l (95% confidence interval [CI] –15.39 to –10.23) reduction in AUC_{0-4} compared to Eremfat RMP. Age, sex, HIV status or administration by nasogastric tube had no impact on the model describing the effect of formulation on AUC_{0-4}. In the multivariate model adjusted for the effect of RMP dose per kg of body weight (AUC_{0-4} increased by 0.48 mg.h/l, 95% CI –0.03 to 0.10, for each 1 mg/kg increase in the dose), the strength of association slightly increased, with administration of R-Cin being associated with a 13.30 mg.h/l (95% CI

–16.40 to –10.20) reduction in AUC_{0-4} compared to the administration of Eremfat RMP.

The RMP content of the two suspensions from the respective batches used during the study were equivalent, with RMP peak areas on the chromatogram for Eremfat and R-Cin solutions of respectively 2180 and 2047.

DISCUSSION

Under the National TB Control Programme, dispersible FDCs are used to treat the majority of South African children with TB. However, commercial suspensions may be useful in the most vulnerable of children requiring individualised care, including the very young or critically ill.

RMP exposures were dependent on the paediatric product used and were 76% lower among children who received the R-Cin suspension than in those who received Eremfat. While the children who received R-Cin were younger and weighed less, they received, on average, a higher dose of RMP per kg of body weight, and the differences in AUC_{0-4} between the two groups remained high (79%) even when adjusted for the effect of dose per kg of body weight.

For adults, a 2 h target RMP concentration of 8–20 mg/l has been proposed,[7] and recent studies suggest that even higher exposures may be more effective.[8–10] Despite the administration of a 15 mg/kg dose, as recommended in the most recent WHO guidelines, the median 2 h concentration achieved was only 1.59 mg/l (IQR 0.89–2.38) for children receiving R-Cin. Those receiving Eremfat RMP had a median 2 h concentration of 6.54 mg/l (IQR 4.47–8.84), in keeping with concentrations reported in adults with TB on standard anti-tuberculosis treatment,[11] but lower than the proposed target of 8–20 mg/l for patients on standard treatment.

Although RMP may be adsorbed to certain plastics, the results of the multivariate analysis found that administration by nasogastric tube did not exert a detectable effect on RMP exposures in the children. We also measured RMP concentrations in an RMP suspension passed through a nasogastric tube. Aliquots of suspension left to stand for 30 min at room
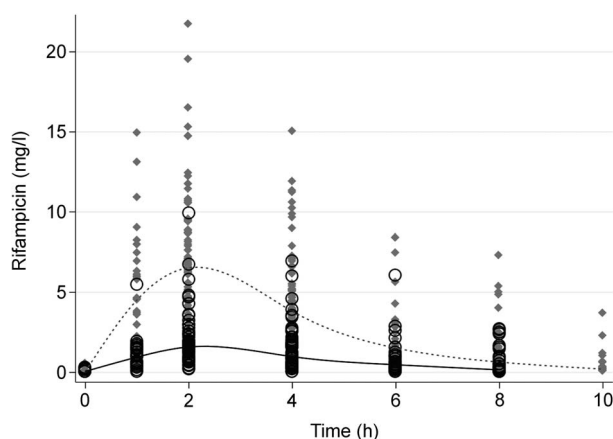

Figure Plasma RMP concentrations in 146 children during the pharmacokinetic sampling interval for children who received Eremfat® RMP (grey diamonds) and R-Cin® RMP (black circles). The dotted line and solid lines track the median splines for the concentrations after doses of Eremfat and R-Cin, respectively, over time. RMP = rifampicin.

Table 2 Truncated RMP AUC (AUC_{0-4}, mg.h/l) by age group and RMP formulation

Age group years	Eremfat® RMP median [IQR]	R-Cin® RMP median [IQR]	P value
<1	14.33 [8.31–18.48] [$n = 26$]	4.65 [3.26–6.68] [$n = 31$]	<0.001
1–<2	18.49 [14.16–23.24] [$n = 20$]	2.53 [2.32–4.69] [$n = 8$]	<0.001
2–<5	21.72 [15.90–30.15] [$n = 25$]	4.23 [3.17–8.56] [$n = 11$]	<0.001
5–<12	15.65 [11.47–17.55] [$n = 21$]	4.02 [2.50–8.60] [$n = 4$]	0.014

RMP = rifampicin; AUC = area under the curve; IQR = interquartile range.

temperature in three nasogastric tubes were found to have similar RMP concentrations to a control aliquot of the suspension (data not shown).

