Epidemiología de la tuberculosis en la población infantil de Manhiça, Mozambique

Paediatric TB epidemiology in Manhiça, southern Mozambique

Elisa López Varela

TESIS DOCTORAL

Epidemiología de la tuberculosis en la población infantil de Manhiça, Mozambique

Elisa López-Varela

TESIS DOCTORAL

Epidemiología de la tuberculosis en la población infantil de Manhiça, Mozambique

---

Paediatric TB epidemiology in Manhiça, southern Mozambique

Elisa López-Varela

A los niños de ITACA

Paediatric TB epidemiology in Manhiça

Epidemiología de la tuberculosis en la población infantil de Manhiça, Mozambique

Tesis presentada por **Elisa López Varela** para optar al grado de Doctora en Medicina

Dirigida por: **Pedro L. Alonso Fernández** y **Denise Naniche**
Línea de investigación: Salud internacional
Grupo de investigación en epidemiología, salud pública y salud internacional

Julio 2016

Barcelona Institute for Global Health (ISGlobal)
&
Centro de Investigação em Saúde de Manhiça (CISM)

Thesis submitted by Elisa López Varela to aspire to the degree of Doctor in Medicine by the University de Barcelona under the direction of Dr Pedro Alonso and Dr Denise Naniche.

The present Doctoral Thesis is presented following the University´s recommendations on presentation of Doctoral Thesis by compendium of publications, as certified by the directors of this thesis.

Dr Denise Naniche

Dr Pedro Alonso

Barcelona, 14th July 2016

TABLE OF CONTENTS:

FIGURES and TABLES

ABBREVIATIONS AND ACRONYMS

ART	Anti-retroviral therapy
BCG	Bacille Calmette-Guerin
CDR	Case detection rate
CISM	Centro de Investigação em Saúde de Manhiça
CXR	Chest radiograph
DOTS	Directly observed therapy
GA	Gastric aspirate
HBC	High burden countries
HDSS	Health and demographic surveillance system
HIV	Human immunodeficiency virus
ISGlobal	Barcelona Institute for Global Health
IPT	Isoniazide preventive therapy
IS	Induced sputum
LTBI	Latent TB infection
MD	Manhiça District
MDG	Millenium Development Goals
MCBIR	Minimum community based incidence rate
MDRTB	Multi-drug resistant tuberculosis
MDH	Manhiça District Hospital
MOH	Ministry of Health
MTBC	*Mycobacterium tuberculosis* complex
NTM	Non-tuberculous mycobacteria
NTP	National Tuberculosis Program
TB	Tuberculosis
TST	Tuberculin skin testing
SDG	Sustainable Development Goals
SSA	Sub-Saharan Africa
WHO	World Health Organization

RESUMEN (Español)

A medida que entramos en la era de los Objetivos de Desarrollo Sostenible una de las metas de salud es acabar con la epidemia de tuberculosis (TB) para el año 2030. Por su parte la Organización Mundial de la Salud (OMS) ha publicado su nueva estrategia de la de lucha contra la TB ("End TB Strategy") que incluye el ambicioso objetivo de reducir la incidencia de TB en un 90% entre el 2015 y 2035. La OMS publicó en 2015 su último informe sobre la situación mundial de la TB que ha servido para mostrar los importantes avances registrados en los últimos años en la lucha contra la TB pero que también ha servido para identificar las principales áreas que desafían el poner fin a la epidemia. La meta de conseguir una inversión en la tendencia de la incidencia de TB para el 2015, uno de los Objetivos de Desarrollo del Milenio, se ha alcanzado en todas las regiones de la OMS, incluidos dieciséis de los veintidós países que agrupan el 80% de los casos mundiales de tuberculosis. Sin embargo el actual ritmo de reducción anual de la incidencia mundial de TB es demasiado lento para alcanzar la erradicación de la epidemia (definida como una tasa de incidencia menor o igual a 10 nuevos casos por cada 100 000 personas por año).

Mozambique es uno de los pocos países endémicos donde la situación epidemiológica de la TB no ha mejorado en la última década. Los estudios epidemiológicos llevados a cabo en el distrito de Manhiça, en el sur de Mozambique, que además tiene una prevalencia comunitaria de VIH particularmente alta, han mostrado una carga de TB extremadamente elevada entre la población adulta, que presenta 2.796 casos por cada 100.000 varones entre los 40 y 45 años de edad.

Existe una relación directamente proporcional entre la carga total de TB en una comunidad y la proporción de esa carga que se da en los niños. En el caso de los países donde la TB es endémica, los niños representan entre el 20 y el 40% de los casos totales. La OMS estima que de los 9,6 millones de casos de TB a nivel mundial que se produjeron en 2014, un millón tuvieron lugar en niños menores de 15 años y 136 000 murieron a causa de la tuberculosis. A pesar del reciente aumento en el interés suscitado por la TB pediátrica, ésta continúa siendo una importante causa de

morbimortalidad en zonas endémicas de TB. Los niños pequeños, así como la personas con inmunodeficiencia secundaria al VIH o a la desnutrición severa, se encuentran en mayor riesgo de desarrollar la enfermedad tras ser infectados por el *Mybocaterium tuberculosis*. El retraso en el diagnóstico y tratamiento de estas personas, especialmente los niños, aumenta el riesgo de una progresión rápida de la enfermedad y consecuentemente de mortalidad. El diagnóstico de la tuberculosis es particularmente difícil en los niños, debido a la falta de especificidad de los síntomas clínicos, a la dificultad de obtener muestras para realizar análisis microbiológicos y a la naturaleza paucibacilar de la enfermedad. El rendimiento diagnóstico de las muestras es con frecuencia inferior al 20% en condiciones programáticas. Las micobacterias no tuberculosas (MNT) son una causa común de falsos positivos en la baciloscopía, lo que lleva a errores en el diagnóstico de TB y, como consecuencia directa, se producen retrasos en el diagnóstico, contribuyendo a la epidemia oculta de la tuberculosis infantil. Para agravar la dificultad en el diagnóstico, se produce el hecho de que la enfermedad afecta a los niños de las comunidades con menos recursos económicos y con acceso limitado a los servicios de salud, a lo que se suma la falta de información y el desconocimiento de la naturaleza de la enfermedad. Por otra parte, incluso cuando se diagnostica debidamente, la notificación de los casos de TB pediátrica es a menudo incompleta. Tanto el infra-diagnóstico como la sub-notificación limitan todavía más nuestro conocimiento de la auténtica incidencia de la tuberculosis entre la población infantil.

Como consecuencia de las dificultades antes mencionadas, la estimación de casos de tuberculosis infantil sigue siendo poco precisa. Sin embargo estas estimaciones son cruciales. En primer lugar, porque la tuberculosis infantil refleja la transmisión en curso dentro de una población y por lo tanto es un útil indicador centinela de la eficacia de Programa Nacional de Tuberculosis. Por otra parte la cuantificación de la carga de enfermedad es importante para orientar las intervenciones en salud, para el establecimiento de objetivos y para la asignación de recursos de manera adecuada.Las primeras estimativas de TB pediátrica de la OMS no estuvieron disponibles hasta 2012, en parte debido a la falta de datos de notificación desagregados por edad en muchas países endémicos. La OMS computó una incidencia de 490 000 casos totales de

tuberculosis infantil. Las estimaciones iniciales se basaron en el uso de los datos de notificación pediátricos y cálculos de la tasa de detección, asumiendo que ésta era igual para niños y adultos. Desde entonces se han realizado grandes esfuerzos y se han producido avances significativos en la mejora de las estimaciones epidemiológicas de TB infantil. En el último informe sobre la situación global de la TB de la OMS (2015), las estimaciones han combinado nuevas metodologías publicadas basadas en modelos matemáticos. La falta de coincidencia entre las estimaciones más recientes (un millón) y las anteriores ilustra la dificultad para producir tales estimaciones y pone de relieve la necesidad de obtener datos de vigilancia regional y local de alta calidad que permitan nutrir los modelos matemáticos y contribuir a la mejora de las estimativas globales de TB pediátrica.

El objetivo general de este proyecto de investigación es aumentar la base de conocimiento sobre la TB pediátrica con el fin de contribuir al fin de la epidemia de TB. Esta tesis tiene por objeto mejorar las estimativas epidemiológicas de TB infantil, así como caracterizar a la enfermedad desde diferentes perspectivas con el fin de vencer barreras críticas para la detección de casos.

El primer artículo de esta tesis proporciona nuevos datos de incidencia poblacional de TB infantil que no están disponibles actualmente en la mayoría de los países del África subsahariana. La tasa de incidencia mínima comunitaria (TIMC) de TB en niños menores de tres años en el distrito de Manhiça fue de 470 por cada 100.000 persona-año, consistentemente alta para todos los grupos de edad. Estos datos confirman la magnitud de la epidemia en esta región y ponen de manifiesto el alto nivel de transmisión comunitaria.El VIH está íntimamente ligado a la tuberculosis. Casi la mitad de los niños menores de 3 años con TB estaban coinfectados de VIH. Los niños con sospecha de TB e infectados por el VIH presentaron una probabilidad de tener tuberculosis seis veces mayor que los no infectados, aunque la probabilidad de obtener confirmación bacteriológica fue más baja en este grupo. La mortalidad en los niños VIH positivos fue significativamente mayor que en los no infectados, (el 14,4% frente al 3,8%, p <0,001) y podría reflejar casos de mortalidad por TB no diagnosticada. Desde el punto de vista operacional, este estudio llama la atención sobre el alto

porcentaje de oportunidades perdidas de quimioprofilaxis ya que menos de la mitad de los casos con criterios (VIH o contactos de TB) iniciaron tratamiento preventivo con isoniazida.

En el segundo estudio, se proporcionan estimaciones de la tasa de detección de casos (TDC) de la tuberculosis infantil utilizando datos comunitarios. Se utilizó la TIMC generada previamente como la estimación más precisa disponible. Para calcular la TDC de tuberculosis en los niños menores de tres años en el Distrito de Manhiça, se comparó la TMIC de 2011-2012 con los casos notificados durante el período 2006-2010. La tasa de detección de casos fue del 40,8%. Es probable que sea una estimación conservadora y que la verdadera TDC sea más baja, lo que confirma la gran carga oculta de la enfermedad.

En el tercer estudio reportamos una alta tasa (88%) de éxito terapéutico al tratamiento de TB en menores de tres años en el Distrito de Manhiça. A pesar de ello casi un tercio de los casos presentaron adherencia incompleta al tratamiento (definido como casos perdidos durante el tratamiento o con un retraso de tres o más semanas para completar el tratamiento). La desnutrición infantil y la historia de migración materna se asociaron significativamente a la adherencia incompleta al tratamiento, lo que a su vez puede conducir a un aumento de la mortalidad. Observamos una mejoría significativa en el éxito terapéutico en 2011-2012 (88%) comparado con el periodo 2006-2010 (67.3%).

Los siguientes cuatro estudios de la tesis contribuyen a caracterizar mejor el problema de la TB pediátrica desde diferentes perspectivas: antropológica, clínica, microbiológica y radiológica. En el cuarto artículo, documentamos que la lesión más frecuentemente encontrada en los casos confirmados y probables de TB en menores de tres años es la consolidación del espacio aéreo, que dificulta la distinción entre la TB y la neumonía bacteriana en niños. La linfadenopatía hiliar (el patrón típico radiológico de la TB pediátrica), fue la segunda lesión más común, pero sólo detectada en una minoría de casos y podría explicarse por la alta prevalencia de VIH y la desnutrición de la población. Estos resultados ponen de relieve las dificultades para diagnosticar la TB en ausencia de confirmación bacteriológica y la necesidad de combinar la información

clínica, radiológica y epidemiológica. Este estudio pone de relieve la importancia de no descartar la TB a pesar de la ausencia de hallazgos radiológicos característicos y la necesidad de mejora de los scores diagnósticos en la población pediátrica.

Esta tesis subraya de relieve el desafío que plantea, a la hora de diagnosticar la TB, el frecuente aislamiento de micobacterias no tuberculosas (MNT) en las muestras pediátricas de esputo o jugo gástrico. Esto conlleva varias implicaciones. En primer lugar, en los países de alta carga de TB donde los métodos moleculares no suelen estar disponibles y el diagnóstico aún se basa en la baciloscopía, este aislamiento es fácilmente interpretado erróneamente como un caso de TB, lo que puede suponer una sobreestimación de la carga de enfermedad. Por otro lado, a la hora de diseñar los "endpoints" de ensayos clínicos de nuevas vacunas contra la TB, es importante considerar la epidemiología de las MNT en niños, incluyendo su exposición medioambiental. Finalmente, parece existir una posible asociación entre las MNT y la reducción de la eficacia de la vacuna Bacillus Calmette-Guérin (BCG) que necesita de mayor investigación.

En el quinto artículo de esta tesis, reportamos una tasa de aislamiento de MNT en las muestras de aspirado gástrico y esputo inducido de niños con sospecha de TB de 26%. La distribución de las especies de fue similar a lo reportado en Sudáfrica: *Mycobacterium intracellulare* fue la micobacteria aislada con mayor frecuencia, seguida de *M. scrofulaceum* y *M. gordonae*. En nuestra cohorte, las MNT parecían no tener relevancia clínica: ninguno de los casos recibió tratamiento específico para MNT y la mortalidad a los 2 años fue comparable a la presentada por los casos con cultivo negativo. Además, la proporción de niños con MNT aisladas en las visitas de seguimiento fue similar a la proporción aislada en la primera vista, independientemente del resultado del cultivo inicial. En comparación con los niños en los que se aisló *Mycobacterium tuberculosis*, aquellos con MNT presentaban menos sintomatología clínica y radiológica, así como una menor mortalidad.

El documento siguiente es una revisión de las MNT en la población infantil. Se trata de la primera revisión en analizar detenidamente el rol de las MNT en niños con una perspectiva de salud pública en países donde la TB es endémica. La revisión ha puesto

de manifiesto que todavía hay muchas lagunas en determinadas áreas de conocimiento, en particular con respecto a la epidemiología de las MNT en países con alta carga de TB. Otras áreas identificadas donde se necesita mayor investigación son: la comprensión de la interacción entre el patógeno MNT y el huésped (incluyendo el efecto de la exposición a MNT sobre la eficacia de la BCG); el desarrollo de nuevas pruebas así como estrategias diagnósticas que permitan el correcto diagnóstico de la TB y NTM en los países de bajos recursos; el desarrollo de guías clínicas que incluyan criterios de tratamiento específico para el niño y el desarrollo de nuevos regímenes de menor duración para el tratamiento de la MNT.

En el último artículo, presentamos los resultados de un estudio cualitativo cuyo objetivo es describir las interpretaciones locales de los signos, etiología, transmisión y prevención de la TB pediátrica. Los resultados de este estudio indican que los cuidadores de niños pequeños presentan en general un bajo nivel de conocimiento sobre la tuberculosis infantil. Muy pocos de ellos sospechaban de que la TB pudiera ser la causa de los síntomas de los niños, incluso a pesar de tener ellos antecedentes de TB. Existe una percepción local de que la TB es una enfermedad de adultos y no infantil. Por otro lado, la TB se interpreta con frecuencia como el resultado de la transgresión de determinados ritos sociales y culturales de purificación. Sin embargo, los procedimientos de diagnóstico pediátrico son altamente aceptados y tolerados. Este estudio mostró además que el comportamiento de búsqueda de cuidados de salud en relación con el tratamiento de TB parece seguir un itinerario circular y complejo entre los sistemas de atención sanitaria convencional y los tradicional. Los resultados de este estudio son útiles para poder informar a las campañas de promoción de la salud con un enfoque en la prevención de los retrasos en el inicio de tratamiento y su adherencia.

Más allá de los objetivos específicos de esta tesis, los estudios reportados muestran una mejoría significativa tanto en la TDC como en el éxito de la TB en niños pequeños del distrito de Manhiça entre el periodo 2011-2012 y el período anterior (2006-2010). El avance se debe en parte a la mejora progresiva en los servicios de salud tanto de la TB como el VIH. Sin embargo también sugieren que en un ámbito semi rural de África

subsahariana con alta carga de VIH y TB, el diagnóstico de la tuberculosis infantil, la TDC y los resultados de tratamiento pueden mejorar lo suficiente como para alcanzar los objetivos de erradicación de TB.

SUMMARY

As the world enters the era of the Sustainable Development Goals, ending the tuberculosis (TB) epidemic by 2030 is one the health targets. The latest World Health Organization (WHO) TB control strategy, the "End TB strategy" has set the ambitious goal of reducing by 90% the TB incidence rate from 2015 to 2035.

In 2015 the WHO global TB report has shown important advances in the fight against TB, but has also served to identify major areas of challenge that urgently need to be addressed to end the epidemic. The Millennium Development Goal target (MDG 6, Target 8) to halt and reverse TB incidence has been achieved worldwide in all WHO regions including 16 of the 22 high burden countries (HBC) which comprise 80% of the world TB cases. However the current annual reduction in the global TB incidence is too slow to achieve an end to the epidemic in the foreseeable future (defined as achieving an incidence rate of 10 new people with TB per 100 000 population per year).

Mozambique is one of the few HBC countries where the burden of TB has not improved over the last decade. At a country level, none of 3 targets linked to the MDG and endorsed by the Stop TB Partnership (reversing incidence, halving mortality and prevalence) were met. Epidemiological studies performed recently in the Manhiça District in Southern Mozambique, which has a particularly high prevalence of HIV in the community, have shown a huge burden of adult TB, reaching 2796/100.000 in males 40-45 years.

A direct relationship exists between the total TB burden in a community and the proportion of that burden found in children. In HBC, children account for up to 20-40% of the global burden. The WHO estimated that of the 9.6 million TB cases globally in 2014, 1 million cases, or 10%, were in children (<15 years), and that 136 000 children died due to TB. Thus, despite a renewed impetus on putting childhood tuberculosis in the spotlight, it remains a potential important cause of morbidity and mortality in TB endemic settings. Infants and young children as well as those with immunodeficiency caused by HIV or severe malnutrition are at highest risk of developing TB disease

following infection. Delay of diagnosis and treatment in these children increases the risk of rapid disease progression and mortality.

TB diagnosis is particularly challenging in children, given the lack of specific symptoms, the difficulty in obtaining samples for microbiological examination and the often paucibacillary nature of the disease. The diagnostic yield of samples is often <20% under TB program conditions. Non-tuberculous mycobacteria (NTM) are a common cause of false positive smear results thus leading to TB misdiagnosis. As a result, the frequent under-diagnosis, misdiagnosis or delayed diagnosis of the disease contribute to the hidden epidemic of tuberculosis in children. Compounding this difficulty in diagnosis is the fact that the disease affects children from the poorest communities with limited access to health service and frequent lack of knowledge on the disease. Moreover, even when diagnosed, paediatric TB reporting is often incomplete. Thus, both under-ascertainment and under-reporting further limit our understanding on the burden of TB in children.

As a consequence of the aforementioned difficulties, global estimates of the burden of childhood tuberculosis remain weak. However, numbers do matter. Firstly, childhood TB reflects ongoing transmission within a population and thus is a useful sentinel indicator of the effectiveness of National Tuberculosis Programs. Moreover, an accurate understanding of the burden is important for identifying problems in program delivery, targeting interventions, monitoring trends, setting goals, allocating resources appropriately and providing strong advocacy. The first WHO estimates of the burden of childhood TB were only available in 2012, partly due to the lack of available age-disaggregated notification data in many HBC. WHO reported a total number of 490 000 incident childhood TB cases. The initial estimates were based on the use of paediatric notification data and case detection rates. The estimates assumed an equal case detection rate in children and adults due to the lack of a robust figure on paediatric TB case detection rate. Since then, great efforts have been made and significant progress achieved in improving TB burden estimates. In the latest WHO report, new methodology has been used combining results from a dynamic model, a statistical approach based on a recent study, and methods previously used by WHO in a

statistical ensemble model. The lack of overlap between the latest estimates (1 million of total incident cases) and the previous ones illustrates the difficulties in producing such estimates. Most importantly, it highlights the need for high quality regional and local surveillance data as well as population based data to feed into the newly produced global estimates of childhood TB.

The overarching goal of this research project is to provide with a knowledge base on paediatric TB that can help inform the End TB strategy. This thesis seeks to improve current estimates on the burden of TB in young children and characterize the disease from different perspectives in order to understand critical barriers to case detection.

The first paper in this thesis provides unique population-based incidence estimates which are currently unavailable in most Sub-Saharan African countries. The minimum community based incidence rate (MCBIR) of TB in children under the age of three from the Manhiça District in 2011-2012 was found to be 470/100.000 person years, consistently high across all ages. These data confirm the magnitude of the epidemic in this region and highlight the high level of ongoing TB community transmission.

HIV is intimately linked to TB. We report that 44% of TB cases in children under the age of three were HIV co-infected in 2011-2012, with a similar rate in the previous years. HIV infected children evaluated for TB were 6 times more likely to have TB than those uninfected, although fewer HIV infected children had bacteriological confirmation. Their mortality was also significantly higher (14.4% vs 3.8%, $p<0.001$) and could reflect missed preventable child TB deaths. From operational point of view, this study raises alert on the high proportion of missed opportunities for chemoprophylaxis in HIV and/or TB exposed, as less than half those patients eligible initiated isoniazid preventive treatment.

In the second study, we have estimated the magnitude of under-detection of childhood TB using population based data. We used the previously generated MCBIR as the most accurate estimate available. To calculate the case detection rate (CDR) of TB among children under three in Manhiça District, we compared the MCBIR (2011-2012) to notified cases during 2006-2010. The case detection rate was 40.8%. This

estimate is likely to be a conservative one and the true CDR can be even lower, confirming the large hidden burden of disease.

In the third study, we have reported that the overall treatment success rate among paediatric TB cases under the age of three in Manhiça District is high (88%).However, incomplete adherence, defined as lost to follow-up or delay of three or more weeks to treatment completion, among patients who did not die, was detected in almost one third of cases. Child malnutrition and the history of a migrant mother were significantly associated with incomplete adherence, which in turn could lead to increased mortality. We have observed a significant improvement in the treatment success rate in 2011-2012 (88%) compared to the period of 2006-2010 (67.3%).

The following four papers of the thesis contribute to better characterizing the problem of paediatric TB from different perspectives: clinical, microbiological, radiological and anthropological.

In the fourth paper, we documented that the most frequent lesion found in confirmed and probable TB cases under three years is air space consolidation, which further complicates the distinction between TB and bacterial pneumonia in children. Hiliar lymphadenopathy, the radiological hallmark of paediatric intrathoracic TB, was the second most common lesion but was only seen in a minority of case and could be explained by the high prevalence of HIV/malnutrition in our population. These findings highlight the difficulties in diagnosing TB in the absence of bacteriological confirmation and the need of combining clinical, radiological and epidemiological information. This study underscores the importance of not ruling out TB despite the absence of characteristic radiological findings in the context of high HIV burden and the need for improved scoring systems for paediatric population.

This thesis highlights the challenge posed by the frequent isolation of NTM in TB diagnosis. In HBC, where molecular methods are often unavailable and TB diagnosis is still based on sputum smear, the common finding of an NTM isolate in childhood samples is likely to be misinterpreted as tuberculosis, potentially overestimating the burden of TB. Thus, understanding the epidemiology of NTM in children, including

environmental exposure is important when designing vaccine trial endpoints. At the same time, NTM may interfere with Bacillus Calmette–Guérin (BCG) vaccine efficacy and this association needs further research. In the fifth article, we reported a high rate of NTM isolation (26%) among gastric aspirates and induced sputum samples of children evaluated for TB. The distribution of NTM species was similar to what has been reported in South Africa, where *Mycobacterium intracellulare* was the most frequent isolate followed by M. *scrofulaceum* and M. *gordonae*. In our cohort, NTM isolates did not seem to have clinically significance. First, neither of the cases received specific treatment and 2 year mortality was comparable to those cases with negative culture. Besides, the proportion of children with an NTM isolate was similar at admission and follow-up visits, regardless of the initial culture result.When compared to children with *Mycobacterium tuberculosis*, those with NTM had better clinical and radiological presentations and lower mortality.

The sixth paper is a comprehensive review of NTM in children and to our knowledge, is the first one to look at NTM in children with a public health perspective of HBC. The review has evidenced that there are still many gaps in knowledge, particularly regarding disease burden in high TB burden settings. Other areas that need further research are: the understanding of the interaction between NTM and the host, (including the effect of NTM exposure on BCG efficacy); the development of new diagnostic tests, strategies and guidelines that allow clinicians from low resource settings to correctly diagnose both TB and NTM disease; the development of child specific treatment criteria and shortened regimens for NTM treatment.

In the final article, we report the results of a qualitative study aiming to describe local understandings of the signs, aetiology, transmission and prevention of paediatric TB. Overall, the results of this study indicate a very low level of awareness regarding TB in children amongst caretakers of small children, including those vulnerable or exposed to TB. Paediatric TB is practically never suspected even among caretakers with TB and there is a local understanding of TB as an illness of adults as an outcome of impurity derived from the transgression of social and cultural norms. However, TB diagnostic procedures were unanimously accepted and tolerated. Health seeking behaviour for

treatment purposes seems to follow a circular and complex itinerary between the conventional and traditional health care systems. The results of this study are useful for informing health promotion messages in order to overcome the potential delays in treatment and adherence.

The first three papers allow for comparison of case detection and treatment outcomes in children <3 yr between the period 2011-2012 and the previous period (2006-2010). The observed increase in treatment success rate and notified incidence rate are partially due to the improvement in care and treatment services both for HIV and TB over time. However, it also demonstrates that in a rural Sub-Saharan African setting with high HIV prevalence, diagnosis of child TB, case detection and surveillance data can be improved to a level necessary for HBC to reach the zero TB targets.

Introduction

A. INTRODUCTION

1.1. GENERAL INTRODUCTION

As the world enters the era of the Sustainable Development Goals (SDGs) (1,2), ending the tuberculosis (TB) epidemic by 2030 is one the health targets under Goal 3, which is to ensure health and well-being for all (2). TB continues to be one of the major global health problems and ranks first alongside HIV as the leading cause of death worldwide (3,4). It causes ill health among millions of people perpetuating the vicious circle of poverty where poverty fuels TB and TB fuels poverty(5).

i. Historical overview of TB control strategies

Tuberculosis (TB) is one of the oldest diseases in the history of mankind. Its agent, *Mycobacterium tuberculosis* complex (MTBC) has been detected in animal and human skeletons that are thousands of years old(6,7). The disease has been known for centuries, receiving different names throughout the history(6): schachepheth in the Old Testament, phthisis in Greek Hippocrates literature, cunsumptio in Latin reports and consumption in the nineteenth century. **Box 1** describes basic facts on the disease. The German Physician Robert Koch isolated MTBC in 1882, and was granted a Nobel Prize for this discovery(8). At that time, one out of seven people were dying of TB worldwide(6). In the first decades of the 20th century, the work by Leon Charles Albert Calmette together with Camille Guerin led to the development of the first TB vaccine, the *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) vaccine (9–11). This vaccine, which is still used today, was first administered in 1921 and helped prevent a large number of deaths(12). In 1943, sixty five years after the isolation, the discovery of streptomycin allowed the disease to be treatable(13). Soon after, TB was disappearing from the global public health agenda(14).

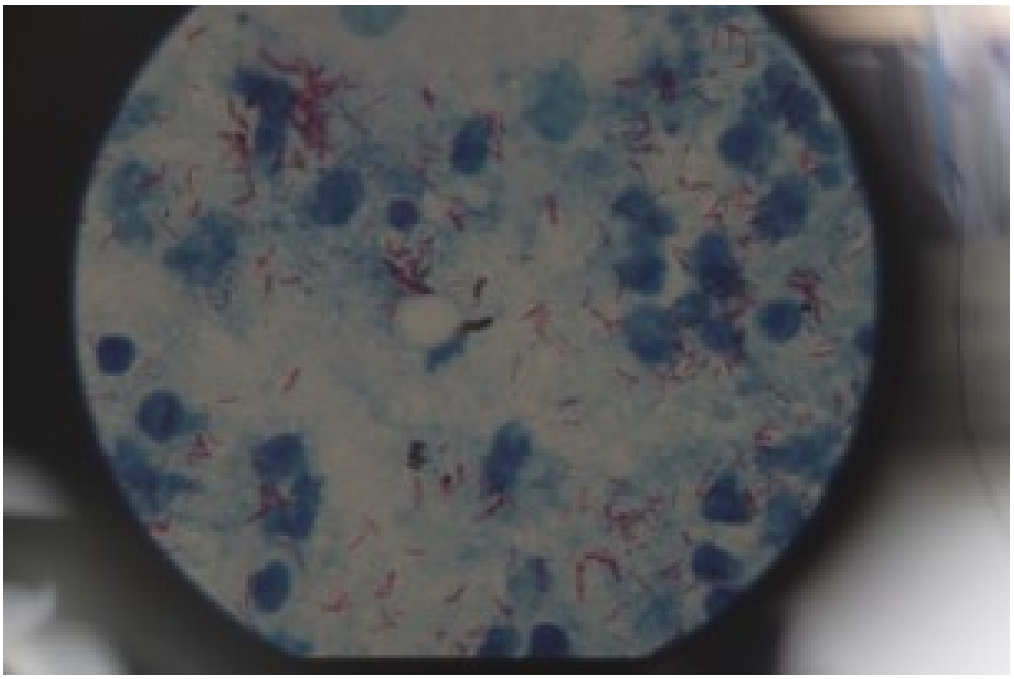
BOX 1. Basics on TB (adapted from WHO TB factsheet) (3)

- Bacterial infection caused by *Mycobacterium tuberculosis* complex
- Transmitted through the air through coughs, sneezes, spits, speaks, or sings
- 1/3rd of the world population is infected with *Mycobacterium tuberculosis* [latent TB infection (LTBI)]
- Around 5-10% of those with LTBI develop the disease. Increased risk is observed among people who have been recently infected with the bacteria or those with immunosuppression caused by HIV (20-30 times more likely to develop the disease), malnutrition, young age, diabetes, among others.
- Mostly affects adults in their most productive years and 95% of the cases are in developing countries
- Primarily targets the lungs (pulmonary disease) but also blood, spine, kidney, brain among others(extrapulmonary TB)
- The symptoms of TB can be mild for many months causing frequent delays in seeking care
- Each undiagnosed and untreated person can infect 15 individuals/year
- Diagnosis is mostly based in sputum smear microscopy in many countries.
- TB is treatable and curable with a standard 6 month course of 4 antimicrobial drugs administered with supervision and support. Between 2000 and 2014 43 million lives were saved through TB diagnosis and treatment.
- Without TB treatment 45% of HIV-negative people with TB on average and nearly all HIV-positive people with TB will die. TB Claims three lives every minute
- Multidrug-resistant tuberculosis (MDRTB) is a form of TB resistant to, at least, isoniazid and rifampicin, the 2 most powerful, first-line anti-TB drugs

At the beginning of the 1990´s, microscopes had already been in use for over 100 years to detect the bacilli and an inexpensive and effective short course combined drug treatment regimen was available(15,16). However, the HIV epidemic emerged and 8 million new TB cases appeared annually. In 1991, the 44th World Health Assembly (WHA) recognized the importance and previous neglect of tuberculosis and set two key

global targets to be reached by 2000: 70% case detection of acid-fast bacilli smear-positive TB patients, and 85% treatment success for those detected(17,18) . In 1995, the global TB monitoring and evaluation system was established in order to follow the progress towards these targets. Based on the work of Karel Styblo and the International Union Against Tuberculosis and Lung Disease, WHO promoted a new strategy named DOTS (directly observed therapy short-course strategy), characterized by a package of the following five elements: 1. Political commitment with increased and sustained financing; 2. Case detection through quality-assured bacteriology; 3. Standardized treatment, with supervision and patient support; 4. An effective drug supply and management system; 5. Monitoring and evaluation system, and impact measurement.

FIGURE1. *Mycobacterium tuberculosis visualization* using the Ziehl–Neelsen stain. TB laboratory at CISM (photo courtesy of Alberto L. García-Asteiro)



This approach allowed 56 million people to be successfully treated between 1995 and 2012, saving 22 million lives. However the results fell short of the targets. In 1999, only 27% of smear-positive cases worldwide were reported and managed by DOTS programs and only a few countries had reached the initial targets(14). As the HIV

epidemic progressed, it soon became the number one risk factor for TB disease, resulting in increased disease burden and posing substantial challenges in Sub-Saharan African (SSA) countries(19). At the same time, the development of multidrug resistance (MDRTB) emerged as a new rising problem(20). As a response to these new epidemiological challenges, in 2000 a new target was put forth in the Millennium Development Goals (MDG) to turn the focus on halting and reversing TB incidence by 2015. The Stop TB Partnership and the First Global Plan to Stop TB (2001-2005) were thus launched and implemented, concentrating on: the expansion of the DOTS strategy particularly in the poorest countries; the emerging challenge of MDRTB and HIV infection; and the need for innovative research on new diagnostics, vaccines and treatment(21,22).

By 2006, 59% of all TB patients were detected and 85% successfully treated. Although this was considered progress, the HIV epidemic kept growing at an alarming speed in areas of SSA and contributed to hinder the MDG (23). In order to accelerate progress, that same year, the Global Plan to Stop TB 2006-2016 was launched in Davos, Switzerland at the World Economic Forum. The Plan set out to reduce TB incidence in line with the MDGs and reach the Partnership's targets for 2015 of halving TB prevalence and deaths compared with 1990 levels (**Box 2**). It emphasized the prioritization of actions for vulnerable populations such as children, the need to foster community and private sector involvement and the need to address the health system(14,24).

In parallel to the public health targets, over recent years major milestones in TB diagnosis and care have been achieved. One of the most exciting examples has been the development and implementation of the rapid test Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, USA), a fully automated NAAT (nucleic acid amplification test) which allows for rapid diagnosis of TB and rifampicin resistance. Five years after its endorsement by WHO(25,26) , Xpert MTB/RIF is recommended as the initial diagnostic test for people at risk of MDRTB and/or people living with HIV by 60% of countries worldwide.

As a result of the intensified efforts, in 2015 the MDG target of halting and reversing the TB incidence was achieved on a worldwide basis. This has allowed for a very

important conceptual transition in the fight against TB from a disease controlling approach to one of elimination. The post-2015 WHO End TB strategy has the vision of making the world free of tuberculosis, with zero deaths, disease, and suffering due to the disease. It envisions a 90% reduction in tuberculosis incidence and 95% reduction in tuberculosis deaths by 2035. Building on the opportunities provided by the new 2015 Sustainable Development Goals (SDG), especially those aiming at universal health coverage and social protection, the End TB Strategy aims to address 4 basic barriers in the fight against TB: weak health systems, underlying determinants, lack of effective tools and unmet funding needs. The costed plan for implementing the first five years of the End TB Strategy is defined under the Global Plan to End TB 2016-2020. Interestingly, the Plan calls attention to differential needs across diverse settings. It proposes different "investment packages" tailored to the local characteristics of the epidemic, health system constrains and socio-economic situations.

BOX 2: Overview of WHO TB control strategies: A. Stop TB Strategy

BOX 2: Overview of WHO TB control strategies: B. End TB Strategy

THE END TB STRATEGY

VISION | A world free of tuberculosis-zero deaths, disease and suffering due to tuberculosis

GOAL | End the global tuberculosis epidemic

INDICATORS	MILESTONES		TARGETS	
	2020	2025	SDG 2030	End TB 2035
Reduction in number of TB deaths compared with 2015 (%)	35%	75%	90%	95%
Reduction in TB incidence rate compared with 2015 (%)	20% (<85/100 000)	50% (<55/100 000)	80% (<20/100 000)	90% (<10/100 000)
TB-affected families facing catastrophic costs due to TB (%)	zero	zero	zero	zero

PRINCIPLES

1. Government stewardship and accountability, with monitoring and evaluation
2. Strong coalition with civil society organizations and communities
3. Protection and promotion of human rights, ethics and equity
4. Adaptation of the strategy and targets at country level, with global collaboration

PILLARS AND COMPONENTS

1. INTEGRATED, PATIENT-CENTRED CARE AND PREVENTION
   - A. Early diagnosis of tuberculosis including universal drug-susceptibility testing, and systematic screening of contacts and high-risk groups
   - B. Treatment of all people with tuberculosis including drug-resistant tuberculosis, and patient support
   - C. Collaborative tuberculosis/HIV activities, and management of co-morbidities
   - D. Preventive treatment of persons at high risk, and vaccination against tuberculosis

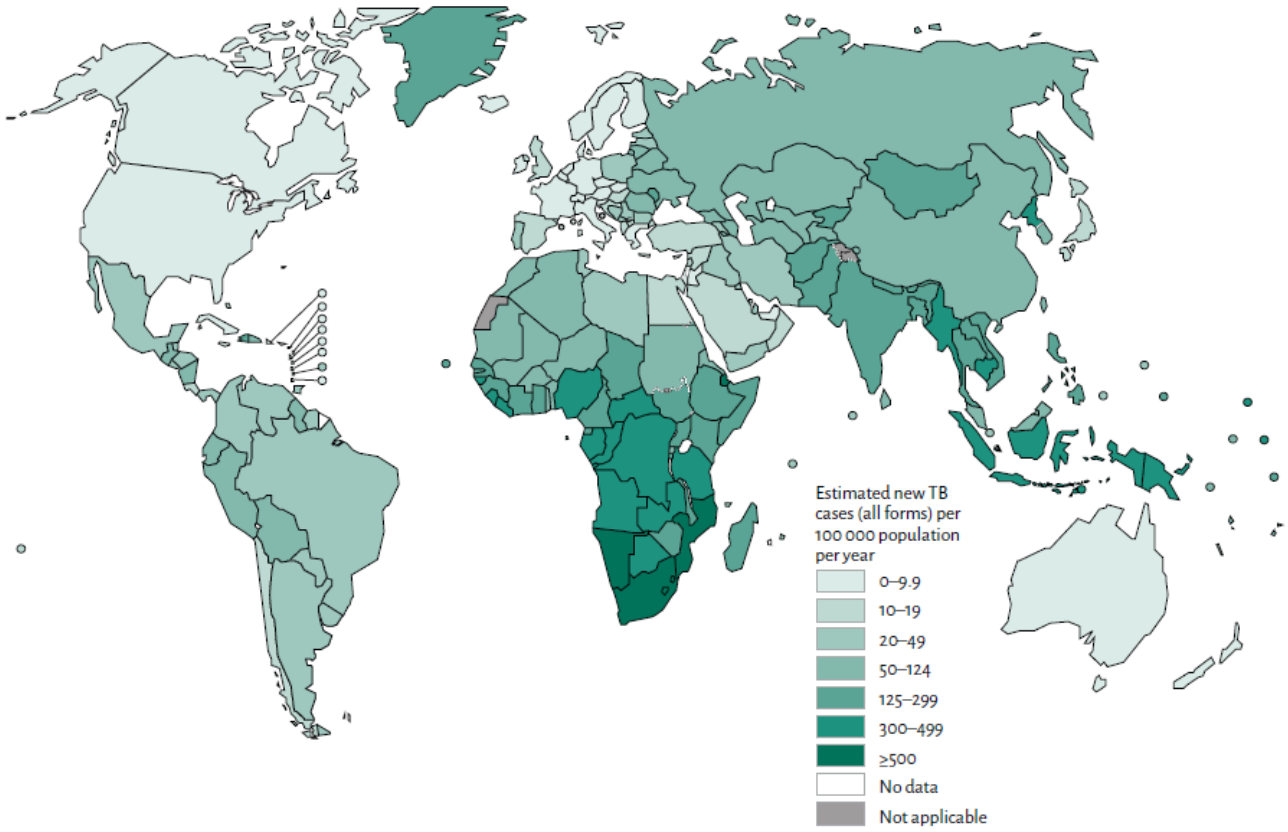
2. BOLD POLICIES AND SUPPORTIVE SYSTEMS
   - A. Political commitment with adequate resources for tuberculosis care and prevention
   - B. Engagement of communities, civil society organizations, and public and private care providers
   - C. Universal health coverage policy, and regulatory frameworks for case notification, vital registration, quality and rational use of medicines, and infection control
   - D. Social protection, poverty alleviation and actions on other determinants of tuberculosis

3. INTENSIFIED RESEARCH AND INNOVATION
   - A. Discovery, development and rapid uptake of new tools, interventions and strategies
   - B. Research to optimize implementation and impact, and promote innovations

ii. Current global situation of the TB epidemic

The latest WHO global TB report published in 2015 has reported important advances in diagnosis and treatment, but has also served to identify major areas that need to be addressed to end the epidemic.

FIGURE 2. WHO estimates of tuberculosis incidence rates (2014)



Disease burden: incidence, prevalence and mortality

In 2014, TB prevalence and mortality were 42 and 47% lower than in 1990, and there was a 32% specific decrease among HIV-TB co-infected patients. The 2006 Stop TB target of a 50% reduction in TB prevalence and mortality was not achieved globally nor in the in the African Region. TB continues to kill 1.5 million per year, including 140 000

children. Ninety percent of the total TB deaths occurred in the Africa and South East Asia Regions. Nearly one in three HIV related deaths was due to TB.

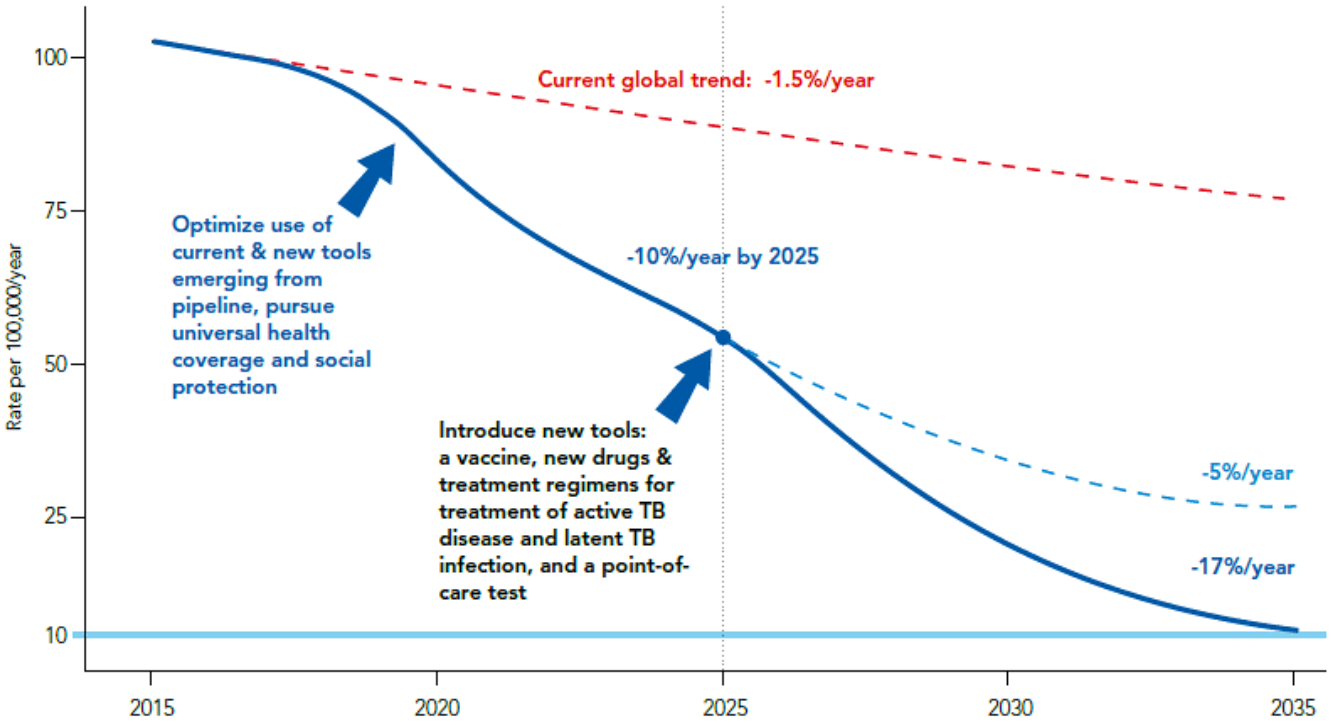
Of the 9.6 million estimated new TB cases in 2014, 1 million were children and 12% occurred in HIV infected individuals. Globally, TB incidence has fallen 1.5% in the last 15 years. The MDG target to halt and reverse TB incidence was achieved worldwide in all WHO regions including 16 of the 22 high burden countries which harbor 80% of the world TB cases. Despite this major achievement, paradoxically TB incidence continues to rise in some countries in Africa, which has 28% of global TB burden and the most severe burden relative to population (281 compared to the 133/100.000 global average).

The WHO End TB strategy must be reinforced in order to achieve an end to the epidemic in the foreseeable future. Ending TB requires a TB incidence rate lower than 10 cases/100 000 population. Thus, the current annual reduction in the global tuberculosis incidence is too slow to reach this threshold. In addition, an estimated 2 billion people with LTBI form a reservoir that sustains the global epidemic(27). The targets can thus only be reached if new tools, including better diagnostics, shorter treatment regimens, effective vaccines and treatment for latent TB infection are introduced.

Figure 3 shows the projection of TB incidence rate according to different scenarios. In the next ten years the targets can be on track if the use of current existing TB tools is optimized and efforts are made towards universal health coverage and increased social protection. However, beyond 2025, increased funding, research and development play a key role. The targets will only be reached if new tools, including better diagnostics, shorter treatment regimens, effective vaccines and treatment for LTBI are introduced. These tools need to be suitable for children and HIV infected populations. Furthermore, once developed, access for the most vulnerable populations needs to be ensured in a timely manner. The recent introduction and scale–up, of Xpert MTB/RIF in HBC was successful in increasing diagnosis of MDRTB but not in ensuring successful treatment outcomes. Thus the introduction of new tools must be conducted without overlooking other steps in the cascade (28,29).

For the first time in many years, the research pipeline looks promising. There are 15 new vaccines candidates in clinical trials, although the first candidate vaccine developed and tested in Phase III trials failed to protect infants against the disease. Several diagnostic assays are under development to detect TB, drug resistance and both combined. Two new drugs (Bedaquiline and Delamanid) have recently been recommended for the treatment of MDRTB under certain conditions, representing the first novel approved drugs in 40 years.

FIGURE 3: Projected acceleration of the decline in the global TB incidence rates (from the end TB strategy, World Health Organization (30))



Limitations in current TB burden estimates

The reported TB data necessary to plan and reach above mentioned health targets have serious limitations particularly in low income HBC. There has been a substantial improvement in the quantity and quality of the reported TB data in the last decade and

currently more than more than 200 countries report consistently notification data. However, experts agree that strengthening surveillance systems continues to be a priority(31). Incidence rate of TB should ideally be calculated based on direct measures. However, this has never been done at a national level due to the inherent difficulties in setting up such a large, expensive and challenging studies. Thus, most HBC derive their estimates from a combination of case notification data and expert opinion. The problem with this system is that notification rates are not a good proxy in countries with poor surveillance systems and poor access to quality health care. In these settings, both under-diagnosis and under-reporting is common. Expert opinion about case detection gap is thus used to estimate under-reporting and under-diagnosis. Methodologies such as inventory studies can be implemented to estimate under-reporting but under-detection is very difficult to estimate in the absence of prevalence or community based studies.

TB prevalence surveys provide the most accurate estimate for burden of disease but not all HBC have yet conducted a prevalence survey. When surveys are not available, prevalence is indirectly derived from incidence and is almost always less precise.

TB mortality among HIV negative people can be measured using data from national or sample vital registration, mortality surveys, or estimated as the product between incidence and case fatality. TB mortality among HIV positive individuals is more complex since HIV is usually indicated as the underlying cause of death according to the international classification of diseases system. Most of the TB deaths in HIV-infected individuals occur in the African region which accounts for 74% of the world TB-HIV burden. Thus the frequent lack of vital registration and the difficulties in ascertaining the actual cause of death are responsible for the large uncertainties in death estimates in this region. In a recent study, Xpert MTB/RIF was proved accurate for TB diagnosis in tissue samples from autopsy cases of unknown cause of death and its use could potentially improve the accuracy of global TB mortality statistics in the future(32).

TB notification and treatment outcomes

In 2014, 6.3 million TB cases were reported globally, of which 6 million were new diagnoses. Children accounted for 6.5% of the global burden of disease, although not all countries, including Mozambique, reported age disaggregated data(3).

At the global level, it is estimated that approximately 37% of people who develop TB are never diagnosed, and/or their cases are not reported. Although the goal to increase CDR is included within the MDG framework, the 70% CDR target established in the 1990s is currently believed to be unreachable. Under-reporting and under-diagnosis are the two main causes of low CDR. The latter is more frequent SSA settings where poor access to health care, failure to recognize TB and lack of appropriate diagnostic test are frequent.

Globally, treatment success rate reached 86% in 2015 and has remained high over the last decade. This rate is lower for HIV infected individuals (73%), who have three fold increased mortality compared to the HIV negative population (11 versus 3.5%)(3).

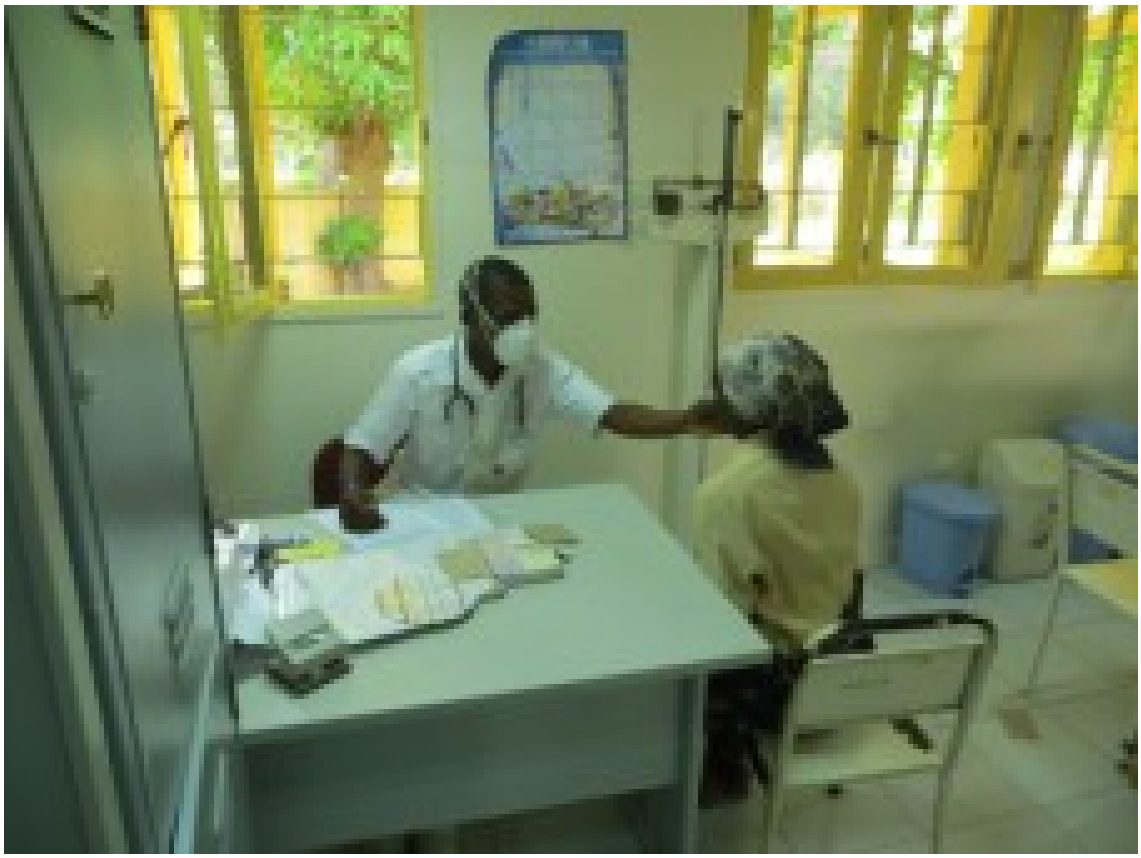
iii. TB epidemiology in Mozambique

Burden of disease

Mozambique stands among the 22 high burden countries which accounted for 80% of the global TB burden in 2014. It is one of the few countries where the burden of TB has not improved over the last decade(33). At a country level, none of 3 targets of the Stop TB Partnership were met. The TB mortality target of 50% reduction was the closest, with a 40% reduction. According to the latest available WHO global estimates(3), Mozambique ranks first, second and third in HIV+TB mortality, TB incidence and overall TB mortality rates respectively **(Box 4).** It is one of the few HBC which has not yet conducted a prevalence study. As it has been the case with the recent prevalence studies conducted in Indonesia or Nigeria, the upcoming survey in Mozambique planned for 2016 may also reveal a higher true burden of disease than previously estimated. In Mozambique, as in most low income HBC, the main source of global

surveillance data comes from notification data from NTP and it is estimated that almost 2/3^{rds} of cases go undetected. As a result, WHO estimates carry large lower and upper uncertainty bounds.