Several reports of substandard and counterfeit RMP-containing products have been published. In most instances, these reports are based on in vitro tests such as the concentration of the active pharmaceutical ingredient and disintegration.[12–14] An analysis of the RMP concentrations in the two formulations studied revealed almost identical RMP content. This indicates that the amount of RMP in R-Cin was adequate, and that the drug was stable in the suspension. We therefore hypothesise that the differences observed in bioavailability are due to the mixture of polymorphic forms of RMP in R-Cin that was not favourable for absorption. Low RMP concentrations attributed to formulation effects have been reported in adults with TB.[15,16]

Production of the active pharmaceutical ingredient is complex and can lead to forms of the molecule with variable solubility.[17] RMP exists in anhydrous polymorphic forms (Form I and Form II), and also in amorphous form.[18] As Form II is metastable, suspension my result in phase transition to a more stable form with subsequent crystal growth. The water solubility of RMP is reported to vary eight-fold depending on the crystalline state of the material,[19] altered particle size affects solubility,[20] and altered solubility is likely to affect bioavailability. However, the relationship between solid-state RMP, dissolution and bioavailability characteristics is poorly understood. Regulatory authorities, including the FDA and the EMA, would generally require in vivo bioequivalence testing for a suspension of a drug such as RMP.[21,22] Likewise, in vivo bioequivalence studies are generally required for suspensions under the South African Medicines Control Council (MCC) guidelines. Although the guidelines state that waivers based on comparative dissolution studies may be acceptable,[23] this condition should not be applied to a Biopharmaceutics Classification System Class II drug such as RMP.[24] The methods used to test the active pharmaceutical ingredient and final product, R-Cin, and the results of such tests that were used to obtain MCC approval, are unknown to the investigators, as both the manufacturer and the regulatory authority regard this information as confidential.

Our study was not designed to compare the bioavailability of the formulations used, and the estimates of the effect of formulation type on the bioavailability of RMP were limited by a relatively small sample size for accurate adjustment for potentially confounding factors such as age, weight and nasogastric tube use. Nor were accurate measures of disease severity available for inclusion in the multivariate analysis. However, the massive impact of formulation on RMP exposures in these children is clear.

CONCLUSIONS

Our incidental findings reveal extremely low RMP concentrations in children as a result of very poor RMP bioavailability in a suspension licensed in South Africa. The findings raise important questions about the quality of the RMP-containing formulations available for children and the procedures in place to protect children from products that do not deliver adequate drug exposures.

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RESUME

CONTEXTE : Afin d'évaluer la dose révisée recommandée par l'Organisation Mondiale de la Santé de 10–20 mg/kg de rifampicine (RMP), nous avons étudié la pharmacocinétique stable de la RMP chez des enfants d'Afrique du Sud qui ont reçu un traitement standard pour une tuberculose (TB) pharmacosensible.

OBJECTIF : Déterminer l'effet de la forme galénique sur la pharmacocinétique de la RMP.

SCHÉMA : Les concentrations plasmatiques de RMP ont été mesurées chez 146 enfants (âge médian 1,4 ans ; fourchette 0,2–10,2 ans). La dose matinale le jour de l'évaluation pharmacocinétique a été administrée sous forme de l'une des deux suspensions orales contenant uniquement de la RMP.

RÉSULTATS : Tandis qu'une formulation a abouti à une concentration à 2 h dans la même fourchette que les adultes (médiane 6,54 mg/l ; intervalle interquartile [IQR] 4,47 –8,84), l'autre a atteint une biodisponibilité médiane de seulement 25% de celle-ci, avec une concentration médiane à 2 h de 1,59 mg/l (IQR 0,89–2,38).

CONCLUSION : La RMP est un médicament essentiel pour le traitement de la TB. Il est crucial que la qualité des suspensions de RMP utilisées pour le traitement des enfants soit assurée.

RESUMEN

MARCO DE REFERENCIA: Con el propósito de evaluar la dosis revisada de 10–20 mg/kg de rifampicina (RMP) que recomienda la Organización Mundial de la Salud, se estudió el estado de equilibrio farmacocinético en los niños surafricanos que recibían un tratamiento corriente por tuberculosis (TB) normosensible.

OBJETIVOS: Determinar el efecto de la forma farmacéutica en la farmacocinética de la RMP.

MÉTODOS: Se determinaron las concentraciones plasmáticas de RMP en 146 niños (mediana de la edad 1,4 años; intervalo 0,2–10,2). El día de la evaluación farmacocinética la toma matinal se administró en una de dos suspensiones que contienen solo RMP.

RESULTADOS: Con una de las presentaciones se alcanzaron en 2 h las concentraciones correspondientes al intervalo observado en los adultos (mediana de 6,54 mg/l; intervalo intercuartil (IQR) 4,47–8,84); con la otra suspensión se obtuvo solo el 25% de esta biodisponibilidad, con una concentración mediana a las 2 h de 1,59 mg/l (IQR 0,89–2,38).

CONCLUSIÓN: La RMP es un medicamento primordial del tratamiento antituberculoso. Es fundamental garantizar la calidad de las suspensiones de RMP que se administran a los niños durante el tratamiento de la TB.

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