FIGURE 4: The National TB Program at Manhiça District Hospital, Mozambique (photo by Elisa López-Varela)



Few quality surveillance or population based studies have been conducted in the country. Those available show that the burden of disease is higher than estimated (33–40) at least in certain regions of the country. Our group has previously evaluated routine surveillance TB data and reported estimates on TB burden and outcomes in the Manhiça District (MD), in Southern Mozambique(33,38,40). The MD has an estimated community prevalence of HIV among adults aged 18-47 years of 39.9% in 2012(41). According to 2011-2012 surveillance data from the NTP in Manhiça, 75% of TB cases were HIV co-infected, compared to the WHO 52% rate reported in 2014 (3,40). The

incidence rate of TB in 2012 was 571/100.000 and maximum among males 40-45 years (2796/100.000).The incidence of laboratory confirmed TB among HIV infected adults aged 18-47 was extremely high (847/100 000) and the population-attributable fraction of TB due to HIV (61.5%) is one of the highest ever reported in Africa (37). The fatality rate among patients on TB treatment was 15.1 %, varying considerably depending on HIV status and ART (8.4, 12.5 and 20.2% among uninfected, infected on ART and infected without ART individuals respectively). Besides, the contribution of TB to the overall death burden of the district reached 20% among adults in their mid-thirties, suggesting that TB remains one of the major public health problems in the country.

TB-HIV and treatment outcomes

Despite numerous longstanding health system challenges, the country has made remarkable progress in the scale up of HIV services, including TB/HIV integration. HIV testing has increased from a reported 3.8 million tested in 2012 to an estimated 6.6 million tested in 2015, representing over $1/4^{th}$ of the population(42). In 2014, over 96% of TB patients knew their HIV status and in 87% of HIV positive TB patients were on antiretroviral therapy (ART). There are two areas were major improvement is still needed. First, the systematic TB screening of HIV infected people was at 54% in 2015 and should reach 100% of HIV-infected individuals. Second, the provision of isoniazid preventive therapy (IPT) to infected HIV individuals reached 45% in 2015 (42). This is a great improvement from earlier estimations from nationally representative retrospective cohort study of adults initiating ART during 2004-2007 which showed that less than 1% of ART enrollees not on TB treatment were prescribed IPT (34). The national treatment success rate has been high over the last years estimated at 88% in 2014. However, there continues to be a high proportion of patients reported as lost to follow-up (43). In Manhiça treatment success in 2011-12 71.2%, was lower than the national average. This is likely to be due to the high TB HIV rate as well as the worse outcomes frequently reported in HIV co-infected individuals(40). The HIV/TB model of integrated care was implemented in the district in 2012 and led to dramatic improvements in ART provision [from 20% in 2011 to 62% in 2012(40)].

MDRTB

In Mozambique, the estimated prevalence of MDRTB in 2014 was reported to be 3.5% of new cases and 11% among retreated cases,. However, only 22% of retreated cases were tested for drug resistance thus casting doubt over comparability of the prevalence of MDRTB in retreated cases to the globally reported prevalence of 20%. Lack of resistance testing is largely due to the low availability of Xpert MTB/RIF and /or drug sensitivity testing in rural areas outside the main cities. Treatment success for MDRTB cases was 28% in 2014.

Operational research literature

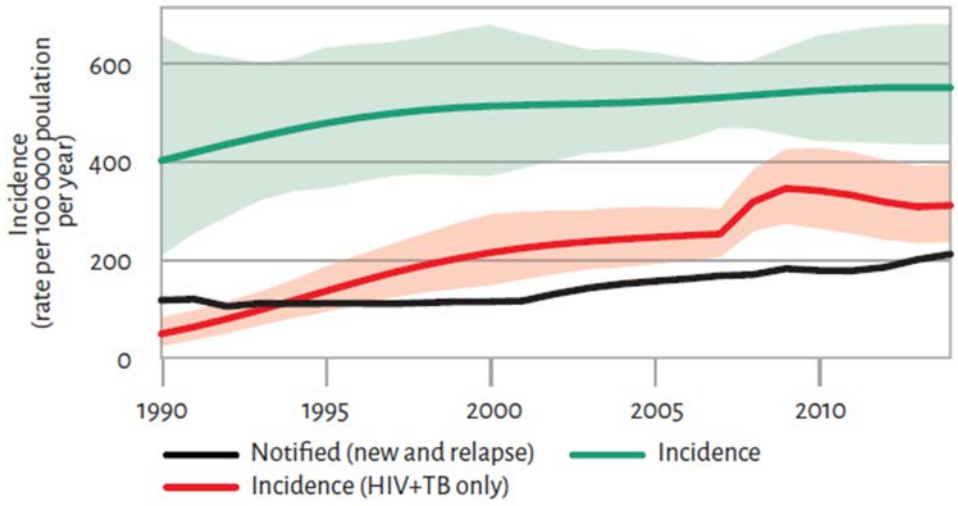
There is increasing operational research literature addressing some of the specific challenges that the country faces in TB control. The common feature is the reference to a huge existing gap between policy recommendations and practice. For example, ensuring proper TB diagnosis, the first step in the care cascade, continues to be regarded as challenging and is a national priority(40). The Mozambican Ministry of Health and National Tuberculosis Program (NTP) began introducing the use of Xpert MTB/RIF for TB diagnosis in smear negative samples in 2012(44). As in many other Sub-Saharan African settings, the implementation has been logistically challenging and has limited the translation into improved health outcomes (28,29). According to a recent publication, Mozambique saw a 69% increase in the number of bacteriologically confirmed cases. However, only 67% of patients diagnosed with the Xpert MTB/RIF initiated treatment and the maintenance of the machines turned out to be costly and challenging(44). Finally, evidence suggests that despite available updated specific guidelines, paediatric TB control continues to lag behind their adult counterparts(45). The 2014 WHO recommendation of scaling up the use of Xpert in children(46,47) is far from being implemented in a country which still faces serious challenges in quantifying paediatric TB cases. Almost half of the population of Mozambique is under the age of 15 years. Yet Mozambique was the only HBC country which did not report age disaggregated data of notified TB cases in 2014.

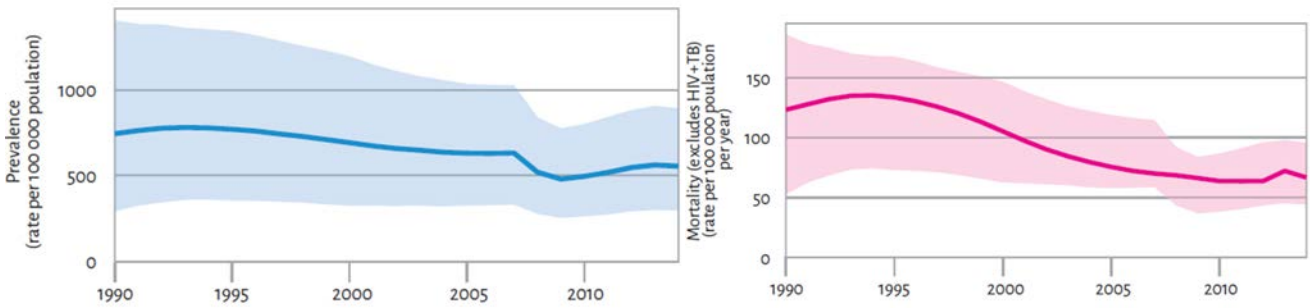
BOX3. WHO TB estimates for Mozambique (2014)(3)

	Number* (thousands)	Rate* (per 100 000 population)	Rank**
Incidence	150 (120-180)	551 (435-680)	2nd
Prevalence	150 (80-240)	554 (295-893)	4th
Mortality (excludes HIV+TB)	18 (12‑26)	67 (44–96)	3rd
Mortality (HIV+TB only)	37 (29-45)	134 (106-165)	1st
Case detection rate (all forms)	39 (31-49)		4th lowest

Population of Mozambique 2014: 27 million
*Numbers and rates are followed by the upper and lower bounds of the 95% uncertainty intervals.
**Ranks of the estimated rates among the 22 hih burden countries

- MDRTB (%, uncertainty intervals): 3.5 (2.2-4.8) new cases; 11 (0-25) retreatment
- Cases tested for MDRTB: 4% new, 22% retreatment
- HIV positive TB patients: 52%; 81% on ART
- Success rate among new cases registered 2013: 88%
- No disaggregated data for TB case notification
- Financing TB control: 28% unfunded



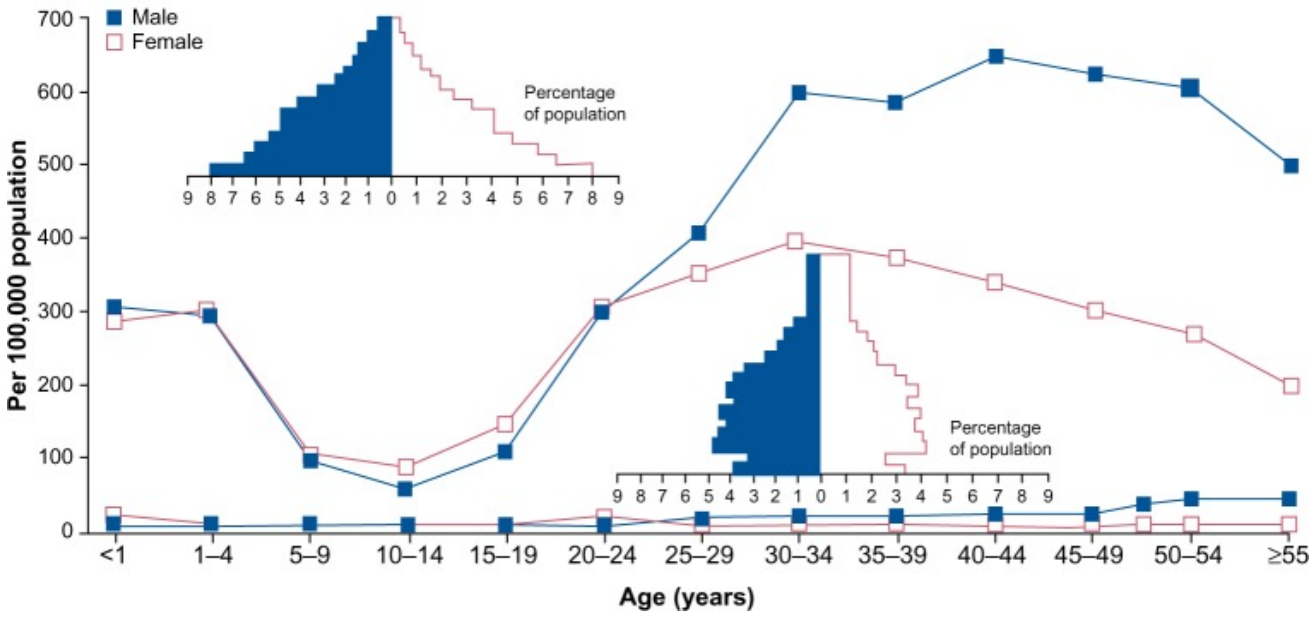


1.2. SPECIFIC INTRODUCCIÓN: PAEDIATRIC TB

i. The problem

In 2014, there were one million new cases of TB in children and 140.000 deaths, most of them were preventable (48). Children constitute a significant proportion of the burden of TB, particularly in HBC where they represent 10-20% of the global burden of disease(49). Factors influencing high rates of paediatric TB include population structure and increased exposure (**Figure 2**). High TB endemic settings typically have 40-50% of their population under the age of 15 (**Figure 5**). In addition, HBC carry increased exposure and infection with TB at a younger age, when TB progression is more likely(50). Box 5 shows key facts about paediatric TB.

FIGURE 5: Age and gender related differences of tuberculosis incidences in hypothetical high and low incidence populations. (Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright © The Union. Donald PR. Childhood tuberculosis: the hidden epidemic. Int J Tuberc Lung Dis. 2004 May;8(5):627-9)(49)



BOX 4: Key facts about TB in children. Adapted from the Roadmap for Childhood Tuberculosis: Towards Zero Deaths(24)

- 10% of children with LTBI develop disease. Risk factors for developing the disease include age (young children, particularly <3yr and adolescents), immunodeficiency due to HIV, malnutrition, measles etc.
- Most children develop TB disease within one year of becoming infected
- Extrapulmonary TB occurs in approximately 20–30% of all cases in children. The most common forms are: TB adenitis and TB pleural effusion
- The presentation of TB disease in children is age-related and dependent on immune response. Infants and young children are at particular risk of developing severe, disseminated and often lethal disease, which may present as TB meningitis or miliary TB. Adolescents are at particular risk of developing adult-type disease (that is, they are often sputum smear-positive and highly infectious)

Children fulfill the three conditions that define a key population at risk of TB according to the End TB Strategy. First, they represent a vulnerable population with increased exposure to TB. Second, in most HBC children are underserved. They have an even more limited access than their adult counterparts to quality TB services, especially diagnostic services. Finally, younger children have increased risk of developing TB (51).

Reaching children and other key populations is essential for ending TB and yet our current **understanding of the TB epidemic in children is incomplete**. The following section summarizes some of the reasons why paediatric TB can be considered a neglected disease and the reasons that have favored this.

Historical neglect of childhood TB

Paediatric tuberculosis has been historically neglected due to several reasons. Firstly, due to the limited focus of the DOTS strategy in targeting smear positive cases (50). Secondly, due to the erroneous perception that children are rarely infectious when

indeed older children and adolescents can transmit TB to contacts(52–54). Thirdly, the skepticism about the reliability of diagnosis given the lack of effective diagnostic tools in children. Additionally, several misconceptions have contributed to attaching lesser importance to paediatric TB including misconceptions about childhood TB severity and the misperception that childhood TB would disappear if TB were contained in adults.

However, there is increased evidence on the contribution of tuberculosis to child mortality(55). Furthermore, child TB represents ongoing transmission and provides the reservoir for future cases. The occurrence of TB in children indicates a system failure to prevent the disease through existing and effective prevention strategies. Thus, addressing childhood is essential in the fight towards TB elimination. But beyond epidemiological reasons, there´s a moral and human rights imperative to reduce preventable deaths and protect this vulnerable population.

Fortunately, in the last years, childhood TB is receiving more attention(56). In 2012, for the first time ever, WHO started including child TB estimates the Global TB Report(57). The first international meeting on childhood TB took place in 2011 and led to the development of an International Roadmap for Childhood Tuberculosis in 2013 (see Box 7). The roadmap identifies key actions that must be taken to reach the goal of zero TB deaths in children. The Global Plan to End TB 2016-2020 specifically emphasizes the need to address children and other key populations. It aims to reach at least 90% of key populations and place them on appropriate therapy (first line, second line and preventive therapy, as required).It encourages countries to define, reach and report progress on key populations (58) and most importantly, it aims to place paediatric TB as a cross-cutting national health priority.

Challenge in Diagnosis

As previously discussed, diagnosis of TB is complex in the general population. However, specificities of paediatric TB present additional diagnostic challenges, especially in infants and young children.

- Bacteriological confirmation

Bacteriological confirmation of TB in children is only obtained in a minority of cases due to several reasons. Firstly, paediatric TB is a paucibacillary disease, which means that few bacilli are usually present. Thus, traditional microbiologic tests perform poorly. Smear microscopy is typically negative even in children with culture confirmed TB(59). Second, non-tuberculous mycobacteria (NTM) are a common cause of false positive smear results thus leading to TB misdiagnosis (60–62). Mycobacterial culture remains the gold standard but is expensive, time consuming and not widely available in most SSA settings. Even when combining smear and culture, the diagnostic yield of samples is <50%(63–65). Since 2013, WHO recommends the use of Xpert MTB/RIF as the initial diagnostic test in children suspected of having MDRTB or HIV-associated TB(47). A recent meta-analysis has shown that sensitivity remains suboptimal compared to culture (62% in expectorated or induced sputum) and is even lower in children younger than 5 years of age (53%)(46). Although a recent study has shown that the performance of Xpert MTB/RIF on a combination of nasopharyngeal aspirate and stool sample is a promising alternative, it detects only very few culture-negative, clinically defined cases of childhood tuberculosis (66). Transcriptional signatures on host blood could eventually lead to a possible diagnostic test but we are still many years away(67).

Secondly, collecting respiratory specimens in infants and in young children less than seven years of age can be challenging because they are usually unable to expectorate spontaneously, as can be done in older children/adults. Moreover, there are operational challenges under current TB program conditions of most HBC (including human resources, training and laboratory capacity) which add further difficulties(68). Three consecutive gastric aspirations (GA) continue to be the reference standard for paediatric TB diagnosis. However, this presents logistical challenges in settings without the capacity to keep a patient in the hospital just for diagnostic purposes (unless also ill enough to justify the hospitalization), and in which parents must therefore take their child to the health post or hospital to have the specimen collection.

TABLE 1. Summary of the types of respiratory specimens used for pulmonary TB diagnosis, their characteristics and limitations

Specimen	**Collection procedure**	**Characteristics: age group, collection time, minimum volume**	**Limitations/Advantages**
Spontaneous sputum	Cough up sputum	- > 7 years -Early morning - 3ml	Only older children
Induced sputum/ laryngo-pharyngeal aspirate	Hypertonic saline nebulization before cough up sputum	- Any age - Early morning - 3ml	Highly operator dependent (results have not been consistent in community settings)
Gastric aspirate	Nasogastric aspiration of gastric juice containing swallowed sputum	-< 7 years - Early morning before out of bed - 5ml	-Loss of volume secondary to stomach emptying (after waking up/sitting/standing), may require gastric lavage 3 consecutive samples recommended (not always feasible in low resource settings)
Gastric lavage	Nasogastric instillation of solution to wash off and recover sputum adhered to walls of stomach	-< 7 years -Early morning - 10 ml	
String test	Oesophageal placement of nylon yarn that can absorb swallowed sputum	-> 4 years -Unknown, duration probably more important -N/A	Not affected by stomach emptying
Naso-pharyngeal aspirate	Nasopharyngeal suctioning to collect secretions from upper respiratory tract, but may also collect from lower tract if cough reflex is stimulated	-<6years -Unknown, probably higher yield in morning -2ml	Yield tends to be similar to or lower than that of induced sputum or gastric aspirate/lavage

Alternative specimen collection strategies have been proposed although none of them have consistently demonstrated a higher diagnostic yield than the gastric aspirate under community-based programmatic conditions (see **Table 1**) (69). While induced sputum (IS) has been found to have a similar (70) if not greater (71) yield than the

traditional GA under research conditions, these specimen collection strategies are highly operator dependent, require more equipment, training, and time to collect than a gastric aspirate, and the quantity of sample of induced sputum is often quite small. Using culture as the detection method, and GA as the specimen collection method, bacteriological confirmation of pulmonary TB in young children is disappointingly low, ranging from 2-10% to 20-40% depending on disease severity(59,72).

- Clinical and score-based diagnosis

Consequently, in most cases, TB is clinically diagnosed based on a combination of non-specific signs and symptoms together with other diagnostic tools such as tuberculin skin testing (TST) and chest radiography (CXR) (47,73,74). These diagnostic approaches have multiple limitations. First, the spectrum of intrathoracic TB disease is broad in children, with a significant confounding effect of HIV on the clinical presentation (59), and overlap with other common conditions such as pneumonia or severe malnutrition(75,76). Additionally, atypical presentation of TB as an acute severe pneumonia, which contradicts traditional standard case management, may be more common than previously thought (77,78). In fact, the relationship between TB and child pneumonia has been a matter of recent research, which suggests that TB may be a cause or contributor to bacterial pneumonia in high TB endemic settings(77,79). Second, CXR which continues to be a critical tool for diagnosing intrathoracic TB, has low specificity, high inter- and intra-observer differences and its reporting lacks standardization (80). Some authors have proposed CXR scoring systems with high negative predictive values in adults but the performance is suboptimal in HIV positive patients and may not be applicable to children(81). Finally, TST is characterized by its low sensitivity. Up to 10% to 20% of immunocompetent children with confirmed TB disease can have a false negative result, due to the use of corticosteroids, recent viral infections or administration of live viral vaccines, skin anergy and incorrect administration (82). This rate is even higher in immunocompromised patients, including HIV infected or malnourished, but also younger children with immature immune systems, due to a reduced response of T helper cells, on which TST is

dependent (83). Most importantly, TST cannot differentiate between LTBI and active TB disease. Furthermore, false positive results are observed in the context of previous BCG vaccination and exposure to environmental mycobacteria. Alternatively, Interferon gamma release assays (IGRAs), which appear to provide a higher specificity thanks to the use of specific *Mycobacterium tuberculosis* antigens, are expensive and sophisticated techniques not available in most HBC.

As a result, diagnostic scores based on the previous approaches are of limited utility (74,84–87). This is particularly true for children infected with HIV, severely malnourished or <5 years of age, who in turn have the highest risk of developing TB disease following infection(24) and of rapid disease progression and mortality when diagnosis is delayed (59). This is proven by the fact that TB accounts for a major proportion of facility-based HIV-related deaths, half of which remained undiagnosed at the time of death, according to autopsy studies (88). On the other hand, over-diagnosis and inappropriate treatment is also common(67).

- Operational challenges

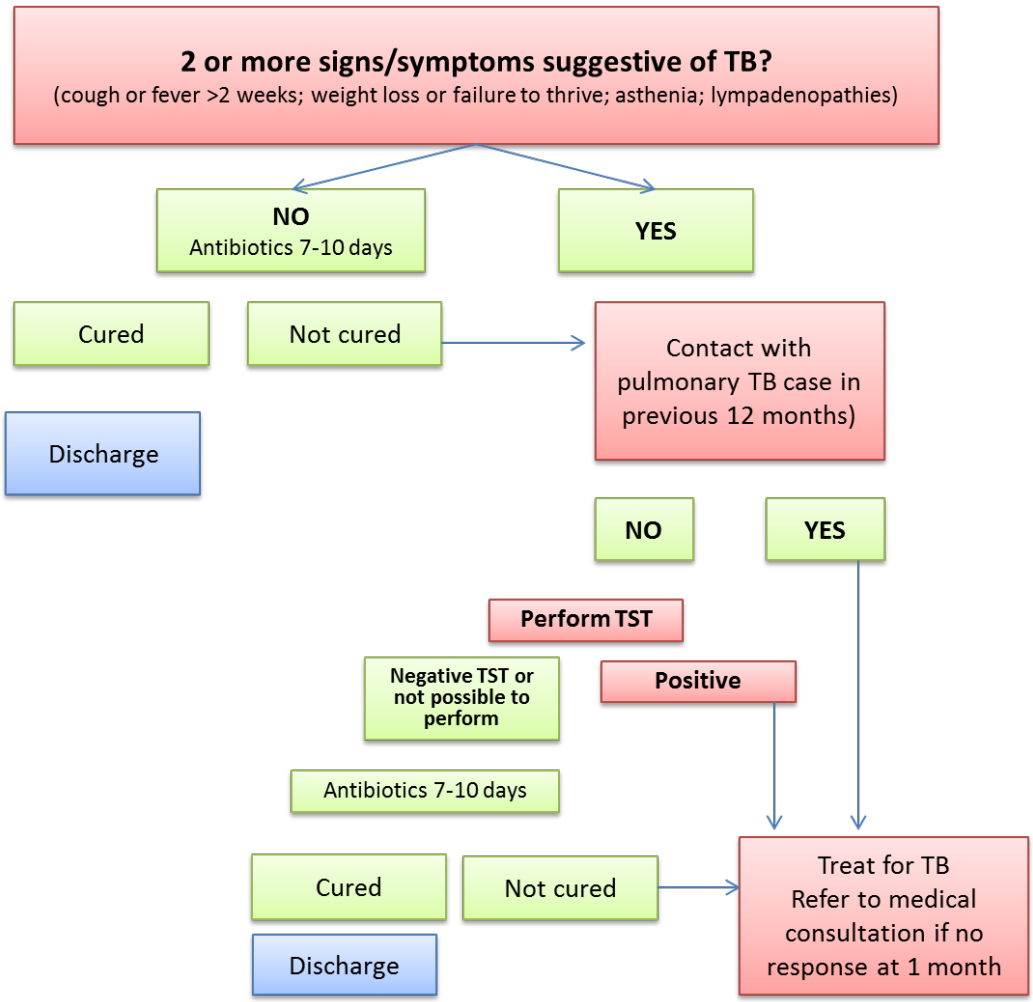
As in many other SSA settings, in Mozambique most paediatric TB cases first seek care at peripheral /rural facilities, which often have limited infrastructure and human resource capacity and training. TST and CXR are usually not available (89)and even when they are the quality of test performance and the ability to correctly interpret the finding is suboptimal. At peripheral health centers, health workers are usually overloaded with different clinical activities including triage, child care activities while responsible at the same time for specific vertical programs such as malnutrition, TB and HIV. There´s a frequent lack of specific training in childhood TB which is often not considered a priority health problem. There is also widespread pessimism among health workers regarding the ability to diagnose the disease at primary health settings(90). As a result, defining the roles and responsibilities for those taking care for paediatric TB cases is sometimes difficult. However, what is definitely a must is the integration of paediatric TB services into the wider primary care, HIV care, maternal and child care.

Although specific paediatric guidelines often exist at a national level **(Figure 6** shows the algorithm for paediatric TB diagnosis in Mozambique), translating them into actual routine procedures is more challenging. For example, despite widespread evidence of the effectiveness of contact investigation and preventive therapy with IPT for high risk contacts (91,92), this is hardly ever done in most HBC. The differential diagnosis of LTBI and TB disease is not always easy in the context of previous BCG vaccination. Plus, up to half the TB disease cases are thought to be asymptomatic in the early stages of the disease. Active screening in the community is often not feasible due to the aforementioned work overload and lack of means. On the other side, few parents bring their children for screening due to economic constrains and perceived low priority if child asymptomatic. Furthermore there are difficulties in establishing a history of exposure particularly among orphan child, and in settings where transmission is not limited to the household. Additional challenges include the lack of procedures for documentation and follow-up of contact screening and chemoprophylaxis in national program(93). WHO also recommends routine screening of TB among HIV infected children and adults and the provision of IPT to those in whom TB is ruled out. However, many clinicians are not confident when ruling out TB, especially in HIV malnourished children, and manifest concerns regarding the risk of treating active cases with monotherapy and the subsequent contribution to creating drug resistance.

- Case definition in the context of research

Even in the context of research, case definition of microbiologically unconfirmed TB in children has been subject to extensive debate. Research in the field of childhood TB diagnostics has traditionally used varying definitions, which have made it difficult to compare between studies and complicated evidence based recommendations. Recent published guidelines (76,94) are not extant of limitations, namely the ability to categorize presumptive TB cases who die before the required criteria for categorization can be established(95).

FIGURE 6: Algorithm for diagnosing TB in children under 14 years in Mozambique



Disease under-ascertainment

We thus have a situation of extremely difficult diagnosis of TB which is compounded by the fact that the disease affects children from the poorest communities with limited access to health service and frequent lack of knowledge on the disease. Paediatric TB cases are likely to develop in a household already affected by an adult TB case Consequently, TB in this family may cause loss of income and perpetuate poverty(96). In addition, most aspects of a child´s health depend on their caregivers, who determine when and where he or she receives assistance, what they eat etc.(97). This is in turn conditioned by availability of the caregivers ´resources, knowledge and

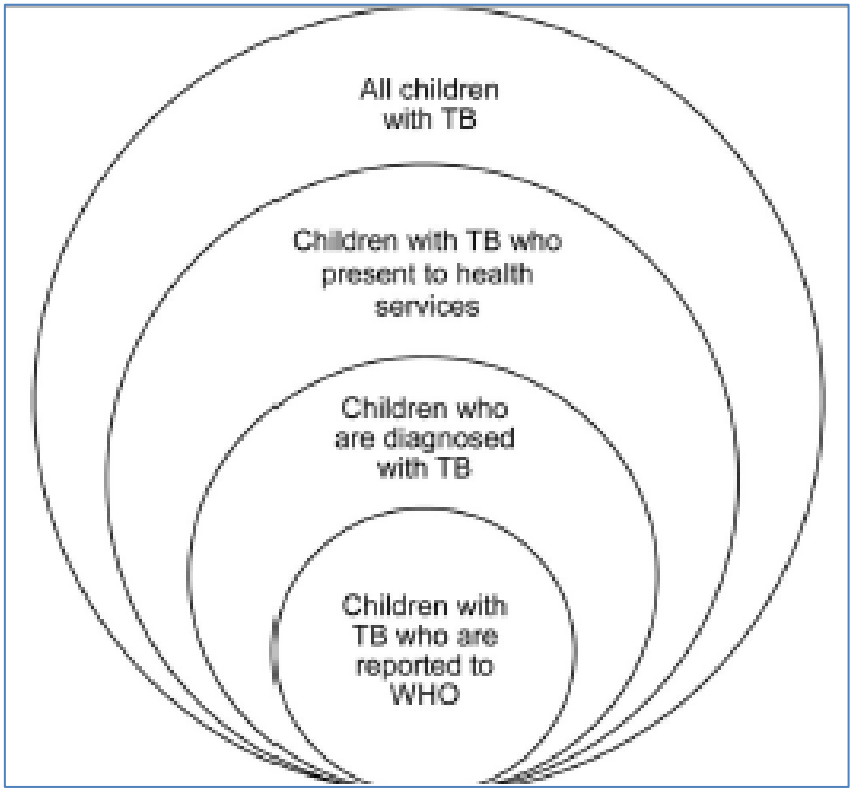
awareness of the disease and cultural factors(89,97). Stigma has been shown to influence health seeking behavior for children with TB(98). As a result, some TB cases may never reach the health system. Improved understanding on the extent of this problem and the barriers faced by vulnerable populations is necessary in order to develop innovative strategies to ensure that this population is not left out of the health system.

Paucity of data

The aforementioned diagnostic challenges result in under-diagnosis, misdiagnosis or delayed diagnosis of the disease and eventually contribute to the hidden epidemic of TB in children. But even when diagnosed, paediatric TB reporting is often incomplete. This results in further limitations in our understanding of the burden of TB in children(73). Under-reporting is particularly frequent in countries with large private health sectors, but can also happen in public non-NTP facilities. For example, in South Africa up to 1/3rd of confirmed cases diagnosed in a public hospital setting were not reported. Underreporting was more frequent in the child had severe forms of the disease or had died prior to referral (99,100). **Figure 7** represents the steps in the cascade from symptoms to notification.

Although determining the epidemiological burden of paediatric TB may not appear to contribute to solving the problem, numbers matter. From a clinical point of view, knowing the local epidemiology helps clinicians assess the likelihood of a TB diagnosis(50). From a programmatic perspective, childhood TB reflects ongoing transmission within a population and thus is a useful sentinel indicator of the effectiveness of the NTP. Moreover, an accurate understanding of the burden is important for setting and monitoring targets, allocating resources appropriately, identifying problems in programme delivery as well as for providing adequate childhood advocacy(73).

FIGURE 7: The cascade from symptoms to reporting in children with tuberculosis (Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright © The Union. Seddon JA, Jenkins HE, Liu L, Cohen T, Black RE, Vos T, et al. Counting children with tuberculosis: why numbers matter. Int J Tuberc Lung Dis [Internet]. 2015;19 Suppl 1(0 1):9–16 (73)



The first WHO estimates of the burden of childhood TB were only available in 2012(101), partly due to the scarce age-disaggregated notification data available in many HBC. The main source of global surveillance data for childhood TB initially came from notification data collected by NTP. Initial estimates showed an incidence of 490 000 (470,000-510,000) equivalent to 6% of the total burden in 2011 and 64.000 deaths in HIV negative children, equivalent to 6% of the TB deaths. Importantly, the uncertainty intervals relative to the best paediatric TB incidence estimate were twice as large as the relative uncertainty for all ages(101). Incidence estimates were based on the use of paediatric notification data and case detection rates. However, these estimates were an educated guess. First, notification data has serious limitations which have been explained in the previous sections and include: the frequent

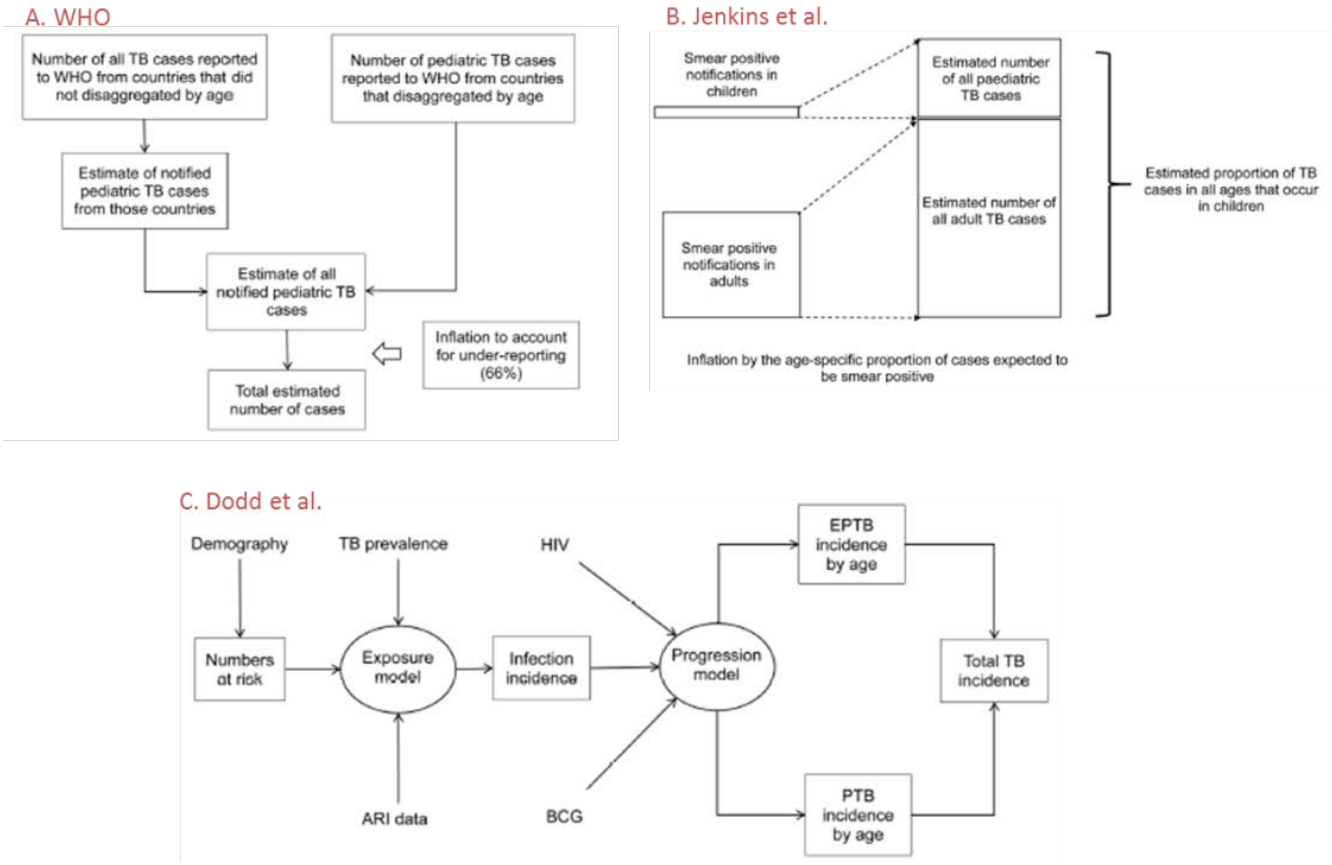
misclassification arising from the diagnostic challenges; the lack of resources for active case finding through contact tracing; limitations in the notification process itself etc. Second, initial estimates assumed an equal CDR in children and adults, whereas under-detection is children is more common(99). Finally, the proportion of TB burden among children was assumed to be equal regardless of whether countries disaggregated notifications or not. However, as previously explained, we expect a higher proportion of TB cases in HBC which in turn are more likely to be unable to report disaggregated data(50). Mortality estimates were calculated among HIV negative children, using data from age-disaggregated vital registration systems when available and ecological statistical predictive models for low income countries.

Recently, several groups have provided new methodologies for estimating TB incidence in children which have substantially improved the estimates(4,102,103). In 2014, following a global consultation on estimates of TB disease burden in children, WHO reported incidence estimates using a different ensemble approach(104). First, it calculated the 2013 child:adult ratio for new and relapse case notifications and assumed this ratio to be the same as the ratio among incident cases. This ratio was then used to disaggregate global TB incidence among children and adults. The second approach was based on dynamic mathematical modeling using adult TB prevalence combined with aspects of the natural history of the disease published by Dodd et al (103). The resulting estimate of global TB incidence did not differ much from that of previous years (550,000 total incident cases equivalent to about 6% of the total incident cases). The latest 2015 WHO report has provided incidence estimates that double the ones from the previous years: 1 million cases. This time, the approach was a statistical ensemble combining the previously mentioned dynamic model by Dodd et al. with child specific CDR based on a study published by Jenkins et al. in 2014 (102).See **Box 5** for details on the latest methodologies.

In general, these recent new methodological approaches have brought substantial progress to the understanding of the epidemic. However, we still do not completely understand which children get TB and how we should reach them(73). Increased use of modelling as well as better data on which to build models are needed to improve the

accuracy of estimates. This includes population based data and data from sentinel sites within national prevalence studies.

BOX 5: Methodologies used to estimate the burden of paediatric TB. (Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright © The Union. Seddon JA, Jenkins HE, Liu L, Cohen T, Black RE, Vos T, et al. Counting children with tuberculosis: why numbers matter. Int J Tuberc Lung Dis [Internet]. 2015;19 Suppl 1(0 1):9–16 (73)



Age-specific surveillance data on treatment outcomes is not routinely reported. However, understanding the outcomes of TB treatment according to different variables such as age, malnutrition or HIV is important to define how we should treat these children. This is due to the aforementioned particularities of the disease in children, including the fact that children are dependent upon their parents/caretakers for every aspect of their lives, including the management of tuberculosis. There are few studies reporting paediatric TB treatment outcomes in SSA(105–108). An

unpublished 2014 meta-analysis by Hunain Khawaja from the UK reported a success and death rate of 81 and 3% respectively for children treated for TB with the pre-2010 WHO dosage recommendations. The authors conclude that there is a need to assess what patient characteristics (including nutrition status, HIV etc) affect treatment outcome(109).

Lack of sufficient research and development, funding and political will

The economic case for ending TB is compelling. It has been estimated that an investment of 1US$ in TB care yields a return of 30 US$(51). Effective treatment gives 20 additional years of life to an individual in the middle of his or her productive life(110). However, the WHO has estimated that, the ongoing shortage of funding to fight TB is close to 2 billion USD per annum globally excluding research(51).

Since resources are limited, priority should be given to new advances in research and development, which traditionally have been too slow in the last decades (including new diagnostics, new treatment options and vaccines).

ii. The opportunity

The International Roadmap for Childhood TB published in 2013(24) laid out the strategic framework in order to achieve zero TB deaths in children. **Box 7** shows the 10 key actions towards reaching zero paediatric TB deaths . Now, the End TB strategy provides a unique chance to address childhood TB to a greater extent than previous approaches (111). First, it advocates for an integrated family centered approach to contact screening for active case detection and provision of preventive therapy following the diagnosis of an index TB case. Second, it urges for a greater collaboration with the maternal and child health sector, which as we have explained before, is very much needed for improved case detection and management of childhood TB. Finally, it calls for intensified research and innovation, in particular improved diagnostics. The Global Plan to End TB aims to reach 90% of people in key populations. In order to

achieve it, countries must first define their key populations, understand the barriers that they face, set specific targets and measure progress.

There is thus an opportunity to build on the increased attention and recent knowledge gained in paediatric TB in order to reduce the burden of TB (24).

The research in this thesis is aligned with the needs identified in the Roadmap and mainly targets two of the key actions. Action number 2 refers to the need of generating better data, including measurements of under-reporting and diagnosis, as well as analysis of disaggregated data (Articles 1, 2 and 3 in this thesis). Action 8 highlights the need to address research gaps in the areas of epidemiology (Article 1), operational and public health (Article 4, 5, 6 and 7). Altogether, the articles included in this thesis contribute to defining a key population in Mozambique (children<3 years), quantifying the burden and case detection rate and understanding specific barriers that this key population encounters.

BOX 6: Overview of the WHO roadmap for childhood TB

The Childhood TB Roadmap – an overview

This roadmap for addressing childhood TB outlines 10 key actions to be taken at both the global and national levels:

1. Include the needs of children and adolescents in research, policy development and clinical practice.
2. Collect and report better data, including on preventive measures.
3. Develop training and reference materials on childhood TB for health care workers.
4. Foster local expertise and leadership among child health workers at all levels of the health care system.
5. Do not miss critical opportunities for intervention (e.g. use strategies such as intensified case-finding, contact tracing and preventive therapy); implement policies for early diagnosis; and ensure there is an uninterrupted supply of high-quality anti-TB medicines for children).
6. Engage key stakeholders, and establish effective communication and collaboration among the health care sector and other sectors that address the social determinants of health and access to care.
7. Develop integrated family- and community-centred strategies to provide comprehensive and effective services at the community level.
8. Address research gaps in the following areas: epidemiology, fundamental research, the development of new tools (such as diagnostics, medicines and vaccines); and address gaps in operational research, and research looking at health systems and services.
9. Close all funding gaps for childhood TB at the national and global levels.
10. Form coalitions and partnerships to study and evaluate the best strategies for preventing and managing childhood TB, and for improving tools used for diagnosis and treatment.

Objectives

B. OBJECTIVES

The overarching goal of this research project is to improve current estimates of childhood TB and inform public health policy to achieve zero paediatric TB deaths.

i. General objective

- Provide community based estimates of the true burden of TB in children under three years of age and treatment outcomes.
- Identify critical barriers to obtaining accurate TB estimates from different perspectives: anthropological, clinical, microbiological and radiological.

ii. Specific objectives

- To determine the minimum community based incidence rate of TB among children under 3 years of age in rural Southern Mozambique (**Article 1**).
- To estimate the paediatric CDR in Mozambique and provide reference methodology and evidence for countries (**Article 2**).
- To describe the treatment outcomes and adherence to TB treatment and evaluate associated factors to poor adherence. (**Article 3**).
- To describe the clinical and radiological findings in children investigated for TB, comparing TB cases with those considered not to have TB (**Article 1 and 4**).
- To report the prevalence of NTM isolation in presumptive TB cases and its clinical characteristics (**Article 5**).
- To review the epidemiological and clinical features of NTM infection in children with a specific focus on the public health implications in high childhood TB burden settings (**Article 6**).
- To describe caretakers' perspectives of paediatric TB and implications on care-seeking behaviors (**Article 7**).

Materials and Methods

C. MATERIALS AND METHODS

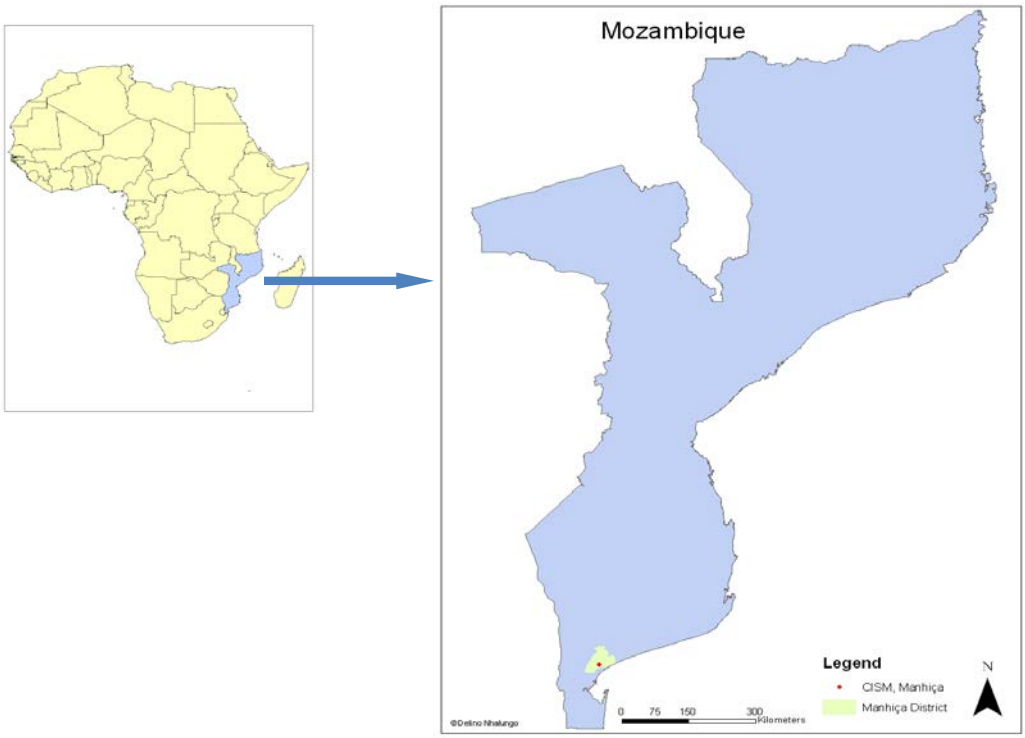
i. Study area and population

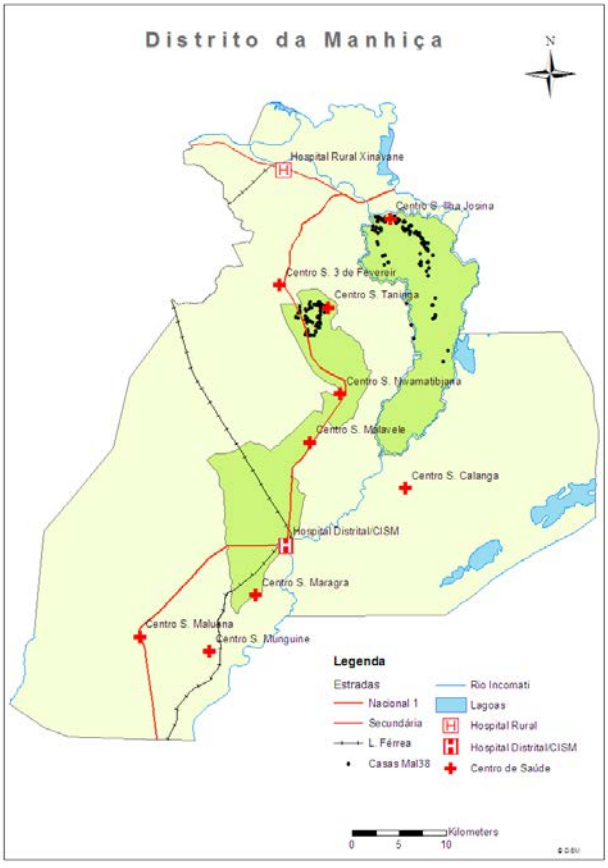
The studies of this thesis were conducted at the Centro de Investigação em Saúde de Manhiça (CISM), located in the District of Manhiça, Southern Mozambique. Established in 1996, the CISM´s mission is to improve health and development through provision of health care, and carrying out research in priority health problems. Its research agenda is focused on the most pressing public health problems in the country such as malaria, HIV, tuberculosis, diarrheal diseases, pneumonias, maternal and reproductive health.

The District of Manhiça (MD) is located in the North of the Maputo province (Figure 5). The capital of the MD, Manhiça, is a semi-rural town, set on a plateau that borders the flood plains of the Incomati River. It is estimated that currently there are 164,500 inhabitants and 37,300 households in the district´s 2,380 square. The age and sex structure of the Manhiça population has a predominance of young people (almost half its population is under the age of 15 years). A particular feature of the population in southern Mozambique is the limited proportion of adult males from age 20 onwards. This may be due to labor migration to Maputo city and the neighboring South Africa, especially in the gold mines.

The Manhiça District Hospital (MDH) is the main health facility in the area, used for primary health care by the nearby population, and is one of the two referral health centers for the MD. It has a 110-bed inpatient ward, an outpatient clinic, a maternal and child health clinic with a small surgical room, and an emergency room. There are a number of peripheral health posts in the area, used only for primary health care. Both the hospital and the health posts are easily accessible and all paediatric outpatient consultations are free, except for a standard subsidized fee for outpatient medication that is taken home.

Figure 8: Africa and the location of Mozambique and Manhiça.





Since 1996, the CISM is running a continuous health and demographic surveillance system (HDSS) for vital events, migrations and morbidity surveillance at the Manhiça Health Center and other peripheral health posts in the area. By linking the information obtained through its morbidity surveillance system to the demographic data, CISM has provided in recent years detailed descriptions of the health status of the community. A full description of the geographic and socio-demographic characteristics of the study community has been presented elsewhere(112).

The demographic component of the HDSS is updated at least once a year. It is based on the Manhiça study area, which at the time of this thesis covered 500 km2 (one fifth of the whole Manhiça district) and 92,000 inhabitants (36,000 of whom live within a 10 km distance from the center of Manhiça town). A permanent identification number is issued for every individual of the study area, and their households (around 20,000) are identified and geopositioned with GPS. In Manhiça DSS, a household is defined as a group of people living under the "same roof", sharing food and other expenses and acknowledge one of them as their leader. In most of the cases the head is a man (71% in Maputo City and 80% in Sub-Saharan Africa). Average number of persons in a household is 4. The surveillance activities consist mainly in visiting every household at least once a year to collect data on vital events such as births, deaths, migration, marriages, divorces, pregnancies and their outcomes, household assets, and other. These yearly visits are complemented by daily visits to all the health facilities to record events which are registered after confirmation at home. In addition, a team of supervisors with motorbike visit weekly the key informants to record all community events occurred during the week. This triangulation of visits or sources of capturing events serve to avoid missing events if the one year-visit was the only source.

The health component of the HDSS is a passive case detection done at health facilities within the study area, targeting children under the age of 15 years who are seen in the inpatient or outpatient services. Whenever a child goes to a health facility the health information is linked to the demographic data through the HDSS permanent identification number.

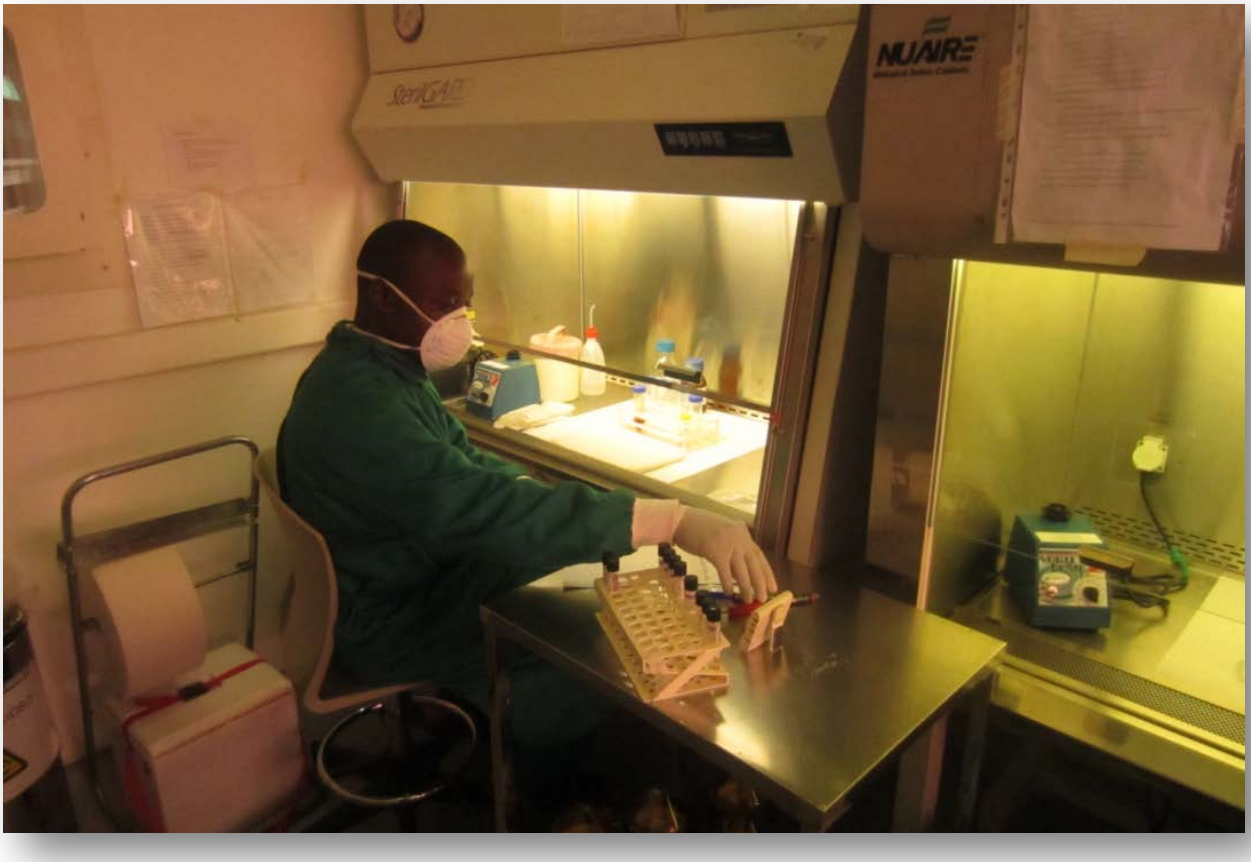
FIGURE9: The Manhiça Health Research Center (CISM)

In Mozambique, newborns are routinely vaccinated at birth with BCG, with estimated coverage ranging from 86% to 95% (113,114). TB and HIV services are offered free of charge by the NTP at all health units (primary, secondary and tertiary level). Overall, TB diagnosis is mostly based in smear microscopy although Xpert MTB/RIF is being slowly deployed in the country(44). Children with presumptive TB are frequently derived from the peripheral health posts to one of the two hospitals in the district for specific evaluation by health workers from child health services. Although GA is sometimes attempted, childhood TB diagnosis is currently achieved via clinical methods. In 2013, specific paediatric TB guidelines were published (see **Figure 4** for the specific algorithm used for TB diagnosis in Mozambique)(115). Since 2012, TB and HIV services are integrated and patients diagnosed with TB receive care and treatment for both diseases at the NTP. Paediatric combined formulations for TB treatment are available.

FIGURE 10: The paediatric TB procedure room at the Manhiça's district hospital. Medical technician and health assistant performing a gastric aspiration (photo by Elisa López Varela).

In the field of Tuberculosis, CISM has participated in large multicenter studies for the evaluation of drugs [Study C(116), EBA01(117)], vaccines (Aeras 402)(118), diagnostics (TB-TRIAGEM) and multiple observational studies(32,37,40,45). Since 2010 it counts with a fully equipped Biosafety Level 3 TB laboratory and processes samples for solid (Lowenstein Jensen, LJ) and liquid culture (MGIT), Line Probe Assays (Hain Lifesciences), Xpert MTB/RIF, ZN and Light Microscopy, as well as QuantiFeron for detection of LTBI. At the MDH, the CISM has helped set up a negative pressure room for the performance of TB sampling procedures which is working since 2010.

FIGURE11: Biosafety TB laboratory at CISM (photo courtesy of Alberto García-Basteiro)



ii. Methodology of studies

The work is presented as a collection of 5 articles published or accepted for publication in peer-reviewed international journals, and 2 manuscripts that are currently under review. Published studies include i) a community-based study to determine the incidence of TB in children under 3 yr in Manhica; ii) an epidemiological analysis aimed at estimating the case detection rate of paediatric TB; iii) four related descriptive studies evaluating: a) the radiological characteristics in children investigated for TB, b) the rate and predictor factors of adherence to TB treatment , c)the prevalence and clinical characteristics associated to NTM isolation in children , d) the caretakers' perspectives of paediatric TB and; v) a literature review article on the current evidence

and interplay of NTM and TB in children-. The specific methodology of each study is described inside each paper.

The studies received support from the European and Development Clinical Trials Partnership (EDCTP) [IP_07_32080_003]. The CISM receives core funding from the Spanish Agency for International Cooperation and Development (AECID) and the HIV/AIDS day hospital (at the MDH) from the Catalan *Agència Catalana de Cooperació al Desenvolupament* (ACCD).

Results

D. ARTICLES

1. Incidence of Tuberculosis among young children in rural Mozambique

López-Varela E, Joaquim Augusto O, Gondo K,García-Basteiro AL, Fraile O, Ira T, Ribó Aristizabal JL, Bulo H, Muñoz Gutierrez J, Aponte J, Macete E, Sacarlal J, Alonso P.. Pediatr Infect Dis J. 2015 Jul;34(7):686-92.

2015 Impact factor 2.72 (Q1)

Incidence of Tuberculosis Among Young Children in Rural Mozambique

Elisa López-Varela, MD, MPH,† Orvalho Joaquim Augusto, MD,* Kizito Gondo, MD,* Alberto L. García-Basteiro, MD, MS,*† Oscar Fraile, MD,*† Tasmiya Ira, BS,* Jose Luis Ribó Aristizabal, MD,‡ Helder Bulo,* José Muñoz Gutierrez, MD, PhD,*† John Aponte, MD, PhD,*† Eusebio Macete, MD, PhD,* Jahit Sacarlal, MD, PhD,* and Pedro L. Alonso, MD, PhD*†*

Background: Tuberculosis (TB) contributes significantly to child morbidity and mortality. This study aimed to estimate the minimum community-based incidence rate of TB among children <3 years of age in Southern Mozambique.

Methods: Between October 2011 and October 2012, in the Manhiça District Health and Demographic Surveillance System, we enrolled prospectively all presumptive TB cases younger than 3 years of age through passive and active case finding. Participants included all children who were either symptomatic or were close contacts of a notified adult smear-positive pulmonary TB. Children were clinically evaluated at baseline and follow-up visits. Investigation for TB disease included chest radiography, HIV and tuberculin skin testing as well as gastric aspirate and induced sputum sampling, which were processed for smear, culture and mycobacterial molecular identification.

Results: During the study period, 13,764 children <3 years contributed to a total of 9575 person-year. Out of the 789 presumptive TB cases enrolled, 13 had TB culture confirmation and 32 were probable TB cases. The minimum community-based incidence rate of TB (confirmed plus probable cases) was 470 of 100,000 person-year (95% confidence interval: 343–629 of 100,000). HIV co-infection was present in 44% of the TB cases.

Conclusion: These data highlight the huge burden of pediatric TB. This study provides one of the first prospective population-based incidence data of childhood tuberculosis and adds valuable information to the global effort of producing better estimates, a critical step to inform public health policy.

Key Words: tuberculosis, pediatrics, epidemiology, incidence, Mozambique

(*Pediatr Infect Dis J* 2015;34:686–692)

Tuberculosis (TB) is an underrecognized but potentially important cause of morbidity and mortality in children in TB endemic settings.[1,2] Infants and young children (<3 years) and those with immunodeficiency caused by HIV or severe malnutrition are at highest risk of developing TB disease following infection.[3] Delay of diagnosis and treatment in these children increases the risk of rapid disease progression and mortality.[4] TB diagnosis is particularly challenging in this population, given the lack of specific symptoms, the difficulty in obtaining samples for microbiological examination and the often paucibacillary disease. The diagnostic yield of samples is often <20% under TB program conditions.[5,6] These diagnostic difficulties result in delayed and underdiagnosis of the disease, contributing to the hidden burden of TB in children.

Child TB is receiving more attention[3] as the World Health Organization (WHO) post-2015 TB strategy seeks to engage the wider health sector including the child health-care sector.[7] The WHO Global Tuberculosis Report 2014 estimates that 550,000 children developed tuberculosis during 2013, representing 6% of the global TB burden.[8] However, several factors suggest that the true burden of disease may be higher as these estimates assume an equal ratio of notified cases in children and adults (whereas underreporting in children is very common[9]), and estimated deaths only include those in HIV-negative children.[3] As a setting's total TB burden increases, there tends to be a rise in the proportion of TB cases attributable to children.[10] Thus, in high TB burden settings, children may represent up to 10–20% of TB cases, with increased TB incidence in <5 and >15 years.[4,8,11]

Mozambique is one of the high TB burden countries listed by the WHO but has a very low reported case-detection rate of 37%.[8] Improved reliable estimates are required to quantify the hidden burden of disease and measure future progress toward the control of TB in the country, especially for vulnerable populations such as children.[8,12,13] We, therefore, aimed to determine the minimum community-based incidence rate (IR) of childhood TB.

MATERIALS AND METHODS

Setting

The study was conducted in the Manhiça District (rural southern Mozambique), where the Manhiça Health Research Center (Centro de Investigação em Saúde de Manhiça) runs a Health and Demographic Surveillance System (HDSS) including the Manhiça District Hospital (MDH) and other peripheral health posts in the area. The HDSS links demographic and clinical data and covers a population of around 92,000 inhabitants, of which approximately 11% are <3 years.[14] A full description of the site can be found elsewhere.[14] In 2011, the <5 years mortality rate was 70 of 1000 live births. Severe malnutrition is common with an estimated IR of 35 of 1000 person-year among children from 1 to 2 years.[15]

TB treatment is offered free of charge at the health units, and children are routinely vaccinated at birth with Bacille Calmette–Guérin (BCG), with estimated coverage ranging from 86% to 90%.[16,17] The 2013 WHO TB incidence estimates for the country is 552 of 100,000 population.[8] The HIV prevalence in the district is among the highest in the world, reaching 39.9% in the community among individuals aged 18–47 years and 29.4% for women attending the antenatal clinic.[18]

Accepted for publication December 11, 2014.

From the *Manhiça Health Research Centre (CISM), Maputo, Mozambique; †ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain; and ‡Radiology Department, Hospital Sant Joan de Déu Barcelona, Passeig Sant Joan de Déu, Barcelona, Spain.

This work was supported by the European & Developing Countries Clinical Trials Partnership [IP_07_32080_003]. The authors have no other funding or conflicts of interest to disclose.

Address for correspondence: Elisa López-Varela, MD, MPH, Centro de Investigação em Saúde de Manhiça (CISM), CP 1929 Maputo, Moçambique. E-mail: elisa.lopez@cresib.cat.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

ISSN: 0891-3668/15/3407-0686
DOI: 10.1097/INF.0000000000000710

Study Design and Participants

A prospective study was designed to recruit participants through passive and active case finding in the community, MDH and peripheral health centers during a 1-year period (2011–2012). Participants included all children from the HDSS who were <3 years at the time of enrolment and had either TB symptoms or were close contacts of a notified adult smear-positive pulmonary TB (PTB) case. Relapse or recurrent cases were excluded.

Clinical Procedures

Presumptive TB cases were identified through 2 strategies: (a) passive case detection of children presenting to the health facility with ≥1 symptoms compatible with TB (see Table 1 for a complete list of symptoms). Those not recruited at the time of the visit to the clinic were later identified through the clinical data collected at the health unit by the HDSS. (b) Active case finding consisted of linking the adult smear-positive PTB cases registered at the district National TB Program (NTP) in the previous 24 months to the HDSS database to identify all household contacts <3 years. At enrolment, demographic and clinical information was collected through interviewing of parents and physical examination. Participants had a chest radiography (CXR) performed, followed by HIV antibody testing and tuberculin skin testing (TST). CXR were performed with a digital radiogram machine and included posteroanterior and lateral projections. For clinical purposes, an initial reading was performed on site by the clinician. Subsequently, all CXR were reviewed and reassessed by an experienced pediatric radiologist (J.R.) who was blinded to the clinical information. TST was performed with intradermal injection of 2 units (Serum Staten´s Institute, Denmark) and reading at 48–96 hours, according to the study protocol. For symptomatic cases, in the same day, 2 ambulatory samples were obtained in a negative pressure facility available at the MDH: 1 gastric aspirate (GA) and 1 induced sputum (IS) with nasopharyngeal suction, following WHO recommendations.[19] Asymptomatic cases with abnormal CXR did not undergo sampling but were reevaluated at further visits. For suspected extrapulmonary TB (EPTB), appropriate samples were obtained.

All case managements were performed by the NTP according to established national clinical guidelines. Those patients with clinical or microbiological diagnosis of TB were started on TB treatment at the NTP with the standard 3 or 4 first-line regimens according to WHO category. Other symptomatic patients were referred for specific treatment and follow-up including antibiotics or nutritional supplementation if indicated. Presumptive cases had a follow-up visit within the next 6 months regardless of initial disease classification to assess resolution of symptoms without anti-TB treatment and/or clinical response to alternative therapy (if any). If persistently symptomatic, further evaluation and testing including CXR and samples were performed to rule out TB. Contacts had a follow-up visit, which included physical examination and CXR, as well as GA and IS samples for those symptomatic or with an abnormal CXR.

TABLE 1. Baseline Characteristics of Presumptive TB Cases (n = 789), n (%)

Sex (male)	430 (54.5)
Age in months [Median (IQR)]	19.8 (13.8–25.9)
Age group	
<1 yr	146 (18.5)
1–2 yr	403 (51.1)
>2 yr	240 (30.4)
BCG scar (n = 785)	686 (87.4)
> 1 hospitalization in previous year	206 (26.1)
≥ 10 consultations in previous year	131 (16.6)
TB contact (documented or reported)	87 (11)
Symptoms*	
Cough ≥2 weeks	156 (19.8)
Fever≥2 weeks	50 (6.3)
Malnutrition (chronic or acute)	668 (84.7)
Wheeze or lower respiratory infection	43 (5.5)
Adenopathy	3 (0.4)
Number of presenting symptoms	
One symptom	608 (77.1)
Only malnutrition	565 (71.6)
Hospitalized at time of enrolment	101 (12.8)
Physical examination	
Stunting (n = 775)†	404 (52.1)
Undernutrition (n = 777)‡	184 (23.7)
Wasting (n = 775)§	92 (11.9)
Kwashiorkor (n = 780)	33 (4.2)
Febrile (n = 780)	44 (5.6)
Crackles on chest examination	27 (3.4)
TST positive (n = 787)	74 (9.4)
HIV positive	104 (13.2)
Radiological changes suggestive of TB (n = 766)	160 (20.9)

*Compatible TB symptoms: cough for ≥ 14 days not responding to appropriate course of antibiotics; fever greater than 38°C ≥14 days. After common causes like malaria or pneumonia were excluded; malnutrition defined as under 60% weight for height, failure to gain weight for more than 2 months or any loss of weight and not responded to nutritional interventional; unexplained wheeze ≥14 days not responding to standard treatments; lower respiratory tract infection ≥14 days not responding to antibiotics after 72 hours; TB exposure in the last 12 months; symptoms compatible with EPTB, such as painless enlarged lymph nodes with or without fistula formation ≥14 days, arthritis, gibbus, meningitis, effusion or unexplained hematuria, dysuria or polaquiuria for ≥21 days.

† Stunting: height for age Z score <2.

‡ Undernutrition: weight for age Z score <2.

§Wasting: weight for height <2.

Laboratory Procedures

Samples were transported within 4 hours of collection and processed in the Biosafety Level III TB laboratory at Centro de Investigação em Saúde de Manhiça. Following NaCl/NaOH digestion and concentration through centrifugation, all samples were processed for acid-fast bacilli smear testing using LED Microscopy and Ziehl–Neelsen staining and inoculated into liquid culture media (BACTEC MGIT 960-automated; Becton Dickinson Microbiology Systems, Sparks, MD) and solid media (Lowenstein–Jensen). Positive cultures were confirmed using Ziehl–Neelsen staining and rapid test as well as Xpert MTB/RIF and identified through mycobacterial molecular identification (GenoType Mycobacterium CM/AS; Hain Lifescience). First-line drug sensitivity testing was performed either on liquid culture or line probe assays. The laboratory is subject to an external quality assurance program.

Study Definitions

- Exposure to TB was defined as either documented (identified through active case finding) or reported contact (household or regular contact during child lifetime).
- Positive TST was defined as an induration >5 mm for HIV or malnourished children and >10 mm for the rest of participants.
- HIV infection was defined as positive antibody test in children >18 months (Determine, Abbott Laboratories and confirmed with Unigold, Trinity Biotech); or positive HIV polymerase chain reaction in those <18 months; or a strong clinical suspicion with positive antibody test in the absence of a polymerase chain reaction result.
- CXR were classified as compatible if presented ≥1 of the following radiographic abnormalities: airway compression, lymphadenopathy, opacification, nodular picture, effusion, cavities, spondylitis or Ghon focus.[20]

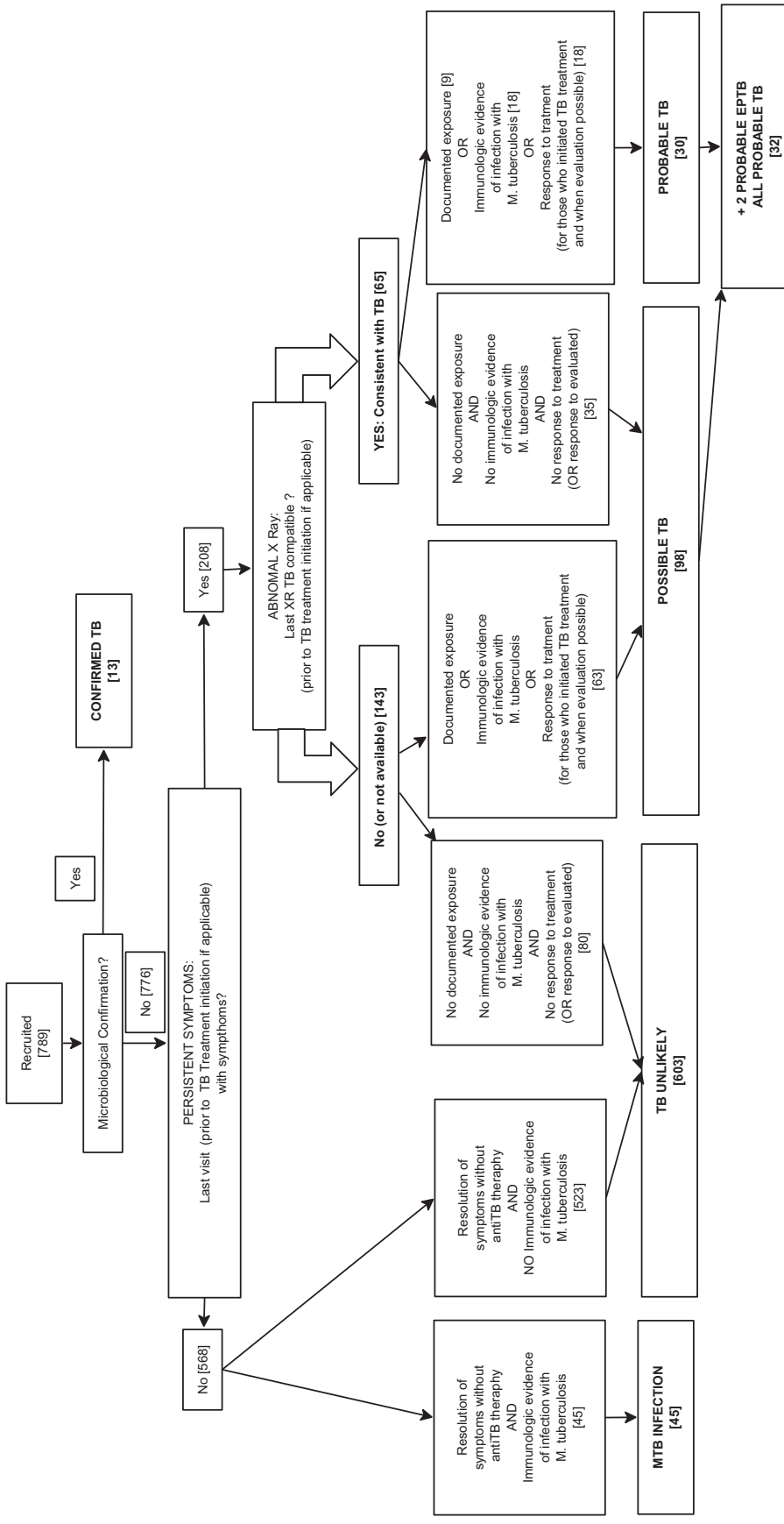


FIGURE 1. Algorithm for study case classification adapted with permission from Graham et al.[21] Definitions: Confirmed TB, compatible symptoms plus a positive culture with MTBC; persistent symptoms, compatible symptoms unresolved at last clinical follow-up visit (before any TB treatment initiation); consistent CXR: CXR read by blinded experienced pediatric radiologist with one or more radiographic abnormalities (airway compression, lymphadenopathy, opacification, nodular picture, effusion, cavities, spondylitis or Ghon focus), for children with more than one CXR, the latter was used given the likelihood of seeing resolving pneumonias; positive response to TB treatment; determined by the clinician at first possible follow-up visit and defined as total/partial resolution of clinical features suggestive of TB present at baseline with no new clinical features suggestive of TB for those patients adherent to TB treatment. Resolution of symptoms without anti-TB treatment was evaluated by the clinical at first follow-up visit as total/partial resolution of clinical symptoms suggestive of TB present at baseline and no new clinical features suggestive of TB in the absence of TB treatment. Description: Of the initial 789 presumptive TB cases, 568 had symptoms resolved at follow-up visits, leaving 208 presumptive cases with persistent TB symptoms, of which 65 had an abnormal CXR at last visit. Probable cases include 30 children who fulfilled the definition plus an additional 2 extrapulmonary cases. Contacts not enrolled as presumptive TB cases are not included in the algorithm.

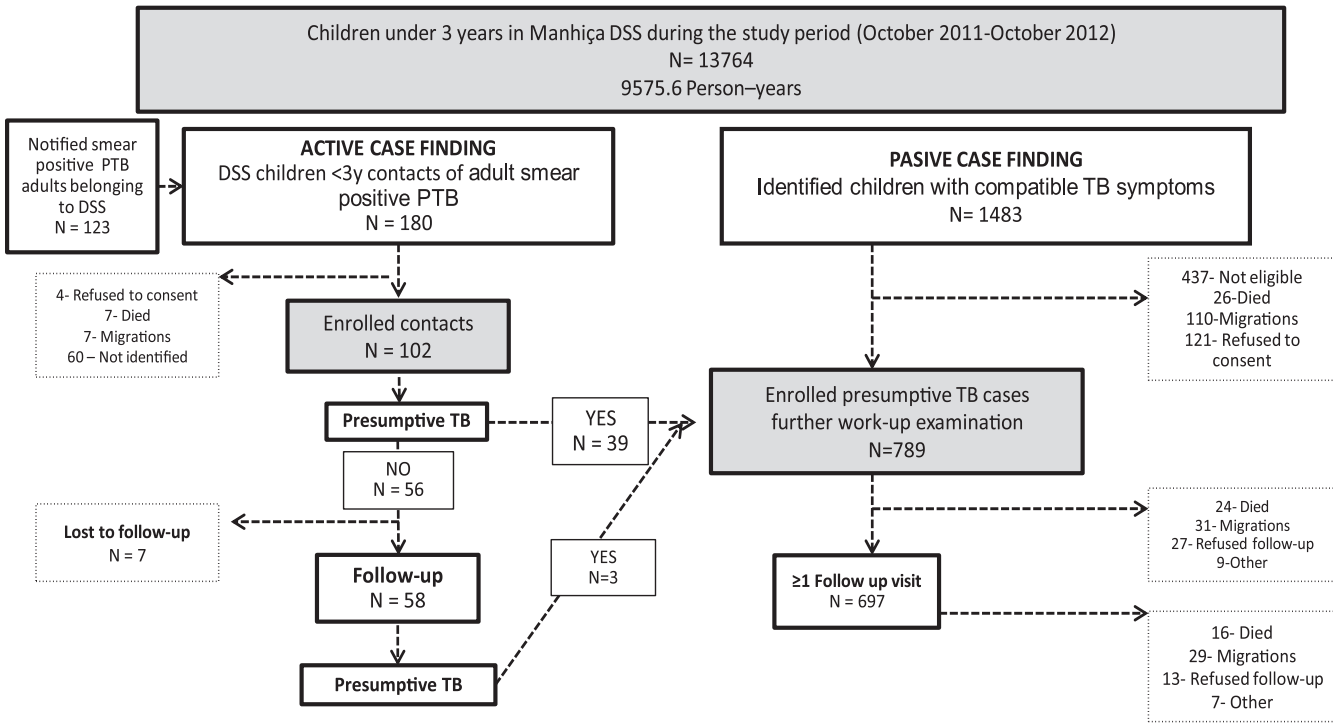


FIGURE 2. Study profile. Flowchart showing the number of children younger than 3 years of age in the study area and those enrolled in the study. A total of 1483 children were identified with at least 1 compatible TB symptom, and 747 presumptive TB cases were enrolled in the study for further workup. Among the 329 adult smear-positive cases registered at the NTP between October 2010 and October 2012, we identified 123 belonging to the study area and 180 contacts <3 years, of whom 102 accepted to participate in the study yielding an additional 42 presumptive TB cases. Among the remaining 60 contacts, 7 were lost to follow-up and the rest had at least 1 follow-up visit. Eighty-eight percent presumptive TB cases enrolled had at least 1 follow-up visit and 632 of 697 completed follow-up (had follow-up visits until alternative diagnosis was made or became asymptomatic).

- Presumptive TB cases included all children <3 years of age with compatible TB sign or symptoms.
- Confirmed TB cases included those with compatible symptoms plus a positive culture with *Mycobacterium tuberculosis*. Probable TB cases were defined as those with (1) compatible symptoms unresolved at last clinical follow-up visit (before any TB treatment initiation) plus (2) compatible CXR (for children with ≥1 CXR, the latter was used given the likelihood of seeing resolving pneumonias) plus (3) at least one of the following: TB exposure, positive TST or positive response to TB treatment. EPTB cases followed the same definition except for the requirement of having an abnormal CXR. The study TB case definition was adapted a standardized clinical case definition of intrathoracic TB disease and included confirmed plus probable cases[21] (see Fig. 1 for complete case definition).

Ethical Approval

The study protocol was approved by the Mozambican National Bioethics Committee and the Hospital Clinic of Barcelona Ethics Review Committee.

Data Analysis and Statistical Considerations

Clinical data were double entered in an electronic data capture system (OpenClinica, www.openclinica.org) and checked for discrepancies. Statistical software for analysis was Stata 13.0 (StataCorp. 2013. Stata: Release 13, StataCorp LP, Statistical Software, College Station, TX). We calculated Z scores for weight-for-age, height-for-age and weight-for-height using WHO 2006 reference data.[22]

The minimum community-based IR was calculated as a density rate with the age-specific yearly number of TB cases (according to the study case definition) among study participants divided by the total age-specific population at risk during a period of 12 months (person-time at risk). Time at risk was individually measured using demographic surveillance system (DSS) data taking into account demographic events (births, deaths and migrations) of all children included in the study. The IR is considered to be minimum as the case detection system cannot ensure that all TB cases are detected. For each IR, 95% exact Poisson confidence interval (CI) was calculated. Proportions were compared using the Pearson or Fisher exact χ^2 test, and odds ratio and 95% CIs were estimated using logistic regression. Variables at a significance level below 0.2 were chosen and placed on stepwise backward multivariate logistic regression. Only factors with a *P* value on likelihood ratio tested were retained on the model.

RESULTS

During the study period, 13,764 children <3 years contributed to a total of 9575 person-year in the Manhiça DSS (Fig. 2). A total of 789 presumptive TB cases were enrolled (42 and 747 identified through active and passive case finding, respectively). Forty-five children fulfilled the TB case definition—13 microbiologically confirmed plus 32 probable TB (Fig. 1). Thus, the minimum community-based IR was 470 of 100,000 person-year (95% CI: 343–629 of 100,000) for confirmed plus probable cases and 135 of 100,000 person year for confirmed cases (95% CI: 72–232 of 100,000; Table 2).

TABLE 2. Community-Based Incidence Rate of Confirmed and Probable Tuberculosis Cases by Age Group

	Cases	Person-Year	IR per 100,000 Person-Year	95% CI	
All tuberculosis cases					
Confirmed	13	9575.6	135.8	72.3	232.2
Probable	32	9575.6	334.2	228.6	471.8
Total	45	9575.6	470.0	342.8	628.8
Confirmed cases by age group					
Age (yr)					
<1	4	3265.6	122.5	33.3	313.3
1–2	5	3199.4	156.3	68.9	407.8
2+	4	3110.5	128.6	52.2	374.5
All confirmed	13	9575.6	135.8	72.3	232.2
Probable cases by age group					
Age (yr)					
<1	6	3265.6	183.7	67.4	399.9
1–2	12	3199.4	375.1	193.8	655.2
2+	14	3110.5	450.1	246.1	755.2
All probable	32	9575.6	334.2	228.6	471.8
All cases by age group					
Age (yr)					
<1	10	3265.6	306.2	146.9	563.2
1–2	17	3199.4	531.4	309.5	850.7
2+	18	3110.5	578.7	343.0	914.6
All TB cases	45	9575.6	470.0	342.8	628.8

Baseline characteristics of presumptive TB cases are presented in Table 1. Fifty-four percent were males, and the age distribution showed a predominance of children between the ages 12 and 24 months (51%). The most frequent clinical feature at enrolment was severe malnutrition, which was the only symptom in 72% of cases. Nutritional assessment found that almost a quarter had severe undernutrition (weight for age Z score <3). Of the 1347 total CXR performed during the study, 27% had only one projection. Twenty-one percent of all presumptive TB cases had a CXR compatible with TB. Thirty percent of presumptive cases had a second TST of which 9% had a positive TST. Among all presumptive TB cases, 9 had a positive smear, although none of the 9 had a positive Mycobacterium tuberculosis culture (4 were non-TB mycobacteria and 5 were culture negative). Non-TB mycobacteria were isolated in 27% of all cultures of presumptive cases. We found 7 EPTB cases—4 lymph node and 3 disseminated—and no TB meningeal cases. A total of 104 children were diagnosed as HIV positive (13%).

We identified 13 confirmed TB cases (7 in GA, 4 in IS and 2 both in GA and IS). The percentage of confirmed cases among TB cases was highest for those <1 year (40% vs. 29% and 22% among children with 1–2 and 2–3 years, respectively), and statistically significantly lower for HIV–TB coinfected cases (10% vs. 44%, $P = 0.02$). Confirmed cases presented a higher frequency of cough or fever when compared with probable cases. Furthermore, the confirmed cases appeared to be more symptomatic at enrolment than did the probable cases (53.8% vs. 15.6% presenting with ≥1 TB symptom, respectively, $P < 0.001$). Probable cases had a higher proportion of HIV infection ($P = 0.01$), positive TST ($P = 0.001$) or BCG scarring ($P = 0.08$) when compared with confirmed cases.

Multivariate logistic regression analysis for TB risk factors showed that HIV infection and number of previous outpatient consultations were predictors of TB disease when compared with unlikely TB cases. After adjusting for other variables, HIV-infected children were 6 times more likely to have TB disease than uninfected ones (odds ratio: 8.4; 95% CI: 4–17; see Table, Supplemental Digital Content 1, http://links.lww.com/INF/C101).

Fifty-two patients were started on TB treatment based on clinical or microbiological criteria, and 67% fulfilled the study TB case definition (Table 3). A total of 97 children initiated isoniazide preventive treatment (IPT; 71 based on exposure history, 21 on TST results and 5 unspecified) and 5 were later diagnosed as TB cases while on IPT. Because of drug supply shortages, isoniazide was not always available, and 47% of children with criteria did not initiate IPT. The mortality rate for all presumptive cases at 12 months after enrolment was 5.2% and increased with decreasing age (10.9%, 5.7% and 0.8% of children in the first, second and third year of life, respectively, $P < 0.001$). Mortality was also higher in TB cases when compared with non-TB (13% vs. 5%, respectively, $P = 0.02$) as it was in HIV-infected children when compared with HIV-uninfected (14.4% vs. 3.8%, $P < 0.001$). The case fatality rate was 9% (n = 4 of 45 TB cases), all deaths taking place in the first 6 months after enrollment.

DISCUSSION

This study provides one of the first prospective population-based incidence estimates of childhood tuberculosis in a high TB–HIV endemic setting and shows a consistently high IR across all ages. These results underscore the hypothesis of a gross underdetection and underreporting of childhood TB in Mozambique and globally.[23]

Mozambique has almost half its population below the age of <15 years, and yet, pediatric TB only accounted for 7% of all new cases notified in 2012, much lower than the expected 10–20% of the total burden of TB disease seen in high burden countries. In the Manhiça District, the notified IR for children <1 year and between 1 and 4 years in 2011 was 163 and 399 of 100,000, respectively (García-Basteiro et al, personal communication, 2014[24]). This corresponds to half the TB IR reported in this study and may suggest underdetection and underdiagnosing. Furthermore, underdetection is common in the wider Mozambican context, where WHO

TABLE 3. Outcome by Group Classification, n (%)

	Presumptive (n = 789)	Definite (n = 13)	Probable (n = 32)	Possible (n = 96)	Unlikely (n = 603)	Mycobacterium tuberculosis Infection (n = 45)
Sex (male)	430 (54.5)	5 (38.5)	15 (46.9)	57 (59.4)	322 (53.4)	31 (68.9)
HIV positive	104 (13.2)	2 (15.4)	18 (56.3)	24 (25)	54 (9)	6 (13.3)
IPT	64 (8.1)	1 (7.7)	4 (12.5)	4 (4.2)	24 (4)	31 (68.9)
TB treatment	52 (6.6)	9 (69.2)	26 (81.3)	14 (14.6)	1 (0.2)	2 (4.4)
Median time to diagnosis (d), n (IQR)	115 (35–224)	41 (35–115)	184 (55–224)	60 (35–235)	14 (14–14)	148 (89–207)
Mortality at 12 mo	41 (5.2)	3 (23.1)	1 (3.1)	13 (13.5)	23 (3.8)	1 (2.2)

IQR indicates interquartile range.

estimates that only 37% of actual TB cases are detected.[8] Although the latest WHO country incidence estimates are 552 per 100,000 population, data from Manhiça suggest that the burden of disease in Southern Mozambique might be much higher. In fact, while the national 2011 notified rate was 186 of 100,000 population, same-year data from the Manhiça suggest the TB IR of smear-positive cases could be as high as 456 per 100,000 population among adults aged 18–47 years.[25]

Globally, several pediatric TB incidence estimates have been published recently, with results varying from less than 200,000 new cases in 2013[26] to 970,000 in 2010.[6] The large variation in the estimates highlights the challenges in estimating the burden of pediatric TB and the need for population-based data to inform predictive models.

There are few studies reporting age-specific pediatric TB incidence in high burden countries. Most studies are based on hospital-based retrospective reviews of notification rates, and to our knowledge, none have reported community IR using DSS.[11,27–35] However, in areas where health seeking behavior strongly modifies the pattern of attendance, community-based studies that use active case detection rather than notified TB rates are necessary to provide accurate estimates. Moreover, childhood mortality and frequent migration are potential causes of disease underestimation if DSS person-years are not available. Inconsistency in TB clinical definitions among studies is a challenge for comparability and has been a limitation in obtaining data for meta-analyses. The recently proposed definition, applied in this study, may pave the road for future comparisons.[9] The IR we report is significantly higher than in other high burden African countries, such as Malawi (notified IR <1 year of 78 of 100,000) or Tanzania (theoretical IR <5 years based on likelihood of disease progression of 134.5–308.5 of 100,000) and similar to data from Gabon (extrapolated IR <15 years 366 of 100,000) or neighboring South Africa (notified IR <5 years 770 of 100,000).[30–32]

There are several limitations to this study, mostly leading to a possible underestimation of the true TB incidence. First, only single-day samples were obtained as most patients would not accept overnight admission, decreasing the chances of microbiological confirmation. Second, for study purposes CXR were read by a single blinded experienced pediatric radiologist rather than the 2 independent CXR readers are often recommended to prevent bias, given the pivotal role of CXR in case definition, and the poor interobserver and intraobserver agreement among reviewers.[36] Third, contact tracing could not be fully implemented mainly because of difficulties in patient identification and poor recording. Fourth, the percentage of EPTB cases was lower than the 20–30% expected and reported by others.[11,30,31,37–39] Although BCG protection may have a role, it is likely that some EPTB cases in this study were missed; the reason may be because of a stronger focus on PTB in the study design, errors in classification (disease localization, including disseminated TB, may be confounding in young children in the absence of CT-scan) or lost cases because of the fact that severely ill children are often transferred to the tertiary reference hospital in the capital for specific diagnostic procedures. Finally, there is a risk of overestimating TB IR by either including TB prevalent cases at enrollment or adding new incident cases during follow-up beyond the 1-year enrollment period. Although this possibility cannot be ruled out, we believe the effect would be minimal and probably overweighed by the above-mentioned risk of underestimation.

In this study, HIV prevalence was high regardless of disease classification, reaching 56% of probable cases. The fact that significantly fewer HIV-infected children had TB confirmation reflects the diagnostic difficulties in this group. Given the overlap between symptoms from both TB and HIV, HIV-infected cases of TB pose the greatest ascertainment bias with the highest risk of over or under estimation. Even though IPT is indicated in all HIV-positive children, the implementation of IPT among African NTP remains very poor,[40] and in this study, the high proportion of missed opportunities for chemoprophylaxis in HIV and/or TB exposed should raise alert. There may also be missed preventable child deaths in HIV-infected presumptive TB cases and possible TB cases. It has been reported that many children who die of diseases such as malnutrition or respiratory infections may have, in fact, undiagnosed TB.[41] There is thus a need for widespread recognition that TB control is crucial for childhood survival.[1]

This study highlights the huge burden of pediatric TB under detection in children younger than 3 years of age. These data add valuable information to the global effort of producing better estimates of childhood TB burden, a critical step to inform public health policy.

ACKNOWLEDGMENTS

The authors thank the participants and their families. The authors are grateful to the National TB Program, health staff at the Manhiça District Hospital and peripheral health centers for their contribution. The authors thank Dr. Bassat and Dr. Naniche (Barcelona Centre for International Health Research) for reviewing the paper.

REFERENCES

1. Graham SM, Sismanidis C, Menzies HJ, et al. Importance of tuberculosis control to address child survival. *Lancet* 2014; 6736: 5–7.
2. Marais BJ, Schaaf HS, Graham SM. Child health and tuberculosis. *Lancet Respir Med*. 2014;2:254–256.
3. World Health Organization. *Roadmap for childhood tuberculosis: towards zero deaths;* 2013. Available at: http://apps.who.int/iris/handle/10665/89506. Accessed on November 18, 2013.
4. Perez-Velez CM, Marais BJ. Tuberculosis in children. *N Engl J Med*. 2012;367:348–361.
5. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. *Paediatr Respir Rev*. 2011;12: 16–21.
6. Jenkins HE, Tolman AW, Yuen CM, et al. Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. *Lancet* 2014; 6736: 1–8.
7.. *WHO | WHO End TB Strategy*. Available at: http://www.who.int/tb/post2015_strategy/en/. Accessed on November 15, 2014.
8. WHO. *Global Tuberculosis Report 2014*. Geneva, Switzerland: World Health Organization; 2014. Available at: http://www.who.int/tb/publications/global_report/en/. Accessed on June 24, 2013.
9. Marais BJ, Graham SM, Maeurer M, et al. Progress and challenges in childhood tuberculosis. *Lancet Infect Dis*. 2013;13:287–289.
10. Donald PR. Childhood tuberculosis: out of control? *Curr Opin Pulm Med*. 2002;8:178–182.
11. Moyo S, Verver S, Mahomed H, et al. Age-related tuberculosis incidence and severity in children under 5 years of age in Cape Town, South Africa. *Int J Tuberc Lung Dis*. 2010;14:149–154.
12. López-Varela E, García-Basteiro AL. World Health Organization guidelines for childhood tuberculosis management: successes achieved and challenges ahead. *Pediatr Infect Dis J*. 2014;33:1310–1311.
13. García-Basteiro AL, López-Varela E, Manhiça I, et al. Mozambique faces challenges in the fight against tuberculosis. *Lancet*. 2014;383:215–216.
14. Sacoor C, Nhacolo A, Nhalungo D, et al. Profile: Manhiça Health Research Centre (Manhiça HDSS). *Int J Epidemiol*. 2013;42:1309–1318.
15. Nhampossa T, Sigaúque B, Machevo S, et al. Severe malnutrition among children under the age of 5 years admitted to a rural district hospital in southern Mozambique. *Public Health Nutr*. 2013;16:1565–1574.
16. Consonni D, Montenegro Agorostos Karagianis MM, Bufardeci G. Immunisation with BCG in the Maringue District, Sofala Province, Mozambique. *Tuberc Res Treat*. 2013;2013:312065.

17. WHO vaccine-preventable diseases: monitoring system. 2013 Global Summary. Mozambique. Available at: http://apps.who.int/immunization_monitoring/en/globalsummary. Accessed on January 2014.
18. González R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med*. 2012;13:581–588.
19. World Health Organization. Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children; 2006.
20. Cuevas LE, Browning R, Bossuyt P, et al. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. *J Infect Dis*. 2012;205 (Suppl 2):S209–S215.
21. Graham SM, Ahmed T, Amanullah F, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis*. 2012;205 (Suppl 2):S199–S208.
22. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development. Geneva: World Health Organization, 2006.
23. Lestari T, Probandari A, Hurtig AK, et al. High caseload of childhood tuberculosis in hospitals on Java Island, Indonesia: a cross sectional study. *BMC Public Health*. 2011;11:784.
24. García-Basteiro A, López-Varela E, Joaquim Augusto O, et al. Determining the minimum tuberculosis incidence risk among adults in the district of Manhiça, Mozambique [abstract PC-441-01]. In: 44th Union World Conference on Lung Health, Paris, France, November 2013; 2013.
25. García-Basteiro AL, López-Varela E, Respeito D, et al. High tuberculosis burden among people living with HIV in southern Mozambique. *Eur Respir J.* 2014;45:547–549.
26. Murray CJ, Ortblad KF, Guinovart C, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:1005–1070.
27. Donald PR, Maher D, Qazi S. A research agenda to promote the management of childhood tuberculosis within national tuberculosis programmes. *Int J Tuberc Lung Dis*. 2007;11:370–380.
28. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intrathoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis*. 2004;8:392–402.
29. Marais BJ, Hesseling AC, Gie RP, et al. The burden of childhood tuberculosis and the accuracy of community-based surveillance data. *Int J Tuberc Lung Dis*. 2006;10:259–263.
30. Harries AD, Hargreaves NJ, Graham SM, et al. Childhood tuberculosis in Malawi: nationwide case-finding and treatment outcomes. *Int J Tuberc Lung Dis*. 2002;6:424–431.
31. Praygod G, Todd J, McDermid JM. Early childhood tuberculosis in northwestern Tanzania. *Int J Tuberc Lung Dis*. 2012;16:1455–1460.
32. van Rie A, Beyers N, Gie RP, et al. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Arch Dis Child*. 1999;80:433–437.
33. Mahomed H, Ehrlich R, Hawkridge T, et al. TB incidence in an adolescent cohort in South Africa. *PLoS One*. 2013;8:e59652.
34. Nantongo JM, Wobudeya E, Mupere E, et al. High incidence of pulmonary tuberculosis in children admitted with severe pneumonia in Uganda. *BMC Pediatr*. 2013;13:16.
35. Waako J, Verver S, Wajja A, et al. Burden of tuberculosis disease among adolescents in a rural cohort in Eastern Uganda. *BMC Infect Dis*. 2013;13:349.
36. Swingler GH, du Toit G, Andronikou S, et al. Diagnostic accuracy of chest radiography in detecting mediastinal lymphadenopathy in suspected pulmonary tuberculosis. *Arch Dis Child*. 2005;90:1153–1156.
37. Cavanaugh J, Genga K, Marigu I, et al. Tuberculosis among children in Kenya: epidemiology and impact of HIV in two provinces. *J Trop Pediatr*. 2012;58:292–296.
38. Kapata N, Chanda-Kapata P, O'Grady J, et al. Trends in childhood tuberculosis in Zambia: a situation analysis. *J Trop Pediatr*. 2013;59:134–139.
39. Flamen A, Bélard S, Kokou C, et al. Childhood tuberculosis in Lambaréné, Gabon: tuberculosis control in its infancy? *Infection* 2014;42:161–164.
40. Schaaf HS, Marais BJ. Management of multidrug-resistant tuberculosis in children: a survival guide for paediatricians. *Paediatr Respir Rev*. 2011;12:31–38.
41. Chintu C, Mudenda V, Lucas S, et al; UNZA-UCLMS Project Paediatric Postmortem Study Group. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet*. 2002;360:985–990.

Table: Supplemental digital content

	TB Unlikey (N=603)		TB Case (N=45)		Univariate		Adjusted OR (95% CI)	
					OR (95% CI)	p	OR (95% CI)	p
Sex								
Male	322	53,4%	20	44,4%	-			
Female	281	46.6%	25	55,6%	1.43 (0.78-2.64)	0.246	1.52 (0.79-2.92)	0.210
Age in months (Median [IQR])	19.8 (14.1 - 26.0)		19.5 (13.8 - 26.4)					
Age category, N (%)								
< 1	100	16,6%	10	22,2%	-			
1-2	318	52,7%	17	37,8%	0.53 (0.24 - 1.21)		0.51 (0.21-1.23)	0.133
2-+	185	30,7%	18	40,0%	0.95 (0.43 - 2.19)	0.153	0.94 (0.39-2.26)	0.890
BCG Scar								
Absent	74	12,3%	7	15,6%	-			
Present	527	87,7%	37	82,2%	0.74 (0.32 - 1.73)	0.488		
TB contact								
No	557	92,4%	32	71,1%	-			
Yes	46	7,6%	13	28,9%	4.92 (2.38 - 10.15)	< 0.001		
Nº consultations in previous year								
Median (IQR)	5 (3 - 8)		7 (2 - 11)		-	0.074		
< 10	505	83,7%	29	64,4%	-			
10 - +	98	16,3%	16	35,6%	2.84 (1.48 - 5.47)	0.001	2.72 (1.35-5.49)	0.005
Nº of hospitalizations in previous year								
None	446	74,0%	26	57,8%	-			
At least one	157	26,0%	19	42,2%	2.08 (1.11 - 3.87)	0.019		
Symptoms at enrollment								
Cough ≥2 weeks								
no	489	81,1%	36	80,0%	-			
yes	114	18,9%	9	20,0%	1.07 (0.50 - 2.29)	0.857		
Fever≥2 weeks								
no	567	94,0%	40	88,9%	-			
yes	36	6,0%	5	11,1%	1.97 (0.73 - 5.30)	0.172		
Chronic or Acute Malnutrition malnutrition								
no	82	13,6%	12	26,7%	-			
yes	521	86,4%	33	73,3%	0.43 (0.21 - 0.88)	0.016	0.42 (0.19-0.89)	0.024
Physical Exam								

Stunting †								
no	281	46,6%	16	35,6%	-			
yes	312	51,7%	28	62,2%	1.58 (0.83 - 2.98)	0.158		
Undernutrition ‡								
no	460	76,3%	30	66,7%	-			
yes	134	22,2%	14	31,1%	1.60 (0.82 - 3.11)	0.161		
Wasting ¥								
no	531	88,1%	37	82,2%	-			
yes	62	10,3%	7	15,6%	1.62 (0.69 - 3.80)	0.262		
Crackles on chest examination								
no	584	97,0%	39	90,7%	-			
yes	18	3,0%	4	9,3%	3.32 (1.07 - 10.36)	0.052*		
Tuberculin skin test								
Postive	0	0,0%	23	51,1%	-	< 0.001µ		
Negative	603	100,0%	21	46,7%	-			
HIV Reported								
Not positive	549	91,0%	25	55,6%	-			
Postive	54	9,0%	20	44,4%	8.13 (4.12 - 16.04)	< 0.001	8.42(4.26-16.62)	< 0.001

Footnote:

Abbreviations: IQR, interquartile range; BCG, Bacille Calmette Guerin; HIV, human immunodeficiency virus; TST, tuberculin skin test

† Stunting: Height for age Z score <2.

‡ Undernutrition: Weight for age Z score < 2.

¥ Wasting: Weight for height <2

µ This variable was not included in multivariate analysis as a positive TST is part of the TB definition

* Exact Chi-squared test

2. Low paediatric tuberculosis case detection rate in Southern Mozambique.

López-Varela E, Augusto OJ, Guerra L, Respeito D, Sacoor C, Sacarlal J, Migliori GB, Sotgiu G, Alonso PL, García-Basteiro AL.

Eur Respir J. 2015 Dec 23.

2015 Impact factor 8.332 (D1)

Low paediatric tuberculosis case detection rate in Southern Mozambique

To the Editor:

Two core indicators adopted for evaluating tuberculosis (TB) control programmes are treatment outcome and case detection rate (CDR). While the former is easy to report, the CDR can only be estimated (calculated as notifications of new and relapse cases divided by estimated incidence). According to the World Health Organization (WHO), Mozambique has one of the lowest CDRs among the high TB burden countries (HBCs), with 37% in 2013 [1]. In children, calculating CDR is even more challenging, given the difficulty in diagnosing TB and the lack of accurate estimates for paediatric incidence [2]. Several paediatric TB incidence estimates have been published recently [3–5], showing higher figures than those provided by WHO. The large variation in estimates and the lack of population-based data from HBCs (particularly from Sub-Saharan Africa) highlights the urgent need for new data to inform predictive models necessary to implement the "End TB Strategy" and achieve elimination [6]. The objective of this study was to calculate the paediatric CDR in Mozambique and to provide reference methodology and evidence for other countries.

The study was conducted in the Manhiça District, a high TB-HIV burden district in southern Mozambique [7], where the Manhiça Health Research Centre runs a health and demographic surveillance system (HDSS) [8]. It was a retrospective, population-based analysis, which estimated the TB CDR by comparing the routine TB incidence rate in children aged <3 years reported in the district between 2006 and 2010 with the incidence rate in the study area computed during a prospective study (the ITACA study: determination of the minimum incidence rate of tuberculosis in infants and children in the Manhiça District, Mozambique; October 2011–October 2012) [9]. The latter was considered as the "most accurate incidence rate estimate" available. Prior to the ITACA study, no routine active case finding was performed and bacteriological confirmation was mostly based on smear microscopy. During the ITACA period, all presumptive TB cases were identified through an active and passive case detection system. Children with TB-related symptoms and close contact with a sputum smear-positive TB patient were evaluated through physical and radiological examination, HIV and tuberculin skin testing, as well as smear microscopy and culture of both induced sputum and gastric aspirate samples [9, 10]. For the purpose of this analysis, TB cases were defined as those who started anti-TB treatment. Relapse patients were included.

We calculated the TB incidence rate during the ITACA period as the number of cases in the HDSS area divided by the mid-year population at risk. Pre-ITACA incidence rates were calculated yearly using the number of TB cases in the whole district divided by the district mid-year population, using the Mozambican National Statistics Institute (INE) latest official census data (2007) and taking into account the estimated age-specific population growth for the whole period. Yearly confidence intervals were calculated assuming a Poisson distribution. A pooled incidence rate was calculated for the pre-ITACA period using a weighting scheme through a Poisson regression with random effects and jackknife 95% CI. According to the 2000–2013 WHO data, there has been a slight increase in incidence during this time-frame in Mozambique (figure 1) [1]. Using a log-linear regression and WHO data, we estimated an overall 0.6% yearly increasing trend in TB incidence rate and applied this correction factor to the final CDR to mitigate overestimation.

A total of 217 TB cases aged <3 years were diagnosed in the pre-ITACA period (2006–2010), with a pooled incidence rate of 251 per 100 000 people (95% CI 227–276 per 100 000). During ITACA, 57 TB cases aged <3 years initiated anti-TB treatment in the HDSS area, equivalent to an incidence rate of 615 per 100 000 people (95% CI 466–797 per 100 000 people) (figure 1). The estimated CDR was 40.8% (95% CI 36.6–45.1%), and 41.8% (95% CI 37.2–46.4%) after correction. In the hypothetical case of a 5% increase in the national incidence, sensitivity analysis showed that the CDR would increase to (49.1%; 95% CI 41.3–57.0%).

The HIV prevalence among the study population was 47% and 46% during the pre-ITACA and ITACA periods, respectively. During the ITACA period, fewer patients aged <1 year initiated anti-TB treatment (8.8% *versus* 35.9%), more TB cases were extrapulmonary (12.3% *versus* 7.4%) and the treatment success rate was significantly better (82.5% *versus* 67.3%; p=0.025).

To our knowledge, this is the first study providing estimates of TB under-detection using population-based data in the paediatric population in Africa and one of the few worldwide. We found a low CDR regardless of age and sex, which underscores the urgent need to close the gap in case detection and reporting, in order to better assess new control interventions [6, 11].

Our findings are in line with the low CDR for Mozambique reported by WHO [1]; however, the rate we report is probably a maximum, given that the ITACA incidence rate is a conservative estimate (single-day

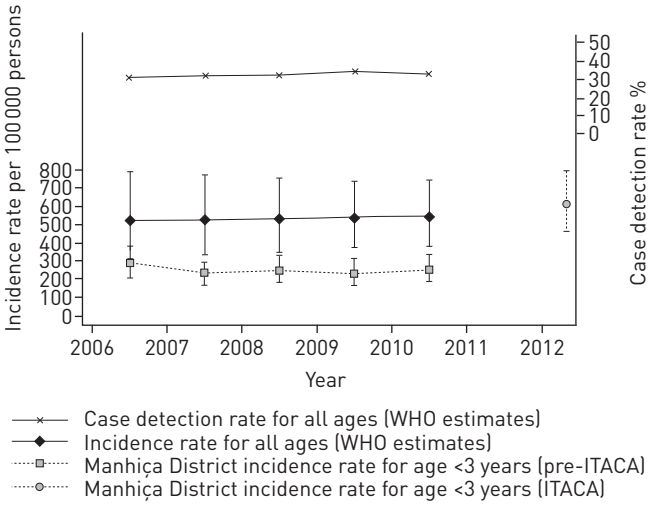


FIGURE 1 Yearly tuberculosis incidence rate in the population aged <3 years in the Manhiça District. World Health Organization (WHO) estimates for all-age incidence rate and case detection rate for Mozambique are shown for the pre-ITACA period. ITACA: study for determination of the minimum incidence rate of tuberculosis in infants and children in the Manhiça District, Mozambique; October 2011–October 2012.

samples were obtained in contrast to the recommended 3-day consecutive sampling; contact tracing could not be fully implemented mainly due to difficulties in patient identification; and some TB cases could have been missed due to mortality prior to treatment initiation or transfers of severely ill patients to the tertiary reference hospital) [9].

The CDR higher than the 35% estimated by Dodd *et al.* [5] for children aged <15 years in all HBCs (based on mathematical modelling of 2010 data) could be due to several reasons.

Given that Mozambique has one of the lowest CDRs among all HBCs and that under-estimation is more frequent in children aged <5 years, the difference observed between our CDR and the one reported by Dodd *et al.* [5] could support the hypothesis that the true CDR in Manhiça is probably lower than 40.8%. Besides, TB incidence and CDR vary greatly across countries and regions (depending on the local epidemiology of TB/HIV and healthcare system characteristics, among others) and Manhiça could show an improved CDR compared with other settings.

Although under-ascertainment, under-reporting and under-diagnosis can all contribute to TB under-estimation [12], the latter is the most probable in Mozambique, where broad paediatric TB case definitions, lack of clear clinical algorithms, low referral rates and difficulties in obtaining samples all contribute to under-diagnosis. Under-reporting is not common, as the private sector has a small role in TB diagnosis and management. Under-ascertainment alone, although common in Manhiça, cannot explain the low CDR, given the small impact of the active case-finding component of this study.

This study has several limitations. First, the use of a historical control for the calculation of CDR may be imperfect as the incidence rate and CDR vary over time. Although we expected that the 0.6% yearly correction could compensate, we acknowledge that the extent of increase in incidence reported for adults may not be the same for children aged <3 years. Furthermore, we compared incidence rates calculated with different denominators (the intervention HDSS population, based on real annual census data for a smaller urban-shaped area *versus* the whole district population, based on projections from the INE 2007 census and considering age-specific annual population growth). We did, however, verify that the CDR did not vary substantially when we calculated the ITACA incidence based on INE district data (and using as denominator the percentage of the INE population belonging to HDSS).

This study provides a novel population-based CDR for paediatric TB in a HBC. Although this estimate is probably a maximum, differences in local detection rates can explain a higher CDR than those reported by others [1, 5]. Given the multiple downstream ramifications of inaccurate estimates [13] and the high mortality of undiagnosed TB in younger children [14], this finding calls for urgent public health interventions to ensure that all TB cases are promptly identified and treated.

@ERSpublications
In this study, the case detection rate of TB in children <3 years in Manhiça, Mozambique was estimated to be 40.8% http://ow.ly/UQg0g

Elisa López-Varela[1,2], Orvalho Joaquim Augusto[1], Luis Guerra[1], Durval Respeito[1], Charfudin Sacoor[1], Jahit Sacarlal[1], Giovanni Battista Migliori[3], Giovanni Sotgiu[4], Pedro L. Alonso[1,2] and Alberto L. García-Basteiro[1,2,5]
[1]Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. [2]Instituto de Salud Global de Barcelona (ISGLOBAL), Barcelona, Spain. [3]WHO Collaborating Centre for TB and Lung Diseases, Fondazione S. Maugeri, Care and Research Institute, Tradate, Italy. [4]Clinical Epidemiology and Medical Statistics Unit, Dept of Biomedical Sciences, University of Sassari – Research, Medical Education and Professional Development Unit, AOU Sassari, Sassari, Italy. [5]Amsterdam Institute for Global Health and Development, Academic Medical Centre, Amsterdam, The Netherlands.

Correspondence: Elisa López-Varela, Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique.
E-mail: elisa.lopez@manhica.net

Received: Sept 01 2015 | Accepted after revision: Nov 09 2015 | First published online: Dec 23 2015

Conflict of interest: None declared.

Acknowledgements: The authors thank the participants and their families. The authors are grateful to the National TB Program (Maputo, Mozambique), Denise Naniche (ISGLOBAL, Barcelona, Spain), Alberto Bila Junior (CISM, Manhiça, Mozambique), health staff at the Manhiça District Hospital (Manhiça, Mozambique) and peripheral health centres for their contribution.

References

1 World Health Organization. Global Tuberculosis Report 2014. WHO/HTM/TB/2014.08. Geneva, World Health Organization, 2014. Available from: http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf
2 Marais BJ, Graham SM, Maeurer M, *et al.* Progress and challenges in childhood tuberculosis. *Lancet Infect Dis* 2013; 13: 287–289.
3 Murray CJ, Ortblad KF, Guinovart C, *et al.* Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; 384: 1005–1070.
4 Jenkins HE, Tolman AW, Yuen CM, *et al.* Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. *Lancet* 2014; 383: 1572–1579.
5 Dodd PJ, Gardiner E, Coghlan R, *et al.* Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. *Lancet Glob Health* 2014; 2: e453–e459.
6 Lönnroth K, Migliori GB, Abubakar I, *et al.* Towards tuberculosis elimination: an action framework for low-incidence countries. *Eur Respir J* 2015; 45: 928–952.
7 García-Basteiro AL, López-Varela E, Respeito D, *et al.* High tuberculosis burden among people living with HIV in southern Mozambique. *Eur Respir J* 2015; 45: 547–549.
8 Sacoor C, Nhacolo A, Nhalungo D, *et al.* Profile: Manhiça Health Research Centre (Manhiça HDSS). *Int J Epidemiol* 2013; 42: 1309–1318.
9 López-Varela E, Augusto OJ, Gondo K, *et al.* Incidence of tuberculosis among young children in rural Mozambique. *Pediatr Infect Dis J* 2015; 34: 686–692.
10 García-Basteiro AL, López-Varela E, Augusto OJ, *et al.* Radiological findings in young children investigated for tuberculosis in Mozambique. *PLoS One* 2015; 10: e0127323.
11 Rudolf F, Wejse C. Tuberculosis case detection revisited: better testing might not improve outcomes. *Lancet Glob Health* 2015; 3: e424–e425.
12 Gibbons CL, Mangen MJ, Plass D, *et al.* Measuring underreporting and under-ascertainment in infectious disease datasets: a comparison of methods. *BMC Public Health* 2014; 14: 147.
13 Cruz AT, Starke JR. What's in a number? Accurate estimates of childhood tuberculosis. *Lancet Glob Health* 2014; 2: e432–e433.
14 Perez-Velez CM, Marais BJ. Tuberculosis in children. *N Engl J Med* 2012; 367: 348–361.

Eur Respir J 2016; 47: 1003–1005 | DOI: 10.1183/13993003.01454-2015 |

Polymers of Z α_1-antitrypsin are secreted in cell models of disease

To the Editor:

The α_1-antitrypsin (α_1-AT) is a 52 kDa glycoprotein that is predominantly synthesised in the liver and secreted into the circulation, where it protects the lungs from the enzyme neutrophil elastase. α_1-AT deficiency (α_1-ATD) is caused by mutations in the α_1-AT gene, with most cases resulting from homozygous inheritance of the Z allele (Glu342Lys). This leads to low levels of circulating α_1-AT, uncontrolled elastase activity and emphysema [1]. The Z mutation destabilises the native α_1-AT and causes the formation of aberrant polymers that accumulate within the endoplasmic reticulum (ER) of

3. Adherence to Childhood Tuberculosis Treatment in Mozambique

López-Varela E, Sequera VG, García-Basteiro AL, Joaquim Augusto O, Munguambe K, Sacarlal J, Alonso.

Status: Accepted for publication in J trop ped

2015 Impact factor 1.059 (Q2)

Journal of Tropical Pediatrics, 2016, 0, 1–11
doi: 10.1093/tropej/fmw048
Original Paper

Adherence to Childhood Tuberculosis Treatment in Mozambique

by Elisa Lopez-Varela,[1,2] Victor Guillermo Sequera,[1,3] Alberto L. García-Basteiro,[1,2,4] Orvalho Joaquim Augusto,[2] Khatia Munguambe,[2,5] Jahit Sacarlal,[2,5] and Pedro L. Alonso[1,2]

[1]ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat De Barcelona, Barcelona, Spain c/ Rosselló, 132, 5° 2ª (08036).
[2]Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique Rua 12, Cambeve, Vila de Manhiça, CP 1929,
[3]Preventive Medicine and Epidemiology Service, Hospital Clínic of Barcelona, Barcelona, SpainVillarroel, 170 08036 Barcelona
[4]Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, the Netherlands Pietersbergweg 17 1105 BM Amsterdam ZO
[5]Faculdade De Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique Av. Julius Nyerere-Campus Universitário, C.P. 254 Maputo
Correspondence: Elisa López-Varela, Centro de Investigação em Saúde de Manhiça (CISM), CP 1929 Maputo, Moçambique.
E-mail <elisa.lopez@manhica.net>

ABSTRACT

Background: There is limited literature regarding adherence rates for the treatment of tuberculosis (TB) in children. We aimed to describe TB treatment outcomes and adherence as well as to evaluate associated factors to poor adherence in Mozambican children.

Methods: This is a sub-study of a community TB incidence study among children <3 years of age. Incomplete adherence included the sum of lost-to-follow-up cases plus those with a delay of > 3 weeks to treatment completion.

Results: Fifty TB treatments were assessed. Forty-four (88.0%) patients completed treatment, two (4.0%) died during treatment and four (8.0%) were lost to follow-up. Incomplete adherence was observed in 31.3% (15 of 48) of cases and was associated with malnutrition or history of a migrant mother.

Conclusion: Although treatment outcome is overall good, there is still a significant proportion of incomplete adherence. Further larger paediatric TB cohorts and qualitative approaches are needed to assess and confirm potential factors for non-adherence.

KEYWORDS: tuberculosis, childhood, adherence, treatment.

BACKGROUND

According to studies performed in the smear-positive adult population, tuberculosis (TB) therapy requires a high adherence rate of >90% to facilitate cure [1, 2]. In adults, poor adherence has proven to increase the risk of morbidity, mortality and drug resistance at an individual and population level [3, 4]. Incomplete adherence to long-term therapy is one of the greatest challenges towards implementing the World Health Organization (WHO) End TB strategy, especially in Africa where the treatment success rate (81% in 2013) has not reached the Stop TB 90% target [5, 6].

Children could account for 20–40% of all TB cases in high-burden settings [7]. Young children are at highest risk of developing TB disease as well as rapid disease progression and mortality if diagnosis and treatment are delayed [8]. Adherence to TB

treatment in children is complex and is influenced by patient and healthcare system factors, among others [9]. It depends on the understanding and motivation of caretakers, who frequently have limited awareness of the disease [3, 10].

Studies on therapy for latent TB infection in children have shown low completion rates ranging from 44 to 78% [11]. Treatment outcomes for paediatric TB disease in the African region have also shown high rates of poor outcomes (deaths, treatment failures and lost to follow-up [LTFU]) ranging from 10 to 19% [12–17]. However, there are few studies that report adherence and treatment duration [11]. In addition, there is limited data on associated barriers to anti-TB treatment in young children [11, 18–20]. Therefore, the aim of this study was to describe the treatment outcomes and adherence to TB treatment and to evaluate factors associated with poor adherence in Mozambican children aged <3 years.

METHODOLOGY

Settings

This study was conducted at the Manhiça District, Southern Mozambique, where the Manhiça Health Research Center runs a health and demographic surveillance system (HDSS) [21]. This setting has a high incidence of both TB and human immunodeficiency virus (HIV), with an estimated community-based incidence rate of TB among children < 3 years of 470/100 000 person-years [22–24] and an estimated case detection rate of 41% [25]. TB treatment is offered at no cost to patients at the health units, and paediatric fixed-dose combinations are available following WHO 2010 dose recommendations [26]. At the time of the study implementation, paediatric TB treatment for smear-negative pulmonary cases and non-severe forms of extrapulmonary TB, included an intensive phase of 2 months of daily Isoniazid, Rifampicin and Pyrazinamide, followed by 4 months of daily Isoniazid and Rifampicin, with weekly and monthly clinical checks and drug collection, respectively [27]. All TB cases co-infected with HIV are managed in an integrated manner with the provision of cotrimoxazole preventive therapy and anti-retroviral therapy at TB clinics.

Study design

This is a sub-study of a larger prospective cohort study that assessed the minimum community incidence of TB among young children (<3 years of age) over a 1 year period (October 2011–12), whose detailed methodology and findings have previously been published [22]. Briefly, all presumptive TB cases were evaluated through physical and radiological examination, HIV and tuberculin skin testing, as well as smear microscopy and culture of both induced sputum and gastric aspirate samples. All participants had at least one follow-up visit arranged within 6 months of recruitment. All TB cases were registered with the National Tuberculosis Program (NTP) and managed according to established national clinical guidelines. Treatment was always initiated at the Manhiça District Hospital (MDH) and patients' care was then transferred to their corresponding peripheral health unit, if applicable. For the purpose of this analysis, TB cases were defined as any case registered to initiate TB treatment at the NTP during the study period. In the incidence study, TB cases followed the National Institute of Health case definition for childhood TB [28] (See Box 1).

Data collection and analysis

Demographic and clinical data were obtained at the initial visit, and follow-up clinical data were recorded at every subsequent visit. Other socio-demographic data were obtained through the HDSS 2012 data. Information on the WHO treatment category, follow-up visits, treatment outcome and adherence were retrospectively extracted from registers of the NTP into a structured data collection tool. Delays in treatment completion were calculated based on the registered date of treatment initiation and treatment completion.

Proportions were compared using the Fisheŕs exact chi-squared test. Prevalence ratio (PR) and its 95% confidence intervals (CI) were calculated from poison regression with robust standard errors to measure the strength of the association between clinical and demographic factors and adherence categories. Programmatic data from the NTP at the MDH for other age groups during the same period were used for comparison. Statistical software for analysis was Stata 11.2 (StataCorp. 2013. Stata: Release 11, StataCorp LP, Statistical Software, College Station, TX).

Box 1. Relevant study definitions

TB case: Any child registered to initiate TB treatment at the NTP during the study period.

Study TB case: Includes microbiologically confirmed plus probable cases (adapted from the standardized NIH case definition for childhood TB [28], full details on the classification are described elsewhere [22]).

Confirmed TB case: Compatible symptoms plus a positive culture with *Mycobacterium tuberculosis*.

Probable TB case: Fulfilling the following three criteria:

1. Compatible symptoms unresolved at last clinical follow-up visit (before any TB treatment initiation).
2. Compatible chest radiograph: ≥1 of the following radiographic abnormalities: airway compression, lymphadenopathy, opacification, nodular picture, effusion, cavities, spondylitis or Ghon focus [29].
3. At least one of the following: TB exposure, positive TST (induration >5 mm for HIV or malnourished children and >10 mm for the rest of participants) or positive response to TB treatment.

HIV infection: Positive antibody test in children >18 months (Determine, Abbott Laboratories and confirmed with Unigold, Trinity Biotech); or positive HIV PCR in those <18 months; or a strong clinical suspicion with positive antibody test in the absence of a PCR result.

Treatment outcomes for drug-susceptible TB patients [5, 30]:

Treatment success: The sum of patients:

1. Cured: A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear or culture negative in the last month of treatment and on at least one previous occasion) AND
2. Treatment completed: A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.

Treatment failed: A TB patient whose sputum smear or culture is positive at month 5 or later during treatment.

Died: A TB patient who dies for any reason before starting or during the course of treatment.

Lost to follow-up: A TB patient whose treatment was interrupted for two consecutive months or more.

Not evaluated: A TB patient for whom no treatment outcome is assigned. This includes cases 'transferred out' to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.

Adherence categories were defined as:

Incomplete adherence. The sum of the following two exclusive categories and calculated over those patients who did not die:

1. **Lost to follow-up** (patients whose treatment was interrupted for 2 consecutive months or more) AND
2. **Delayed completion** (patients with a delay of ≥ 3 weeks beyond the expected date (calculated as 6 months after treatment initiation)).

Full adherence. All treatment success cases with no delay in treatment completion.

NIH, National Institute of Health; NTP, National TB Program; TB, tuberculosis; TST, tuberculin skin test; PCR, polymerase chain reaction.

Ethical considerations

This study was approved by the Mozambican National Bioethics Committee (Ref.15/CNBS/2010). Written informed consent was obtained from the parents/caregivers of all children.

RESULTS

Fifty children <3 years old consented to participate and initiated TB treatment in the district of Manhiça (Tables 1 and 2). All were treated for drug-susceptible TB; nine cases were microbiologically confirmed on the basis of culture, although none of them was smear positive on microscopy for acid-fast bacilli. Of all children starting treatment, 26 (52.0%) were male and 24 (48.0%) were HIV infected. Although patients were followed up in 10 different peripheral healthcare centres, >64.0% of cases were managed by two single health centres, one of which was the MDH (30.0%).

All 50 cases had documented treatment outcomes: 44 (88.0%) children successfully completed treatment,

Table 1. Clinical and socio-demographic characteristics of TB cases <3 year at the time of TB diagnosis

Clinical	*n*	(%)
Sex (male)		
Male	26	(52)
Female	24	(48)
Age in months, (Median [IQR])	19.8	[14.6-26.1]
Age at diagnosis (months)		
<12	10	(20)
12–24	23	(46)
>24	17	(34)
BCG Scar[a]		
Present	43	(87.8)
Absent	6	(12.2)
HIV coinfected		
Yes	24	(48)
No	26	(52)
TST[b]		
Positive	21	(43.8)
Negative	27	(56.2)
Hospitalizations in previous year to TB diagnosis		
Yes	20	(40)
No	30	(60)
N° outpatient consultations in previous year to TB diagnosis		
<10	36	(72.0)
≥10	14	(28)
Study TB cases definition		
Confirmed	9	(18)
Probable	25	(50)
Possible	13	(26)
MTB infection/TB unlikely	3	(6)
TB compatible CXR		
Yes	18	(36)
No	32	(64)
Symptoms		
Cough >2 weeks		
Yes	14	(28)
No	36	(72)
Fever >2 weeks		
Yes	6	(12)
No	44	(88)
Wheeze		
Yes	3	(6)
No	47	(94)
Chronic or acute malnutrition		
Yes	11	(22)
No	39	(78)
Adenopathy		
Yes	1	(2)
No	49	(98)
Contact of pulmonary TB case (documented or reported)		
Yes	14	(28)
No	36	(72)
Hospitalized at time of enrolment		
Yes	9	(18)
No	41	(82)
Number of follow-up visits to the cohort study during course of TB treatment		
<2	11	(22)
≥2	39	(78)

Denominator is $n = 50$ except for
[a]($n = 49$) and
[b]($n = 48$).
BCG, Bacille Calmette Guerin; HIV, human immunodeficiency virus; IQR, interquartile range; CXR, chest X ray; TST, tuberculin skin test; MTB, *Mycobacterium tuberculosis*.
Definitions: Positive TST was defined as an induration >5 mm for HIV or malnourished children and >10 mm for the rest of participants. HIV infection was defined as positive antibody test in children >18 months (Determine, Abbott Laboratories and confirmed with Unigold, Trinity Biotech); or positive HIV polymerase chain reaction in those <18 months; or a strong clinical suspicion with positive antibody test in the absence of a polymerase chain reaction result. CXR were classified as compatible if presented ≥1 of the following radiographic abnormalities: airway compression, lymphadenopathy, opacification, nodular picture, effusion, cavities, spondylitis or Ghon focus.

2 (4.0%) died before treatment completion and 4 (8.0%) were LTFU. There were no treatment failures nor transferred cases. Among treatment success cases, 11 (25.0%) had a delay in treatment completion, eight of which were males, over half HIV infected and one microbiologically confirmed TB case. Among the LTFU, three were males, all lived >2 km distance from the MDH, none fulfilled the study TB case definition and all were HIV-infected. Overall incomplete adherence (delayed plus LTFU) was reported in

Table 2.

Sociodemographic	*n*	(%)
Distance to peripheral healthcare centres in Km (Median [IQR])	1.68	[1.18–2.50]
Distance to peripheral healthcare centres		
<1	30	(60)
2–5	15	(30)
>5	5	(10)
Distance to Manhiça Health Center Km (Median [IQR])	12.46	[3.9–17.4]
Distance to Manhiça Health Center		
<5 km	16	(32)
≥5 km	34	(68)
Number of people living in the house (median [IQR])	6	[4–9]
Number of people living in the house		
<6	21	(42)
≥6	29	(58)
Number of children <15 years living in the house[a]		
1 a 3	35	(80)
≥4	9	(20)
Children's birth order[a]		
1st or 2nd	30	(68.2)
≥3rd	14	(31.8)
Children <15 years at home[a]		
1–4	26	(59.1)
≥5	18	(40.9)
Orphan (death of mother)	1	(2.0)
Orphan (death of father)	4	(8)
Death in the household in the previous year	14	(28)
Migration of the mother in previous 2 year	8	(16)
Migration of the father in previous 2 year	16	(32.0)
Migration in the household member	20	(40)
SES[b]		
Poorest	15	(30)
Less poor	20	(40)

Denominator is $n = 50$ except for
[a]($n = 44$) and
[b]($n = 35$).
IQR, interquartile range; SES, socio-economic status.
Definitions: A household asset-based wealth index was used to categorize the household SES into five wealth quintiles. The two lowest quintiles were grouped as 'poorest' and the remaining three quintiles were renamed 'less poor'.

31.3% (15 among the 48 patients who did not die) (Table 3). Figure 1 shows the distribution of the number of days from treatment initiation to treatment completion. Eleven cases finished treatment before the expected date, eight of them 1–2 days earlier. One patient had a 74 day delay in treatment completion.

Compared with other age groups registered at the MDH NTP during the same period, we found a higher treatment success rate in children <3 years (88% vs. 68.1% and 72.5% among patients aged 3–15 years and >15 years, respectively). However, incomplete adherence was similar in all groups. Figure 2 shows adherence results for these three age categories (final numbers exclude deaths).

Being malnourished at enrolment and having a mother with a history of migration in the previous 2 years to TB diagnosis were shown to be potential risk factors for incomplete adherence (PR: 2.9; 95% CI: 1.4–6.1 and PR: 2.9; 95% CI: 1.4–6.0, respectively, $p < 0.05$) (Table 3).

DISCUSSION

Data on adherence to TB treatment in children are scarce. To our knowledge, this is the first study describing the profile and treatment outcomes among paediatric TB cases in Mozambique and one of the few reporting adherence and treatment outcomes in a well-characterized cohort of young children. Although the overall treatment success rate (88%) was close to the 90% Stop TB target [6], there were still a significant proportion of paediatric TB cases with incomplete adherence (31.3%). Being malnourished and having a migrant mother were potential risk factors for incomplete adherence.

We have previously reported a treatment success rate of 67.3% among children aged <3 years in Manhiça during 2006–10 [25]. The significant improvement observed in the current study compared with the previous years can be owing to several reasons. First, to the substantial recent decrease in mortality, as improved TB/HIV care and treatment services are available at the health facilities. The proportion of patients who die during TB treatment has decreased from 17% during 2006–10 to 4% in this study 31]. Secondly, improved outcomes may be owing to a slight decrease in the number of LTFU (from 9.6% in 2006–10 to 8%) that could be

Table 3. Univariate analysis of predictor factors for incomplete adherence

	Full adherence[a]		Incomplete adherence[b]		PR (95% CI)	*p* value
	n	(%)	*n*	(%)		
Total	**33**	**(68.8)**	**15**	**(31.3)**		
Sex*						
Female	19	(82.6)	4	(17.4)	Reference	
Male	14	(56)	11	(44)	2.5 (0.9-6.9)	0.065
HIV-coinfected						
No	20	(80.0)	5	(20.0)	Reference	
Yes	13	(56.6)	10	(43.5)	1.9 (0.8-4.4)	0.080
Study TB case definition						
Possible-unlikely	7	(50.0)	7	(50.0)	Reference	
Confirmed-probable	26	(76.5)	8	(23.5)	0.5 (0.1-1.1)	0.072
Number of outpatient consultations in previous year*						
0–9	21	(61.8)	13	(38.2)	Reference	
≥10	12	(85.7)	2	(14.3)	0.4 (0.1-1.5)	0.171
Hospitalized at time of enrolment*						
No	30	(75.0)	10	(25.0)	Reference	
Yes	3	(37.5)	5	(62.5)	2.5 (1.2-5.4)	0.088
Symptom at enrolment: malnutrition*						
No	30	(76.9)	9	(23.1)	Reference	
Yes	3	(33.3)	6	(66.7)	2.9 (1.4-6.1)	0.018
Migration of the mother in previous 2 years*						
No	31	(75.6)	10	(24.4)	Reference	
Yes	2	(28.6)	5	(71.4)	2.9 (1.4-6.0)	0.024
Death in the household in the previous year*						
No	22	(61.1)	14	(38.9)	Reference	
Yes	11	(91.7)	1	(8.3)	0.2 (0.1-1.5)	0.073
Number of people living in the house						
<6	11	(55.0)	9	(45.0)	Reference	
≥6	22	(78.6)	6	(21.4)	0.5 (0.2-1.1)	0.082
Distance to the nearest healthcare centre						
<2 km	23	(76.7)	7	(23.3)	Reference	
≥2 km	10	(55.6)	8	(44.4)	1.9 (0.8-4.4)	0.127

[a]Full adherence: treatment completed on time with full adherence.
[b]Incomplete adherence includes (a) delayed treatment completion plus (b) treatment non-completion.
p values obtained through chi-squared test or Fisher's exact test (when applicable*).
Only those variables with *p*-values < 0.2 are shown.
A significant association was found between the variable malnutrition and hospitalization ($p = 0.02$), migration of the mother ($p = 0.001$) and TB case definition ($p = 0.002$).

influenced by the fact of being included in the epidemiological incidence study. Although the study had active follow-up visits, which could have positively influenced treatment adherence, the univariate analysis did not show an association.

The treatment success rate reported in this study is similar to the 87% estimated by WHO for all new cases in 2012 for Mozambique [5]. It decreased, however, in other age groups during the same period, reaching 68.1% and 72.5% in patients aged

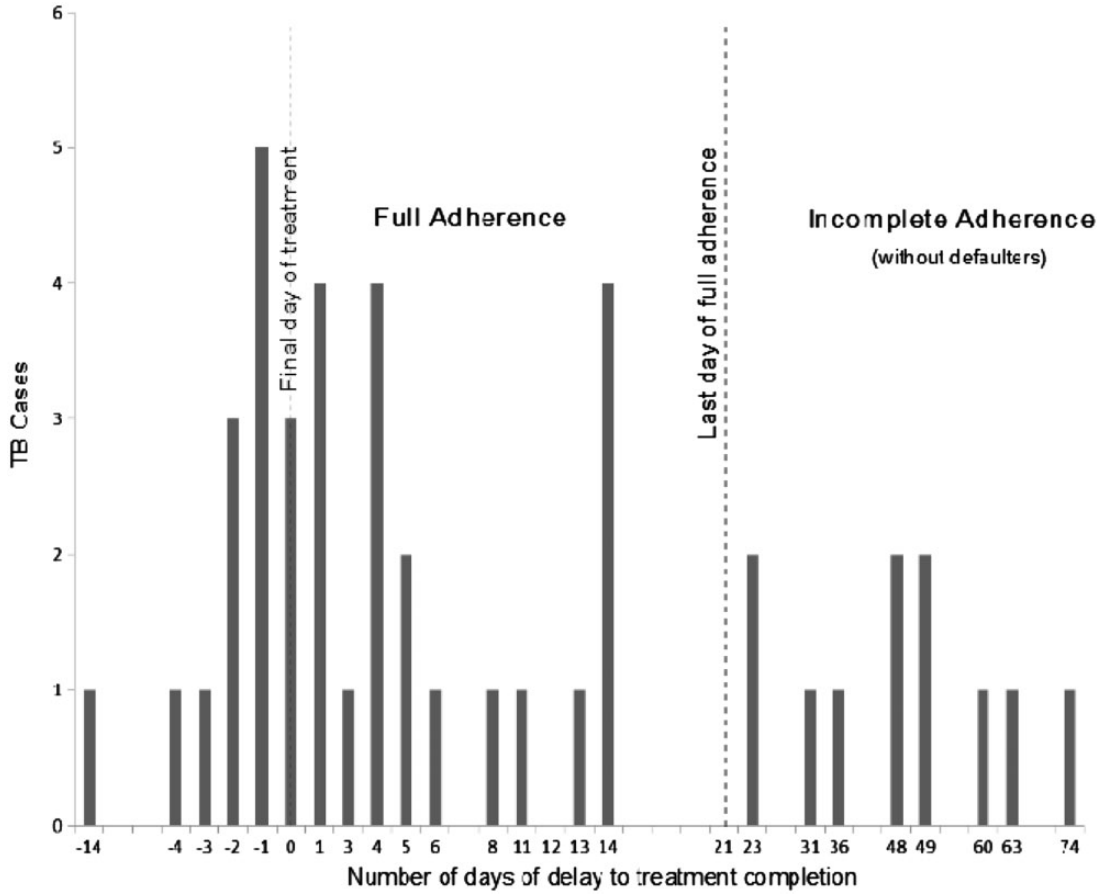


Fig. 1. Duration of treatment among patients with treatment completion. Definitions: Days of delay in treatment completion were calculated based on a standard treatment duration of 180 days for retreatment cases, final day was adjusted). Number of days in X axis are not in scale.

3–15 years and >15 years, respectively. While other authors have also found higher success rates with younger ages [11], Hailu *et al.* [18] found that being >5 years of age was a predictor of treatment success.

Comparing TB treatment adherence results among different studies is difficult owing to the large variations in the definitions of adherence found in the literature, particularly for childhood TB [11, 17, 19, 32–35]. As stated by Chang *et al.* [36], it seems reasonable to consider LTFU and incomplete adherence as part of the same problem, with different levels of severity. Thus, for the purpose of this study, we used a definition of incomplete adherence that includes LTFU plus delayed completion. Despite the differences in the definitions, the results in this study are similar to other paediatric reports from high burden countries [17, 34, 35, 37]. Unpublished results from a recent meta-analysis show a treatment success rate of 81% with 3% mortality, with large variations among the studies included [12].

We have identified several factors associated with incomplete adherence, many of them were expected and have been previously cited by other authors as predictors of poor outcome [13, 38, 39]. Evidence of chronic or acute malnutrition at diagnosis was associated with incomplete adherence. Several studies have also reported poor TB outcomes among malnourished children [38, 39] but in this study malnutrition was associated both with LTFU and death as well as with a delay in treatment completion. This suggests that beyond the deleterious immunological impact of malnutrition on TB progression, other aspects such

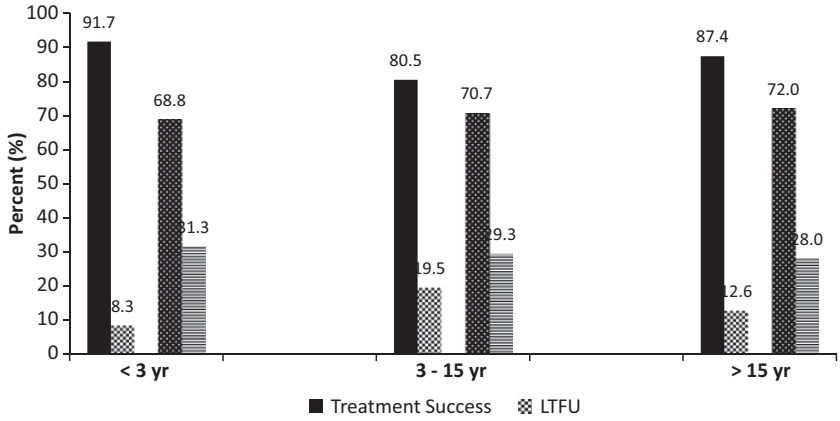


Fig. 2. Treatment and adherence outcomes among different age groups (2011–12). This figure shows treatment outcome (treatment success vs. LTFU) among patients who did not die and adherence results (full vs. incomplete adherence) among all age groups initiating TB treatment at the NTP of the Manhiça Health Center ($n = 867$ to ≥ 15 years; $n = 82$ to < 15–3 years; $n = 56$ to < 3 years). Excluded from this analysis are: dead and transferred cases as well as TB cases with number of treatment days missing.

as tolerance to drugs or, more importantly, the social context have an impact on adherence and outcome. The importance of the caregiver–child relationship has been shown to impact overall child survival, and thus, the history of migration of the mother seems to impact treatment adherence and outcomes [40]. Malnutrition was significantly associated with a history of a migrant mother, hospitalization at the time of diagnosis and TB case definition, and thus the specific impact of these other variables on adherence is difficult to interpret.

HIV co-infection is also a well-known risk factor for incomplete adherence in adults [2, 41–44], and children [19, 38], partly owing to the increased pill burden and secondary effects. Because of the high treatment compliance rate (90%) required to facilitate cure and reduce the risk of rapid disease progression in children, the poor adherence observed among HIV-TB co-infected cases is cause for concern, as it could lead to increased mortality. Given the inherent difficulties in diagnosing paediatric TB, caregivers may sometimes reflect their uncertainty in the diagnosis by not fulfilling the treatment recommendations. This may be the underlying cause of the poorer adherence observed among cases not fulfilling the study TB definition. Moreover, TB diagnostic algorithms do not perform well among HIV co-infected children [28]. Given the HIV co-infection rate observed among confirmed and non-confirmed TB cases (10% and 51%, respectively), this may have played a role in hesitance of diagnosis and adherence to treatment. The gender difference observed in the current study was not statistically significant and needs to be further evaluated with larger sample sizes. Some adult studies have also noted a higher rate of incomplete adherence among males although this association needs to be further evaluated and may not be necessarily extrapolated to the paediatric population [44–47].

There are several limitations to this study. First, the small sample size of the cohort limits the ability to reach statistical significance for several potential associations (no multivariable logistic regression analysis was possible). Secondly, the analysis of adherence was performed using data measured indirectly with a retrospective design. In addition, the fact that most children starting treatment were part of a research study could have had a potential positive influence on adherence, although the study was focusing on case detection. Moreover, we did not register other common factors reported to influence adherence such as major side effects, who the main caregiver for the child was or the exact phase of the treatment where the main delay occurred. Furthermore, the results may be biased given that TB under-reporting is more common in severe forms

of the disease in which the patients die before treatment initiation. Finally, we did not capture common system failures such as drug stock rupture or health personnel absenteeism, which might lead to non-patient-originated poor adherence.

In conclusion, although paediatric treatment outcome is overall good, there is still a significant proportion of incomplete adherence cases. This study setting may have represented an improved health system scenario, so the true program performance may show worse indicators. Reinforcing the importance of timely treatment completion should remain a high priority. Successful treatment of paediatric TB requires the commitment and involvement of the corresponding caregiver. Further larger paediatric TB cohorts and qualitative research are needed to assess and eventually address potential risk factors for non-adherence.

ACKNOWLEDGEMENTS

The authors thank the participants and their families. The authors are grateful to the National TB Program, health staff at the Manhiça District Hospital and peripheral health centres for their contribution. The authors thank Prof Steve Graham and Dr Denise Naniche for their comments to the manuscript.

FUNDING

This work was supported by the European & Developing Countries Clinical Trials Partnership [IP_07_32080_003].

REFERENCES

1. Awofeso N. Anti-tuberculosis medication side-effects constitute major factor for poor adherence to tuberculosis treatment. Bull World Health Organ 2008;86:B–D. http://www.ncbi.nlm.nih.gov/pubmed/18368191 (6 July 2016)
2. Adane AA, Alene KA, Koye DN, *et al.* Non-adherence to anti-tuberculosis treatment and determinant factors among patients with tuberculosis in northwest Ethiopia. PLoS One 2013;8:e78791.
3. World Health Organization (WHO). Adherence to long-term therapies: evidence for action. WHO/MNC/03.01. 2003, Geneva : World Health Organization, 2003.
4. Burman WJ, Cohn DL, Rietmeijer CA, *et al.* Noncompliance with directly observed therapy for tuberculosis. Epidemiology and effect on the outcome of treatment. Chest 1997;111:1168–73. http://www.ncbi.nlm.nih.gov/pubmed/9149565 (5 November 2015, date last accessed)
5. World Health Organization (WHO). Global Tuberculosis Report 2014. Geneva, Switzerland: World Health Organization, 2014. WHO/HTM/TB/2014.08. [cited 2013 Jun 24]; Available from: http://www.who.int/tb/publications/global_report/en/
6. Stop TB Partnership. Stop TB Partnership and World Health Organization. Global Plan to Stop TB 2006– 2015. Geneva: World Health Organization, 2006 (WHO/HTM/STB/2006.35).
7. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med 2012;367:348–61. http://www.ncbi.nlm.nih.gov/pubmed/22830465 (3 March 2013, date last accessed).
8. World Health Organization (WHO). Roadmap for childhood tuberculosis: towards zero deaths. Geneva: World Health Organization, 2013 (WHO/HTM/TB/2013.12). http://apps.who.int/iris/handle/10665/89506 (18 November 2013, date last accessed).
9. Munro SA, Lewin SA, Smith HJ, *et al.* Patient adherence to tuberculosis treatment: a systematic review of qualitative research. PLoS Med 2007;4:e238. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1925126&tool=pmcentrez&rendertype=abstract (2 October 2015, date last accessed)
10. Craig SR, Adams LV, Spielberg SP, *et al.* Pediatric therapeutics and medicine administration in resource-poor settings: a review of barriers and an agenda for interdisciplinary approaches to improving outcomes. Soc Sci Med 2009;69:1681–90. http://www.ncbi.nlm.nih.gov/pubmed/19796859 (5 November 2015, date last accessed).
11. van Zyl S, Marais BJ, Hesseling AC, *et al.* Adherence to anti-tuberculosis chemoprophylaxis and treatment in children. Int J Tuberc Lung Dis 2006;10:13–18. http://www.ncbi.nlm.nih.gov/pubmed/16466031
12. Khawaja H. Treatment outcomes in children with tuberculosis: a systematic review and meta-analysis. BSc Dissertation in Reproductive and Developmental Sciences. Imperial College London, 2014.
13. Adejumo OA, Daniel OJ, Adebayo BI, *et al.* Treatment outcomes of childhood TB in Lagos, Nigeria. J Trop Pediatr 2016;62:131–8. http://www.ncbi.nlm.nih.gov/pubmed/26705331 (25 June 2016, date last accessed).
14. Osman T, El Sony A. Case management of childhood tuberculosis in children's hospitals in Khartoum. East Mediterr Heal 2014;20:442–9. http://www.ncbi.nlm.nih.gov/pubmed/25023771 (25 June 2016, date last accessed).
15. Oeltmann JE, Chengeta B, Mboya JJ, *et al.* Reported childhood tuberculosis treatment outcomes, Gaborone and Francistown, Botswana, 1998-2002. Int J Tuberc Lung Dis 2008;12:186–92. http://www.ncbi.nlm.nih.gov/pubmed/18230252 (25 June 2016, date last accessed)
16. Mtabho CM, Irongo CF, Boeree MJ, *et al.* Childhood tuberculosis in the Kilimanjaro region: lessons from and for the TB programme. Trop Med Int Health 2010;15:

496–501. http://www.ncbi.nlm.nih.gov/pubmed/20230572 (25 June 2016, date last accessed)
17. Harries AD, Hargreaves NJ, Graham SM, *et al.* Childhood tuberculosis in Malawi: nationwide case-finding and treatment outcomes. Int J Tuberc Lung Dis 2002;6:424–31. http://www.ncbi.nlm.nih.gov/pubmed/12019918
18. Hailu D, Abegaz WE, Belay M. Childhood tuberculosis and its treatment outcomes in Addis Ababa: a 5-years retrospective study. BMC Pediatr 2014;14:61.
19. Wilkinson D, Davies GR. Pediatric tuberculosis in rural South Africa–value of directly observed therapy. J Trop Pediatr 1998;44:266–9. http://www.ncbi.nlm.nih.gov/pubmed/9819487 (4 November 2015, date last accessed)
20. Osman Yusuf K, Fromsa Seifu M, Kefale Gelaw B, *et al.* Non Adherence and its Contributing Factors to Anti-TB Drug in Children's at Adama Referral Hospital, Oromia, Ethiopia. Glob J Med Res 2015;15(2). http://medicalresearchjournal.org/index.php/GJMR/article/view/878.
21. Sacoor C, Nhacolo A, Nhalungo D, *et al.* Profile: Manhica Health Research Centre (Manhica HDSS). Int J Epidemiol 2013;42:1309–18. http://www.ncbi.nlm.nih.gov/pubmed/24159076 (8 November 2013, date last accessed).
22. López-Varela E, Augusto OJ, Gondo K, *et al.* Incidence of Tuberculosis among young children in rural Mozambique. Pediatr Infect Dis J 2015;34:686–92. http://www.ncbi.nlm.nih.gov/pubmed/26069945 (22 March 2016, date last accessed).
23. García-Basteiro AL, López-Varela E, Respeito D, *et al.* High tuberculosis burden among people living with HIV in southern Mozambique. Eur Respir J 2015;45:547–9. http://erj.ersjournals.com/content/early/2014/11/13/09031936.00145714.extract
24. González R, Munguambe K, Aponte J, *et al.* High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med 2012;13:581–8. http://www.ncbi.nlm.nih.gov/pubmed/22500780 (24 June 2013, date last accessed).
25. López-Varela E, Augusto OJ, Guerra L, *et al.* Low paediatric tuberculosis case detection rate in Southern Mozambique. Eur Respir J 2016;47:1003–5. http://www.ncbi.nlm.nih.gov/pubmed/26699721 (14 January 2016, date last accessed)
26. World Health Organization. Rapid advice. Treatment of tuberculosis in children. WHO/HTM/TB/2010.13; 2010, Geneva : World Health Organization, 2003.
27. Graham SM. Guidance for National Tuberculosis Programmes on the management of tuberculosis in children - an update. Malawi Med J 2007;19:82–6.
28. Graham SM, Ahmed T, Amanullah F, *et al.* Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis 2012;205:S199–208. http://www.ncbi.nlm.nih.gov/pubmed/22448023 (5 March 2013, date last accessed)
29. García-Basteiro AL, López-Varela E, Augusto OJ, *et al.* Radiological findings in young children investigated for tuberculosis in Mozambique. PLoS One 2015;10:e0127323. http://dx.plos.org/10.1371/journal.pone.0127323
30. World Health Organization. WHO revised definitions and reporting framework for tuberculosis. WHO/HTM/TB/2013.2. 2013. http://www.ncbi.nlm.nih.gov/pubmed/23611033
31. Auld AF, Alfredo C, Macassa E, *et al.* Temporal trends in patient characteristics and outcomes among children enrolled in Mozambique's national antiretroviral therapy program. The Pediatr Infect Dis J 2015;1. http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006454-900000000-97756
32. Biddulph J. Short course chemotherapy for childhood tuberculosis. Pediatr Infect Dis J 1990;9:794–801.
33. Al-Dossary F, Ong LT, Correa AG. Treatment of childhood tuberculosis with a six month directly observed regime of only two weeks of daily therapy. Pediatr Infect Dis J 2002;21:91–7.
34. Te Water N, Donald P. Twice weekly vs daily chemotherapy for childhood tuberculosis. Pediatr Infect Dis J 2000;19:405–10.
35. Meissner PE, Musoke P, Okwera A, *et al.* The value of urine testing for verifying adherence to anti-tuberculosis chemotherapy in children and adults in Uganda. 2002;6:903–8.
36. Chang KC, Leung CC, Tam CM. Risk factors for defaulting from anti-tuberculosis treatment under directly observed treatment in Hong Kong. Int J Tuberc Lung Dis 2004;8:1492–8.
37. Schaaf HS, Marais BJ, Hesseling AC, *et al.* Surveillance of antituberculosis drug resistance among children from the Western Cape Province of South Africa–an upward trend. Am J Public Health 2009;99:1486–90.
38. Aketi L, Kashongwe Z, Kinsiona C, *et al.* Childhood tuberculosis in a sub-Saharan tertiary facility: Epidemiology and factors associated with treatment outcome. PLoS One 2016;11:1–13.
39. Hicks RM, Padayatchi N, Shah NS, *et al.* Malnutrition associated with unfavorable outcome and death among South African MDR-TB and HIV co-infected children. Int J Tuberc Lung Dis 2014;18:1074–83. http://www.ncbi.nlm.nih.gov/pubmed/25189555 (25 June 2016, date last accessed)
40. World Health Organization. The importance of caregiver-child interactions for the survival of child and healthy development of young children: a review. 2004, Geneva : World Health Organization, 2003.
41. Kayigamba FR, Bakker MI, Mugisha V, *et al.* Adherence to tuberculosis treatment, sputum smear conversion and mortality: a retrospective cohort study in 48 Rwandan clinics. PLoS One 2013;8:e73501.
42. Garrido MDS, Penna ML, Perez-Porcuna TM, *et al.* Factors associated with tuberculosis treatment default in

an endemic area of the Brazilian Amazon: a case control-study. PLoS One 2012;7:e39134.
43. Daftary A, Padayatchi N, O'Donnell M. Preferential adherence to antiretroviral therapy over tuberculosis treatment: a qualitative study of drug-resistant TB/HIV co-infected patients in South Africa. Glob Public Health 2014;9:1107–16.
44. Muture BN, Keraka MN, Kimuu PK, *et al*. Factors associated with default from treatment among tuberculosis patients in Nairobi province, Kenya: a case control study. BMC Public Health 2011;11:696.
45. Kulkarni P, Akarte S. Non-adherence of new pulmonary tuberculosis patients to anti-tuberculosis treatment. Ann Med Health Sci Res 2013;3:67–74.
46. Boyle SJO, Power JJ, Ibrahim MY, *et al*. Factors affecting patient compliance with anti-tuberculosis chemotherapy using the directly observed treatment, short-course strategy (DOTS) SUMMARY. 2002;6:307–12.
47. Comolet TM, Rakotomalala R, Rajaonarioa H. Factors determining compliance with tuberculosis treatment in an urban environment, Tamatave, Madagascar. Int J Tuberc Lung Dis 1998;2:891–7.

4. Radiological findings in young children investigated for tuberculosis in Mozambique.

García-Basteiro AL, López-Varela E, Augusto OJ, Gondo K, Muñoz J, Sacarlal J, Marais B, Alonso PL, Ribó JL.

PLoS One. 2015 May 28;10(5)

2015 Impact factor 3.234 (D1)

PLOS | ONE

RESEARCH ARTICLE

Radiological Findings in Young Children Investigated for Tuberculosis in Mozambique

Alberto L. García-Basteiro[1,2], Elisa López-Varela[1,2]*, Orvalho Joaquim Augusto[2], Kizito Gondo[1], José Muñoz[2], Jahit Sacarlal[1], Ben Marais[3], Pedro L. Alonso[1,2], José L. Ribó[4]

1 Centro de Investigação em Saude de Manhiça (CISM). Rua 12, Cambeve CP 1929 Maputo, Mozambique, **2** ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic—Universitat de Barcelona, Rossello, 132, 08036, Barcelona, Spain, **3** Marie Bashir Institute for Infectious Diseases and Biosecurity Institute (MBI) and The Children's Hospital at Westmead, The University of Sydney, Australia. Westmead NSW 2145 Australia, **4** Radiology Department, Hospital San Juan de Dios. Passeig Sant Joan de Déu, 2. 08950 Esplugues de Llobregat, Barcelona, Spain

* elisa.lopez@manhica.net

Citation: García-Basteiro AL, López-Varela E, Augusto OJ, Gondo K, Muñoz J, Sacarlal J, et al. (2015) Radiological Findings in Young Children Investigated for Tuberculosis in Mozambique. PLoS ONE 10(5): e0127323. doi:10.1371/journal.pone.0127323

Academic Editor: Pere-Joan Cardona, Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol. Universitat Autònoma de Barcelona. CIBERES, SPAIN

Received: December 5, 2014

Accepted: April 13, 2015

Published: May 28, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This study is an ancillary study of a larger project funded by European and Developing Countries Clinical Trial Partnership (EDCTP). The funders had no no role in the study design, data collection and analysis.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Introduction

Chest radiography remains a critical tool for diagnosing intrathoracic tuberculosis (TB) in young children who are unable to expectorate. We describe the radiological findings in children under 3 years of age investigated for TB in the district of Manhiça, southern Mozambique, an area with a high prevalence of TB and HIV.

Methods

Digital antero-posterior and lateral projections were performed and reviewed by two independent readers, using a standardized template. Readers included a local pediatrician and a pediatric radiologist blinded to all clinical information. International consensus case definitions for intra-thoracic TB in children were applied.

Results

A total of 766 children were evaluated of whom 43 (5.6%) had TB. The most frequent lesion found in TB cases was air space consolidation (65.1%), followed by suggestive hilar lymphadenopathy (17.1%) and pleural effusion (7.0%). Air space consolidation was significantly more common in TB cases than in non-TB cases (odds ratio 8.9; 95% CI: 1.6-50.5), as were hilar lymphadenopathy (OR 17.2; 95% CI: 5.7-52.1). The only case with miliary infiltrates and 3 with pleural effusions occurred in HIV-infected children.

Conclusion

Frequent air space consolidation complicates radiological distinction between TB and bacterial pneumonia in young children, underscoring the need for epidemiological contextualization and consideration of all relevant signs and symptoms.

Introduction

Childhood tuberculosis (TB) is a leading cause of respiratory disease in TB endemic areas. The World Health Organization (WHO) estimates that 550 000 children developed TB in 2013[1], but recent modeling studies suggest that the burden could be much higher [2,3]. Young children, and immunocompromised individuals, have an increased risk of developing active disease following *M. tuberculosis* infection [4].

The diagnosis of TB is particularly challenging in young children, given the non-specific nature of their symptoms, difficulties in obtaining samples for microbiological examination and the often pauci-bacillary nature of their disease [4]. Liquid culture, the accepted diagnostic reference standard, is positive in less than 50% of children treated for TB [5,6], although this varies depending on the degree of lung involvement [7]. Moreover, liquid culture or molecular diagnostic tests are not available in many low resource-limited settings[8]. In everyday practice, TB diagnosis in young children relies heavily on exposure to an infectious source case or immunological evidence of *M. tuberculosis* infection, together with findings suggestive of tuberculosis (TB) on the chest radiograph (CXR).

CXR remains a critical tool for diagnosing intrathoracic TB which is the most common presentation of TB in children [9]. In fact, CXR signs suggestive of TB are considered essential to establish a diagnosis of probable intrathoracic TB, according to international consensus clinical case definitions [10]. The most common radiological finding associated with TB in children is perihilar or mediastinal lymphadenopathy [11,12]. Cavitary lesions are rare [12], except in very young infants and HIV infected children [13], or with the emergence of adult-type disease during adolescence.

Few studies have described CXR findings in young children evaluated for TB, comparing TB cases with those considered not to have TB. We describe radiological findings in children under 3 years of age investigated for TB in Mozambique, in an area endemic for both TB and HIV[14]

Methods

A prospective descriptive study of young children (<3 years of age) evaluated for TB was conducted at the Manhiça Health Research Center (CISM), located in Southern Mozambique, over a 1-year period (October 2011–2012).[15] Socio-demographic characteristics of the population living in the district of Manhiça have been described in detail elsewhere [16]. In 2011, the incidence rate of confirmed TB among HIV infected adults aged 18–47 was 847 per 100.000 [14]. The overall estimated TB incidence rate in Mozambique is 552 per 100.000 population [17]. Most children in the country receive Bacille Calmette-Guerin (BCG) vaccination at birth; coverage is around 90% [18].

Children with symptoms suspicious of TB and those in close contact with a sputum smear-positive TB case were evaluated. Symptoms suspicious of TB included: cough for ≥14 days not responding to a course of antibiotics; referred fever for ≥14 days after common causes like malaria were excluded; weight loss/failure to thrive(defined as under 60% weight for height, failure to gain weight for >2 months, any loss of weight unresponsive to nutritional rehabilitation). Full details on inclusion criteria are described elsewhere.[15]

Evaluation included a physical examination, HIV rapid antibody test (Determine, Abbott Laboratories), tuberculin skin test (TST) and CXR. A positive TST was defined as an induration of ≥5mm for HIV-infected or malnourished children and ≥10mm for the rest of participants. HIV infection was defined as a positive antibody test in children >18 months (Determine, Abbott Laboratories and confirmed with Unigold, Trinity Biotech); or positive

HIV PCR in those <18 months; or a strong clinical suspicion with positive antibody test in the absence of a PCR result.

One induced sputum (IS) and one gastric aspirate (GA) were collected from each participant and evaluated by smear microscopy and culture. All participants included had at least one follow up visit arranged within six months of recruitment regardless of initial disease classification, to assess symptom resolution with or without TB treatment. Those who remained symptomatic were re-assessed, by repeated CXR and collection of new samples. TB cases received 6-months of supervised treatment according to the National TB Control Program protocol. Non diseased-contacts were referred to the NTP for isoniazid preventive treatment (IPT) initiation.

Chest radiograph reading and reporting

CXRs were performed with a digital X-ray machine (Philips—Optimus 50). Antero-posterior (AP) and lateral projections were performed. Chest X rays were reviewed by two observers. Initial reading was done by a local pediatrician (ELV) and second reading by an experienced pediatric radiologist in Barcelona, Spain, (JLR) who was blinded to all clinical information. For the purpose of this study, the blinded reading was used. CXRs were evaluated and reported using the CXR tool developed by Andronikou [10]. This tool (**annex 1**) first assesses the quality of the CXR and then evaluates the presence and location of airway compression and/or tracheal displacement, soft tissue density suggestive of hilar lymphadenopathy, air space opacification (consolidation), nodular images (miliary or larger; widespread and bilateral), pleural effusion, cavities, calcified parenchyma or vertebral spondylitis. If at least one of the above features is found, the CXR is classified as "consistent with tuberculosis".

Case definitions

We applied international consensus case definitions for intra-thoracic TB in children, which differentiates definite (microbiologically-confirmed), probable and possible TB, as well as unlikely TB and not TB [10]. All children with definite and probable TB were recognized as a "TB case". Children with definite TB had compatible symptoms and positive *M. tuberculosis* culture. Probable TB cases had compatible symptoms, suggestive CXR and at least one of the following: TB exposure, positive tuberculosis skin test (TST) or positive response to TB treatment. For the purpose of this study all other children were regarded as "non TB cases".

Data analysis and ethics

Clinical data from participants was double entered using OpenClinica software and laboratory information was retrieved using Servolab platform. DICOM software was used to visualize and read the chest X rays. Statistical analysis was done using Stata 13.0 (StataCorp 2013, College Station, TX). The study protocol was approved by the Mozambican National Bioethics Committee and the Hospital Clinic of Barcelona Ethics Review Committee. Written informed consent was obtained from the caretakers of all study participants. The individuals whose chest X rays are shown in this manuscript have given written informed consent (as outlined in PLOS consent form) to publish these case details.

Results

A total of 766 TB presumptive cases had at least one CXR at admission. Of them, 752 (98.1%) cases had the AP projection and 481 (62.8%) had the lateral view (467 cases had both). The technical quality of the chest X ray was generally good. At admission, 76.4% of all chest X rays

were classified as acceptable (71.9% and 79.3% of AP and lateral views respectively) and only 1.9% as illegible. Around 55.1% of the participants were male and 50.8% were between 12 and 23 months of age. There were 43 Tb cases, 13 of them (30.2%) were microbiologically confirmed (7 in GA, 4 in IS and 2 both in GA and IS). The prevalence of HIV infection in children admitted into the study was 13.1% and 46.5% among TB cases. Baseline characteristics of these TB presumptive cases can be found in Table 1.

Air space opacification was the most frequent parenchymal abnormality observed at admission, present in 18.5% (142/766) of all presumptive cases, followed by airway compression (or tracheal displacement), which was observed in 5.5% of all cases (45/766). Soft tissue density suggestive of lymphadenopathy was seen in 2.1% of all presumptive cases.

Table 1. Clinical characteristics of children less than 3 years of age evaluated for TB.

	TB cases[1] (n = 43[#])		Non-TB cases[2] (n = 723[#])	
	n	%	n	%
Sex				
Male	19	44.2	403	44.3
Female	24	55.8	320	55.7
Age (months)				
< 12	9	20.9	132	18.3
12–23	16	37.2	373	51.6
24–35	18	41.9	218	30.1
Cough				
Yes	8	18.6	144	19.9
No	35	81.4	579	80.1
Fever				
Yes	5	11.6	45	6.2
No	38	88.4	678	93.8
Malnutrition				
Yes	31	72.1	616	85.2
No	12	27.9	107	14.8
HIV status				
Infected	20	46.5	80	11.1
Uninfected	23	53.5	643	88.9
TST result				
Positive	23	54.8	51	7.1
Negative	19	45.2	671	92.9
BCG scar				
Present	42	97.7	720	99.6
Absent	1	2.3	3	0.4

Definitions: cough for $\geq$ 14 days not responding to appropriate course of antibiotics; referred fever 14 days after common causes like malaria or pneumonia were excluded; weight loss/failure to thrivedefined as under 60% weight for height, failure to gain weight for more than 2 months or any loss of weight not responding to nutritional intervention

[1]TB cases—includes confirmed and probable TB cases

[2]Non-TB cases—includes possible, unlikely and not TB as defined in the consensus case definitions for intra-thoracic TB in children by an expert panel.[10]

[#] Missing values implies that the observation was not recorded.

doi:10.1371/journal.pone.0127323.t001

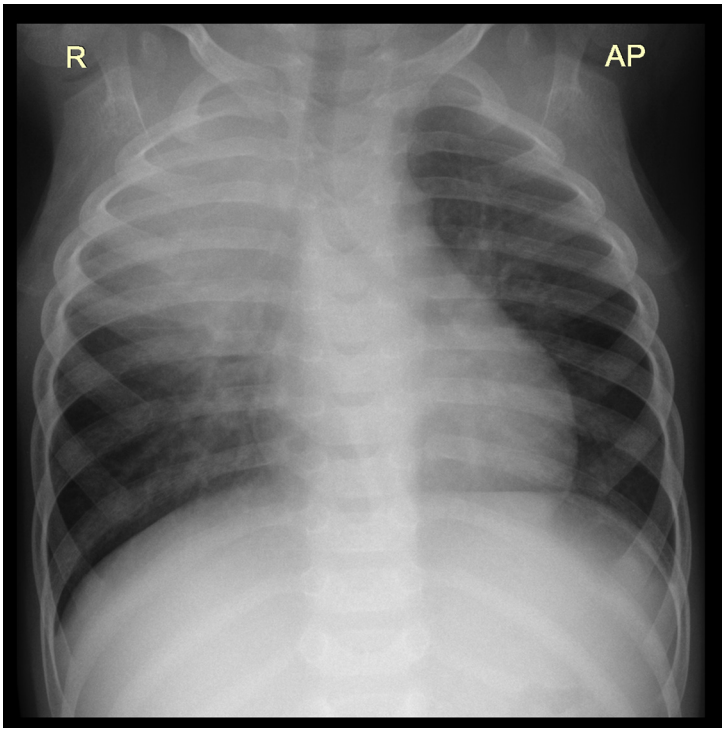


Fig 1. Air space opacification in the left upper lobe in a 20 month old male HIV-uninfected infant.

doi:10.1371/journal.pone.0127323.g001

The most frequent lesion found in TB probable and confirmed cases was air space opacification (65.1%, Fig 1) followed by soft tissue density suggestive of lymphadenopathy (17.1%, Figs 2 and 3), pleural effusion (7.0%,Fig 4) and airway compression (5% Fig 5). These findings were much more common in TB cases than in non TB cases: OR 8.9 (95% CI: 1.6–50.5), 17.2 (95% CI: 5.7–52.1) and 11.0 (95% CI: 2.5–48.6) respectively. Radiological characteristics of TB cases are described in Table 2.

All 3 cases pleural effusion cases, as well as the only miliary case (Fig 6) occurred in HIV positive cases. Soft tissue suggestive of lymphadenopathy was more frequently found in HIV positive TB cases than in HIV negative (21.1% vs 13.6% respectively), although the association of this finding to HIV status was not statistically significant.

Among TB cases, the most frequent location of soft tissue density suggestive of lymphadenopathy was the mediastinal region of the upper right lobe, followed by mediastinal portion of the middle right lobe and anterior paratracheal region (as seen in the lateral view). The two TB cases with air compression signs affected the right bronchus. Air space opacification was more frequent in middle right lobe, followed by lower right and lower left lobe. Out of the three TB cases presenting pleural effusion, two of them were observed in the right lobe.

Out of the 43 TB cases, only 20 had a chest X ray during the last month of treatment or the next 4 months after treatment was finished. Air space opacification, present in 65% of children at diagnosis was still present in 50% of cases with available follow up CXR, with no difference depending on HIV status. Lymphadenopathy which was observed in 7 cases at diagnosis, was still present in 2 cases at follow up. Of the three cases with pleural effusions at diagnosis, two of them had follow up CXR and in one of them, the effusion persisted at 5 month follow up (Table 3).

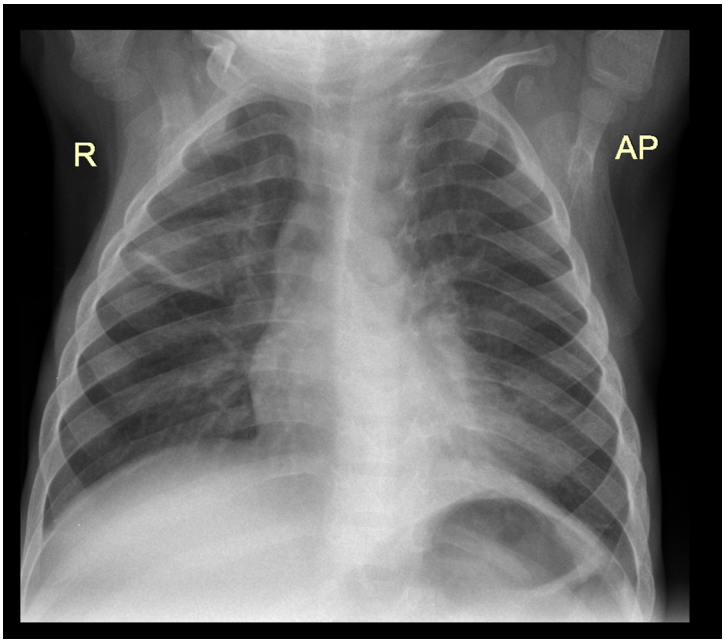


Fig 2. Bilateral bronchoneumonia with perihilial lymph node enlargement and possible left bronchius compression in an 5 month old male HIV infected TB case. AP view.

doi:10.1371/journal.pone.0127323.g002

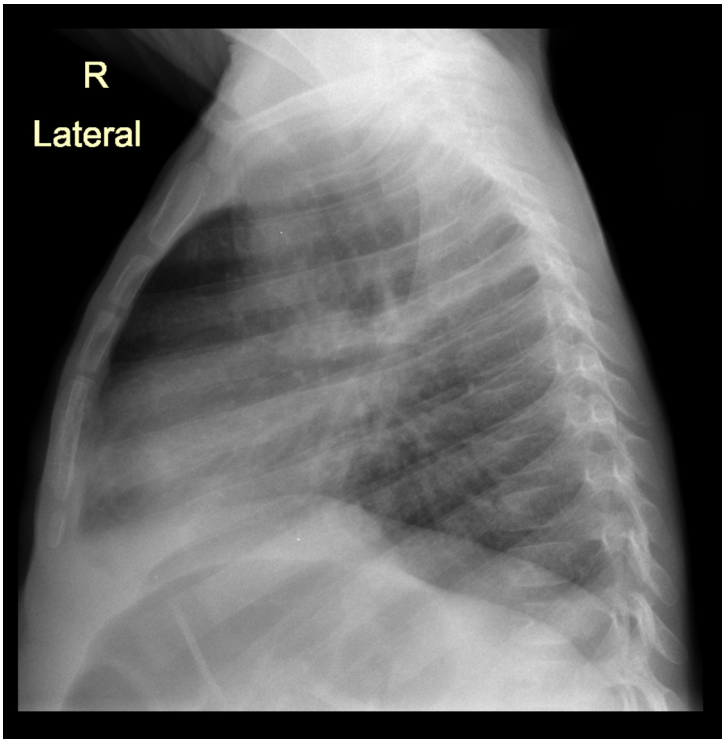


Fig 3. Bilateral bronchoneumonia with perihilial lymph node enlargement and possible left bronchius compression in an 5 month old male HIV infected TB case. Lateral View.

doi:10.1371/journal.pone.0127323.g003

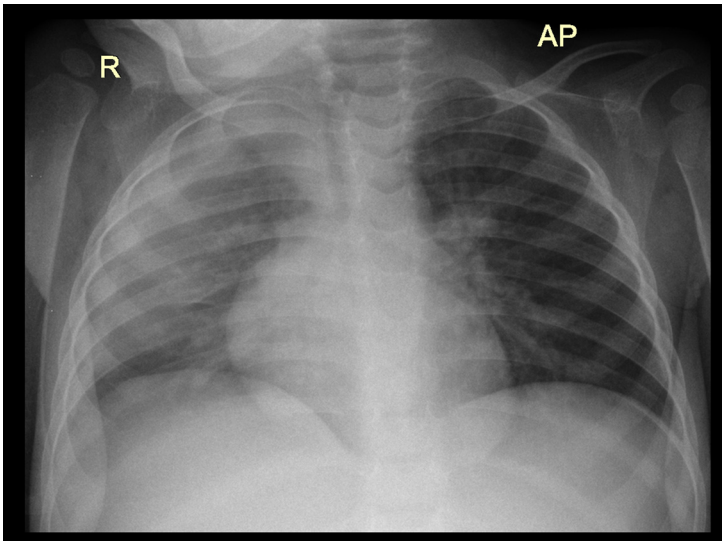


Fig 4. Right sided pleural effusion with no signs of primary disease in a 19 month old female HIV positive patient.

doi:10.1371/journal.pone.0127323.g004

Discussion

To our knowledge this is one of the few studies from a high TB burden country reporting in a systematic manner the results of digital CXR from young TB presumptive infants, following most recommendations from the Expert Panel for evaluation of TB diagnostic tools in children.[10] Although the CXR review tool developed by S. Andronikou and the South African Tuberculosis Vaccine Initiative (SATVI) has already been used for TB case classification

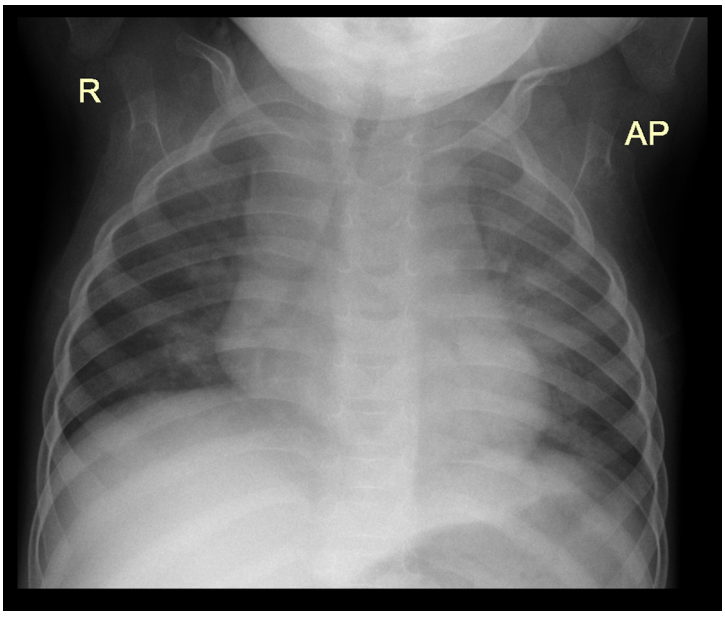


Fig 5. Airway compression and displacement of the left main bronchus with some consolidation in the left inferior lobe in a 17 month old HIV negative female.

doi:10.1371/journal.pone.0127323.g005

Table 2. Chest X Ray characteristics according to TB disease status.

	All TB presumptive cases	TB cases[1] (n = 43)		Non TB cases[2] (n = 723)		OR (95% CI)	p valpue
		n	%	n	%		
Age (months)							
< 12	141	9	(20.9)	132	(18.3)	1.00	
12–23	389	16	(37.2)	373	(51.6)	1.59(0.69–3.68)	0.028
24–35	236	18	(41.9)	218	(30.2)	1.92(0.96–3.85)	0.06
Airway compression or tracheal displacement							
Yes	6	2	(4.8)	4	(0.6)	8.88 (1.56–50.48)	0.039
No	750	40	(95.2)	710	(99.4)	1.00	
Soft tissue density suggestive of lymphadenopathy							
Yes	15	7	(17.1)	8	(1.2)	17.17 (5.66–52.06)	<0.001
No	701	34	(82.9)	667	(98.8)	1.00	
Air space opacification							
Yes	142	28	(65.1)	127	(17.8)	8.61 (4.36–17.03)	<0.001
No	614	15	(34.9)	586	(82.2)	1.00	
Nodular Picture (miliary or larger widespread and bilateral)							
Yes	1	1	(2.3)	0	(0.0)	—	
No	761	42	(97.7)	719	(100.0)	1.00	
Pleural Effusion							
Yes	8	3	(7.1)	5	(0.7)	11.02 (2.50–48.57)	0.007
No	755	39	(92.9)	716	(99.3)	1.00	
Other abnormalities (calcified parenchyma)							
Yes	2	0	(0.0)	2	(0.3)	—	
No	760	42	(100.0)	718	(99.7)	1.00	

[1]TB cases—includes confirmed and probable TB cases

[2]non-TB cases—includes possible, unlikely and not TB as defined in the consensus case definitions for intra-thoracic TB in children by an expert panel. [10]

When cases for all variables do not add up, it means that the observation was not visible in the radiography.

* P value obtained through χ^2 (chi-squared) test or Fisher's exact test (when applicable).

doi:10.1371/journal.pone.0127323.t002

purposes in at least one large vaccine clinical trial [19], this is the first report providing detailed results using this standardized template.

In our study, the most frequent lesion found in both TB cases and presumptive cases in was air space opacification, although the odds for of presenting lymphadenopathy among TB cases compared to non TB cases, was the highest in comparison to the other lesions. Thus, this finding goes in line with the general expert opinion that states that mediastinal/hilar lymphadenopathy is the radiological hallmark in childhood tuberculosis [12,20,21]. However, given that the observation of lymphadenopathy is more frequent in younger children compared to adolescents, as reported by other authors [11,21,22], our results clearly show a lower prevalence of this typical finding among TB cases. This could be due to various reasons. Parenchymal lesions such as air space opacification (whether due to TB or other concomitant pathologies), could hinder the visualization of enlarged lymph nodes, although both findings could be present at the same time. This could be enlightened by the performance of US or CT scan [23]. It could

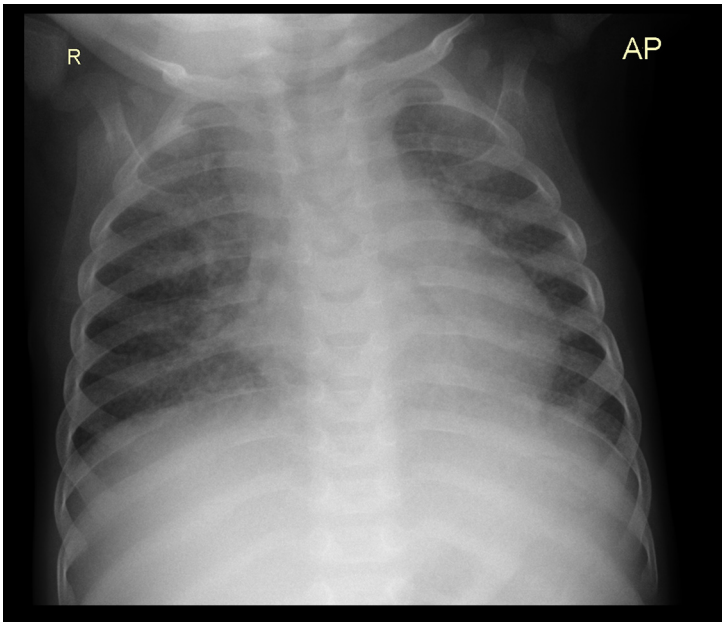


Fig 6. Millet sized nodules of a miliary tuberculosis in an 13 month old HIV infected male.

doi:10.1371/journal.pone.0127323.g006

also happen that the high level of malnutrition among our TB cases (72%) and the presence of other immune deficiencies (HIV infection), which are known factors affecting disease progression, might have favored the bronchopneumonic consolidation, as an evolved lesion from regional lymph node disease. Nonetheless, the fact that parenquimal consolidation was the most frequent radiological finding among TB cases complicates the radiological distinction from common bacterial pneumonia, hindering further TB diagnosis.

The analysis of RX patterns depending on HIV status did not show any relevant difference on radiological findings. Although there is no literature comparing Rx findings depending on HIV status for this age group, since 46% of our TB cases were HIV positive, we expected to have more atypical presentations of TB for this group, with more cases of miliary TB and massive mediastinal glands. Clearly, the CXR characterization on HIV positive infants with tuberculosis, which depends on their immune status (CD4 counts), presence of reconstitution syndrome or HAART treatment, needs to be further assessed with larger studies

Table 3. Chest X-ray characteristics, before and after TB treatment, in child TB cases less than 3 years of age.

		On Admission		5–10 month after treatment initiation	
		HIV infected	HIV uninfected	HIV infected	HIV uninfected
	Number of cases with Chest X Ray	20	23	11	9
Radiographic findings	Airway compression or tracheal displacement	1	1	0	0
	Soft tissue density suggestive of lymphadenopathy	4	3	1	1
	Air Space Opacification	13	15	6	4
	Nodular/Miliary Picture	1	0	0	0
	Pleural Effusion	3	0	1	0
Abnormal X ray		16	16	7	4

* Total number of cases by HIV status.

doi:10.1371/journal.pone.0127323.t003

Although by the end of treatment it was expected that 66% of abnormal chest radiographs at diagnoses would have disappeared [24], we found a lower proportion of disease free compatible CXR (50%). In our study, in 50% of children with available follow up, consolidation persisted, although with different characteristics and sometimes locations. It could be that disease resolution is slower in a population with high level of undernutrition and HIV or some consolidations might be explained by a paradoxical reaction due to immune reconstitution. Another explanation might be that some of the cases were misdiagnosed as TB when other causal pathogens could have caused the pathological image (ie, pneumonias).

This study had several methodological limitations. First, although most patients had an AP projection, some of them lacked the lateral projection, thus, given the pivotal role of lateral views for lymphadenopathy evaluation, some of them could have been missed. Second, due to the limited number of TB cases identified in these studies, the conclusions, especially on the association of HIV status to different radiological findings among TB cases, are not robust, and studies with greater sample sizes are needed. Second, although we have not observed any cavities, calcified parenchyma (Ghon focus) or vertebral spondylitis among TB cases, we have observed that the template does not allow differentiating among these lesions, and reporting of the location might not be possible. We recommend that these lesions, which have been found in other CXR evaluations, could be reported separately. Third, although it has been recommended that the readings from the two reviewers were masked to clinical data and discrepancies resolved by a third reader, this was not possible due to logistical and personnel constraints (only one was masked). Poor inter observer agreement among reviewers regarding lymphadenopathy evaluation in children has been reported, so this limitation, together with the inadequate capturing of differences in opinion, could have reduced the accuracy of the findings which are being reported.

Chest X ray evaluation remains a crucial tool for TB diagnosis in childhood due to the difficulty of isolating TB from sputum or other human samples and the unavailability of reliable TB diagnostic methods in this age group. The correct interpretation of CXR for diagnostic purposes in both clinical practice and research makes standardization of reporting critical. There are some scoring systems for adults which are especially useful to discard TB disease [25,26], but this type of scoring systems are inexistent for children, which have distinct radiological manifestations and where HIV infection, severe malnutrition adds another level of complexity given the absence of atypical presentation of radiological manifestations. Thus, there is a need for improved scoring systems for pediatric populations.

Conclusions

Hilar lymph node enlargement, often regarded as the typical radiological feature of TB in children, was seen in a minority of TB cases. Parenchymal consolidation was the most common finding, complicating radiological distinction from common bacterial pneumonia in children less than 3 years of age. These findings underscore the importance of not ruling out TB despite the absence of the most characteristic radiological findings, and the need of combining radiological information with other signs, symptoms or epidemiological information in cases where laboratory confirmation is not possible. The role of HIV infection and malnutrition in the radiological presentation of TB among young children, highly present in this rural population of Southern Mozambique, deserves to be further studied.

Author Contributions

Conceived and designed the experiments: PLA JS JM. Performed the experiments: EL KG JS. Analyzed the data: EL ALGB OJA JLR. Wrote the paper: EL ALGB BM.

References

1. World Health Organization. Global Tuberculosis Report 2014. Geneva, Switzerland: WHO/HTM/TB/2014.08; 2014.
2. Jenkins HE, Tolman AW, Yuen CM, Parr JB, Keshavjee S, Pérez-Vélez CM, et al. Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. Lancet. 2014; 6736: 1–8. doi: 10.1016/S0140-6736(14)60195-1
3. Dodd PJ, Gardiner E, Coghlan R, Seddon J. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Glob Heal. Dodd et al. Open Access article distributed under the terms of CC BY; 2014; 2: e453–e459. doi: 10.1016/S2214-109X(14)70245-1 PMID: 25103518
4. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med. 2012; 367: 348–61. doi: 10.1056/NEJMra1008049 PMID: 22830465
5. Newton SM, Brent AJ, Anderson S, Whittaker E, Kampmann B. Paediatric tuberculosis. Lancet Infect Dis. 2008; 8: 498–510. doi: 10.1016/S1473-3099(08)70182-8 PMID: 18652996
6. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. Paediatr Respir Rev. 2012; 12: 16–21. doi: 10.1016/j.prrv.2010.09.008.New
7. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis. 2006; 42: e69–71. doi: 10.1086/502652 PMID: 16575719
8. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2014.
9. Theart a C, Marais BJ, Gie RP, Hesseling C, Beyers N. Criteria used for the diagnosis of childhood tuberculosis at primary health care level in a high-burden, urban setting. Int J Tuberc Lung Dis. 2005; 9: 1210–4. PMID: 16333926
10. Graham SM, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis. 2012; 205 Suppl: S199–208. doi: 10.1093/infdis/jis008
11. Leung AN, Müller NL, Pineda PR, FitzGerald JM. Primary tuberculosis in childhood: radiographic manifestations. Radiology. 1992; 182: 87–91. doi: 10.1148/radiology.182.1.1727316 PMID: 1727316
12. Marais BJ, Gie RP, Schaaf HS, Starke JR, Hesseling AC, Donald PR, et al. A proposed radiological classification of childhood intra-thoracic tuberculosis. Pediatr Radiol. 2004; 34: 886–94. doi: 10.1007/s00247-004-1238-0 PMID: 15300340
13. Schaaf HS, Marais BJ, Whitelaw A, Hesseling AC, Eley B, Hussey GD, et al. Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. BMC Infect Dis. 2007; 7: 140. doi: 10.1186/1471-2334-7-140 PMID: 18047651
14. García-Basteiro AL, López-Varela E, Respeito D, González R, Naniche D, Manhiça I, et al. High tuberculosis burden among people living with HIV in southern Mozambique. Eur Respir J. 2014; doi: 10.1183/09031936.00145714
15. Lopez-Varela E, Joaquim Augusto O, Gondo K, García-Basteiro AL, Fraile O, Ira T, et al. Incidence of Tuberculosis among young children in rural Mozambique. Pediatr Infect Dis J. 2015;In press. doi: 10.1097/INF.0000000000000710
16. Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, et al. Profile: Manhica Health Research Centre (Manhica HDSS). Int J Epidemiol. 2013; 42: 1309–18. doi: 10.1093/ije/dyt148 PMID: 24159076
17. World Health Organization. Global Tuberculosis Report 2013. WHO/HTM/TB/2013.11; 2013.
18. Consonni D, Montenegro Agorostos Karagianis MM, Bufardeci G. Immunisation with BCG in the Maringue District, Sofala Province, Mozambique. Tuberc Res Treat. 2013; 2013: 312065. doi: 10.1155/2013/312065 PMID: 23738066
19. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet. Elsevier Ltd; 2013; 381: 1021–8. doi: 10.1016/S0140-6736(13)60177-4
20. Kim WS, Choi J-I, Cheon J-E, Kim I-O, Yeon KM, Lee HJ. Pulmonary tuberculosis in infants: radiographic and CT findings. AJR Am J Roentgenol. 2006; 187: 1024–33. doi: 10.2214/AJR.04.0751 PMID: 16985152

21. Boloursaz MR, Khalilzadeh S, Baghaie N, Khodayari AA, Velayati AA. Radiologic manifestation of pulmonary tuberculosis in children admitted in pediatric ward-Massih Daneshvari Hospital: a 5-year retrospective study. Acta Med Iran. 2009; 48: 244–9.

22. Salazar GE, Schmitz TL, Cama R, Sheen P, Franchi LM, Centeno G, et al. Pulmonary Tuberculosis in Children in a Developing Country. Pediatrics. 2001; 108: 448–453. doi: 10.1542/peds.108.2.448 PMID: 11483814

23. Bosch-Marcet J, Serres-Créixams X, Zuasnabar-Cotro A, Codina-Puig X, Català-Puigbó M, Simon-Riazuelo JL. Comparison of ultrasound with plain radiography and CT for the detection of mediastinal lymphadenopathy in children with tuberculosis. Pediatr Radiol. 2004; 34: 895–900. doi: 10.1007/s00247-004-1251-3 PMID: 15365649

24. Gie R. Diagnostic Atlas of Intrathoracic Tuberculosis in Children. Gie R, editor. Paris: International Union Against Tuberculosis and Lung Disease; 2003.

25. Pinto LM, Pai M, Dheda K, Schwartzman K, Menzies D, Steingart KR. Scoring systems using chest radiographic features for the diagnosis of pulmonary tuberculosis in adults: a systematic review. Eur Respir J. 2013; 42: 480–94. doi: 10.1183/09031936.00107412 PMID: 23222871

26. Pinto LM, Dheda K, Theron G, Allwood B, Calligaro G, van Zyl-Smit R, et al. Development of a simple reliable radiographic scoring system to aid the diagnosis of pulmonary tuberculosis. PLoS One. 2013; 8: e54235. doi: 10.1371/journal.pone.0054235 PMID: 23349835

5- High rates of non-tuberculous mycobacteria isolation in mozambican children with presumptive tuberculosis.

López-Varela E,García-Basteiro AL , Joaquim Augusto O, Fraile O, Bulo H, Ira T, Gondo K, Von Ingen K, Naniche D, Sacarlal J, Alonso P.

Status: submitted for publication

Title:

High rates of Non-Tuberculous Mycobacteria isolation in Mozambican Children with Presumptive Tuberculosis

Authors and affiliations:

Elisa López-Varela1,2, Alberto L. García-Basteiro 1,2,3 Orvalho Joaquim Augusto1, Oscar Fraile1, 2, Helder Bulo1, Tasmiya Ira1, Kizito Gondo1, Jakko van Ingen4, Denise Naniche 2, Jahit Sacarlal1, Pedro Alonso 1

(1) Centro de Investigação em Saude de Manhiça (CISM). CP 1929 Maputo, Mozambique

(2) ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain. Barcelona Centre for International Health Research (CRESIB) -Hospital Clínic-Universitat de Barcelona. Barcelona, Spain

(3) Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, The Netherlands

(4) Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherland

Corresponding author:

Elisa López-Varela, MD MPH

Centro de Investigação em Saude de Manhiça (CISM). CP 1929 Maputo, Mozambique

Elisa.lopez @isglobal.org

Preliminary results from this study were presented at the 44th Union World Conference on Lung Health, 2013, Paris, France

Abstract

Introduction

Nontuberculous mycobacteria (NTM) can cause disease which can be clinically and radiologically undistinguishable from tuberculosis (TB), posing a diagnostic and therapeutic challenge in high TB settings. We aim to describe the prevalence of NTM isolation and its clinical characteristics in children from rural Mozambique.

Methods

This study was part of a community TB incidence study in children <3 years of age. Gastric aspirate and induced sputum sampling were performed in all presumptive TB cases and processed for smear testing using LED Microscopy, liquid and solid culture, and mycobacterial molecular identification by GenoType® Mycobacterium CM/AS.

Results

NTM were isolated in 26.2% (204/775) of children. The most prevalent NTM species was *M. intracellulare* (N=130), followed by *M. scrofulaceum* (N=35) and *M. fortuitum* (N=8). Children with NTM were significantly less symptomatic and less likely to present with an abnormal chest radiograph than those with *M. tuberculosis*. NTM were present in 22% of follow-up samples and 25 children had the same species isolated from ≥2 separate samples. All were considered clinically insignificant and none received specific treatment. Children with NTM isolates had equal mortality and likelihood of TB treatment as those with negative culture although they were less likely to have TB ruled out.

Conclusion

NTM isolation is frequent in presumptive TB but not clinically significant in this patient cohort. However, it can overestimate TB burden. Further studies are needed to understand the epidemiology and the clinical significance of NTM in children.

INTRODUCTION

Nontuberculous mycobacteria (NTM) are a large family of acid-fast bacteria, widespread in the environment and common in soil and water (1). In children, the most frequent NTM disease is cervicofacial lymphadenitis, followed by skin and soft tissue infections. It can occasionally produce lung disease and disseminated infection, although the latter are extremely rare in the absence of genetic disorders (cystic fibrosis and mendelian susceptibility to mycobacterial disease) or acquired immunodeficiency (2–4).

Childhood TB is a frequent cause of lung disease in high TB endemic countries(5). However, underdetection is common (6), partially due to the inherent difficulties in obtaining respiratory samples in children, coupled to the paucibacillary nature of TB in this age group (5). An additional layer of complexity is posed by the fact that NTM lung disease and tuberculosis (TB) have overlapping clinical and radiological manifestations. This poses a diagnostic and therapeutic challenge in high TB settings where sophisticated laboratory services are unavailable(7). Moreover, it has been suggested that NTM may have an effect on the response to BCG vaccination and a better understanding of this association is needed in the context of novel tuberculosis vaccines assessment(8).

The epidemiology of NTM varies by world region, however there are few studies reporting NTM isolation in the pediatric population, especially in low resource TB endemic settings(9–11). Little data for the epidemiology and clinical burden is available for this neglected cousin of TB. The objective of this study is to determine the prevalence and describe the clinical characteristics associated with NTM isolation in young children in a rural area of Southern Mozambique.

MATERIALS AND METHODS

Study setting

This study was conducted at the Manhiça Health Research Center (CISM), located in Southern Mozambique, a semi-rural area with a high burden of high HIV and TB(12). The region is a farming area where 38 % of the households have formal housing and 55% are supplied with piped water (13). The Bacille Calmette-Guerin (BCG) vaccination is above 95%(14).

Study design

This study was part of a larger prospective descriptive study assessing the minimum community incidence of TB among young children (<3 years of age) over a 1-year period (October 2011- 2012)(15). TB cases were classified according to the NIH definitions (16). This study showed an incidence rate of TB of 470/100,000 among children aged less than 3, with a low estimated case detection rate of 40.8% (95% CI 36.6–45.1%)(6,15).

Clinical procedures

Children with symptoms suspicious of TB and those in close contact with a sputum smear-positive TB case were recruited through active and passive case detection system and evaluated through physical examination, HIV rapid antibody test (Determine®, Abbott Laboratories), tuberculin skin test (TST) and a chest radiograph (CXR). For symptomatic cases, upon admission to the study, one induced sputum with nasopharyngeal suction and one gastric aspirate same-day ambulatory samples were collected from each participant and evaluated by smear microscopy and culture. All participants included had at least one follow up visit arranged within six months of recruitment regardless of initial disease classification, to assess symptom resolution with or without TB treatment. Those who remained symptomatic were re-assessed, by repeated CXR and collection of new samples. Full details on study procedures are described elsewhere(15,17). Clinical management of new TB cases diagnosed was performed by the National Tuberculosis Program (NTP) according to established national clinical guidelines.

Laboratory procedures

Sterile single use consumables were used for each clinical procedure. Gastric aspirates were neutralized with bicarbonate on the spot. Samples were transported within 4 hours of collection and processed in the TB laboratory at CISM The laboratory is subject to an External Quality Assurance program provided by the National Health Laboratory Service in South Africa and is certified by the International Organization for Standardization (ISO 90001:2008 for Quality Management). Gastric aspirates were *buffered* with sodium bicarbonate, and immediately sent to the laboratory Following n-acetyl-l-cysteine/NaOH digestionand decontamination and concentration through centrifugation, all samples were processed for AFB smear testing using auramine/rhodamine staining and LED fluorescence microscopy followed up by Ziehl-Neelsen (ZN) staining and inoculated into liquid culture (MGIT)

media and solid media (Lowenstein Jensen; Beckton Dickison (BD). Positive cultures were confirmed using ZN staining and immunochromatographic assay, BD MGIT TBc Identification Test (TBc ID, Becton Dickinson, Sparks, MD) as well as Xpert MTB/RIF (Cepheid, Sunnyvale, CA) and identified through mycobacterial molecular identification (HAIN GenoType® Mycobacterium CM/AS).

Ethical approval

The study protocol was approved by the Mozambican National Bioethics Committee and the Hospital Clinic of Barcelona Ethics Review Committee. Written informed consent was obtained from the caretakers of all study participants.

Data Analysis and Statistical Considerations

Clinical data was double entered in an electronic data capture system (OpenClinica ™ www.openclinica.org) and checked for discrepancies. Statistical software for analysis was Stata 13.0 (StataCorp. 2013. Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP).

The prevalence of NTM was calculated as the proportion of NTM positive cultures at initial admission visit over those who underwent bacteriological investigation. Two children had TB isolated at a follow-up visit (N=2) and were excluded from the follow-up analysis.

RESULTS

Culture results

A total of 789 presumptive TB cases were admitted for investigation in the study. Of them, 775 children had at least one mycobacterial culture result available at the initial visit: 11 had a positive culture for MTB (1.42%) and 204 had an NTM isolate (26.2%) (**Table 1**). The prevalence of NTM isolate findings was 16% (118/738) for gastric aspirate and 15.3% (111/726) for induced sputum. The diagnostic yield of liquid culture alone was 15.7% (109/695) for GA and 14.8% (103/698) for IS compared to 3.9% (25/641) for GA and 3.8% (25/662) for IS in solid media alone. The overall rate of sample contamination was larger among GA vs IS (3.8% vs 1.8%) and solid vs liquid culture (13.5% vs 7.5). Among all presumptive TB cases, seven had a positive smear (3 had an NTM isolated, one did not have culture results available and the remaining three had negative cultures). Gastric aspiration was able to identify a larger number of *M. tuberculosis* (MTB) isolates than IS (7 vs 4) All MTB cases were positive on liquid culture and only 4 of them were also positive on solid media.

Mycobacterial culture yielded 187 identifiable NTM isolates comprising 8 different species and 35 unidentifiable NTM. The most prevalent NTM was *M. intracellulare* (N=130), followed by *M. scrofulaceum* (N=35) and *M. fortuitum* (N=8) (**Table2**). NTM were isolated both in GA and IS in 25 children, 9 of them had the same NTM species (all of them were *M. intracellulare*). Two or more NTM species were isolated in 18 children, two cases had both isolates identified in the same sample.

Clinical characteristics associated with NTM isolation

Among those with NTM isolates, 42% were female and 52% were between 12 and 23 months of age (**Table3**). The prevalence of NTM isolates slightly increased with age (from 22% in the first year to 27% in the third year of life) and a BCG scar was present in 84.3% of the cases. The most frequent clinical feature at enrolment was malnutrition (89.7%) followed by prolonged cough (18.1%). At physical examination, two children had lymphadenopathy (one was cervical and one was a fistulized inguinal node) and 4 had an abnormal lung examination. Fifteen percent had a positive TST and 12% were HIV positive. At admission, 35 children had abnormal CXR (17.6%).

Compared to children with MTB isolates, those with NTM were healthier: they had less number of outpatient department visits in the previous year (OR 5.97, 95% CI 1.64-21.69, p=0.01), presented fewer symptoms (OR 4.92, p=0.006), and were less likely to present with an abnormal CXR (OR 16.4, p<0.001). There were no differences in TST nor HIV positivity.

There was no significant difference between the prevalence of the NTM at admission in HIV-infected and uninfected TB presumptive cases (25 vs 30% respectively).

Follow-up data and outcomes

Twenty-five children were identified as having the same NTM species isolated from at least two separate IS or GA samples (all except for two were *M. intracellulare*). Three fulfilled the microbiological criteria for diagnosing NTM lung disease(18) and had pulmonary symptoms plus abnormal CXR. However, these isolates were considered clinically insignificant. None of the children were treated for NTM disease, one received TB treatment and there were no deaths registered two years post admission.

We did not find any difference regarding mortality or likelihood of TB treatment in those where NTM were isolated compared to culture negative children, supporting the clinical insignificance of NTM isolates (**Table 4**). However, children with NTM were more likely to be classified as probable or possible TB, more likely to initiate isoniazid preventive therapy (IPT), and time to treatment initiation was longer.
Most patients (88%), regardless of the initial culture result (204 NTM, 11 MTB, 567 negative), had at least one follow-up visit. Among them, 14% had follow-up samples collected with an overall prevalence of NTM of 22%. This number was the same for those children with an NTM compared to a negative sample at admission.

DISCUSSION

To our knowledge, this is the first study reporting the rate of NTM isolation in children in Mozambique and one of the few in Sub-Saharan Africa. Our findings suggest that NTM isolation in GA and IS samples of presumptive childhood TB cases is very frequent but may not be clinically significant in this patient cohort. Thus, in high TB endemic countries, NTM isolation complicates patient management as the underlying diagnosis is most often assumed to be TB.

Recent studies in TB endemic countries have reported high rates of NTM isolation in presumptive TB cases(10,19–22). Adult studies in Nigeria and Uganda have shown a significant increase in NTM isolation rate from 1-4% in older studies using solid media to 15% in more recent ones in which liquid media were used (23). In our cohort the yield of NTM was significantly higher in liquid than solid culture in agreement with the results of a meta-analysis showing that liquid culture alone has a 66% sensitivity compared with 51% for solid media alone (24). The single published paper reporting NTM isolates in Mozambique detected NTM isolates in the sputum of 3 out of 320 HIV infected adults (25). In children tested for tuberculosis, several reports have described frequent NTM isolates both in gastric aspirates and in sputum, ranging from 4 to 10%(9,10,26,27). Although some authors detected a higher yield of induced sputum versus gastric lavage(10), in our study the rate was similar for both types of samples.

Once isolated in a respiratory sample, it is often difficult to distinguish whether the NTM are causally related to the clinical disease, simply a reflection of recent environmental exposure, or a contaminant in biological specimen related to the sampling technique or laboratory equipment(28). Environmental exposure to NTM has been hypothesized to increase as children grow older (9,10) ;differences in prevalence among children of different age were also observed in the current study,but there was no clear increase with increasing age. In our study, the rate of isolation and the distribution of species did not vary substantially throughout the study period, which renders systematic NTM laboratory contamination unlikely. Although sterile water was used for the sampling procedures, we cannot rule out the possibility of contamination of the mouth/gastrointestinal tract immediately prior to the sampling process.

A symptomatic child with a positive culture yielding NTM does not indicate pulmonary disease per se (1) and does not necessarily require treatment (10). In this cohort, we believe that the isolated NTM were not clinically significant. Firstly, none of the children received NTM specific treatment and all 3 symptomatic cases that fulfilled the microbiological and radiological criteria for NTM lung disease were alive and well two years later. Secondly, we observed that the proportion of children with an NTM isolate at a follow-up visit was the same regardless of the initial culture result. Finally, and although we could not compare our results to a control group, the 2 year mortality did not increase as compared to those children with negative cultures. On the other hand, children with MTB isolates had a worse clinical and radiological presentation and a higher two year mortality than those with NTM. These results are similar to those published by Hatherill et al, who reported that presumptive TB cases with NTM isolates were less likely to demonstrate radiological features compared to MTB. However, they did see and association between NTM and older age, constitutional symptoms and lower rates of positive TST(10).

We did observe that isolating an NTM decreased the odds of ruling out TB according to NIH definition as compared to children with negative culture at admission (Table 4). Besides, children with NTM isolates were more likely to initiate IPT and although there were no differences in the proportion of children initiating treatment, time to treatment initiation was much shorter if an NTM was found on admission. Without molecular methods, often unavailable in TB endemic countries, a positive sputum smear for NTM is likely to be misinterpreted as

tuberculosis. In this study almost half of the positive smears were due to NTM. These findings highlight that NTM isolates can pose a clinically significant obstacle to the accurate diagnosis of childhood tuberculosis, potentially overdiagnosing the TB cases. This is particularly important when designing TB vaccine trials for two reasons: firstly, because NTM exposure may affect the efficacy of BCG vaccination (8,29); secondly, because the presence of NTM in respiratory samples can interfere with the NIH diagnosis of TB as shown in this study

The distribution of NTM species in this study is similar to what has been reported in South Africa in a collaborative NTM-NET study(30), where *M. intracellulare* was the most frequent isolate, followed by *M. scrofulaceum* and *gordonae*. Similar to the South African data in the NTM NET study, *M. malmoense*, which historically was considered to be restricted to Scandinavia and north-western Europe, was identified in our cohort.

Several challenges are presented when a clinician is managing a suspected case of childhood NTM pulmonary disease. Besides being clinically and radiologically indistinguishable from TB, current guidelines do not provide specific advice for diagnosis of NTM in children. The clinical significance is difficult to establish and often requires longer follow-up and obtainment of several confirmatory samples. In our setting, even if NTM disease was accurately diagnosed in children, treatment is challenging as the availability of rifampicin or ethambutol are limited outside the National TB Program which only manages tuberculosis cases. Moreover, child friendly formulations are often unavailable and treatment is lengthy and with common side effects(18).

This study had several limitations. Firstly, we do not have a control group to provide data on NTM isolation rates in healthy children. Culture negative presumptive TB cases can be a heterogeneous group which can potentially include unidentified culture negative TB. Secondly, only pulmonary samples were obtained and thus, there is no information regarding possible NTM lymphadenitis. Thirdly, as stated before, laboratory contamination, although unlikely, cannot be ruled out completely.

In summary, NTM isolation is frequent in children evaluated for presumed TB in Mozambique and can contribute to overdiagnosing TB. Further studies are needed to understand the epidemiology of NTM in children, including the environmental exposure in other rural Sub-saharan African settings and the relationship between NTM isolation and BCG efficacy. There is a need for pediatric TB guidelines to discuss the role of NTM isolates and better orient the clinician attending presumptive TB children from high burden TB and low resource countries.

The authors declare no conflicts of interest.

Acknowledgement

The authors thank the participants and their families. The authors are grateful to the National TB Program, health staff at the Manhiça District Hospital and peripheral health centers for their contribution.

References

1. López-varela E, García-basteiro AL, Santiago B, Wagner D, Ingen J Van, Kampmann B, et al. Non-tuberculous mycobacteria in children : muddying the waters of tuberculosis diagnosis. Lancet Respir [Internet]. Elsevier Ltd; 2015;3(3):244–56. Available from: http://dx.doi.org/10.1016/S2213-2600(15)00062-4
2. Blyth CC, Best EJ, Jones C a, Nourse C, Goldwater PN, Daley AJ, et al. Nontuberculous mycobacterial infection in children: a prospective national study. Pediatr Infect Dis J. 2009 Sep;28(9):801–5.
3. Cruz AT, Ong LT, Starke JR. Mycobacterial infections in Texas children: a 5-year case series. Pediatr Infect Dis J [Internet]. 2010 Aug [cited 2014 Oct 27];29(8):772–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20661106
4. Pham-Huy A, Robinson JL, Tapiéro B, Bernard C, Daniel S, Dobson S, et al. Current trends in nontuberculous mycobacteria infections in Canadian children: A pediatric investigators collaborative network on infections in Canada (PICNIC) study. Paediatr Child Health [Internet]. 2010 May [cited 2014 Oct 27];15(5):276–82. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2912635&tool=pmcentrez&rendertype=abstract
5. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med [Internet]. 2012 Jul 26 [cited 2013 Mar 3];367(4):348–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22830465
6. López-Varela E, Augusto OJ, Guerra L, Respeito D, Sacoor C, Sacarlal J, et al. Low paediatric tuberculosis case detection rate in Southern

Mozambique. Eur Respir J [Internet]. 2015 Dec 23 [cited 2016 Jan 14];ERJ – 01454–2015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26699721

7. Gopinath K, Singh S. Non-tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger? PLoS Negl Trop Dis [Internet]. 2010 Jan [cited 2016 Apr 8];4(4):e615. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2860495&tool=pmcentrez&rendertype=abstract
8. Thom M, Howard C, Villarreal-Ramos B, Mead E, Vordermeier M, Hope J. Consequence of prior exposure to environmental mycobacteria on BCG vaccination and diagnosis of tuberculosis infection. Tuberculosis (Edinb) [Internet]. 2008 Jul [cited 2014 Nov 16];88(4):324–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18329343
9. Asiimwe BB, Bagyenzi GB, Ssengooba W, Mumbowa F, Mboowa G, Wajja A, et al. Species and genotypic diversity of non-tuberculous mycobacteria isolated from children investigated for pulmonary tuberculosis in rural Uganda. BMC Infect Dis [Internet]. 2013 Jan [cited 2014 Dec 5];13:88. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3599115&tool=pmcentrez&rendertype=abstract
10. Hatherill M, Hawkridge T, Whitelaw A, Tameris M, Mahomed H, Moyo S, et al. Isolation of non-tuberculous mycobacteria in children investigated for pulmonary tuberculosis. PLoS One [Internet]. 2006 Jan [cited 2013 Mar 5];1(1):e21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1762386&tool=pmcentrez&rendertype=abstract
11. Workalemahu B, Berg S, Tsegaye W, Abdissa A, Girma T, Abebe M, et al. Genotype diversity of Mycobacterium isolates from children in Jimma, Ethiopia. BMC Res Notes [Internet]. BMC Research Notes; 2013 Jan [cited 2014 Apr 20];6(1):352. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3766673&tool=pmcentrez&rendertype=abstract
12. García-Basteiro AL, López-Varela E, Respeito D, González R, Naniche D, Manhiça I, et al. High tuberculosis burden among people living with HIV in southern Mozambique. Eur Respir J [Internet]. 2014;04:09031936.00145714 – . Available from: http://erj.ersjournals.com/content/early/2014/11/13/09031936.00145714.extract
13. Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, et al. Profile: Manhiça Health Research Centre (Manhiça HDSS). Int J Epidemiol [Internet]. 2013 Oct [cited 2013 Dec 21];42(5):1309–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24159076
14. Lanaspa M, Balcells R, Sacoor C, Nhama A, Aponte JJ, Bassat Q. The performance of the expanded programme on immunization in a rural area of Mozambique. Acta Trop [Internet]. 2015 Sep [cited 2016 Mar 9];149:262–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26095045
15. López-Varela E, Augusto OJ, Gondo K, García-Basteiro AL, Fraile O, Ira T, et al. Incidence of Tuberculosis Among Young Children in Rural Mozambique. Pediatr Infect Dis J [Internet]. 2015 Jul [cited 2016 Mar 22];34(7):686–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26069945
16. Graham SM, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis [Internet]. 2012 May 15 [cited 2013 Mar 5];205 Suppl (Suppl 2):S199–208. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22448023
17. García-Basteiro AL, López-Varela E, Augusto OJ, Gondo K, Muñoz J, Sacarlal J, et al. Radiological Findings in Young Children Investigated for Tuberculosis in Mozambique. PLoS One [Internet]. 2015;10(5):e0127323. Available from: http://dx.plos.org/10.1371/journal.pone.0127323
18. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med [Internet]. 2007 Feb 15 [cited 2014 Jul 10];175(4):367–416. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17277290
19. Hoza AS, Mfinanga SGM, Rodloff AC, Moser I, König B. Increased isolation of nontuberculous mycobacteria among TB suspects in Northeastern, Tanzania: public health and diagnostic implications for control programmes. BMC Res

Notes [Internet]. 2016 Jan [cited 2016 Apr 8];9(1):109. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4756402&tool=pmcentrez&rendertype=abstract

20. Buijtels PC a. M, van der Sande M a. B, de Graaff CS, Parkinson S, Verbrugh H a., Petit PLC, et al. Nontuberculous Mycobacteria, Zambia. Emerg Infect Dis [Internet]. 2009 Feb [cited 2013 Mar 5];15(2):242–9. Available from: http://wwwnc.cdc.gov/eid/article/15/2/08-0006_article.htm
21. Buijtels PCAM, van der Sande MAB, Parkinson S, Verbrugh HA, Petit PLC, van Soolingen D. Isolation of non-tuberculous mycobacteria at three rural settings in Zambia; a pilot study. Clin Microbiol Infect [Internet]. 2010 Aug [cited 2013 Jun 27];16(8):1142–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19832715
22. Maiga M, Siddiqui S, Diallo S, Diarra B, Traoré B, Shea YR, et al. Failure to recognize nontuberculous mycobacteria leads to misdiagnosis of chronic pulmonary tuberculosis. PLoS One [Internet]. 2012 Jan [cited 2013 Mar 5];7(5):e36902. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3353983&tool=pmcentrez&rendertype=abstract
23. Aliyu G, El-Kamary SS, Abimiku A, Brown C, Tracy K, Hungerford L, et al. Prevalence of Non-Tuberculous Mycobacterial Infections among Tuberculosis Suspects in Nigeria. PLoS One [Internet]. 2013 Jan [cited 2013 Jun 6];8(5):e63170. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3650061&tool=pmcentrez&rendertype=abstract
24. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. J Clin Microbiol [Internet]. 2004 May [cited 2014 Oct 1];42(5):2321–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=404614&tool=pmcentrez&rendertype=abstract
25. Nunes EA, De Capitani EM, Coelho E, Panunto AC, Joaquim OA, Ramos M de C. Mycobacterium tuberculosis and nontuberculous mycobacterial isolates among patients with recent HIV infection in Mozambique. J Bras Pneumol publicação Of da Soc Bras Pneumol e Tisilogia [Internet]. 2008 Oct [cited 2016 Mar 10];34(10):822–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19009216
26. Workalemahu B, Berg S, Tsegaye W, Abdissa A, Girma T, Abebe M, et al. Genotype diversity of Mycobacterium isolates from children in Jimma, Ethiopia. BMC Res Notes [Internet]. 2013;6:352. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3766673&tool=pmcentrez&rendertype=abstract
27. Kordy F, Richardson SE, Stephens D, Lam R, Jamieson F, Kitai I. Utility of Gastric Aspirates for Diagnosing Tuberculosis in Children in a Low Prevalence Area: Predictors of Positive Cultures and Significance of Non-Tuberculous Mycobacteria. Pediatr Infect Dis J [Internet]. 2014 Aug 6 [cited 2014 Oct 22]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25101762
28. Raju RM, Raju SM, Zhao Y, Rubin EJ. Leveraging Advances in Tuberculosis Diagnosis and Treatment to Address Nontuberculous Mycobacterial Disease. Emerg Infect Dis [Internet]. 2016 Mar [cited 2016 Mar 8];22(3):365–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4766907&tool=pmcentrez&rendertype=abstract
29. Poyntz HC, Stylianou E, Griffiths KL, Marsay L, Checkley AM, McShane H. Non-tuberculous mycobacteria have diverse effects on BCG efficacy against Mycobacterium tuberculosis. Tuberculosis (Edinb) [Internet]. 2014 May [cited 2014 Nov 15];94(3):226–37. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4066954&tool=pmcentrez&rendertype=abstract
30. Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: A NTM-NET collaborative study. Eur Respir J Off J Eur Soc Clin Respir Physiol [Internet]. 2013 Apr 18 [cited 2013 Jun 27]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/23598956

Table 1 : Mycobacterial culture results per sample available at admission

	Gastric Aspirate (N, %)		Induced Sputum (N, %)	
Performed	773		750	
Smear positive	3	0,39%	6	0,80%
Culture results available	767	99,22%	739	98,53%
Liquid culture				
Contaminated	72	9,39%	41	5,55%
Negative	579	75,49%	591	79,97%
NTM	109	15,71%	103	14,76%
MTB	7	1,01%	4	0,57%
Solid culture				
Contaminated	126	16,43%	77	10,42%
Negative	615	80,18%	634	85,79%
NTM	25	3,90%	25	3,78%
MTB	1	0,16%	3	0,45%
Liquid plus solid culture				
Contaminated	29	3,78%	13	1,76%
Negative	613	79,92%	611	82,68%
NTM	118	15,99%	111	15,29%
MTB	7	0,95%	4	0,55%

Abbreviations: NTM: Nontuberculous mycobacteria; MTB: *M. tuberculosis*
Footnote: % of NTM and MTB among uncontaminated samples

Table 2: Frequency of different specimens according to sample type

	SAMPLES				PATIENTS (Gastric aspirate plus induced sputum)		
NTM species	Gastric aspirate (N=118) N, %		Induced sputum (N=111) N %		Single isolate (N)	Coinfection (N, %)	
M. intracellulare	67	56,8%	70	63,1%	114	14	10,9%
M. scrofulaceum	17	14,4%	18	16,2%	27	8	22,9%
Mycobacterium Sp.	20	16,9%	12	10,8%	28	4	12,5%
M. fortuitum	6	5,1%	3	2,7%	8	1	11,1%
M. malmoense	2	1,7%	3	2,7%	2	3	60,0%
M. interjectum	5	4,2%	0	0,0%	2	3	60,0%
M. gordonae	1	0,8%	1	0,9%	2	0	0,0%
M. chelonae	0	0,0%	3	2,7%	1	2	66,7%
M. abscessus	0	0,0%	1	0,9%	1	0	0,0%

Table 3: Baseline characteristics of presumptive TB cases with at least one sample at admission according to mycobacterial culture results

	NTM (N, %) 204		MTB (N, %) 11		Univariate OR (95% CI)	p
Sex						
male	118	57,84%	5	45,45%		
female	86	42,16%	6	54,55%	0.53 (0.48-5.59)	0.42*
Age in months (Median [IQR])	20.7 (14.9-26.2)		21.4 (14.5-33.6)			
Age category, N (%)						
< 1	32	15,69%	2	18,2%	1	
1-2	107	52,45%	5	45,5%	0.75 (0.14-4.06)	
2-+	65	31,86%	4	36,4%	0.98 (0.17-5.71)	0.84*
BCG Scar						
Absent	31	15,20%	4	36,4%	1	
Present	172	84,31%	7	63,6%	0.31 (0.09-1.16)	0.08
TB contact (documented or reported)						
No	188	92,16%	10	90,9%	1	
Yes	16	7,84%	1	9,1%	1.17 (0.14-9.82)	0.61
Number of consultations in previous year						
Median (IQR)						
< 10	179	87,75%	6	54,5%	1	
10 - +	25	12,25%	5	45,5%	5.97 (1.64-21.69)	0.01
Symptoms at enrollment						
Cough ≥2 weeks						
no	167	81,86%	5	45,5%	1	
yes	37	18,14%	6	54,5%	5.41 (1.52-19.24)	0.01
Fever≥2 weeks						
no	197	96,57%	7	63,6%	1	
yes	7	3,43%	4	36,4%	16.08	< 0.001
Chronic or Acute Malnutrition malnutrition						
no	21	10,29%	5	45,5%	1	
yes	183	89,71%	6	54,5%	0.14	< 0.001
Wheeze						
no	201	98,53%	10	90,9%	1	
yes	3	1,47%	1	9,1%	6.7	0.07
Adenopathy						
no	202	99,02%	10	90,9%	1	
yes	2	0,98%	1	9,1%	10	0.03
Number of positive criteria						
One	164	80,39%	5	45,5%	1	
More than one	40	19,61%	6	54,5%	4.92	0.006
Hospitalized during TB presumption						
no	193	94,61%	8	72,7%	1	
yes	11	5,39%	3	27,3%	6.58	0.004
Physical Exam						
Abnormal lung exam						
no	200	98,04%	8	72,7%	1	
yes	4	1,96%	3	27,3%	18.7	< 0.001
Fever						
no	201	99,50%	9	90,0%	1	

yes	1	0,50%	1	10,0%	22.33	0.002
Lymphadenoapthy						
no	202	99,02%	10	90,9%	1	
yes	2	0,98%	1	9,1%	10	0.03
Tuberculin skin test						
Negative	173	85,22%	10	90,9%	1	
Positive	30	14,78%	1	9,1%	0.58	0.6
HIV Reported						
Not positive	180	88,24%	9	81,8%	1	
Positive	24	11,76%	2	18,2%	1.66	0.53
Radiological changes suggestive of TB						
No	164	82,41%	2	22,22%	1	
Yes	35	17,59%	7	77,78%	16.4	< 0.001

Abbreviations: NTM: Nontuberculous mycobacteria; MTB: *M. tuberculosis;* OR: odds ratio; IQR: Interquartile range; TB: Tuberculosis
Footnote: * = p values calculated using the Fisher's exact test.

Table 4: Outcomes and follow-up information according to culture result at admission

Outcomes	NTM (N, %) 204		MTB (N, %) 11		Negative (N, %) 567	
TB case type*						
Confirmed	0		11		0	
Probable	7	3,43%	0		25	4,41%
Possible	48	23,53%	0		48	8,47%
MTB infection	24	11,76%	0		21	3,70%
Unlikely TB	125	61,27%	0		473	83,42%
Isoniazide preventive treatment						
No	181	88,73%	11	100%	527	92,95%
Yes	23	11,27%	0	0%	40	7,05%
TB treatment						
No	194	95,10%	4	36,4%	534	94,18%
Yes	10	4,90%	7	63,6%	33	5,82%
Median time to treatment initiation (days) N (IQR)	51 (41-100)		35 (33-63)		140 (48-233)	
Mortality at 24 months						
No	190	93,14%	8	72,7%	529	93,30%
Yes	14	6,86%	3	27,3%	38	6,70%

Abbreviations: NTM: Nontuberculous mycobacteria; MTB: *M. tuberculosis;* TB: Tuberculosis; IQR: Interquartile range;
Footnote: * NIH TB case definition

6. Non-tuberculous mycobacteria in children: muddying the waters of TB diagnosis.

López-Varela E,García-Basteiro AL, Santiago B, Wagner D, Van ingen J, Kampmann B. Lancet Respir M. 2015 Mar 3(3):244–256.

2015 Impact factor 15.328 (D1)

Non-tuberculous mycobacteria in children: muddying the waters of tuberculosis diagnosis

Elisa López-Varela, Alberto L García-Basteiro, Begoña Santiago, Dirk Wagner, Jakko van Ingen, Beate Kampmann

Lancet Respir Med 2015; 3: 244–56

See Comment page 179

Centro de Investigação em Saude de Manhiça, Maputo, Mozambique (E López-Varela MD, A L García-Basteiro MD); **ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain** (E López-Varela, A L García-Basteiro); **Unidad Enfermedades Infecciosas Pediátricas, Departamento de Pediatría, Hospital General Univesitario Gregorio Marañón, Madrid, Spain** (B Santiago MD); **Laboratorio Inmunobiología Molecular, Hospital General Univesitario Gregorio Marañón, Madrid, Spain** (B Santiago); **Center of Infectious Diseases and Travel**

Non-tuberculous mycobacteria (NTM) are a large family of acid-fast bacteria, widespread in the environment. In children, NTM cause lymphadenitis, skin and soft tissue infections, and occasionally also lung disease and disseminated infections. These manifestations can be indistinguishable from tuberculosis on the basis of clinical and radiological findings and tuberculin skin testing. A diagnostic and therapeutic problem for respiratory physicians and other clinicians is therefore evident, particularly in settings where childhood tuberculosis is common, and bacteriological confirmation of any mycobacterial disease is difficult because of low availability of laboratory services in low-resource settings and the inherent paucibacillary nature of mycobacterial disease in childhood. The epidemiology of NTM varies by world region, and attempts to understand the burden of NTM disease and to identify risk factors in the paediatric population are hampered by inadequate mandatory NTM reporting and the overlap of clinical presentation with tuberculosis. The immune response to both NTM and *Mycobacterium tuberculosis* is based on cellular immunity and relies on the type-1 cytokine pathway. The disruption of this immune response by genetic or acquired mechanisms, such as mendelian susceptibility to mycobacterial disease or HIV, might result in predisposition to mycobacterial infections. Published diagnostic and management guidelines do not provide specific advice for diagnosis of NTM in children, from whom the quantity and quality of diagnostic samples are often suboptimum. Treatment of NTM infections is very different from the treatment of tuberculosis, depends on the strain and anatomical site of infection, and often involves antibiotic combinations, surgery, or both. In this Review, we summarise the epidemiological and clinical features of NTM infection in children, with a specific focus on the implications for public health in settings with a high endemic burden of childhood tuberculosis.

Introduction

The *Mycobacterium* genus is divided into three groups: the *Mycobacterium tuberculosis* complex, *Mycobacterium leprae*, and the remainder, collectively labelled non-tuberculous mycobacteria (NTM). NTM are a large family of microorganisms common in soil and water. Most of them are non-pathogenic, but some can cause human disease.[1,2] NTM and *Mycobacterium tuberculosis* complex share microbiological attributes, induce similar immune responses, and have overlapping clinical manifestations, particularly diseases of the lymph node and lung. However, disease caused by NTB is a diagnostic challenge for respiratory physicians, paediatricians, and other clinicians because it cannot readily be distinguished from tuberculosis on the basis of clinical history, tuberculin skin test results, radiological patterns,[3] and initial laboratory reports. In children, NTM infection can result in cervical lymphadenitis, skin and osteoarticular infections, lung disease (although rare in the absence of chronic lung disorders), and disseminated infections in the setting of primary or acquired immune deficiencies.[1,4–6] Diagnosis is especially challenging in children living in regions where tuberculosis is highly endemic and where epidemiological and clinical studies of NTM diseases are scarce.[7–10] The underlying diagnosis is most often assumed to be childhood tuberculosis, and standard tuberculosis treated is started empirically. However, the treatment of NTM is very different from that of tuberculosis, making the initial correct diagnosis essential to ensure appropriate treatment.

When paediatric respiratory samples are available, diagnosis of tuberculosis in most high-burden settings is initially based on smear microscopy, which is often negative in children due to the paucibacillary nature of tuberculosis in children. If present in a smear, *M tuberculosis* and NTM cannot be distinguished

Key messages

- Non-tuberculous mycobacteria (NTM) are environmental organisms that can cause disease in adults and children
- NTM and tuberculosis disease are often clinically indistinguishable
- In settings with a high burden of tuberculosis, little data for the epidemiology and clinical burden of NTM disease are available, the ability to confirm mycobacterial disease microbiologically is poor, and NTM-specific treatment options are not widely available
- Data for the epidemiology of NTM in children, including transmission mechanisms and risk factors, are scarce
- NTM exposure seems to affect the efficacy of Bacillus Calmette–Guérin vaccination, although the mechanism is unclear
- The most common NTM-associated disease in children is NTM lymphadenitis; other clinical manifestations include skin and soft tissue infections, lung disease (predominantly in people with previous lung comorbidities), and disseminated infections (mostly in immune-compromised children)
- Diagnosis of both tuberculosis and NTM disease should be based on clinical, radiological, and microbiological assessment; diagnostic criteria for NTM pulmonary disease specifically for children do not exist
- NTM isolation from non-sterile sites, such as gastric aspirate and sputum, does not necessarily imply disease
- The treatment strategy for NTM disease depends on the species, site, disease severity, and, in most tuberculosis-endemic countries, on the availability of drugs and trained surgical personnel
- NTM frequently develop resistance to antimicrobial drugs; thus, medical management relies on antibiotic combinations, with macrolides as the cornerstone of treatment
- Studies of the clinical and programmatic effect of new diagnostic algorithms for NTM based on the Xpert MTB/RIF assay are needed

microscopically. Newer molecular assays, such as the Xpert MTB/RIF assay, are able to confirm acid-fast bacilli seen in a smear as *M tuberculosis*, but these assays are not widely used and would not be able to identify NTM specifically as they only identify *M tuberculosis* complex. Furthermore, the tuberculin skin test, which is widely used in screening for latent tuberculosis infection, does not have sufficient specificity in the subpopulation of people who have received the Bacillus Calmette–Guérin (BCG) vaccine or had NTM infections.[11,12] Whether or not the BCG vaccine has a role in protection against NTM needs further investigation.[13]

In this Review, we present data of the epidemiology of paediatric NTM diseases, review the host immune response to mycobacteria, expand on NTM clinical syndromes, and elaborate on approaches to diagnosis and treatment of NTM in children, particularly in regions of the world where tuberculosis is endemic. Finally, we review the gaps in knowledge and define future research priorities. The correct identification of NTM disease has important implications for treatment and follow-up, making this topic relevant for respiratory physicians and other health-care professionals who have to distinguish between tuberculosis-like presentations with different causes in clinical practice.

Epidemiology of NTM in children

Burden of disease

National surveillance studies of NTM disease are uncommon, and most countries do not do systematic reporting of the occurrence of NTM disease or isolation of NTM. Thus, geographical distribution is difficult to determine. However, findings from extensive studies[14–16] show epidemiological differences by region and country, which might affect the local incidence and clinical manifestations of NTM disease. Results from some studies[16–19] have shown an increase in NTM isolates and disease in the past decades, but these data are mainly for adult populations in developed countries, and no published data support a true increase in NTM disease in children. An association between increased disease incidence of mycobacterial disease caused by NTM and decreased incidence of tuberculosis has been suggested in adults,[20] although no causal relation has been proven.

In many countries, the absence of diagnostic tests specifically for NTM further hinders the quantification of NTM disease. In countries with a high burden of tuberculosis, a positive sputum smear for NTM is likely to be misinterpreted as tuberculosis. Moreover, the definition of NTM disease or infection has not been consistent for several years,[2,21,22] which adds further complexity to the estimation and comparison of epidemiological trends for NTM disease.

Most estimates of incidence of paediatric NTM disease from high-income countries focus on the incidence of mycobacterial lymphadenitis, the most frequent clinical syndrome in children (table 1). Results of a prospective study[1] in Australia that used an active surveillance network showed an annual incidence of 0·84 NTM infections per 100 000 children younger than 15 years. In Sweden, the incidence of NTM lymphadenitis between 1969–90 varied from a minimum of 0·06 to a maximum of 5·7 per 100 000 children younger than 5 years.[23] In the Netherlands, the annual incidence was estimated to be 0·8 cases per 100 000 children aged 0–18 years, although not all NTM infections were confirmed by laboratory methods.[5] Results of incidence studies from Canada and Finland show similar estimates.[25,27]

In developed countries, *Mycobacterium avium-intracellulare* complex (MAC) is usually the most frequently NTM isolated in children, followed by *Mycobacterium malmoense*, *Mycobacterium haemophilum*, and *Mycobacterium lentiflavum*, although the precise order varies with location and timing of the studies.[1,5,23,27–30] These findings are in line with those from a study by Hoefsloot and colleagues,[14] which included 20 182 NTM pulmonary isolates from different world regions (mostly from adults), the results of which showed that MAC species accounted for almost 50% of all NTM isolates, with substantial variation by region. However, the geographical distribution of specific NTM isolates in children remains unknown.

In low-resource settings, no estimates exist for the incidence of clinical syndromes caused by NTM. The available data are usually a byproduct of studies done to assess the burden of pulmonary tuberculosis in children, from whom NTM were isolated from respiratory specimens. Investigators from Nigeria noted that of 137 patients older than 10 years with acid-fast bacillus infection and receiving tuberculosis treatment, 12 (12%) and 4 (4%) of 97 positive isolates obtained had infections due to non-mycobacteria or NTM, respectively.[31] In Uganda, the prevalence of NTM in sputum samples or gastric aspirates from children tested for tuberculosis was 26 (3·7%) of 710 and 69 (4·6%) of 1490 infants and adolescents, respectively.[9] *Mycobacterium fortuitum* (42% of the total) *Mycobacterium szulgai*, and *Mycobacterium gordonae* were the most frequently isolated NTM.[9] In a study[10] in Ethiopian children, NTM were isolated in 10 (10%) of 101 samples investigated for tuberculosis, and NTM represented 10 (40%) of 25 positive mycobacterial isolates, with *M fortuitum* being the NTM most frequently isolated. In a similar study[8] in South Africa, 109 (6%) of 1732 bacterial cultures (and a third of the positive ones) from paediatric presumptive tuberculosis cases yielded NTM, with a yield ratio of *M tuberculosis* to NTM less than 2:1 (187 *M tuberculosis vs* 109 NTM; 46 (42%) of 109 NTM isolates had been diagnosed as tuberculosis. MAC species were the most frequently isolated NTM (45%), followed by *M gordonae*, *Mycobacterium flavescens*, and *Mycobacterium scrofulaceum*. In the South African and Ugandan studies,[8,9] about a third of NTM isolates were not further identified. Increasing age was associated with a higher prevalence of NTM in both studies.

Medicine and Center for Chronic Immunodeficiency, Freiburg University, Germany (D Wagner MD); **Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands** (J van Ingen MD); **Vaccinology Theme, MRC Unit, The Gambia** (Prof B Kampmann MD); **Department of Paediatrics, Imperial College London, UK** (Prof B Kampmann)

Correspondence to:
Prof Beate Kampmann, Department of Paediatrics, Imperial College London, St Mary's Campus, London W2 1PG, UK
b.kampmann@imperial.ac.uk

	Country	Study period	Age group (years)	Measure of disease frequency	Frequency	Specimen	Most isolated NTM
Blyth et al (2009)[1]	Australia	2004–07	0–14	Annual incidence of infection	0·84/100 000	From relevant extrapulmonary site	MAC
Romanus et al (1995)[23]	Sweden	1986–90†	0–4	Annual incidence of infection	4·5/100 000	From relevant extrapulmonary site	MAC
Romanus et al (1995)[23]	Sweden	1986–90†	5–9	Annual incidence of infection	1·0/100 000	From relevant extrapulmonary site	MAC
Romanus et al (1995)[23]	Sweden	1986–90†	10–14	Annual incidence of infection	0·08/100 000	From relevant extrapulmonary site	MAC
Haverkamp et al (2004)[5]	The Netherlands	2001–03	0–4	Annual incidence of infection	2·3/100 000	From relevant extrapulmonary site	MAC
Haverkamp et al (2004)[5]	The Netherlands	2001–03	5–9	Annual incidence of infection	0·5/100 000	From relevant extrapulmonary site	MAC
Haverkamp et al (2004)[5]	The Netherlands	2001–03	10–14	Annual incidence of infection	0·25/100 000	From relevant extrapulmonary site	MAC
Reuss et al (2008)[24]	Germany	2003–05	0–14	Annual incidence of disease	1·3/100 000	From relevant extrapulmonary site	MAC
Katila et al (1987)[25]	Finland	1977–86	..	Annual incidence rate of cervical adenitis	0·3/100 000	From relevant extrapulmonary site	MAC
Joshi et al (1989)[26]	Australia	1976–85	0–14	Annual incidence rate of lymphadenitis	0·87/100 000	From relevant extrapulmonary site	MAC
Sigalet et al (1992)[27]	Canada	1979–90	0–14	Annual incidence rate of lymphadenitis	1·21/100 000	From relevant extrapulmonary site	MAC
Hatherill et al (2006)[8]	South Africa	2001–05	..	Prevalence of NTM isolation*	6%	Pulmonary samples (gastric aspirate and induced sputum)	MAC
Asiimwe et al (2013)[9]	Uganda	2009–11	Infants	Prevalence of NTM isolation*	3·7%	Pulmonary samples (gastric aspirate and induced sputum)	*Mycobacterium fortuitum*
Workalemahu et al (2013)[10]	Ethiopia	2011	0–14	Prevalence of NTM isolation*	10%	Pulmonary samples (gastric aspirate or expectorated sputum)	*Mycobacterium fortuitum*

MAC=*Mycobacterium avium* complex. NTM=non-tuberculous mycobacteria. *Among presumptive tuberculosis cases. †Only the most recent incidence estimates from this report are shown in the table.

Table 1: **Incidence of paediatric (age <15 years) NTM disease or infection or prevalence of NTM isolates in presumptive tuberculosis cases**

NTM isolates are also a common finding in conjunction with multidrug-resistant tuberculosis. Results of a study[32] in Mali showed that 11 (18%) of 61 adult patients with tuberculosis receiving treatment for multidrug-resistant tuberculosis (after completing the retreatment regimen) were identified as having NTM isolates, whereas only 22 (36%) of the 61 patients had confirmed multidrug-resistant tuberculosis. The investigators estimated that 63% of patients starting second-line tuberculosis treatment would be receiving inappropriate or unnecessary treatment.

An important limitation to the interpretation of these findings is the absence of data for background NTM isolates from healthy individuals, since the prevalence of asymptomatic infections have traditionally been inferred from antibody and skin test studies in countries with a low prevalence of tuberculosis.[2] Additionally, the possibility of concurrent tuberculosis and NTM infection should be taken into account, especially in view of the fairly low sensitivity of bacterial culture-based tuberculosis diagnosis.

Mechanism of transmission

The mechanism of NTM transmission remains incompletely understood. The presence of NTM in natural and constructed drinking-water distribution systems has been widely described.[33] Thus, aerosol inhalation from municipal or private water systems is thought to be an important route of acquisition. Water transmission has also been suggested, based on the results of a study[34] that showed the same genetic composition of MAC isolates in pulmonary secretions and the household water systems. The presence of MAC in shower biofilms in the USA further confirms the constant exposure of people to NTM.[35] Nosocomial NTM outbreaks in paediatric patients who have received haematopoietic cell and bone marrow transplants has been associated with hospital water supply.[36,37] NTM have also been reported in domestic animals, potting soil,[38,39] and soil samples from patients' home.[40] Soil transmission has been suggested by investigators of a case-control study in which patients with MAC infection did more farming or gardening than controls.[41] Although NTM transmission has mostly been associated with environmental sources, probable nosocomial human-to-human transmission has been reported[42] in patients with cystic fibrosis admitted to a health-care centre in which identical strains of *Mycobacterium abscessus* subspecies massiliense were identified by whole-genome sequencing. A possible case of household human-to-human transmission of *Mycobacterium kansasii* has also been reported.[43,44]

Risk factors for NTM disease in children

Cystic fibrosis and mendelian susceptibility to mycobacterial disease (MSMD) are two distinct inborn genetic disorders associated with NTM disease. Among various acquired causes of immunodeficiency, NTM disease is associated with HIV infection and very low CD4 cell counts in both adults and children,[45–48] although the incidence of disseminated NTM disease has reduced with the advent of antiretroviral therapy. NTM diseases have been reported in paediatric patients with cancer, in whom bloodstream NTM infections are associated with the use of central venous catheters,[49,50] and disseminated forms of NTM disease are known to

have occurred in recipients of organ transplants or haematopoietic stem cell transplants and in patients receiving immune therapies directed against T cells.[51–54]

In adults, a previous history of tuberculosis disease is a risk factor for NTM pulmonary disease, probably due to structural damage of the lung (such as in bronchiectasis) altering mucociliary clearance and thereby predisposing the lung tissue to NTM isolation and disease.[55] Although the same phenomenon has not been described in children, bronchiectases are often seen in children with HIV and chronic lung disease,[56] past tuberculosis, recurrent pneumonia, severe immunosuppression, and lymphoid interstitial pneumonitis.[57]

Host–pathogen interactions

In immunocompetent hosts, NTM are conventionally regarded as commensal bacteria and unlikely to cause disease. By contrast with *M tuberculosis*, in the absence of particular primary or secondary immunodeficiencies, the host immune system is usually capable of containing and possibly eradicating NTM via established innate and acquired immune mechanisms.

Important insights into protective immune mechanisms against mycobacteria in general have come from studies of patients affected by disseminated forms of NTM. Results of these studies[58,59] showed an association of interferon γ and interleukins 12 and 23, components of the type-1 cytokine pathway, with MSMD. MSMD is a rare syndrome caused by intracellular pathogens. Most genetic defects underlying MSMD are autosomal recessive mutations in the β1 subunit of the interleukin 12 receptor (IL12RB1) or autosomal mutations in interferon γ receptor 1 (IFNGR1), which affect the interleukin 12/23-mediated and interferon γ-mediated interactions between macrophages and T cells. Ten different gene mutations have already been identified in human beings (figure 1) and have substantially informed our understanding of the human response to mycobacterial infection, which relies on the integrity of the helper T cell type 1-cytokine pathway. Disruptions to this pathway render the host susceptible to disease by otherwise weakly pathogenic mycobacteria. The possibility of an underlying genetic defect ought to be kept in mind when assessing patients with unusual clinical manifestations of NTM disease, BCGosis (dissemination of BCG), or even tuberculosis.

Similarly, acquired immunodeficiencies such as HIV/AIDS and the development of antibodies against interferon γ impair cellular immunity by disrupting the same signalling pathways.[60] In patients with HIV, susceptibility to NTM infection is strongly associated with absolute CD4 T cell count,[61] emphasising the need for intact cellular immune mechanisms to contain these bacteria, equivalent to findings in the context of *M tuberculosis* infection. However, tuberculosis occurs in individuals with HIV with a range of CD4 T cells counts, whereas NTM usually only becomes a clinical problem in advanced stages of HIV with CD4 counts below 50 cells per µl.[61] Such low CD4 cell counts are rarely seen in this era of successful antiretroviral therapy.

Whether the performance of diagnostic tests for latent and active tuberculosis can be affected by the presence of NTM is a subject of debate: the tuberculin skin test, which detects sensitisation to mycobacteria, is confounded by the presence of antigens that are shared by all mycobacteria

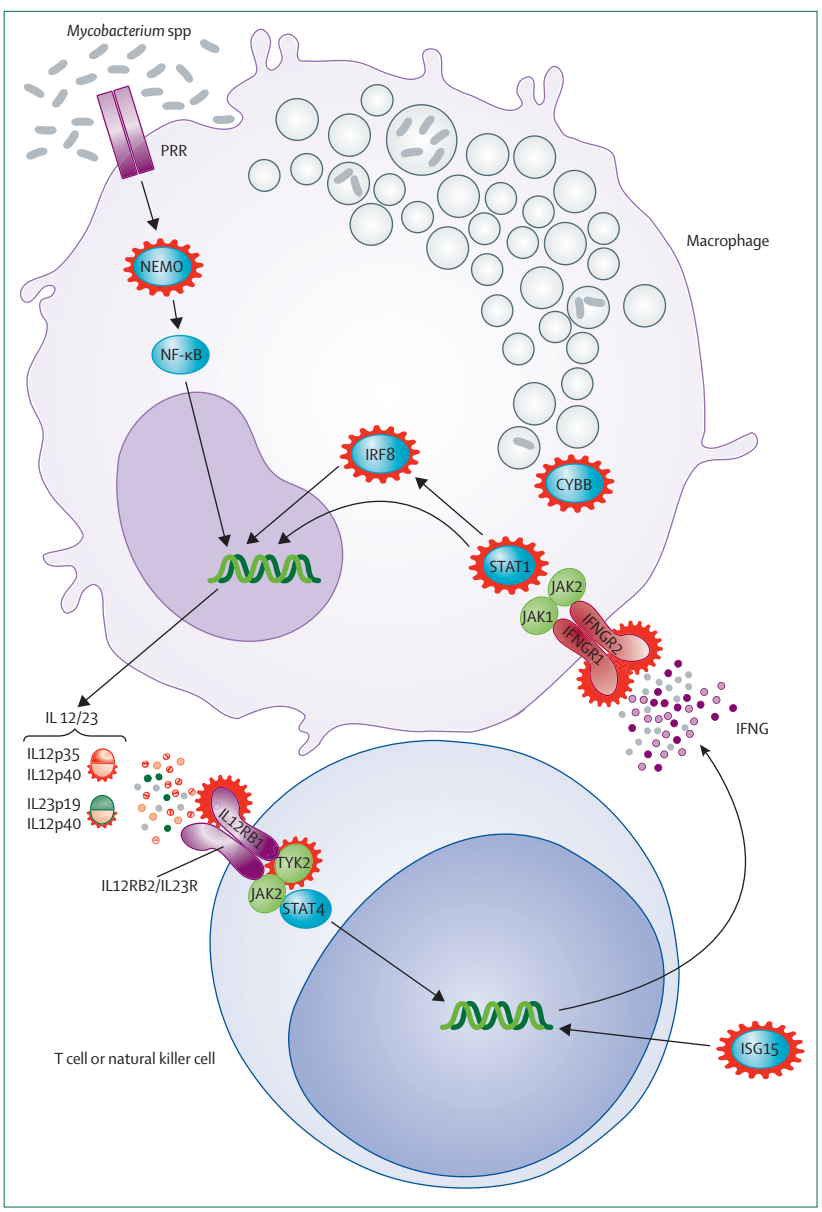


Figure 1: **Mendelian susceptibility to mycobacterial disease**
After phagocytosis of mycobacteria, the interferon γ, interleukin 12, and interleukin 23 signalling pathway between macrophages and natural killer cells or T lymphocytes is activated. Known genetic defects associated with Mendelian susceptibility to mycobacterial disease are highlighted in red. IL12=interleukin 12. IL23=interleukin 23. IL12RB1=β subunit of interleukin 12 receptor 1. IL23R=interleukin 23 receptor. IFNG=interferon γ. IFNGR1=interferon γ receptor 1. IFNGR2=interferon γ receptor 2. IRF8=interferon regulatory factor 8. ISG15=ISG15 ubiquitin-like modifier. JAK1=Janus kinase 1. JAK2=Janus kinase 2. NEMO=NF-κB essential modulator. PRR=pattern recognition receptor. STAT1=signal transducer and activator of transcription 1. STAT4=signal transducer and activator of transcription 4. TYK2=tyrosine kinase 2.

and are not specific for *M tuberculosis*. In populations that have not received the BCG vaccine, the presence of NTM can help to account for discrepancies between results of tuberculin skin tests and interferon-γ release assays.[62] The blood-based interferon-γ release assays, which are based on the immunodominant antigens ESAT-6 and CFP-10, are more specific for the diagnosis of latent tuberculosis infection than is the tuberculin skin test. However, the interferon-γ release assays are not readily available or cost effective in resource-poor settings and are not recommended for the diagnosis of active tuberculosis.[63] Theoretically, discrimination between the effect of NTM on tuberculin skin test reactivity and *M tuberculosis* sensitisation would be possible by comparison of the results of a positive tuberculin skin test with interferon-γ release assay findings. However, *M kansasii*, *M szulgai*, *Mycobacterium marinum*, and *Mycobacterium riyadhense* also express ESAT-6 and CFP-10 antigens.[64] Algorithms that sequentially use results of tuberculin skin tests and interferon-γ release assays to increase the distinction of tuberculosis and NTM lymphadenitis could allow for rational management of this disease, especially in settings where the incidence of tuberculosis is low.[65,66] In countries where tuberculosis is highly endemic and BCG vaccine coverage is high, this approach is probably of little value, since the tuberculin skin test could easily be positive because of previous BCG vaccination.

To eliminate the issue of cross-reactivity, novel skin tests with reagents specific for *M tuberculosis* are undergoing clinical assessment and might be licensed in the near future. However, it is unlikely that these new tests will be able to differentiate between tuberculosis infection and NTM disease. Data[67,68] from settings in which tuberculosis is not endemic have shown the interferon-γ release assay to be highly specific for the differentiation of clinical syndromes due to NTM from those caused by *M tuberculosis*, but this approach is less likely to work in tuberculosis-endemic settings, where widespread pre-existing sensitisation to *M tuberculosis* is expected. Similar issues are likely to affect the performance of NTM-specific skin tests in these settings.

Whether NTM also have an effect on vaccine responses to BCG and its efficacy has been a subject of substantial research and debate. BCG vaccine efficacy against tuberculosis differs between regions, varying from 0–89%.[69–71] Exposure to NTM in the environment might play a part in this variation. Some people have argued that populations with lower BCG vaccine efficacy have higher prevalence of cross-reactive NTM. Malawian and South Indian populations seem to have a higher frequencies and larger NTM-specific tuberculin skin test responses than people in the UK.[72,73] Data[70] from Malawi showed BCG to have an overall lower immunogenicity than it does in the UK. Results of a study[74] from The Gambia have not confirmed these findings, although BCG vaccination in itself has an effect on tuberculin skin test responses at least for a few years. Interestingly, some countries in northern and eastern Europe, where BCG vaccination was abolished, have seen an increase in NTM lymphadenitis in children, although no controlled studies have been done to investigate the prevalence of NTM disease in the presence or absence of the BCG vaccination programme.[25] Findings of studies in animals suggest that the type of NTM exposure (route of exposure, species, and mycobacteria viability) might affect BCG vaccine-mediated protection.[13,75,76] These findings emphasise the need for a better understanding of the contribution of NTM to vaccine responses in tuberculosis-endemic settings where novel tuberculosis vaccines are being assessed.

Clinical features of NTM in children

Clinical syndromes

The most common NTM-associated diseases are NTM-associated lymphadenitis in children and NTM pulmonary disease in adults. *Mycobacterium ulcerans* disease (ie, Buruli ulcer) and skin infections with *M marinum* can also occur in children, but are beyond the scope of this Review. Other skin and soft tissue infections caused by NTM (often caused by rapid-growing NTM, but also sometime by slow-growing NTM) can occur in immunocompetent as well as immunosuppressed patients, often after puncture wounds.[1,4,77–80] Chronic osteomyelitis or arthritis due to infection with slow-growing or rapid-growing mycobacteria have only been described in case reports and case series of children with NTM disease.[1,80,81] Endobronchial NTM disease has been reported in children, can occur in the first year of life, and often leads to bronchial obstruction.[3,82–85] NTM-related peritonitis can be caused by slow or rapid-growing mycobacteria in patients on peritoneal dialysis and should be suspected if leukocytes in the peritoneal fluid are mainly lymphocytes, bacterial cultures stay negative, and empirical antibiotic treatment has failed.[86,87]

Localised (cervicofacial) lymphadenitis

NTM-associated cervical lymphadenitis is the most frequent manifestation of NTM disease in immunocompetent and otherwise healthy children.[1,4,6,88] The clinical presentation is indistinguishable from *M tuberculosis*-associated lymphadenitis, which always needs to be considered in the differential diagnosis. Other differential diagnoses in patients with unilateral lymphadenopathy include streptococcal, *Bartonella*, *Brucella*, and *Toxoplasma* infection, as well as lymphoma.[87] Compared with the usually bilateral tuberculosis lymphadenitis, NTM lymphadenitis occurs earlier in childhood (50% of cases are in children younger than 3 years, and 80% in children younger than 5 years; mean age at diagnosis is about 2·5 years).[24,88] Patients often present with a history of unilateral lymph node swelling, usually affecting the jugulodigastric, parotid or

pre-auricular, submandibular, and posterior triangle lymph nodes, that persists for weeks to months despite antibiotic treatment.[6] Left untreated, these lymph node swellings might regress spontaneously but often progress from a painless, firm mass with increased vascularity (stage I of tubercular lymphadenitis) to a more fluctuant mass due to liquefaction (stage II). Next, often violaceous skin discoloration over the affected lymph nodes occurs and the skin becomes thinner (stage III; figure 2), leading to fistulisation (stage IV).[65] Causative pathogens are usually slow-growing mycobacteria, mostly from the MAC complex or *M haemophilum*. Patients infected with *M haemophilum* tend to be older than those with the more common *M avium* lymphadenitis.[80]

Pulmonary disease

Pulmonary NTM disease is clinically and radiographically indistinguishable from pulmonary tuberculosis[89,90] and therefore might not be suspected in children assessed for pulmonary tuberculosis.[8,9,32,91] However, pulmonary NTM disease has rarely been described in healthy children with no pulmonary predisposition.[1,4,6]

Patients with cystic fibrosis are predisposed to NTM infection, mostly with *M avium* and *M abscessus*. In fact, the prevalence of NTM isolation in patients with cystic fibrosis ranges from 3% to 13%, which rises with increasing age.[92–97] Many patients with cystic fibrosis and a first positive NTM culture do not progress to active disease, and the clinical significance of an NTM isolate is obscured by other respiratory infections. In the context of an initial positive NTM culture, impaired lung function and accelerated decline indicate clinical significance.[98] In a review[97] of the prevalence of NTM in cystic fibrosis centres, about half of the patients with respiratory NTM isolates received treatment, but criteria for starting treatment varied widely between centres.

Disseminated disease

Patients with genetic susceptibility to mycobacterial disease often die early in infancy due to disseminated disease, but some can have an asymptomatic course until adulthood.[99] Infants with disseminated disease usually present with a combination of fever, weight loss, diarrhoea, osteomyelitis or arthritis, generalised lymphadenitis, subcutaneous abscess or dermatitis, and hepatosplenomegaly.[100,101] Disseminated NTM disease can also occur in patients with acquired immunodeficiency (eg, patients who have received organ or bone marrow transplants and those with solid cancers or leukaemia; figure 3), but these patients might also have localised (skin, osteoarticular, or pulmonary) or catheter-related disease manifestations.[1,49,50,102–104] In children infected with HIV, disseminated NTM disease can also occur as a manifestation of immune reconstitution inflammatory syndrome (IRIS). NTM-related IRIS needs to be considered in the first weeks and months after starting antiretroviral therapy.[105,106] However, in tuberculosis-endemic areas it is much more likely that the clinical presentation of IRIS is triggered by *M tuberculosis* or BCG rather than NTM.[107]

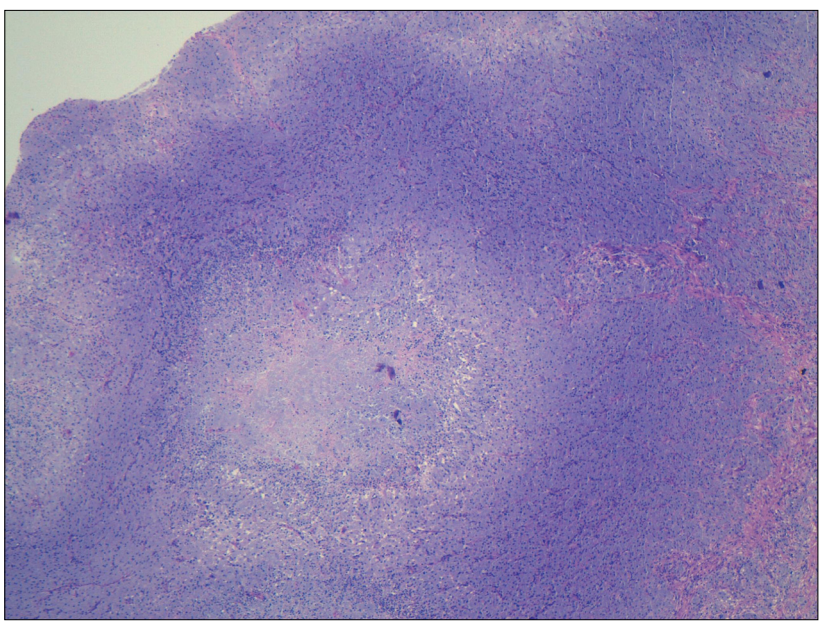


Figure 2: **Non-tuberculous mycobacteria lymphadenitis**
Paraffin section of a lymph node from a girl with HIV and *Mycobacterium genavense* disseminated disease. The image shows a typical granuloma with dense lymphocytic infiltration surrounding a necrotic core. Haematoxylin and eosin staining 10× magnification.

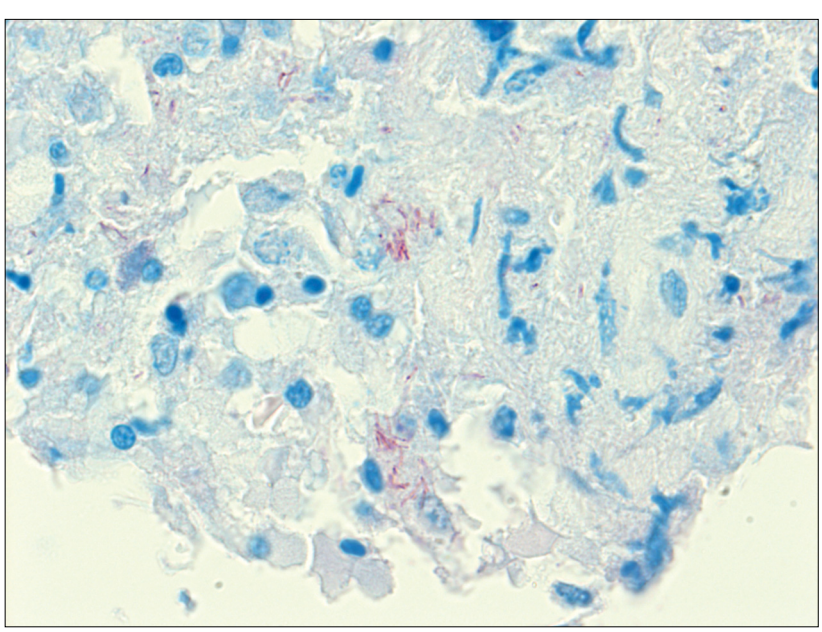


Figure 3: **Disseminated non-tuberculous mycobacteria disease**
Bone marrow biopsy from a 14-year-old girl with disseminated *Mycobacterium genavense* disease complicating an acquired immunodeficiency syndrome. Pink rods are the *M genavense* bacteria. Ziehl-Neelsen staining 630× magnification.

Diagnosis of NTM disease manifestations

Overview

Diagnosis of both tuberculosis and NTM disease manifestations requires clinical, radiological, and microbiological assessment. By contrast with *M tuberculosis*, the isolation of NTM from a non-sterile specimen (such as gastric or nasopharyngeal aspirate) needs to be interpreted in a wider clinical context, because it does not necessarily imply disease.

Microbiological diagnosis of NTM disease is achieved by detection of the causative NTM by PCR or bacterial culture, both of which are rarely widely available in most counties with a high burden of tuberculosis. The role of these methods, their diagnostic accuracy, and their interpretation differ by type of disease. Optimum microbiological diagnosis of NTM disease depends on the use of solid and liquid media, as well as several different incubation temperatures. Identification of cultured mycobacteria is best done by molecular methods because biochemical tests and *M tuberculosis*-specific MPT64 antigen detection can provide a low-cost way to differentiate NTM from *M tuberculosis*.[108,109] Drug-susceptibility testing of NTM is still in its infancy and has been extensively covered in a recent review.[110]

Panel 1: **Clinical and microbiologic criteria for diagnosing non-tuberculous mycobacterial lung disease[2]**

Clinical criteria (both necessary)

- Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution CT scan that shows multifocal bronchiectasis with multiple small nodules (A, I)
- Appropriate exclusion of other diagnoses (A, I)

Microbiological criteria (one or more are necessary)

- Positive culture results from at least two separate expectorated sputum samples (A, II); if the results are non-diagnostic, consider repeat sputum acid-fast bacilli smears and cultures (C, III)
- Positive culture result from at least one bronchial wash or lavage (C, III)
- Transbronchial or lung biopsy with mycobacterial histopathological features (granulomatous inflammation or acid-fast bacilli) and one positive culture for non-tuberculous mycobacteria (NTM), or biopsy showing mycobacterial histopathological features (granulomatous inflammation or acid-fast bacilli) and one or more sputum or bronchial washing that is culture positive for NTM (A, II)

Additional recommendations

- Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination (C, III)
- Patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria should be followed-up until the diagnosis is firmly established or excluded (C, III)
- The diagnosis of NTM lung disease does not, per se, necessitate the use of treatment; this decision should be based on potential risks and benefits of treatment for individual patients (C, III)

Letters indicate the quality of evidence (adapted from the Infectious Disease Society of America/US Public Health Service Rating System), categorised according to the strength of each recommendation for or against its use: A=good evidence to support a recommendation for use; B=moderate evidence to support a recommendation for use; C=poor evidence to support a recommendation for or against use; D=moderate evidence to support a recommendation against use; E=good evidence to support a recommendation against use. Numerals indicate the source of evidence on which quality of evidence is based: I=evidence from at least one properly randomised controlled trial; II=evidence from at least one well designed clinical trial without randomisation, from cohort or case-controlled analytical studies (preferably from more than one centre), from multiple time-series studies, or from dramatic results in uncontrolled experiments; III=evidence from opinion of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Localised (cervicofacial) lymphadenitis

NTM lymphadenitis in children is a paucibacillary disease that often is suspected and diagnosed presumptively without microbiological confirmation. The sensitivity of bacterial culture is suboptimum, in the range of 41–80%.[26,111,112] Molecular detection of NTM in lymph node biopsy samples, with a sensitivity of up to 72%, is more sensitive than bacterial culture.[111] The use of molecular techniques would be especially useful in regions where the important causative agents of NTM lymphadenitis (eg, *M haemophilum*) are difficult to culture and in settings where bacterial culture can only be done on routine solid media at 37°C. Results of a study comparing fine needle aspirates with biopsy specimens showed that fine needle aspirates are better for microbiological diagnosis of NTM lymphadenitis, either by PCR or bacterial culture.[111] However, an excision biopsy can have the advantage of being fully diagnostic and curative at the same time. Some lymphadenitis-causing NTM (eg, *M haemophilum*) grow optimally at about 30°C. It is thus recommended to incubate bacterial cultures from lymph nodes at 30°C and 37°C.[113] For optimum detection of *M haemophilum*, bacterial culture media needs to be supplemented with an iron source, such as hemin or ferric ammonium citrate.[114]

Disseminated disease

Disseminated disease is usually confirmed by blood culture or staining and culture of bone marrow biopsy specimens (figure 2, figure 3); blood and bone marrow culture offer a similar sensitivities (60–80%).[115–117] Findings of two studies showed that staining and culture of liver biopsy specimens can provide a faster and slightly more sensitive alternative to blood and bone marrow culture.[118,119] Automated systems are available for mycobacterial blood culture. The various systems have equal sensitivities of about 80%.[120] In settings where these automated systems are not available, it is best to incubate lysed and centrifuged samples in both a liquid and a solid medium; omission of one medium type decreases sensitivity by 10–15%.[121] The disseminated skin disease associated with rapid growers (eg, *M abscessus* and *Mycobacterium chelonae*) and *M haemophilum* tends to affect patients with haematological malignancies or solid organ transplants, but not patients with HIV/AIDS.[2,114] This disease manifestation is usually diagnosed by culture of skin biopsy specimens. Sensitivities and specificities of bacterial culture and PCR in these biopsies have not been systematically studied. For skin biopsy specimens it is also advised to incubate cultures at 30°C and 37°C, particularly for detection of *M haemophilum* in tuberculosis-endemic areas[113,114] and *M marinum*.[122]

Pulmonary disease

The available scientific literature on microbiological diagnosis of NTM pulmonary disease focuses almost entirely on adults because this disease type is rare in children. However, NTM pulmonary disease might present a complex diagnostic problem to physicians in all settings. In young children, obtaining respiratory samples is more difficult than in adults and older children. The use of gastric aspirates for diagnosis of NTM pulmonary disease has not been studied and is not recommended, since NTM survive the acidic environment of the stomach and can be part of normal commensal flora. Several reports[8–10,123–125] have described frequent NTM isolates both in gastric aspirates and in sputum, which suggests that their presence is probably clinically irrelevant, but can be misleading.

Respiratory samples for NTM culture should be decontaminated with 1% N-acetyl-L-cysteine–sodium hydroxide and concentrated by centrifugation.[126] For samples from patients with cystic fibrosis or samples otherwise heavily colonised by *Pseudomonas aeruginosa* and related bacteria, a second decontamination step with 5% oxalic acid can increase the sensitivity of mycobacterial culture.[127] According to the results of a meta-analysis,[128] liquid culture alone has a 66% sensitivity compared with 51% for solid media alone. If both solid and liquid media are used, sensitivity of NTM culture increases to 76%. Whether incubation of respiratory samples at lower temperatures (ie, 30°C) is useful has not been investigated.

Even if cultured from proper sputum or bronchoalveolar lavage samples, a positive culture yielding NTM does not indicate pulmonary disease per se, and the clinical significance is difficult to establish and often requires longer follow-up and obtainment of several confirmatory samples.

According to the 2007 statement from the American Thoracic Society and the Infectious Diseases Society of America,[2] clinical symptoms and typical radiographic findings have to be present to diagnose NTM pulmonary disease (panel 1). The appropriate exclusion of other diagnoses, including pulmonary tuberculosis, is especially problematic if pre-existing or other concomitant pulmonary diseases are present. A major question is the applicability of these guidelines in settings with a high burden of tuberculosis, partly because of the large number of patients who have been started on empirical tuberculosis treatment by the time an NTM isolate is reported.[129,130] Furthermore, the clinical significance of an NTM isolation in a patient receiving tuberculosis treatment is unknown.[2,91] Even if the isolates are probably clinically insignificant, whether coinfection plays a part in tuberculosis pathogenesis or time to sputum clearance is unclear.[32]

These criteria have only been established in the adult population and fit best with specific NTM species, namely *M avium*, *M kansasii*, and *M abscessus*. At present, whether these criteria can also be applied to children or patients with other NTM species is unclear.[2]

	Recommended treatment
Lymphadenitis	
All species	Complete excision (first choice); if complete excision is not possible, partial excision, incision and drainage, or curettage with or without antibiotics
Lung disease	
Mycobacterium avium complex	Rifampicin plus ethambutol plus macrolide (with or without intravenous amikacin or streptomycin in severe disease); lung surgery for children with localised disease presenting poor response to therapy, macrolide resistance, or severe complications
Mycobacterium kansasii	Rifampicin plus isoniazid plus ethambutol
Mycobacterium abscessus	Amikacin plus cefoxitin or imipenem plus one or two additional drugs on the basis of susceptibilities (macrolide, linezolid, tigecycline); lung surgery, combined with chemotherapy, should be considered in children with localised disease
Mycobacterium chelonae	Macrolide plus one additional agent (tobramycin or imipenem)
Mycobacterium fortuitum	Two agents, on the basis of susceptibilities (co-trimoxazole, fluoroquinolone, amikacin, imipenem)
Mycobacterium malmoense	Identical to *M avium* complex
Mycobacterium xenopi	Rifampicin plus ethambutol plus macrolide, with or without moxifloxacin
Disseminated disease	
M avium complex	Clarithromycin plus ethambutol plus rifampicin or rifabutin*
M kansasii	Rifampicin plus isoniazid plus ethambutol
M chelonae	Macrolide plus one additional agent (tobramycin or imipenem)
M abscessus	Amikacin plus cefoxitin or imipenem plus one or two additional agents, on the basis of susceptibilities (macrolide, linezolid, tigecycline)
M abscessus	Amikacin plus cefoxitin or imipenem plus one or two additional agents, on the basis of susceptibilities (macrolide, linezolid, tigecycline)

*Rifabutin is preferred in patients taking drugs that strongly interact with rifampicin (ie, antiretrovirals).

Table 2: **Treatment recommendations for common NTM diseases in children**

Investigators of some studies have attempted to distinguish the clinical or radiological patterns of tuberculosis and NTM pulmonary disease, although few paediatric data from countries with a high burden of tuberculosis exist, and the existing data are inconsistent.[8,39,89,130,131] Increasing age seems to be a common risk factor for NTM disease in both adults and children. Children with NTM isolates in a South African study[8] were more likely to report constitutional symptoms (including fever and weight loss), but had less tuberculin skin test reactivity and reduced likelihood of supportive radiographic features, compared with tuberculosis cases.

Treatment

The establishment of an accurate diagnosis is essential before the treatment of NTM disease, and risks and benefits of treatment should be weighed on the basis of underlying long-term baseline conditions, disease severity, potential toxic effects, and drug interactions.[2,132] Randomised trials comparing therapeutic strategies for NTM at different anatomical sites in adults and children alike are scarce, and there are no systematically collected data for treatment outcomes. NTM frequently develop resistance to antimicrobial drugs, so medical management relies on combinations of several antibiotics, with macrolides being the cornerstone of treatment.

Panel 2: Research gaps

Disease burden
- Disease burden in children, particularly in settings with a high tuberculosis burden
- Geographical distributions and specific distributions of non-tuberculous mycobacteria (NTM) species
- Risk factors for NTM infection in children
- Transmission mechanisms
- Association between tuberculosis and NTM epidemiology in children

Host factors
- Better understanding of the interactions between NTM and host that determine pathogenesis, both in immunocompetent and immunodeficient hosts
- Effect of NTM on the diagnostic tests for latent and active tuberculosis
- Effect of NTM exposure on the efficacy of the Bacillus Calmette–Guérin vaccine and implications for novel tuberculosis vaccine candidates

Diagnosis
- New, child-friendly diagnostic approaches for NTM (including low-cost but sensitive techniques to discern NTM from *M tuberculosis* in clinical specimens and cultures)
- Child-specific diagnostic criteria for NTM disease (all sites) applicable in low-resource settings (and tuberculosis-endemic countries)
- Paediatric guidelines for the diagnosis and management of NTM
- Clinical significance of NTM isolates in patients with tuberculosis who are on treatment (tuberculosis-NTM co-isolates or co-infections)

Treatment
- Child-specific treatment criteria and algorithms
- New, child-friendly, and shortened regimens for NTM treatment
- Prognosis or outcome of NTM disease in children
- Pharmacokinetic and pharmacodynamic studies for widely used anti-mycobacterial drugs used for the treatment of NTM disease in children

Search strategy and selection criteria

We searched PubMed for articles published in English, French, Spanish, German, and Dutch up to May 31, 2014. We restricted the search to reports of NTM infections in human beings. We used the search terms "nontuberculous mycobacteria", "MOTT", "NTM", "Mycobacteria, atypical", and "Mycobacterium infections, atypical" in combinations with "children", and "tuberculosis". The search algorithm included both MeSH and free text terms. Other relevant articles were identified through reference lists of articles identified from this search, reviews, and additional searches of the authors' personal archives. We assessed the relevance and importance of identified studies on the basis of the sample sizes included in the analyses and the relevance to the topic. Specific relevant case reports were also included. References from all relevant articles were screened and some were included in this Review.

Antibiotics should be tailored specifically to the isolated species (table 2), although in-vitro susceptibility to drugs other than macrolides, fluoroquinolones, and aminoglycosides do not generally correspond with in-vivo response to therapy.[133] Pharmacokinetic data[2,134,135] from adults and children show low drug plasma concentrations for key drugs, such as rifampicin, ethambutol, and clarithromycin, although more studies are needed in the paediatric population. Treatment regimens are lengthy, drug intolerance and side-effects are common, and child-friendly formulations are not available for most drugs, hindering adherence and worsening prognosis.[2] An additional difficulty in regions where tuberculosis is endemic is the low availability of drugs for the treatment of NTM. Even availability of mainstream drugs, such as rifampicin and ethambutol, can be low if they are to be used outside of national tuberculosis programmes, which would only manage patients with a diagnosis of tuberculosis, not those with an NTM disease.

Treatment of NTM adenitis depends on disease stage and severity. The approaches range from conservative strategies for mild presentations (the so-called watch-and-wait approach)[136,137] to complete surgical excision of the affected lymph node. The surgical approach is regarded as the best curative option by most clinicians.[30,138] However, permanent injury of the facial nerve is a major concern with surgery.[139] Incision and drainage, curettage, or partial excision are associated with more recurrences and persistent suppuration.[138] In setting where the burden of tuberculosis is high, the chosen approach is usually determined by the availability of trained surgical personnel and drugs.

Treatment of MAC pulmonary disease consists of a combination of macrolides, ethambutol, and a rifamycin, with streptomycin or amikacin sometimes added for patients with severe disease. Treatment of *M kansasii* lung disease includes rifampicin, isoniazid, and ethambutol. Antibiotics should be given until sputum cultures have been negative for a minimum of 1 year. *M kansasii* pulmonary disease can be limited to 1 year, regardless of the time of sputum conversion.[2,140] Surgery can be useful in patients with localised disease who have shown drug intolerance, drug resistance, or poor microbiological response.[2] In regions where mycobacterial culture or PCR are not available, persistent detection of acid-fast bacilli in children adequately treated against tuberculosis or multidrug-resistant tuberculosis should suggest a potential NTM infection, although empirical treatment without identification of the particular NTM species is not recommended.[2]

Disseminated MAC infection in children with HIV should be treated with a regimen containing at least a macrolide and ethambutol. Rifabutin is often added in the beginning, since a more frequent resolution of bacteraemia and increased survival have been reported.[141,142] Aminoglycosides are alternatives, especially in the setting of breakthrough NTM infections or if macrolide resistance is suspected.[2,143] After clinical improvement, treatment should continue with at least two of the successful drugs included in the initial treatment phase. Secondary prophylaxis to prevent recurrence is recommended for the rest of the patient's life or until immune restoration is achieved.[143,144] Antibiotic regimens for disseminated NTM disease in paediatric patients with other acquired defects of cell-mediated immunity and MSMD are the same as for

children with HIV. Some patients with MSMD are responsive to treatment with subcutaneous interferon γ in addition to antibiotics, although this treatment is of little value to patients with complete interferon γ receptor or STAT1 defects.[101,145] Haematopoietic stem cell transplantation has been used in severe forms of MSMD with varying success.[146,147]

Conclusions

NTM are ubiquitous but not always pathogenic in children. They are a clinically significant obstacle to the accurate diagnosis of childhood tuberculosis, and many patients are probably started on tuberculosis treatment when in fact they had underlying NTM disease, although the extent of this misdiagnosis is unknown. Once diagnosed accurately and estimated to be of clinical relevance, treatment needs to be tailored to the specific NTM strains, but there are no systematic outcome studies or controlled clinical trials of different treatment modalities. The challenge of NTM diagnosis and treatment should be tackled by experienced clinicians and laboratory scientists to allow the development and testing of clinical algorithms and treatment protocols in a sufficient number of patients. Networks such as the TBNET, ptbnet, and NTM-NET can have an important role in combining collective experience in the management of tuberculosis and NTM and assisting clinicians in their decision-making processes.[14,148,149] The diagnostic conundrum between tuberculosis and NTM disease in resource-poor settings will probably remain, and a substantial number of research gaps are yet to be addressed (panel 2). Without advanced capacity in laboratories worldwide, including in resource-poor and tuberculosis-endemic countries, this situation is unlikely to change in the near future. In the meantime, clinical isolates need to be characterised to the best of our abilities to correctly identify at least *M tuberculosis*, when present, since tuberculosis remains a prevalent and treatable condition in children.

Contributors

EL-V, ALG-B, and BK generated the concept and design of the Review. All authors participated in the review of the scientific literature and the writing of the first draft. BS created figure 1. All authors discussed edits of initial drafts and EL-V compiled edits into subsequent drafts. All authors reviewed and approved the final version of the Review.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank M Cebollero, Pathology Department, Hospital Gregorio Marañón, Madrid, Spain, for providing figure 2.

References

1 Blyth CC, Best EJ, Jones CA, et al. Nontuberculous mycobacterial infection in children: a prospective national study. *Pediatr Infect Dis J* 2009; **28:** 801–05.

2 Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; **175:** 367–416.

3 Nolt D, Michaels MG, Wald ER. Intrathoracic disease from nontuberculous mycobacteria in children: two cases and a review of the literature. *Pediatrics* 2003; **112:** e434.

4 Cruz AT, Ong LT, Starke JR. Mycobacterial infections in Texas children: a 5-year case series. *Pediatr Infect Dis J* 2010; **29:** 772–74.

5 Haverkamp MH, Arend SM, Lindeboom JA, Hartwig NG, van Dissel JT. Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. *Clin Infect Dis* 2004; **39:** 450–56.

6 Pham-Huy A, Robinson JL, Tapiéro B, et al. Current trends in nontuberculous mycobacteria infections in Canadian children: a pediatric investigators collaborative network on infections in Canada (PICNIC) study. *Paediatr Child Health* 2010; **15:** 276–82.

7 Shenai S, Rodrigues C, Mehta A. Time to identify and define non-tuberculous mycobacteria in a tuberculosis-endemic region. *Int J Tuberc Lung Dis* 2010; **14:** 1001–08.

8 Hatherill M, Hawkridge T, Whitelaw A, et al. Isolation of non-tuberculous mycobacteria in children investigated for pulmonary tuberculosis. *PLoS One* 2006; **1:** e21.

9 Asiimwe BB, Bagyenzi GB, Ssengooba W, et al. Species and genotypic diversity of non-tuberculous mycobacteria isolated from children investigated for pulmonary tuberculosis in rural Uganda. *BMC Infect Dis* 2013; **13:** 88.

10 Workalemahu B, Berg S, Tsegaye W, et al. Genotype diversity of *Mycobacterium* isolates from children in Jimma, Ethiopia. *BMC Res Notes* 2013; **6: 352**.

11 Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006; **10:** 1192–204.

12 Cobelens F. False-positive tuberculin reactions due to non-tuberculous mycobacterial infections. *Int J Tuberc Lung Dis* 2007; **11:** 934–35.

13 Poyntz HC, Stylianou E, Griffiths KL, Marsay L, Checkley AM, McShane H. Non-tuberculous mycobacteria have diverse effects on BCG efficacy against *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* 2014; **94:** 226–37.

14 Hoefsloot W, van Ingen J, Andrejak C, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: A NTM-NET collaborative study. *Eur Respir J* 2013; **42:** 1604–13.

15 Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 2002; **23:** 553–67.

16 Kendall BA, Winthrop KL. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. *Semin Respir Crit Care Med* 2013; **34:** 87–94.

17 Marras TK, Chedore P, Ying AM, Jamieson F. Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario, 1997–2003. *Thorax* 2007; **62:** 661–66.

18 Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 2010; **182:** 970–76.

19 Ringshausen FC, Apel R-M, Bange F-C, et al. Burden and trends of hospitalisations associated with pulmonary non-tuberculous mycobacterial infections in Germany, 2005–2011. *BMC Infect Dis* 2013; **13:** 231.

20 Brode SK, Daley CL, Marras TK. The epidemiologic relationship between tuberculosis and non-tuberculous mycobacterial disease: a systematic review. *Int J Tuberc Lung Dis* 2014; **18:** 1370–77.

21 Medical Section of the American Lung Association. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. *Am J Respir Crit Care Med* 1997; **156:** S1–25.

22 Epson E, Cassidy M, Marshall-Olson A, Hedberg K, Winthrop KL. Patients with nontuberculous mycobacteria: comparison of updated and previous diagnostic criteria for lung disease. *Diagn Microbiol Infect Dis* 2012; **74:** 98–100.

23 Romanus V, Hallander HO, Wåhlén P, Olinder-Nielsen AM, Magnusson PH, Juhlin I. Atypical mycobacteria in extrapulmonary disease among children. Incidence in Sweden from 1969 to 1990, related to changing BCG-vaccination coverage. *Tuber Lung Dis* 1995; **76:** 300–10.

24 Reuss AM, Wiese-Posselt M, Weissmann B, et al. Incidence rate of nontuberculous mycobacterial disease in immunocompetent children: a prospective nationwide surveillance study in Germany. *Pediatr Infect Dis J* 2009; **28:** 642–44.

25 Katila ML, Brander E, Backman A. Neonatal BCG vaccination and mycobacterial cervical adenitis in childhood. *Tubercle* 1987; **68:** 291–96.

For **TBnet and ptbnet** see www.tb-net.org

For **NTM-NET** see www.ntm-net.org

26 Joshi W, Davidson PM, Jones PG, Campbell PE, Roberton DM. Non-tuberculous mycobacterial lymphadenitis in children. *Eur J Pediatr* 1989; **148:** 751–54.

27 Sigalet D, Lees G, Fanning A. Atypical tuberculosis in the pediatric patient: implications for the pediatric surgeon. *J Pediatr Surg* 1992; **27:** 1381–84.

28 Grange JM, Yates MD, Pozniak A. Bacteriologically confirmed non-tuberculous mycobacterial lymphadenitis in south east England: a recent increase in the number of cases. *Arch Dis Child* 1995; **72:** 516–17.

29 Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. *Clin Infect Dis* 1995; **20:** 954–63.

30 Lindeboom JA, Kuijper EJ, Bruijnesteijn van Coppenraet ES, Lindeboom R, Prins JM. Surgical excision versus antibiotic treatment for nontuberculous mycobacterial cervicofacial lymphadenitis in children: a multicenter, randomized, controlled trial. *Clin Infect Dis* 2007; **44:** 1057–64.

31 Pokam BT, Asuquo AE. Acid-fast bacilli other than mycobacteria in tuberculosis patients receiving directly observed therapy short course in cross river state, Nigeria. *Tuberc Res Treat* 2012; **2012:** 301056.

32 Maiga M, Siddiqui S, Diallo S, et al. Failure to recognize nontuberculous mycobacteria leads to misdiagnosis of chronic pulmonary tuberculosis. *PLoS One* 2012; **7:** e36902.

33 Falkinham JO. Epidemiology of infection by nontuberculous mycobacteria. *Clin Microbiol Rev* 1996; **9:** 177–215.

34 Falkinham JO. Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerg Infect Dis* 2011; **17:** 419–24.

35 Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, Pace NR. Opportunistic pathogens enriched in showerhead biofilms. *Proc Natl Acad Sci USA* 2009; **106:** 16393–99.

36 Iroh Tam P-Y, Kline S, Wagner JE, et al. Rapidly growing mycobacteria among pediatric hematopoietic cell transplant patients traced to the hospital water supply. *Pediatr Infect Dis J* 2014; **33:** 1043–46.

37 Kline S, Cameron S, Streifel A, et al. An outbreak of bacteremias associated with *Mycobacterium mucogenicum* in a hospital water supply. *Infect Control Hosp Epidemiol* 2004; **25:** 1042–49.

38 Kankya C, Muwonge A, Djønne B, et al. Isolation of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: public health significance. *BMC Public Health* 2011; **11:** 320.

39 Buijtels PCAM, van der Sande MAB, Parkinson S, Verbrugh HA, Petit PLC, van Soolingen D. Isolation of non-tuberculous mycobacteria at three rural settings in Zambia; a pilot study. *Clin Microbiol Infect* 2010; **16:** 1142–48.

40 De Groote MA, Pace NR, Fulton K, Falkinham JO. Relationships between *Mycobacterium* isolates from patients with pulmonary mycobacterial infection and potting soils. *Appl Environ Microbiol* 2006; **72:** 7602–06.

41 Maekawa K, Ito Y, Hirai T, et al. Environmental risk factors for pulmonary *Mycobacterium avium-intracellulare* complex disease. *Chest* 2011; **140:** 723–29.

42 Aitken ML, Limaye A, Pottinger P, et al. Respiratory outbreak of *Mycobacterium abscessus* subspecies massiliense in a lung transplant and cystic fibrosis center. *Am J Respir Crit Care Med* 2012; **185:** 231–32.

43 Ricketts WM, O'Shaughnessy TC, van Ingen J. Human-to-human transmission of *Mycobacterium kansasii* or victims of a shared source? *Eur Respir J* 2014; **44:** 1085–87.

44 Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 2013; **381:** 1551–60.

45 Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006; **296:** 292–300.

46 Horsburgh CR. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. *N Engl J Med* 1991; **324:** 1332–38.

47 Alarcón JO, Freimanis-Hance L, Krauss M, et al. Opportunistic and other infections in HIV-infected children in Latin America compared to a similar cohort in the United States. *AIDS Res Hum Retroviruses* 2012; **28:** 282–88.

48 Levine B, Chaisson RE. *Mycobacterium kansasii*: a cause of treatable pulmonary disease associated with advanced human immunodeficiency virus (HIV) infection. *Ann Intern Med* 1991; **114:** 861–68.

49 Reilly AF, McGowan KL. Atypical mycobacterial infections in children with cancer. *Pediatr Blood Cancer* 2004; **43:** 698–702.

50 Levendoglu-Tugal O, Munoz J, Brudnicki A, Fevzi Ozkaynak M, Sandoval C, Jayabose S. Infections due to nontuberculous mycobacteria in children with leukemia. *Clin Infect Dis* 1998; **27:** 1227–30.

51 Nicholson O, Feja K, LaRussa P, et al. Nontuberculous mycobacterial infections in pediatric hematopoietic stem cell transplant recipients: case report and review of the literature. *Pediatr Infect Dis J* 2006; **25:** 263–67.

52 Unal E, Yen C, Saiman L, et al. A low incidence of nontuberculous mycobacterial infections in pediatric hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2006; **12:** 1188–97.

53 Morales P, Gil A, Santos M. *Mycobacterium abscessus* infection in transplant recipients. *Transplant Proc* 2010; **42:** 3058–60.

54 Jordan N, Waghmare A, Abi-Ghanem AS, Moon A, Salvatore CM. Systemic *Mycobacterium avium* complex infection during antitumor necrosis factor-α therapy in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2012; **54:** 294–96.

55 Mirsaeidi M, Hadid W, Ericsoussi B, Rodgers D, Sadikot RT. Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. *Int J Infect Dis* 2013; **17:** e1000–04.

56 Zar HJ. Chronic lung disease in human immunodeficiency virus (HIV) infected children. *Pediatr Pulmonol* 2008; **43:** 1–10.

57 Amorosa JK, Miller RW, Laraya-Cuasay L, et al. Bronchiectasis in children with lymphocytic interstitial pneumonia and acquired immune deficiency syndrome. *Pediatr Radiol* 1992; **22:** 603–06.

58 Bustamante J, Picard C, Boisson-Dupuis S, Abel L, Casanova J-L. Genetic lessons learned from X-linked Mendelian susceptibility to mycobacterial diseases. *Ann N Y Acad Sci* 2011; **1246:** 92–101.

59 Van de Vosse E, Hoeve MA, Ottenhoff THM. Human genetics of intracellular infectious diseases: molecular and cellular immunity against mycobacteria and salmonellae. *Lancet Infect Dis* 2004; **4:** 739–49.

60 Kampmann B, Hemingway C, Stephens A, et al. Acquired predisposition to mycobacterial disease due to autoantibodies to IFN-γ. *J Clin Invest* 2005; **115:** 2480–88.

61 Lawn SD, Myer L, Edwards D, Bekker L-G, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. *AIDS* 2009; **23:** 1717–25.

62 Latorre I, De Souza-Galvão M, Ruiz-Manzano J, et al. Evaluating the non-tuberculous mycobacteria effect in the tuberculosis infection diagnosis. *Eur Respir J* 2010; **35:** 338–42.

63 Kampmann B, Whittaker E, Williams A, et al. Interferon-γ release assays do not identify more children with active tuberculosis than the tuberculin skin test. *Eur Respir J* 2009; **33:** 1374–82.

64 van Ingen J, de Zwaan R, Dekhuijzen R, Boeree M, van Soolingen D. Region of difference 1 in nontuberculous *Mycobacterium* species adds a phylogenetic and taxonomical character. *J Bacteriol* 2009; **191:** 5865–67.

65 Penn R, Steehler MK, Sokohl A, Harley EH. Nontuberculous mycobacterial cervicofacial lymphadenitis—a review and proposed classification system. *Int J Pediatr Otorhinolaryngol* 2011; **75:** 1599–603.

66 Staufner C, Sommerburg O, Holland-Cunz S. Algorithm for early diagnosis in nontuberculous mycobacterial lymphadenitis. *Acta Paediatr* 2012; **101:** e382–85.

67 Hermansen TS, Thomsen VØ, Lillebaek T, Ravn P. Non-tuberculous mycobacteria and the performance of interferon γ release assays in Denmark. *PLoS One* 2014; **9:** e93986.

68 Magdorf K, Schuck SD, Leitner S, Wahn U, Kaufmann SHE, Jacobsen M. T-cell responses against tuberculin and sensitin in children with tuberculosis and non-tuberculosis mycobacterial lymphadenopathy. *Clin Microbiol Infect* 2008; **14:** 1079–83.

69 Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol* 1993; **22:** 1154–58.

70 Colditz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA* 1994; **271:** 698–702.

71 Roy A, Eisenhut M, Harris RJ, et al. Effect of BCG vaccination against *Mycobacterium tuberculosis* infection in children: systematic review and meta-analysis. *BMJ* 2014; **349:** g4643.

72 Weir RE, Black GF, Nazareth B, et al. The influence of previous exposure to environmental mycobacteria on the interferon-γ response to bacille Calmette-Guérin vaccination in southern England and northern Malawi. *Clin Exp Immunol* 2006; **146:** 390–99.

73 Tuberculosis Prevention Trial. Trial of BCG vaccines in south India for tuberculosis prevention: first report. *Bull World Health Organ* 1979; **57:** 819–27.

74 Burl S, Adetifa UJ, Cox M, et al. The tuberculin skin test (TST) is affected by recent BCG vaccination but not by exposure to non-tuberculosis mycobacteria (NTM) during early life. *PLoS One* 2010; **5:** e12287.

75 Orme IM, Roberts AR, Collins FM. Lack of evidence for a reduction in the efficacy of subcutaneous BCG vaccination in mice infected with nontuberculous mycobacteria. *Tubercle* 1986; **67:** 41–46.

76 Thom M, Howard C, Villarreal-Ramos B, Mead E, Vordermeier M, Hope J. Consequence of prior exposure to environmental mycobacteria on BCG vaccination and diagnosis of tuberculosis infection. *Tuberculosis (Edinb)* 2008; **88:** 324–34.

77 Lee WJ, Kang SM, Sung H, et al. Non-tuberculous mycobacterial infections of the skin: a retrospective study of 29 cases. *J Dermatol* 2010; **37:** 965–72.

78 Correa AG, Starke JR. Nontuberculous mycobacterial disease in children. *Semin Respir Infect* 1996; **11:** 262–71.

79 Edeer Karaca N, Boisson-Dupuis S, Aksu G, et al. Granulomatous skin lesions, severe scrotal and lower limb edema due to mycobacterial infections in a child with complete IFN-γ receptor-1 deficiency. *Immunotherapy* 2012; **4:** 1121–27.

80 Lindeboom JA, Bruijnesteijn van Coppenraet LES, van Soolingen D, Prins JM, Kuijper EJ. Clinical manifestations, diagnosis, and treatment of *Mycobacterium haemophilum* infections. *Clin Microbiol Rev* 2011; **24:** 701–17.

81 Williams B, Neth O, Shingadia D, et al. *Mycobacterium kansasii* causing septic arthritis and osteomyelitis in a child. *Pediatr Infect Dis J* 2010; **29:** 88–89.

82 Powell DA, Walker DH. Nontuberculous mycobacterial endobronchitis in children. *J Pediatr* 1980; **96:** 268–70.

83 Dore ND, LeSouëf PN, Masters B, et al. Atypical mycobacterial pulmonary disease and bronchial obstruction in HIV-negative children. *Pediatr Pulmonol* 1998; **26:** 380–88.

84 Osorio A, Kessler RM, Guruprasad H, Isaacson G. Isolated intrathoracic presentation of *Mycobacterium avium* complex in an immunocompetent child. *Pediatr Radiol* 2001; **31:** 848–51.

85 Freeman AF, Olivier KN, Rubio TT, et al. Intrathoracic nontuberculous mycobacterial infections in otherwise healthy children. *Pediatr Pulmonol* 2009; **44:** 1051–56.

86 Ferrara E, Lemire J, Grimm PC, et al. Mycobacterial peritonitis in pediatric peritoneal dialysis patients. *Pediatr Nephrol* 2004; **19:** 114–17.

87 Song Y, Wu J, Yan H, Chen J. Peritoneal dialysis-associated nontuberculous mycobacterium peritonitis: a systematic review of reported cases. *Nephrol Dial Transplant* 2012; **27:** 1639–44.

88 Hatzenbuehler LA, Starke JR. Common presentations of nontuberculous mycobacterial infections. *Pediatr Infect Dis J* 2014; **33:** 89–91.

89 Kim YK, Hahn S, Uh Y, et al. Comparable characteristics of tuberculous and non-tuberculous mycobacterial cavitary lung diseases. *Int J Tuberc Lung Dis* 2014; **18:** 725–29.

90 Koh WJ, Yu CM, Suh GY, et al. Pulmonary TB and NTM lung disease: comparison of characteristics in patients with AFB smear-positive sputum. *Int J Tuberc Lung Dis* 2006; **10:** 1001–07.

91 Kendall BA, Varley CD, Hedberg K, Cassidy PM, Winthrop KL. Isolation of non-tuberculous mycobacteria from the sputum of patients with active tuberculosis. *Int J Tuberc Lung Dis* 2010; **14:** 654–56.

92 Olivier KN, Weber DJ, Wallace RJ, et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003; **167:** 828–34.

93 Roux A-L, Catherinot E, Ripoll F, et al. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in france. *J Clin Microbiol* 2009; **47:** 4124–28.

94 Leung JM, Olivier KN. Nontuberculous mycobacteria in patients with cystic fibrosis. *Semin Respir Crit Care Med* 2013; **34:** 124–34.

95 Esther CR, Henry MM, Molina PL, Leigh MW. Nontuberculous mycobacterial infection in young children with cystic fibrosis. *Pediatr Pulmonol* 2005; **40:** 39–44.

96 Leung JM, Olivier KN. Nontuberculous mycobacteria: the changing epidemiology and treatment challenges in cystic fibrosis. *Curr Opin Pulm Med* 2013; **19:** 662–69.

97 Seddon P, Fidler K, Raman S, et al. Prevalence of nontuberculous mycobacteria in cystic fibrosis clinics, United Kingdom, 2009. *Emerg Infect Dis* 2013; **19:** 1128–30.

98 Martiniano SL, Sontag MK, Daley CL, Nick JA, Sagel SD. Clinical significance of a first positive nontuberculous mycobacteria culture in cystic fibrosis. *Ann Am Thorac Soc* 2014; **11:** 36–44.

99 De Beaucoudrey L, Samarina A, Bustamante J, et al. Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* 2010; **89:** 381–402.

100 Hoshina T, Takada H, Sasaki-Mihara Y, et al. Clinical and host genetic characteristics of Mendelian susceptibility to mycobacterial diseases in Japan. *J Clin Immunol* 2011; **31:** 309–14.

101 Haverkamp MH, van de Vosse E, van Dissel JT. Nontuberculous mycobacterial infections in children with inborn errors of the immune system. *J Infect* 2014; **68** (suppl 1)**:** S134–50.

102 Shachor-Meyouhas Y, Sprecher H, Eluk O, Ben-Barak A, Kassis I. An outbreak of *Mycobacterium mucogenicum* bacteremia in pediatric hematology-oncology patients. *Pediatr Infect Dis J* 2011; **30:** 30–32.

103 Arlotta A, Cefalo MG, Maurizi P, Ruggiero A, Dodi I, Riccardi R. Critical pulmonary infection due to nontuberculous mycobacterium in pediatric leukemia: report of a difficult diagnosis and review of pediatric series. *J Pediatr Hematol Oncol* 2014; **36:** 66–70.

104 Kornreich L, Goshen Y, Horev G, Grunebaum M. Mycobacterial respiratory infection in leukemic children. *Eur J Radiol* 1995; **21:** 44–46.

105 Lawn SD, Lipman MC, Easterbrook PJ. Immune reconstitution disease associated with mycobacterial infections. *Curr Opin HIV AIDS* 2008; **3:** 425–31.

106 Steenhoff AP, Wood SM, Shah SS, Rutstein RM. Cutaneous *Mycobacterium avium* complex infection as a manifestation of the immune reconstitution syndrome in a human immunodeficiency virus-infected child. *Pediatr Infect Dis J* 2007; **26:** 755–57.

107 Orikiiriza J, Bakeera-Kitaka S, Musiime V, Mworozi EA, Mugyenyi P, Boulware DR. The clinical pattern, prevalence, and factors associated with immune reconstitution inflammatory syndrome in Ugandan children. *AIDS* 2010; **24:** 2009–17.

108 Giampaglia CMS, Martins MC, Inumaru VTG, Butuem IV, Telles MAS. Evaluation of a rapid differentiation test for the *MycobacteriuM tuberculosis* complex by selective inhibition with rho-nitrobenzoic acid and thiophene-2-carboxylic acid hydrazide. *Int J Tuberc Lung Dis* 2005; **9:** 206–09.

109 Martin A, Bombeeck D, Mulders W, Fissette K, De Rijk P, Palomino JC. Evaluation of the TB Ag MPT64 Rapid test for the identification of *Mycobacterium tuberculosis* complex. *Int J Tuberc Lung Dis* 2011; **15:** 703–05.

110 van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat* 2012; **15:** 149–61.

111 Bruijnesteijn van Coppenraet ES, Lindeboom JA, Prins JM, Peeters MF, Claas ECJ, Kuijper EJ. Real-time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of mycobacterial lymphadenitis in children. *J Clin Microbiol* 2004; **42:** 2644–50.

112 Danielides V, Patrikakos G, Moerman M, Bonte K, Dhooge C, Vermeersch H. Diagnosis, management and surgical treatment of non-tuberculous mycobacterial head and neck infection in children. *ORL J Otorhinolaryngol Relat Spec* 2014; **64:** 284–89.

113 Bruijnesteijn van Coppenraet LES, Kuijper EJ, Lindeboom JA, Prins JM, Claas ECJ. *Mycobacterium haemophilum* and lymphadenitis in children. *Emerg Infect Dis* 2005; **11:** 62–68.

114 Saubolle MA, Kiehn TE, White MH, Rudinsky MF, Armstrong D. *Mycobacterium haemophilum*: microbiology and expanding clinical and geographic spectra of disease in humans. *Clin Microbiol Rev* 1996; **9:** 435–47.

115 Kilby JM, Marques MB, Jaye DL, Tabereaux PB, Reddy VB, Waites KB. The yield of bone marrow biopsy and culture compared with blood culture in the evaluation of HIV-infected patients for mycobacterial and fungal infections. *Am J Med* 1998; **104:** 123–28.

116 Akpek G, Lee SM, Gagnon DR, Cooley TP, Wright DG. Bone marrow aspiration, biopsy, and culture in the evaluation of HIV-infected patients for invasive mycobacteria and histoplasma infections. *Am J Hematol* 2001; **67:** 100–06.

117 Ker C-C, Hung C-C, Huang S-Y, et al. Comparison of bone marrow studies with blood culture for etiological diagnosis of disseminated mycobacterial and fungal infection in patients with acquired immunodeficiency syndrome. *J Microbiol Immunol Infect* 2002; **35:** 89–93.

118 Prego V, Glatt AE, Roy V, Thelmo W, Dincsoy H, Raufman JP. Comparative yield of blood culture for fungi and mycobacteria, liver biopsy, and bone marrow biopsy in the diagnosis of fever of undetermined origin in human immunodeficiency virus-infected patients. *Arch Intern Med* 1990; **150:** 333–36.

119 Cappell MS, Schwartz MS, Biempica L. Clinical utility of liver biopsy in patients with serum antibodies to the human immunodeficiency virus. *Am J Med* 1990; **88:** 123–30.

120 Crump JA, Tanner DC, Mirrett S, McKnight CM, Reller LB. Controlled comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for detection of mycobacteremia. *J Clin Microbiol* 2003; **41:** 1987–90.

121 Morgan MB, Reves RR, Wilson ML, Stone BL, Burman WJ. Comparison of BACTEC 12B vs solid media for the recovery of *Mycobacterium avium* complex from blood cultures in AIDS patients. *Diagn Microbiol Infect Dis* 1997; **28:** 45–48.

122 Alfa MJ, Manickam K, Sepehri S, Sitter D, Lenton P. Evaluation of BacT/Alert 3D automated unit for detection of nontuberculous mycobacteria requiring incubation at 30 degrees C for optimal growth. *J Clin Microbiol* 2011; **49:** 2691–93.

123 Kordy F, Richardson SE, Stephens D, Lam R, Jamieson F, Kitai I. Utility of gastric aspirates for diagnosing tuberculosis in children in a low prevalence area: predictors of positive cultures and significance of non-tuberculous mycobacteria. *Pediatr Infect Dis J* 2014; published online Aug 6. DOI:10.1097/INF.0000000000000498.

124 Oberhelman RA, Soto-Castellares G, Gilman RH, et al. Diagnostic approaches for paediatric tuberculosis by use of different specimen types, culture methods, and PCR: a prospective case-control study. *Lancet Infect Dis* 2010; **10:** 612–20.

125 Sanchini A, Fiebig L, Drobniewski F, et al. Laboratory diagnosis of paediatric tuberculosis in the European Union/European Economic Area: analysis of routine laboratory data, 2007 to 2011. *Euro Surveill* 2014; **19:** 20744.

126 Peres RL, Maciel EL, Morais CG, et al. Comparison of two concentrations of NALC-NaOH for decontamination of sputum for mycobacterial culture. *Int J Tuberc Lung Dis* 2009; **13:** 1572–75.

127 Bange F-C, Böttger EC. Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2002; **21:** 546–48.

128 Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *J Clin Microbiol* 2004; **42:** 2321–25.

129 Corbett EL, Blumberg L, Churchyard GJ, et al. Nontuberculous mycobacteria: defining disease in a prospective cohort of South African miners. *Am J Respir Crit Care Med* 1999; **160:** 15–21.

130 Buijtels PC, van der Sande M, de Graaff CS, et al. Nontuberculous mycobacteria, Zambia. *Emerg Infect Dis* 2009; **15:** 242–49.

131 Al Jarad N, Demertzis P, Jones DJ, et al. Comparison of characteristics of patients and treatment outcome for pulmonary non-tuberculous mycobacterial infection and pulmonary tuberculosis. *Thorax* 1996; **51:** 137–39.

132 Daley CL, Griffith DE. Pulmonary non-tuberculous mycobacterial infections. *Int J Tuberc Lung Dis* 2010; **14:** 665–71.

133 van Ingen J, Kuijper EJ. Drug susceptibility testing of nontuberculous mycobacteria. *Future Microbiol* 2014; **9:** 1095–110.

134 van Ingen J, Egelund EF, Levin A, et al. The pharmacokinetics and pharmacodynamics of pulmonary *Mycobacterium avium* complex disease treatment. *Am J Respir Crit Care Med* 2012; **186:** 559–65.

135 Koh W-J, Jeong B-H, Jeon K, Lee S-Y, Shin SJ. Therapeutic drug monitoring in the treatment of *Mycobacterium avium* complex lung disease. *Am J Respir Crit Care Med* 2012; **186:** 797–802.

136 Zeharia A, Eidlitz-Markus T, Haimi-Cohen Y, Samra Z, Kaufman L, Amir J. Management of nontuberculous mycobacteria-induced cervical lymphadenitis with observation alone. *Pediatr Infect Dis J* 2008; **27:** 920–22.

137 Lindeboom JA. Conservative wait-and-see therapy versus antibiotic treatment for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Clin Infect Dis* 2011; **52**: 180–84.

138 Perdikogianni C, Galanakis E. Non-tuberculous mycobacterial cervical lymphadenitis in the immunocompetent child: diagnostic and treatment approach. *Expert Rev Anti Infect Ther* 2014; **12:** 959–65.

139 Parker NP, Scott AR, Finkelstein M, et al. Predicting surgical outcomes in pediatric cervicofacial nontuberculous mycobacterial lymphadenitis. *Ann Otol Rhinol Laryngol* 2012; **121:** 478–84.

140 Ahn CH, Lowell JR, Ahn SS, Ahn SI, Hurst GA. Short-course chemotherapy for pulmonary disease caused by *Mycobacterium kansasii*. *Am Rev Respir Dis* 1983; **128:** 1048–50.

141 Shafran SD, Singer J, Zarowny DP, et al, for the Canadian HIV Trials Network Protocol 010 Study Group. A comparison of two regimens for the treatment of *Mycobacterium avium* complex bacteremia in AIDS: rifabutin, ethambutol, and clarithromycin versus rifampin, ethambutol, clofazimine, and ciprofloxacin. *N Engl J Med* 1996; **335:** 377–83.

142 Karakousis PC, Moore RD, Chaisson RE. *Mycobacterium avium* complex in patients with HIV infection in the era of highly active antiretroviral therapy. *Lancet Infect Dis* 2004; **4:** 557–65.

143 Siberry GK, Abzug MJ, Nachman S, et al. Guidelines for the prevention and treatment of opportunistic infections in HIV-exposed and HIV-infected children: recommendations from the National Institutes of Health, Centers for Disease Control and Prevention, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *Pediatr Infect Dis J* 2013; **32** (suppl 2)**:** i–KK4.

144 Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the Prevention and Treatment of Opportunistic Infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009; **58:** 1–166.

145 Holland SM. Treatment of infections in the patient with Mendelian susceptibility to mycobacterial infection. *Microbes Infect* 2000; **2:** 1579–90.

146 Chantrain CF, Bruwier A, Brichard B, et al. Successful hematopoietic stem cell transplantation in a child with active disseminated *Mycobacterium fortuitum* infection and interferon-γ receptor 1 deficiency. *Bone Marrow Transplant* 2006; **38:** 75–76.

147 Roesler J, Horwitz ME, Picard C, et al. Hematopoietic stem cell transplantation for complete IFN-γ receptor 1 deficiency: a multi-institutional survey. *J Pediatr* 2004; **145:** 806–12.

148 Sester M, van Leth F, Bruchfeld J, et al. Risk assessment of tuberculosis in immunocompromised patients. A TBNET study. *Am J Respir Crit Care Med* 2014; **190:** 1168–76.

149 Basu Roy R, Sotgiu G, Altet-Gómez N, et al. Identifying predictors of interferon-γ release assay results in pediatric latent tuberculosis: a protective role of bacillus Calmette-Guerin? A pTB-NET collaborative study. *Am J Respir Crit Care Med* 2012; **186:** 378–84.

7. Mothers and caretakers understanding and care-seeking practices for paediatric tuberculosis in a rural district of Southern Mozambique

Mindu C, **Lopez-Varela E**, Joaquim Augusto J, Gondo K, Garcia-Bastero AL, Jahit Sacarlal J, Alonnso PL, Munguambe K.

Status: submitted for publication

Title: Caretakers' perspectives of paediatric TB and implications for care-seeking behaviours in Southern Mozambique.

Authors: Carolina Mindu[1], Elisa Lopez-Varela [1,2], Yara Alonso-Menendez[1], Yolanda Mausse[1], Orvalho Joaquim Augusto[1], Kizito Gondo[1], Jose Múñoz[1,2], Jahit Sacarlal[1,4], Alberto L. Garcia-Basteiro[1,2,3], Pedro L. Alonso[1,2], Khátia Munguambe[1,4]

(1) Centro de Investigação em Saúde de Manhiça (CISM). Rua 12, Cambeve CP 1929 Maputo, Mozambique

(2) ISGlobal, Barcelona Global Health Institute, Hospital Clınic – Universitat de Barcelona, C. Rosselló,132, 08036, Barcelona, Spain

(3) Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, The Netherlands

(4) Faculdade de Medicina, Universidade Eduardo Mondlane (UEM). Av. Salvador Allende 702, Maputo, Mozambique

Corresponding author:

Khátia Munguambe.

Centro de Investigação em Saúde de Manhiça (CISM), Rua 12, Cambeve CP 1929 Maputo, Mozambique.

Email: khatia.munguambe@manhica.net

Abstract

Background: Tuberculosis (TB) continues to be an important public health concern, especially in poor resourced settings. TB diagnosis is challenging, particularly among children, which are the most vulnerable to its´ impacts. Lack of knowledge and awareness of the disease compromises prompt diagnosis and treatment compliance.

Objective: To gain insights regarding caretakers´ knowledge of the aetiology and prevention of paediatric TB in southern Mozambique, to describe their care-seeking behaviours and to assess the acceptability of diagnostic procedures.

Methods: A total of 35 caretakers were interviewed, all of which had children with TB compatible symptoms. Eleven were caretakers of children diagnosed with TB, 11 of children for whom TB was discarded and 13 of undiagnosed children. The first two groups took part in a TB incidence study, whilst the third group did not. All underwent the same semi-structured interviews, the results of which were analysed and compared using content analysis.

Results: Even when confronted by signs suggestive of TB, most caretakers never suspected it or misinterpreted the signs, even among TB positive caretakers and those with TB contacts. There was limited knowledge of TB, except among those undergoing treatment. The transgression of social norms was often presented as explanation for TB in parallel to medically sound causes. The use of traditional

care for prevention is widespread, but for treatment purposes it varied. TB diagnostic procedures were considered painful but were unanimously tolerated.

Conclusions: There is little understanding that TB affects children. Caretakers are poorly equipped with skills to recognize the TB signs and symptoms which are often mixed with other diseases. Moreover, the causes of TB are also not understood and often mixed with traditional concepts. It is probable that health care seeking behaviours are influenced by this conceptualisation of TB.

Implications: To overcome the potential delays in treatment and poor adherence, health promotion messages should be consistent and repetitive, emphasising that TB can also occur in children, differentiating it from HIV, and taking into account that TB is often understood as an outcome of impurity derived from the transgression of social and cultural norms.

Introduction

Tuberculosis (TB) is an under-recognized but important cause of morbidity and mortality among children from high TB burden countries (HBC)(1,2). It is estimated that only one third of paediatric TB cases in these settings are detected and reported to the National TB Control Programs (NTP)(3,4). This is mostly due to the difficulties in diagnosing paediatric TB, which in turn leads to under-diagnosis, misdiagnosis or delayed diagnosis. Not only obtaining samples for microbiological examination is often challenging in young children but also traditional microbiologic tests perform poorly. Additionally, the spectrum of TB disease in children is broad with a significant confounding effect of HIV on the clinical presentation(5), and overlap with other common conditions such as pneumonia or severe malnutrition. Because TB commonly affects children from the poorest communities with limited access to health services, the barriers in reaching an accurate TB diagnosis are aggravated by the complex patterns of care seeking(6,7), increasing the risk of rapid disease progression and mortality in young children(5).

Few studies have examined the perceptions of local communities regarding the aetiology and prevention of TB(8–10). This number lessens further when considering paediatric TB(11,12). Yet understanding how TB is conceptualised is crucial, not only to comprehend the causes behind delays in treatment, but also to inform strategies to increase awareness and change behaviour. Although most of the available qualitative research on TB has focused on the behavioural perspective, it has generally been directed at

improving adherence to health facility based treatment among adults(13–16). In many settings, adult TB patients have reported some form of self-treatment or treatment prescribed by traditional healers (TH) prior to presentation to the formal health services(15,17,18). There is still little understanding about the care-seeking practices particular to paediatric TB as well as the patterns that these itineraries follow. Understanding caregivers' recognition of paediatric TB could thus contribute to improved detection and treatment of paediatric TB cases.

This study sought to describe local understandings of the aetiology and prevention of paediatric TB among caretakers in Southern Mozambique, identify the care-seeking practices related to this disease and explore the relationship between the former and the latter. Moreover, it aimed to evaluate the acceptability of paediatric TB diagnostic procedures among caregivers who seek care at health facilities.

Materials and Methods

Study site and population

The study was conducted in Mozambique, in the districts of Manhiça and Bilene-Macia, both located in the southern region of the country. Mozambique has one of the highest TB and HIV burdens worldwide(19). According to the latest World Health Organization (WHO) Global TB report, it ranks first and second in TB mortality among HIV positive population and overall TB incidence

respectively(20). Most new-born children in the country receive Bacille Calmette-Guerin (BCG) vaccination, which has a coverage of around 86-94%(21,22). TB treatment is offered free of charge by the NTP, down to the level of the peripheral health centres, where paediatric combined formulations are also available.

Manhiça is a rural District within Maputo Province, where the Centro de Investigação em Saúde de Manhiça (CISM) runs a Health and Demographic Surveillance System (HDSS) of a population served by the Manhiça District Hospital (MDH) and 10 peripheral health centres (23). At the time of the study, the HDSS covered a population of around 92,000 inhabitants (out of a total population of 160,000), of which approximately 11% were <3yr. The<5yr mortality rate was 70/1000 live births(24). The population belongs mainly to the Changana ethnic group and is mostly comprised of subsistence farmers, informal traders, employees of sugar estates, and migrant labourers in South Africa. Community based studies performed at CISM have shown an HIV prevalence of 39.9% among individuals aged 18-47 years(25) and a minimum community based TB incidence rate of 470 per 100.000 person-year under the age of three(26).

The second study site, the neighbouring district of Bilene-Macia located in Gaza Province, is also a rural area belonging to the Changana ethnic group. It has an area of 2.180 Km^2 and 151,548 inhabitants(27), primarily employed in small-scale farming and, to a lesser extent, in informal commerce.. The<5yr mortality rate was 82/1000 live births at the time of the study(27). The reasons for

extending the study area to Bilene-Macia are the following. Firstly, given that it is a neighbouring district of Manhiça, and has an ethnic background similar to Manhiça, it allows for comparison at a cultural level. Secondly, it differs from Manhiça in that it does have a history of clinical research such as that of Manhiça, a factor that may have influenced the care-seeking practices of its inhabitants in favour of formal care. Thirdly, the district of Bilene-Macia has lower formal health-care coverage than Manhiça and could thus potentially differ regarding the care-seeking practices of its population.

Study design and procedures

This was a qualitative study which recruited caretakers of children with presumptive TB in whom the disease was ruled out after clinical evaluation at the MDH (Group A), caretakers of children diagnosed with TB also at the MDH (Group B), and caretakers of children with TB compatible symptoms identified by TH (Group C). A minimum sample size of 10 caretakers per group was foreseen in order to fulfil the study objectives.

Groups A and B were recruited and interviewed in Manhiça in parallel to a larger prospective childhood TB incidence study(28), which used active and passive case detection strategies to identify cases with TB compatible symptoms (see Box 1). Presumptive cases were then evaluated through physical examination, tuberculin skin and HIV testing, chest X Ray as well as microbiological evaluation of samples obtained through same day induced sputum (IS) with nasopharyngeal suction and gastric aspirate (GA). A sub-sample of these caretakers was selected

to take part in the qualitative study. Recruitment for the qualitative study was done by requesting the clinician for caretakers ≥ 18 years old, accompanying children between 1-3 years with criteria to participate in the incidence study, residing in the district of Manhiça and willing to participate in the qualitative study. Assignment to group A or B took place after being examined for TB and either having the disease ruled out (group A) or receiving a positive TB diagnosis (group B).

A third group (group C) consisting of caretakers of children between 1-3 years with TB compatible symptoms recruited outside the study area of the TB incidence study was included in order to explore the same issues but outside the framework of a clinical research project. For this purpose, a group of TH in the district of Bilene-Macia was trained to screen presumptive TB patients. The training did not focus on TB terminology, instead it skilled TH to identify and recruit caretakers with children presenting a combination of signs, symptoms and other precedents leading to suspicion of TB (Box 1). Caretakers were identified either through their visit to the TH or by fieldworkers, in collaboration with THs and district health authorities, actively seeking them out within the immediate community of the TH.

Data collection and analysis

Data was collected through semi-structured interviews (SSI) by two trained Mozambican social science research assistants (CM and YM), supported by two local interviewers.

Caretakers were all invited for a SSI focused on their knowledge of TB (aetiology and prevention), care seeking practices, including the extent to which they sought alternative care, their initial interpretation of their child's symptoms, their itinerary and experiences from their perceived onset of the illness until the moment of diagnosis, and their assessment of the diagnostic procedure and results[see Box 2 for overview of questions]. Participants were visited at their households, explained the nature of the study, invited to participate and consulted on the appropriate date to conduct the interview. The majority of interviews took place at the participants' homes. The majority of interviews were conducted in Changana language and very few were conducted in Portuguese. Choice of language was determined by participants' convenience. All interviews were tape-recorded, recordings we transcribed verbatim and the data was analysed and compared following content analysis.

Ethical considerations

The study protocol was approved by the National Health Bioethics Committee of Mozambique and the Ethics Review Committee of the Hospital Clinic of Barcelona. Participation was voluntary and written informed consent was obtained from all the caretakers participating in the study .

Results

Characteristics of respondents

A total of 35 participants were included in the study (11 in groups A and B, respectively, and 13 in group C). All caregivers were female and 90% of them

were the mothers themselves. Detailed socio-demographic characteristics of the caregivers and their children are presented in Table 1.

Identification and interpretation of TB compatible signs and symptoms

When responding to the open ended question about what triggered care-seeking at the health facility, the most common complaints were fever, prolonged cough, diarrhoea, respiratory distress and oedema. The most cited suspected illness was asthma also referred to illness of the chest (*xifuva*) (n=13), followed by HIV (n=5), 'lack of blood' (*kuhela ngati*) (n=4), flu (n=4), malaria (n=2) and *mavabji ya n'weti* (n=2) - an locally defined as "illnessof the moon', essentially characterized by convulsions and often translated as epilepsy due to its presentation.

> *"Eh, I thought of all illnesses, even HIV. This is why I had to go test him and they said he didn't have it [HIV]"* – Caretaker, Group B

Only two caretakers suspected TB (one of which was a TB patient herself); the first cited TB by itself and the second cited it along other possibilities, such as HIV and *nkhonlhole* - a locally identified illness, often translated as TB, and described as intensive, prolonged coughing (sometimes with blood expectoration), chills and loss of appetite, that is commonly associated with adults. On the other hand, the rest of the caretakers who were either TB patients themselves or had household TB contacts did not suspect of TB (n=6).

Approximately a third of the caretakers stated they suspected no illness (n=10). Several of them cited reasons for this, including that the symptoms were not

worrisome enough, that it is simply expected that children become ill, or that there was no apparent cause (for example, a death in the household or near vicinity, or the presence of adultery). (See Table 2)

> *"… I didn´t suspect anything […] I wouldn´t think that something like this could have happened because I didn't think that in my house there are problems… The problem is when there is a death and you fail [to follow the rituals]…it may happen that the child falls [ill]."*– Caretaker, Group A

Knowledge and perceptions on the aetiology and prevention of TB

When enquired on their knowledge of the aetiology of TB, many respondents gave several and distinct answers. Amongst groups A and B, over half of the caretakers showed some knowledge about the aetiology of TB (n=12), which was in-line with the usual health promotion messages. These groups´ medically sound knowledge on TB aetiology or transmission modes included coughing (n=4), coughing and sharing of food or utensils (n=4), or contact with a TB infected person (n=4). However, it is important to note that, with the exception of one, all of the caretakers that indicated coughing as a mode of transmission belonged to group B (TB positive children), and all who suggested contact with a TB positive person either were TB patients themselves or had a household contact.

Of note, many of those who revealed perceptions of TB in line with biomedical knowledge simultaneously provided other radically different answers. An

important number of caretakers (n=8) reported that TB results from transgression of social norms, such as adultery (n=3), the neglect of death-related rituals (n=4), or of rituals in general (n=1). In the first case, the deceased is usually a household member, whose death facilitates the transmission of TB, often as a result of not performing *kutxinga* (a purification ceremony, involving among other practices the engagement by a couple of close relatives of the deceased in a sexual act carried out after the death of a relative), which, if not carried out appropriately, is believed to bring misfortune to the family, including TB. In the case of adultery, its presence is perceived to cause the transmission of TB through the sharing of objects.

> [Reflecting on her reaction after being informed her son had TB]
> *"Oh! I became surprised 'eh! Can you get this illness when you never committed adultery?'"* – Caretaker, Group B

Other mentioned forms of acquiring TB were stepping on infected sputum (n=1), through a virus (n=1), suffering from low weight (n=1), exposure to dust and cold (n=1) and the 'separation of lungs' (n=1). On the other hand, a significant amount of the caretakers (n=8) claimed not to know about the aetiology or transmission of TB, despite the fact that three of them did have TB themselves.

Amongst group C, the vast majority of caretakers (n=10) expressed no knowledge of the aetiology of TB, whilst only one indicated coughing as a mode of transmission. On the other hand, one caretaker stated that a possible cause

was sharing food with a mourning person, whilst another claimed close contact with a person that has committed adultery (in this case by sharing objects which become “hot” upon the contact with an adulterous person) as a cause of TB. Both scenarios imply impurity, either by the act itself (adultery) or as a consequence of neglecting a death-related purification ritual.

> *"Yes, I thought maybe her blood had finished [...] Then I thought: her stomach is large. So I said that maybe someone who had committed adultery had given her something, something hot "* – Caretaker, Group C

In terms of prevention, groups A and B pointed out various possible methods. The most cited form of prevention was avoiding the sharing of food or related utensils (n=5), followed by avoiding the cold (n=1), covering the mouth whilst coughing (n=1), avoiding sleeping beside a person infected with TB (n=1), and avoiding sexual intercourse during the lactation period (n=1). The latter refers to the perceived health risk that mothers having sexual intercourse within the first 2-3 months after giving birth entails for their children, including acquiring TB. Finally, a vast majority of caretakers from groups A and B (n=15) stated they did not know how TB could be prevented.

On the other hand, the vast majority (n=11) of caretakers of group C indicated they did not know any possible method of prevention. Only two suggested the consumption of minced garlic as a form of prevention (see Table 3).

Care-seeking practices

The vast majority (n=18) of caretakers of groups A and B sought alternative care at some point during the child's lifetime. Cultural norms require the performance of the *kutsivelela* ritual soon after the birth of a child. This ritual is performed by a TH and precedes the administration of a traditional remedy to prevent the aforementioned illness of the moon. All participants that reported seeking alternative care (N=18) had administered this remedy to their children. Most (n=13) reported to have sought TH solely for the purpose of prevention and claimed this was the sole remedy their children had ever received from a TH. The remaining reported having sought additional care from TH (n=3) or administered homemade remedies (n=2) in order to treat their child's current illness.

> *"He/she may be 2 months old, one month, and has to take that remedy for... to make disappear the illness [n'weti]... that one of old tradition"* – Caretaker, Group B

In the case of group C, the majority (n=9) also reported having sought alternative care through TH at some point during the child's lifetime. However, not all reported administering the remedy for *n'weti* prevention. Approximately half (n=4) claimed to have sought TH solely for the purpose of treating their child's current symptoms. The rest claimed it was for prevention (n=3), or for prevention and treatment purposes (n=2). Thus, almost half (n=6) of the caretakers of group C had sought TH for treatment purposes, in contrast to merely three caretakers (1/3) in groups A and B.

All caretakers in the 3 groups had sought care in the formal system at some point. However, those that had also sought alternative care seemed to approach both systems in no particular order; and often in a circular manner. The following is an excerpt from an interview with a caretaker of group C that suitably illustrates this circularity:

> *Interviewer - When she began to get ill, what was the first thing you did here at home?*
>
> *Respondent - I took her to the hospital [...] First I gave her conventional medicine [...] I saw she was not improving so I gave her traditional medicine and then I saw she was still not responding so I returned to the hospital*

When asked about what care is available to treat TB, over half (n=12) of the caretakers of groups A and B claimed that the only place where it can be treated is the hospital. Few (n=3) had heard that TH could treat it, though they claimed to not believe it. A considerable amount (n=9) believed that both systems could treat TB. Only one caretaker stated not to know if either could treat it. In contrast, only a minority (n=5) of group C claimed that TB can only be treated at hospitals. The majority (n=8) believed that both the formal and alternative care systems can treat TB.

Those caretakers who consider that TH cannot treat TB do so because they believe that since on the one side there are traditional illnesses and on the other

conventional illnesses, each domain has its own sphere of illnesses it can diagnose and treat.

> *"This remedy [for n'weti] works according to that illness that has to be treated when children are young... this medicine cannot cure those other [conventional] illnesses"* – Caretaker, Group A

> *"You get sick with that illness and you go to the TH, but there are illnesses that do not go according to tradition..."* –Caretaker, Group B

In terms of treatment preference, all caretakers of groups A and B claimed to prefer hospitals to TH because they considered them financially accessible, safer and more effective. On the other hand, a significant number (n=4) of caretakers of group C claimed to consider both equally capable and expressed no particular preference. The only concern raised regarding treatment by TH was the financial burden it often supposed, in contrast to public hospitals where HIV, malaria and TB treatment is free of charge (see Table 4).

> *"First I went to the TH but I did not have enough money, so I realised that in the hospital it is simpler"* – Caretaker, Group C

Acceptability of TB diagnosis and diagnostic procedures

Although diagnostic techniques, especially the induced sputum, were perceived by caretakers to be harmful for children, they were in fact tolerated. Table 5 summarises the feelings expressed by mothers and caretakers who witnessed

the procedures. Half of the caretakers (n=11) stated they felt fearful and all felt discomfort whilst watching what seemed to be a very painful procedure.

> *"They did... they did that thing of inserting small tubes and then inserting them in the mouth to take out sputum... Ah! I was sacred! I also cried... I was scared because I had never seen that done to a child..."* – Caretaker, Group A

Despite the procedures being considered invasive, aggressive and painful, all caretakers accepted their need, which was explained in terms of their trust in the formal system and the power of technology.

> *"But it is the machines that detect it. Not even the doctor... I don't disagree with what it detects... So it can still happen that I say my child has no illness but when the machine sees it I have to believe it because once he started to take the pills he became better"* – Caretaker, Group B

In terms of accepting the positive TB result, many (n=4) caretakers of group B did not believe a child under 3 could have TB, and thus expressed surprise upon hearing the result. Further reasons for such surprise were the absence of perceived causes, such as the lack of close deaths and/or adultery. In contrast, those that already had a close TB contact, or were TB positive themselves, found it easier to accept the results.

"In children I did not believe it [...] What made me change my mind, even though I thought that you got the illness when there is a death in the family, was that then I could see that even if there is no death the child can get the illness because I now saw she was ill." – Caretaker, Group B

Discussion

This study substantially adds to the yet limited body of evidence regarding paediatric TB from the socio-behavioural perspective(29–33). Overall, the results of this study indicate a very low level of awareness amongst caretakers of small children, including those vulnerable or exposed to TB, in southern Mozambique regarding the symptoms compatible with paediatric TB.

The fact that misinterpreting symptoms can delay appropriate care seeking has been well documented(8,10,34). In the present study, an insignificant number of caretakers across all groups interpreted their child's symptoms as potential TB, even amongst those that either had TB themselves or had a TB contact in the household. However, the most cited illness under suspicion was asthma, followed by HIV, both of which are illnesses that are easily, and often, confounded with TB, even by health workers(31,34). In line with these results, a recent study conducted in Kenya concluded that TB symptoms are often confused with HIV, malaria and asthma(8). The mixing-up with HIV may discourage caretakers from facing the problem, due to the stigma already attached to it, resulting in inappropriate care seeking.

On the other hand, a third of all caretakers did not suspect of any particular illness. Although this did not mean that they did not eventually seek care, it could have potentially delayed the process. Some of the reasons cited for not suspecting of any illness were that it was deemed natural that a child falls sick, or that the expected causes (adultery, close death) were not present, and thus the child could not become seriously ill.. Moreover, this inability to interpret the child's illness could be further aggravated by the fact that TB symptoms are sub-acute and insidious and can be easily perceived as mundane afflictions. This was also found to be a delaying factor in various studies(6,11,35). In a Peruvian study it was found that 'non-alarming' symptoms common to TB were not considered sufficiently worrisome to justify care seeking. Finally, as caretaker's reactions to diagnosis revealed, the recurrent perception of TB as an illness of adults could further contribute to the misinterpretation of symptoms.

The results also revealed a considerable lack of knowledge regarding the aetiology of TB. As expected, this was especially exacerbated amongst caretakers of group C, given that only one suggested coughing as a mode of transmission. However, in spite of having participated in the incidence study, caretakers of group A presented practically the same level of understanding of TB transmission to that of group C. These findings are in line with those found in a recent study conducted in China, according to which 60% of caretakers of children recently diagnosed with TB were unaware of the modes of transmission(11). In contrast, caretakers of group B presented a relatively high level of understanding, with over half of respondents reporting coughing as a form of transmission. This gap in knowledge could be due to the fact that group

B was more exposed to biomedical knowledge of TB, given that they initiated TB treatment, which implies weekly visits to the NTP accompanied by some counselling. These findings are to some extent corroborated by those of a recent study conducted in India, on the knowledge of TB among caretakers of children undergoing treatment. The study found that, in spite of being under treatment, only 42% of caretakers were aware of TB transmission modes(12).

Medically sound knowledge of TB was mostly presented in parallel to socio-culturally derived knowledge. These 'alternative accounts' of the causes of TB were either related to the presence of a nearby death (either through ritual neglect or contact with a mourning person) or the presence of adultery (usually transmitted through the sharing of objects). These two accounts reflect a perceived connection between the causation of illness and the notion of impurity. This association has been previously observed in cases of contagious diseases in various locations, including Southern Mozambique(36). According to Green, illnesses that are bio-medically classified as contagious are often understood in a quasi-naturalistic fashion since they are associated with pollution through impurity, rather than in terms of supernatural causes such as witchcraft or sorcery.

Previous studies have also identified a variety of misconceptions related to the acquisition of TB(8,34,37). These causes range from being bewitched, to being a victim of 'the evil eye' or simply through sexual intercourse. This association is not only pertinent to understanding the timeliness of care-seeking practices but,

in relation to adultery as a cause, it is also indicative of the perceived association between HIV and TB.

Overall, in this study the response to the results was one of surprise, either due to disbelief at the possibility of children acquiring TB or due to the absence of perceived TB causes. In terms of the acceptability of the diagnostic procedure, the results reveal a high degree of acceptance that is consistent across groups. The majority of caretakers reported feeling fearful and/or considered the procedure to be invasive, aggressive or painful, yet they all claimed to consider it necessary and thus tolerable. This observation correlates with previous studies assessing other diagnostic/prevention tools undertaken in the same setting(38–41). These studies concluded that the hospital is highly regarded and that patients often expressed their trust and obedience towards the procedures in terms of following 'the law of the hospital'. Few studies have aimed to assess the acceptability of TB diagnostic procedures in children, although these findings do correlate with the positive results attained in a study conducted in Peru(42), which found that 84% were willing to undergo a second procedure.

Practically all caretakers seek some form of alternative care through TH, although the results revealed important differences between the two sites; one limiting it to prevention and the fulfilment of social norms and the other emphasising treatment. This discrepancy was expected and thus confirms our initial assumptions, which are in turn based on the aforementioned factors that distinguish the two districts; 1) the lower level of formal health-care coverage

present in the district of Bilene-Macia, and 2) the history of influence of a research centre on the population of Manhiça.

Moreover, the results revealed that the care-seeking pattern of those that did indeed seek treatment in both the formal and alternative systems consists of a rather circular and complex itinerary; the path seems to consist of a continuous exchange between the two until the patient is cured. This reveals three important concerns. Firstly, in cases where the patient begins at the TH, this entails a considerable delay in reaching the formal system, an issue that is particularly concerning in the case of paediatric TB. Secondly, it places considerable pressure on the formal system to successfully treat their patients in order not to lose them, at least temporarily, to the alternative care system. Finally, it implies that those patients that seek both systems of care may be mixing both treatments, which may further delay the curing process. These care-seeking patterns are not only relevant in terms of delaying a first contact with the formal health system but also hold implications for treatment adherence. A previous study of paediatric TB adherence in Manhiça showed that a quarter of cases under treatment failed to adhere adequately, either by ceasing the treatment or delaying its completion by over 3 weeks(44). In this light, the findings of this study could provide an explanation for this failure; TB patients that abandon and/or pause treatment may be being lost to the alternative care system.

Similarly complex patterns of care seeking have been found in studies across Africa(17,35,37,43). In contrast with the present results, these studies claim that, amongst those that seek alternative care, the itinerary always begins with the

TH. However, according to the results of this study the itinerary may equally begin at the formal or alternative system. In line with the results of this study for group C, other studies in Sub-Saharan Africa have found a rate of TH use for the treatment for TB to be as high as 40%(17,18). Several studies also assessed the extent to which this delayed formal care seeking and concluded an average of 2 months(7,35).

This study has some limitations. Firstly, the sample of this study is not representative of those that do not ever seek health care in the formal sector. Due to the recruitment method applied, all participants from groups A and B (with the exception of one) and all caretakers of group C had sought care at the hospital at some point in order to treat their TB compatible symptoms. Secondly, it is unclear for any of the groups, but particularly those in Manhiça, the extent to which the research centre´s interviewer inhibited participants from talking openly about their alternative health-care seeking practices, given that the research centre is intimately related to the district hospital and is often perceived to be aligned with the same formal health-care system objectives. Although this does not explain the discrepancy between the results of the two settings, it must be taken into consideration whilst assessing all interpretations.

Conclusion

The knowledge of TB among caretakers from this area of Southern Mozambique is very low and paediatric TB is seldom suspected and often confounded by other illnesses, even among TB positive caretakers and those with TB contacts. TB is often understood as an outcome of impurity derived from the transgression of

social and cultural norms. The pursuit of traditional medicine for childhood illness prevention seemed almost universal. However, care-seeking practices and the belief in the power of traditional versus conventional medicine to treat the current episode varied between the two study sites. TB diagnostic procedures were unanimously accepted and tolerated by caretakers regardless of the transitory feelings of fear they elicited.

In order to overcome potential delays in treatment and lack of adherence, health promotion messages should be informed by the aforementioned findings. Messages should emphasise that TB is not solely an illness of adults, that although associated with HIV, these are distinct diseases and that due to the possibility of confounding with other illnesses it should be ruled out at first suspicion. Finally, a significant effort should be made to inform people of the lengthy and complex nature of TB diagnosis and treatment in order to encourage compliance with the formal system in favour of alternative care.

Acknowledgements

The authors would like to thank the children and their families participating in the study. They are also grateful to the health staff of Manhica District Hospital.

Tables and Figures

Box 1: Signs, symptoms and history considered TB compatible for the purpose of this study

Symptoms suggestive of TB
Cough for ≥14 days not responding to a course of antibiotics
Referred fever for ≥14 days after common causes like malaria were excluded
Weight loss/failure to thrive (defined as under 60% weight for height, failure to gain weight for >2 months, any loss of weight unresponsive to nutritional rehabilitation)
Unexplained wheeze ≥14 days not responding to standard treatments
Lower respiratory tract infection ≥14 days not responding to antibiotics after 72 hours
TB exposure in the last 12 months
Symptoms compatible with EPTB (such as painless enlarged lymph nodes with or without fistula formation ≥14 days, arthritis, gibbus, meningitis, effusion or unexplained hematuria, dysuria or polaquiuria for ≥21 days)

Box 2: Interview guiding questions

Guiding Questions
How do you think TB is transmitted?
What do you believe causes it?
Is it possible to prevent it? How so?
What made you take the child to the hospital?
What symptoms did s/he present?
How did you interpret these? Did you suspect of any illness?
How did you feel about the diagnostic procedure?
Did you seek any other treatment before the hospital? If so, which kind?
What is your preferred form of treatment?

Table 1: Socio-demographic characteristics of caregivers and children[1]

Characteristics of Caregiver [2]	**Frequency (N, %)**
Relationship	
Parent (mother)	28 (90%)
Aunt	2 (6%)
Grandmother	1 (3%)
Formal education	
Secondary	18 (58%)
Primary	9 (29%)
None	2 (6%)
Unknown	2 (6%)
Occupation	
Formally unemployed	11 (35%)
Farmer	6 (19%)
Unknown	6 (19%)
Formally employed	2 (6%)
Sugar estate worker	1 (3%)
Student	1 (3%)
Seller	1 (3%)
Marital status	
Married	11 (35%)
Unknown	16 (52%)
Divorced	2 (6%)
Single	2 (6%)
Characteristics of Child	
Sex	
Male	18 (51%)
Female	17 (49%)
Characteristics of Household[3]	
Nº people	
<5	4 (22%)
5-10	10 (56%)
>10	4 (22%)
Nº children	
1-5	12 (67%)
>5	6 (33%)
TB contact	7 (32%)
Caretaker	4
Household contact	3

[1] Since the vast majority of participants of the incidence study were recruited passively (only 6% were recruited actively), all participants of group A and B, with the exception of one, were recruited from this group.

[2] We were unable to identify demographic data of 4 of the caregivers from groups A and B

[3] This data was only collected for those that participated in the incidence study (groups A and B)

Table 2: Condition suspected by mothers and caretakers of presumptive TB cases

Suspected condition (nº of caretakers)	Signs described (nº of caretakers)
None (10)	Low weight/loss of appetite (7), fever (5), cough (6), seizures (0), diarrhoea (2), oedema (2), rhinorrhoea (1), headache (1), epistaxis (1), difficulty breathing (1)
HIV (5)	Low weight/loss of appetite (2), cough (3), diarrhoea (4), oedema (1), fever (1), vomiting (1), loss of strength (0), rhinorrhoea (1), difficulty breathing (2), bad general appearance (1)
Asthma (13)	Low weight/loss of appetite (4), cough (13), diarrhoea (2), bad general appearance (2), difficulty breathing (8), fever (4), vomiting (2), chest pain (1)
TB (2)	Low weight/loss of appetite (1), cough (2), diarrhoea (1), oedema (1), rhinorrhoea (0), loss strength (0)
Flu (4)	Cough (4), low weight (1), fever (3), bad general appearance (1), rhinorrhoea (1), difficulties breathing (1), chest pain (1), vomiting (1)
Illness of the Moon (2)	Cough (1), fever (1), difficulty breathing (1), seizures (1), low weight (2)
Lack of blood (4)	Fever (2), oedema (2), difficulty breathing (2), cough (2), vomiting (1), low weight (1), diarrhoea (1)
Malaria (2)	Fever (2), cough (2), difficulties breathing (1)

Table 3: Caretaker´s knowledge and perception on TB aetiology and prevention

Caregivers (N)	Aetiology (N)	Prevention (N)
Group A - non TB cases (11[4])	Unknown (6)	Unknown (9)
	Death (1)	Avoid sharing food (1)
	Dust, cold (1)	Avoid sex during lactation (1)
	Low weight (1)	
	Cough (1)	
	TB contact (1)	
	Virus (1)	
	Separation of the lungs (1)	
Group B - TB cases (11)	Cough, sharing of utensils (4)	Unknown (6)
	Cough (3)	Avoid sharing food/utensils (4)
	TB contact (3)	Avoid sleeping close to TB patient (1)
	Adultery (3)	Having cold drinks (1)
	Death (3)	Cover mouth (1)
	Unknown (2)	
	Stepping on TB infected sputum (1)	
	Ritual neglect (1)	
Group C -non TB cases (13)	Unknown (10)	Unknown (11)
	Cough (1)	
	Death (1)	Consuming minced garlic (2)
	Adultery (1)	

[4] The numbers of group B at times (in the case of causes and prevention) surpass the total number of caretakers because in a few occasions a given respondent would provide various different answers.

Table 4: Care-seeking practices

Practices	Groups A & B (22)	Group C (13)
Sought alternative care	18	9
Prevention only	13	3
Treatment only	0	4
Prevention & Treatment	5	2

Table 5: Different attitudes and reactions of caretakers facing the invasive TB diagnostic

	Category	Nr of respondents	Illustrative quotes
Attitude facing procedure	Scared	9	"When they inserted the tubes I got very scared, I did not have the courage to look…"
	Pity/ Worried about child´s reaction	7	"I didn´t look because my heart hurt…the child cried a lot"
	Angry/ sad/ anguished	7	"I was angry… felt sorry for him, because he screamed…I could tell that they were hurting him"
	Neutral	3	"I don´t know, I just wanted to see my daughter get well"
Acceptability	Acceptable	11	"It is acceptable because the child go well after they diagnosed him"
	Acceptable but necessary	15	"It is too invasive and painful, but it is necessary"
	Neutral	1	N/A

Bibliography

1. Graham SM, Sismanidis C, Menzies HJ, Marais BJ, Detjen AK, Black RE, et al. Importance of tuberculosis control to address child survival. Lancet (London, England) [Internet]. 2014 May 3 [cited 2016 Jun 28];383(9928):1605–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24671079
2. Marais BJ, Graham SM, Maeurer M, Zumla A. Progress and challenges in childhood tuberculosis. Lancet Infect Dis [Internet]. 2013;13(4):287–9. Available from: http://dx.doi.org/10.1016/S1473-3099(13)70031-8
3. Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Glob Heal. 2014 Jul;2(8):e453–9.
4. López-Varela E, Augusto OJ, Guerra L, Respeito D, Sacoor C, Sacarlal J, et al. Low paediatric tuberculosis case detection rate in Southern Mozambique. Eur Respir J. 2015 Dec;ERJ – 01454–2015.
5. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med [Internet]. 2012 Jul 26 [cited 2016 Jun 28];367(4):348–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22830465
6. Paz-Soldan VA, Alban RE, Dimos Jones C, Powell AR, Oberhelman RA. Patient Reported Delays in Seeking Treatment for Tuberculosis among Adult and Pediatric TB Patients and TB Patients Co-Infected with HIV in Lima, Peru: A Qualitative Study. Front Public Heal [Internet]. 2014 Dec 22 [cited 2016 Jun 28];2:281. Available from: http://journal.frontiersin.org/article/10.3389/fpubh.2014.00281/abstract
7. Barker RD, Millard FJC, Malatsi J, Mkoana L, Ngoatwana T, Agarawal S, et al. Traditional healers, treatment delay, performance status and death from TB in rural South Africa. Int J Tuberc Lung Dis [Internet]. 2006 Jun [cited 2016 Jun 28];10(6):670–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16776455
8. Liefooghe R, Baliddawa JB, Kipruto EM, Vermeire C, De Munynck a O. From their own perspective. A Kenyan community's perception of tuberculosis. Trop Med Int Health. 1997;2(8):809–21.
9. Kaona FAD, Tuba M, Siziya S, Sikaona L. An assessment of factors contributing to treatment adherence and knowledge of TB transmission among patients on TB treatment. BMC Public Health. 2004;4(January):68.
10. Melaku S, Sharma HR, Alemie GA. Pastoralist Community's Perception of Tuberculosis: A Quantitative Study from Shinille Area of Ethiopia. Tuberc Res Treat [Internet]. 2013 [cited 2016 Jun 28];2013:475605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24381757
11. Zhang S, Ruan W, Li Y, Wang X, Wang X. Experiences of the parents caring for their children during a tuberculosis outbreak in high school: a qualitative study. BMC Public Health [Internet]. 2014 [cited 2016 Jun 28];14:132. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24507182
12. Yagnavalkya Jani GDBNT. Knowledge and awareness of tuberculosis in caregivers of paediatric tuberculosis patients of north Gujarat region, India: a cross sectional study. Int J Res Med Sci. 2015;3(12):3572–5.

13. Munro SA, Lewin SA, Smith HJ, Engel ME, Fretheim A, Volmink J. Patient Adherence to Tuberculosis Treatment: A Systematic Review of Qualitative Research. Rylko-Bauer B, editor. PLoS Med [Internet]. 2007 Jul 24 [cited 2016 Jun 28];4(7):e238. Available from: http://dx.plos.org/10.1371/journal.pmed.0040238
14. Oeser CC, Escombe AR, Gilman RH, Friedland JS, Evans CAW, Moore DAJ. Does traditional medicine use hamper efforts at tuberculosis control in urban Peru? Am J Trop Med Hyg. 2005;73(3):571–5.
15. Brouwer JA, Boeree MJ, Kager P, Varkevisser CM, Harries AD. Traditional healers and pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis [Internet]. 1998 Mar [cited 2016 Jun 28];2(3):231–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9526196
16. Makanjuola T, Taddese HB, Booth A. Factors associated with adherence to treatment with isoniazid for the prevention of tuberculosis amongst people living with HIV/AIDS: A systematic review of qualitative data. PLoS One. 2014;9(2).
17. Salaniponi FM, Harries AD, Banda HT, Kang'ombe C, Mphasa N, Mwale A, et al. Care seeking behaviour and diagnostic processes in patients with smear-positive pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis [Internet]. 2000 Apr [cited 2016 Jun 28];4(4):327–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10777081
18. Wilkinson D, Gcabashe L, Lurie M. Traditional healers as tuberculosis treatment supervisors: precedent and potential. Int J Tuberc Lung Dis [Internet]. 1999 Sep [cited 2016 Jun 28];3(9):838–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10488894
19. García-Basteiro AL, Respeito D, Augusto OJ, López-Varela E, Sacoor C, Sequera VG, et al. Poor tuberculosis treatment outcomes in Southern Mozambique (2011-2012). BMC Infect Dis [Internet]. 2016 [cited 2016 Jun 28];16(1):214. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27198545
20. (WHO) WHO. WHO | Global tuberculosis report 2015. WHO/HTM/TB/2015.08. 2015. World Health Organization; 2015.
21. World Health Organization (WHO). WHO Vaccine Preventable Disease Monitoring System: 2014 Global Summary. [Internet]. World Health Organization; 2014. Available from: http://apps.who.int/immunization_monitoring/globalsummary
22. WHO/UNICEF. Mozambique: WHO and UNICEF estimates of immunization coverage: 2014 revision Mozambique -BCG. 2015.
23. Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, et al. Profile: Manhiça Health Research Centre (Manhiça HDSS). Int J Epidemiol [Internet]. 2013 Oct [cited 2016 Jun 28];42(5):1309–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24159076
24. Instituto Nacional de Estadistica (INE) M. Estatisticas do Distrito da Manhica. 2012.
25. González R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med. 2012 Nov;13(10):581–8.
26. World Health Organization (WHO). Global Tuberculosis Report 2014 [Internet]. WHO/HTM/TB/2014.08. 2014. Available from:

http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:No+Title#0
27. Instituto Nacional de Estatística (INE) M. III recenseamento geral da população e habitação, 2007: Indicadores Sociodemográficos, província de Gaza. 2010.
28. López-Varela E, Augusto OJ, Gondo K, García-Basteiro AL, Fraile O, Ira T, et al. Incidence of Tuberculosis Among Young Children in Rural Mozambique. Pediatr Infect Dis J [Internet]. 2015 Jul [cited 2016 Jun 28];34(7):686–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26069945
29. Silva T de MV da, Santos MÁ dos, Almeida F de A, Silva T de MV da, Santos MÁ dos, Almeida F de A. Understanding the experiences of caregivers of children with tuberculosis in directly observed therapy. Rev da Esc Enferm da USP [Internet]. 2014 Dec [cited 2016 Jun 28];48(spe2):39–45. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0080-62342014000800039&lng=en&nrm=iso&tlng=en
30. Franck C, Seddon JA, Hesseling AC, Schaaf HS, Skinner D, Reynolds L. Assessing the impact of multidrug-resistant tuberculosis in children: an exploratory qualitative study. BMC Infect Dis [Internet]. 2014 [cited 2016 Jun 28];14:426. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25084990
31. Bjerrum S, Rose M V, Bygbjerg IC, Mfinanga SG, Tersboel BP, Ravn P. Primary health care staff's perceptions of childhood tuberculosis: a qualitative study from Tanzania. BMC Health Serv Res [Internet]. 2012 [cited 2016 Jun 28];12:6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22229965
32. Stop TB Partnership. Key Populations Brief: Children. 2016.
33. Cremers AL, de Laat MM, Kapata N, Gerrets R, Klipstein-Grobusch K, Grobusch MP, et al. Assessing the Consequences of Stigma for Tuberculosis Patients in Urban Zambia. Kumar A, editor. PLoS One [Internet]. 2015 Mar 25 [cited 2016 Jul 3];10(3):e0119861. Available from: http://dx.plos.org/10.1371/journal.pone.0119861
34. Deribew A, Abebe G, Apers L, Jira C, Tesfaye M, Shifa J, et al. Prejudice and misconceptions about tuberculosis and HIV in rural and urban communities in Ethiopia: a challenge for the TB/HIV control program. BMC Public Health [Internet]. 2010 [cited 2016 Jun 28];10:400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20604951
35. Eastwood S V., Hill PC. A gender-focused qualitative study of barriers to accessing tuberculosis treatment in The Gambia, West Africa. Int J Tuberc Lung Dis. 2004;8(1):70–5.
36. Green EC. Purity, pollution and the invisible snake in Southern Africa. http://dx.doi.org/101080/0145974019969966129. 2010;
37. Abebe G, Deribew A, Apers L, Woldemichael K, Shiffa J, Tesfaye M, et al. Knowledge, health seeking behavior and perceived stigma towards tuberculosis among tuberculosis suspects in a rural community in Southwest Ethiopia. PLoS One. 2010;5(10).
38. Boene H, González R, Valá A, Rupérez M, Velasco C, Machevo S, et al. Perceptions of malaria in pregnancy and acceptability of preventive interventions among Mozambican pregnant women: Implications for effectiveness of malaria control in pregnancy. PLoS One. 2014;9(2).

39. Montgomery CM, Munguambe K, Pool R. Group-based citizenship in the acceptance of indoor residual spraying (IRS) for malaria control in Mozambique. Soc Sci Med. 2010;70(10):1648–55.
40. Munguambe K, Pool R, Montgomery C, Bavo C, Nhacolo A, Fiosse L, et al. What drives community adherence to indoor residual spraying (IRS) against malaria in Manhiça district, rural Mozambique: a qualitative study. Malar J [Internet]. 2011;10(1):344. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3339361&tool=pmcentrez&rendertype=abstract
41. Pool R, Munguambe K, Macete E, Aide P, Juma G, Alonso P, et al. Community response to intermittent preventive treatment delivered to infants (IPTi) through the EPI system in Manhiça, Mozambique. Trop Med Int Heal. 2006;11(11):1670–8.
42. Chow F, Espiritu N, Gilman RH, Gutierrez R, Lopez S, Escombe AR, et al. La cuerda dulce--a tolerability and acceptability study of a novel approach to specimen collection for diagnosis of paediatric pulmonary tuberculosis. BMC Infect Dis. 2006 Jan;6:67.
43. Yimer S, Holm-Hansen C, Yimaldu T, Bjune G. Health care seeking among pulmonary tuberculosis suspects and patients in rural Ethiopia: a community-based study. BMC Public Health. 2009;9:454.
44. Saifodine A, Gudo PS, Sidat M, Black J. Patient and health system delay among patients with pulmonary tuberculosis in Beira city, Mozambique. BMC Public Health. 2013;13:559.

Summary of results and discussion

E. SUMMARY OF RESULTS AND DISCUSSION

The research in this thesis contributes to the global effort of measuring TB burden in young children. It is focused in a particularly vulnerable population (children under the age of three) in a high HIV incidence setting with an increased risk of disease progression and mortality. The results of the thesis are important from a national perspective in order to adapt the End TB Strategy according to the local epidemiology of its key populations. However, the results could be generalized to other SSA settings with similar characteristics. The thesis combines epidemiological, clinical and social approaches to delve into the characteristics of the local tuberculosis epidemics. The findings reported point to several barriers which contribute to under-detection as well as under-ascertainment. These findings can inform public health policy and aid in the design of better strategies to find and treat TB cases.

i. Burden of TB in children

The minimum community based incidence rate (MCBIR) of TB in children under the age of three from a high TB-HIV endemic area in Southern Mozambique was found to be 470/100.000 person-years, consistently high across all ages. These results confirm the magnitude of the epidemic in this region and highlight the high level of ongoing TB community transmission. However, this is probably an under-estimate the true burden of disease.

The study has identified young children, which are likely to contribute to a large part of the total national burden, as a priority key population in Mozambique. The current data can be informative in setting childhood specific targets in Mozambique, an important step for embracing the End TB Strategy nationally.

The results of this study add to the existing global effort of providing more accurate estimates on the burden of disease and highlight the need for further paediatric population-based studies from HBC. Targeting different age frames and epidemiological settings will contribute to reducing the uncertainty in the existing

estimates. As a step forward, Mozambique, which has not reported disaggregated data in the latest WHO reports, should consider including children in the upcoming national prevalence survey.

In this study, 28% of cases were culture positive TB cases, in line with the approximate expected value(73). It is important to note two factors. First, the MTBC culture positive cases did not include any of the 9 positive smear cases . Of the nine, four were NTM cases and 5 were smear positive culture negative cases which could reflect excessive decontamination in specimen handling. Our results underscore the limited utility of smear microscopy for TB diagnosis in young children and the need to favor molecular tests and/or culture when possible. At the time of the study and under programmatic conditions, Xpert MTB/RIF, which could have increased the diagnostic yield, was not yet rolled-out and culture was rarely available outside study context. Thus, smear microscopy continued to be the most widely available diagnostic assay nationwide. Second, instead of the 3 consecutive GAs accepted as the reference for paediatric TB diagnosis, only two single-day samples (one IS and one GA) were obtained, decreasing the chances of microbiological confirmation. This was due to the fact that most patients would not accept overnight admission. The results highlight the urgent need to carry out research on specimen collection strategies that will optimize the yield of the microbiological tests that are currently available, but more importantly, to evaluate them under conditions that are as similar as possible to the daily realities of the TB control programs in high-burden settings(59,66). It seems reasonable to consider collecting more than one type of specimen and potentially pooling specimens in order to optimize the yield and lower the total costs by requiring less total number of tests.

The rate of HIV coinfection among TB cases in children under three has been very high over the last years (from 44- 47% during the period 2006-2012). HIV infected children evaluated for TB were 6 times more likely to have TB than those uninfected, although fewer HIV infected children had bacteriological confirmation. These results have several implications. First, these results confirm that HIV infection and/or malnutrition are responsible for the greatest ascertainment bias with the highest risk of over or under estimation. In HBC, where TB disease is usually associated with poverty,

malnutrition and HIV, there is thus a need for improved diagnostic tools and strategies for this particular population. Given the strong association between HIV and TB, it is unlikely that TB elimination can be achieved without a specific emphasis in reaching co-infected children. A second implication of our results is the need for developing new TB prevention tools and improving the implementation of existing ones, particularly for HIV infected children. In this study, despite being part of the national guidelines, less than half of eligible patients , including HIV infected and /or TB contact cases, initiated IPT. It is crucial to understand the barriers from the patient and health system perspective that challenge the translation of this type of high evidence-based policies into practice. In this study, contact tracing could not be fully implemented and finally only 28% of TB cases had a documented TB contact. We found that establishing a history of contact, although important, may not always be feasible in orphan children and in contexts where outside-household TB transmission can occur.

The overall mortality observed among presumptive TB cases was high, particularly in HIV infected children, who had a significant increased risk of death compared to HIV uninfected (14.4% vs 3.8%, p<0.001). The high mortality observed represented a challenge when implementing the standardized case definition, as potential missed TB cases could represent a classification bias(119).Although not all of these deaths are attributable to TB, there is increasing evidence of the major role of TB to child mortality (55,77). These results underscore the need for better tools to ascertain the cause of death, especially in HIV infected children, as a first step to avoid preventable deaths.

In the second paper of this thesis we highlight the large number of missed paediatric TB cases which contribute to the large hidden burden of disease. This epidemiological study aimed at determining the case detection rate in the Manhiça District, using this MCBIR as the most accurate estimate available. To calculate the case detection rate of TB among children under three in Manhiça District, we compared the MCBIR (2011-2012) to notified cases during 2006-2010. The case detection rate was 40.8%, although again, due to methodological limitations, the true CDR is probably even lower. The results of this study support the hypothesis of a huge underestimation of childhood TB in Mozambique and globally. Under-ascertainment, under-reporting and

under-diagnosis can all contribute to low case detection rate. In Mozambique and in the context of this study, under-detection is probably mostly due to under-diagnosis. However, further research should be developed to estimate the likely contribution of each of these steps to the total under-estimation. This data shows that three out of five children <3yr with TB are not accessing appropriate TB health services or are lost to follow up before a diagnosis can be reached. In fact, diagnosing TB in children often requires multiple visits and a long follow-up period, increasing the chances of being lost to follow up. A true point of care diagnostic test would have a significantly impact in the number of children detected and treated.

When diagnosed and treated in a timely manner, TB outcomes are expected to be excellent. In the third paper, we documented treatment outcomes and adherence among paediatric TB cases under the age of three in Manhiça District during 2011-2012. The overall treatment success rate was high (88%), close to the 90% Stop TB target and consistent with the WHO estimates for all ages in Mozambique (88% in 2014). Despite the high treatment success rate, there were still a significant proportion (31.2%) of children with incomplete adherence to TB treatment, defined as lost to follow-up or delay of three or more weeks to treatment completion among patients who did not die. Low adherence can result in an emergence of new strains, drug resistance and poor treatment outcomes. Furthermore, it can result in prolonged infectiousness and affect the timing of symptom resolution. This has implications on the perceived likelihood of the initial TB diagnosis if it is not microbiologically confirmed. In these instances, symptom resolution/treatment response is useful for diagnostic confirmation of childhood tuberculosis and can be clouded by low adherence.

Child malnutrition and the history of a migrant mother were identified as potential risk factors of incomplete adherence. The small sample size of the study could have limited our ability to detect other potential risk factors such as male gender, HIV infection, TB case definition and distance from home to the treatment center. Given the pivotal role of adherence in treatment outcomes, increased surveillance in high risk patients could help improve overall outcomes.

ii. Characterization of the disease and barriers to case detection

This thesis has also documented that the most frequent lesion found in chest radiography of confirmed and probable TB cases less than three years is air space consolidation, which further complicates the distinction between TB and bacterial pneumonia in children. These results support the hypothesis that TB might be a direct cause of pneumonia or an underlying comorbidity that increases the risk of secondary bacterial pneumonia(77). Hiliar lymphadenopathy, the radiological hallmark of paediatric TB, was the second most common lesion but was only seen in a minority of cases. This could be due to the fact that air-space consolidation hinders the visualization of underlying mediastinal/hilar lymphadenopathies, in the absence of an ultrasound or a CT scan. Moreover, the high prevalence of HIV and malnutrition in this cohort could favor the bronchopneumonic consolidation as an evolved lesion from a regional lymph node. However, further studies on the radiological presentation of HIV co-infected children with different levels of immunosuppression are needed to better inform existing guidelines.

In this study, opacification was still present at the end of TB treatment in half the cases, rather than the expected 34%. The high prevalence of HIV/malnutrition in this cohort could explain the slower disease resolution. These findings highlight the difficulties in diagnosing TB in the absence of bacteriological confirmations and, under those circumstances, the need of combining clinical, radiological and epidemiological information. This study underscores the importance of not ruling out TB despite the absence of characteristic radiological findings and the need for improved scoring systems for the paediatric population.

In the fifth and sixth paper of this thesis, we address the microbiological challenge posed by NTM in TB diagnosis. The comprehensive review of NTM in children is the first one to look at NTM in children, with a public health perspective of high TB endemic settings. The review suggests that NTM are a clinically significant obstacle to the accurate diagnosis of childhood tuberculosis. Many patients are probably started

on tuberculosis treatment when in fact they had underlying NTM disease, although the extent of this misdiagnosis is unknown.

We have reported a high rate (26%) of NTM isolation among GA and IS samples of children evaluated for TB. The isolation of an NTM interfered with the diagnosis of TB. In particular, 3 of the 7 positive smear samples were due to NTM rather than MTBC; moreover, isolating an NTM on liquid or solid culture decreased the odds of ruling out TB according to the NIH definition. In high TB endemic settings, where molecular methods are often unavailable and TB diagnosis is still based on sputum smear, the common finding of an NTM isolate in childhood samples is likely to be misinterpreted as tuberculosis, potentially overestimating the burden of TB. Thus, understanding the epidemiology of NTM in children, including environmental exposure is important when designing vaccine trial endpoints. At the same time, NTM may interfere with BCG vaccine efficacy and this association needs further research.

The distribution of NTM species was similar to what has been reported in South Africa, where *M intracellulare* was the most frequent isolate followed by *scrofulaceum* and *gordonae*(120). Once isolated from a respiratory sample and correctly identified, it is often difficult to establish if an NTM isolate is causally related to the clinical disease, a reflection of environmental exposure or biological contaminant. In our cohort, NTM isolates did not seem to be clinically significant. The NTM cases did not receive specific treatment and 2 year mortality was comparable to those cases with negative culture. Besides, the proportion of children with an NTM isolate was similar at admission and follow-up visits, regardless of the initial culture result. When compared to children with MTBC, those with NTM had better clinical and radiological presentations, and lower mortality. Current guidelines do not provide specific advice for diagnosis of NTM in children. A consensus on the role of NTM isolates and their inclusion in paediatric TB guidelines would be useful to orient attending clinicians.

The review identified a substantial number of research gaps yet to be addressed. These include: NTM disease burden in high TB burden settings; the understanding of the interaction between NTM and the host, (including the effect of NTM exposure on BCG efficacy); the development of new diagnostic tests, strategies and guidelines that

allow clinicians from low resource settings to correctly diagnose both TB and NTM disease; the development of child specific treatment criteria and shortened regimens for NTM treatment. The review concludes that without advanced capacity in laboratories worldwide, including in resource-poor and tuberculosis-endemic countries, this situation is unlikely to change in the near future. In the meantime, clinical isolates need to be characterized to the best of our abilities to correctly identify at least MTBC, when present, since tuberculosis remains a prevalent and treatable condition in children.

The final paper of the thesis addressed child TB from the caretaker perspective and contributes to the understanding of disease under-ascertainment. We report the results of a qualitative study aiming to describe local understandings of the signs, aetiology, transmission and prevention of paediatric TB. Overall, the results of this study indicate a very low level of awareness regarding paediatric TB amongst caretakers of small children, including those vulnerable or exposed to TB. Paediatric TB is practically never suspected even among TB positive caretakers and there is a local understanding of TB as an illness of adults stemming from impurity derived from the transgression of social and cultural norms. However, TB diagnostic procedures were unanimously accepted and tolerated. Health seeking behaviour for treatment purposes seems to follow a circular and complex itinerary between the conventional and traditional health care systems. The results of this study are useful for informing health promotion messages in order to overcome the potential delays in treatment and adherence.

Beyond the specific objectives of this thesis, the studies have documented an increase in case detection and treatment success rate in the period of 2011-2012 compared to 2006-2010. These advances could partially be attributed to the improvement in care and treatment services both for HIV and TB over time. However, it also suggests that in a rural SSA setting with high HIV prevalence, diagnosis of child TB, case detection and surveillance data can be improved to a level necessary for HBC to reach the zero TB targets.

Conclusions

F. GENERAL CONCLUSIONS

1. The minimum community based incidence rate of TB in Mozambican children under three years of age was 470/100.000 person years and suggests high ongoing TB community transmission and a huge opportunity for prevention.

2. In the prospective community study, 28% of TB cases were culture positive for *Mycobacterium tuberculosis* and none of them had a positive smear test. Microbiological confirmation was significantly less likely among HIV infected children. This underscores that HIV TB co-infected cases present the highest challenge for TB diagnosis. Point-of-care tests and algorithmic approaches are needed, particularly in these children.

3. The estimated case detection rate for paediatric TB was 40.8% and is probably mostly due to under-diagnosis. This rate confirms the huge hidden burden of disease and demonstrates that a large proportion of young children are not accessing appropriate TB health services. Interventions to improve case detection and early diagnosis should be prioritized.

4. Almost half the TB cases in children under the age of three were co-infected with HIV. Children with HIV were 6 times more likely to have TB than those uninfected and their mortality was higher. In order to prevent unnecessary infant mortality, new TB prevention strategies among HIV infected individuals are needed. Meanwhile, effective strategies such as isoniazid preventive therapy need to be correctly implemented.

5. During the prospective study, the overall treatment success rate was high (88%) although it did not reach the 90% target. However, almost one third of patients had incomplete adherence to treatment and several risk factors for incomplete adherence were identified, including malnutrition and history of migrant mother.

6. In the context of a high prevalence of paediatric HIV and malnutrition, the most frequent lesion observed in chest radiography of TB cases was parenchymal consolidation, complicating radiological distinction from common bacterial pneumonia.

7. The absence of hiliar lymphadenopathy on chest radiography, the radiological hallmark of paediatric TB, should not rule out pulmonary TB. There is a need to further investigate the radiological presentation of TB in HIV co-infected and /or malnourished children with different levels of immunosuppression in order to improve current diagnostic scoring systems.

8. Non tuberculous mycobacteria are frequently encountered in gastric aspirates and /or induced sputum samples of children. Although the isolates are rarely clinically significant, their presence can contribute to overestimating the burden of TB in the absence of available molecular diagnostic methods.

9. There is a need for paediatric TB guidelines to arrive at a consensus on the role of NTM isolates and on recommendations for clinician attending these cases. At the same time, current guidelines should provide specific advice for diagnosis of NTM in children.

10. To overcome the potential delays in treatment and adherence health promotion messages should be consistent and repetitive, emphasize TB can also occur in children, differentiate it from HIV, and take into account that TB is often understood as an outcome of impurity derived from the transgression of social and cultural norms.

Bibliography

G. BIBLIOGRAPHY

1. Sustainable Development Goals: Sustainable Development Knowledge Platform [Internet]. [cited 2016 May 26]. Available from: https://sustainabledevelopment.un.org/
2. Fitchett JR, MacPherson P, Corbett EL. Implementing the end TB strategy and the intersection with the sustainable development goals, 2016-2030. Trans R Soc Trop Med Hyg [Internet]. 2015;110(3):145–7. Available from: http://trstmh.oxfordjournals.org/content/110/3/145.extract
3. World Health Organization (WHO). Global Tuberculosis Report 2015. Geneva, Switzerland. WHO/HTM/TB/2015.08. 2015;1:1689–99.
4. Murray CJL, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet [Internet]. 2014 Jul 21 [cited 2014 Jul 22];384(9947):1005–70. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4202387&tool=pmcentrez&rendertype=abstract
5. World Health Organization (WHO). Addressing Poverty in TB Control. Options for National TB Control Programmes. Geneva, Switzerland. WHO/HTM/TB/2005.352. World Health Organization; [cited 2016 May 26]; Available from: http://www.who.int/tb/challenges/poverty/en/
6. Cambau E, Drancourt M. Steps towards the discovery of Mycobacterium tuberculosis by Robert Koch, 1882. Clin Microbiol Infect [Internet]. 2014 Mar [cited 2016 Jun 11];20(3):196–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24450600
7. Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, et al. Origin, spread and demography of the Mycobacterium tuberculosis complex. PLoS Pathog [Internet]. 2008 [cited 2016 Jun 11];4(9):e1000160. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18802459
8. Koch R. Die aetiologie der tuberkulose. Berliner Klinische Wochenschrift. 1882;19:221–230.
9. Calmette A, Guerin C, Weil-Halle B. Essai d'immunisation contre l'infection tuberculeuse. Bull Acad Med 1924; 91: 787–796.
10. Calmette A, Guerin C, Boquet A, et al. Paris: Masson et cie; 1927. La vaccination préventive contre la tuberculose par le BCG.
11. Calmette A. Prophylactic vaccination of the newborn against tuberculosis. Br Med J. 1927;1(3464):987.
12. Aronson JD, Aronson CF, Taylor HC, Smithburn, K. C., and Lavin GI, Willis HS, Willis HS, et al. A Twenty-Year Appraisal of BCG Vaccination in the Control of Tuberculosis. Arch Intern Med [Internet]. American Medical Association; 1958 May 1 [cited 2016 Jun 11];101(5):881. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.1958.00260170037006
13. Ahmad Z, Makaya NH, Grosset J. Chapter 1: History of Drug Discovery: Early Evaluation Studies and Lessons Learnt from Them. Karger Publishers; 2011 [cited 2016 Jun 11]. p.

2–9. Available from: http://www.karger.com/doi/10.1159/000324894

14. Lienhardt C, Glaziou P, Uplekar M, Lönnroth K, Getahun H, Raviglione M. Global tuberculosis control: lessons learnt and future prospects. Nat Rev Microbiol [Internet]. Nature Publishing Group; 2012 May 14 [cited 2016 Jun 11];10(6):407. Available from: http://www.nature.com/doifinder/10.1038/nrmicro2797

15. Crofton J. Chemotherapy of pulmonary tuberculosis. Br Med J [Internet]. 1959 Jun 27 [cited 2016 Jun 11];1(5138):1610–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13662631

16. Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. Nat Rev Drug Discov [Internet]. Nature Publishing Group; 2013 Apr 30 [cited 2016 Jun 11];12(5):388–404. Available from: http://www.nature.com/doifinder/10.1038/nrd4001

17. World Health Organization (WHO). Forty-fourth World Health Assembly: Resolutions and Decisions. Resolution WHA 44.8. Geneva, Switzerland, 1991. WHA44/1991/REC/1.

18. World Health Organization (WHO). Framework for Effective Tuberculosis Control. WHO Tuberculosis Programme. Geneva, Switzerland, 1994.

19. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis [Internet]. 2010 May 15 [cited 2016 Jun 11];50 Suppl 3:S201–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20397949

20. Pablos-Méndez A, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F, et al. Global surveillance for antituberculosis-drug resistance, 1994-1997. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. N Engl J Med [Internet]. 1998 Jun 4 [cited 2016 Jun 11];338(23):1641–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9614254

21. Kumaresan J, Heitkamp P, Smith I, Billo N. Global Partnership to Stop TB: a model of an effective public health partnership. Int J Tuberc Lung Dis [Internet]. 2004 Jan [cited 2016 Sep 12];8(1):120–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14974755

22. World Health Organization (WHO). Global Tuberculosis Programme. Report of the Ad Hoc Committee on the Tuberculosis Epidemic. London, March 17–19, 1998.

23. Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. JAMA [Internet]. 2005 Jun 8 [cited 2016 Jun 11];293(22):2767–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15941807

24. World Health Organization (WHO). World Health Organization (WHO). Roadmap for childhood tuberculosis: towards zero deaths. Geneva, Switzerland,2013. WHO/HTM/TB/2013.12. [cited 2013 Nov 18]; Available from: http://apps.who.int/iris/handle/10665/89506

25. World Health Organization. World Health Organization (WHO). Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance : Xpert MTB/RIF system : policy statement. Geneva, Switzerland, 2011. WHO/HTM/TB/2011. 28 p.

26. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular

detection of tuberculosis and rifampin resistance. N Engl J Med [Internet]. 2010 Sep 9 [cited 2016 Jun 9];363(11):1005–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20825313

27. Dheda K, Barry CE, Maartens G. Tuberculosis. Lancet (London, England) [Internet]. Elsevier; 2015 Sep 11 [cited 2015 Sep 22];387(10024):1211–26. Available from: http://www.thelancet.com/article/S0140673615001518/fulltext

28. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, et al. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Heal [Internet]. Elsevier; 2015 Aug [cited 2016 Jun 29];3(8):e450–7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2214109X1500100X

29. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet (London, England) [Internet]. Elsevier; 2014 Feb 1 [cited 2016 Jun 29];383(9915):424–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24176144

30. World Health Organization (WHO). The End TB Strategy. Geneva, Switzerland. 2015. WHO [Internet]. World Health Organization; 2015 [cited 2016 Jun 9]; Available from: http://www.who.int/tb/post2015_strategy/en/

31. World Health Organization. WHO Global Task Force on TB Impact Measurement.2015. Available at: www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/global_consultation_meeting_report.pdf.

32. García-Basteiro AL, Ismail MR, Carrilho C, Ussene E, Castillo P, Chitsungo D, et al. The role of Xpert MTB/RIF in diagnosing pulmonary tuberculosis in post-mortem tissues. Sci Rep [Internet]. 2016 [cited 2016 Jun 7];6:20703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26860394

33. García-Basteiro AL, López-Varela E, Manhiça I, Macete E, Alonso PL. Mozambique faces challenges in the fight against tuberculosis. Lancet [Internet]. 2014 Jan 18 [cited 2014 Jan 22];383(9913):215–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24439733

34. Auld AF, Mbofana F, Shiraishi RW, Alfredo C, Sanchez M, Ellerbrock T V, et al. Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy--Mozambique, 2004-2008. PLoS One [Internet]. 2013 Jan [cited 2013 Dec 21];8(1):e54665. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3551849&tool=pmcentrez&rendertype=abstract

35. Saifodine A, Gudo PS, Sidat M, Black J. Patient and health system delay among patients with pulmonary tuberculosis in Beira city, Mozambique. BMC Public Health [Internet]. 2013 [cited 2016 Jun 7];13:559. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24499197

36. Bos JC, Smalbraak L, Macome AC, Gomes E, van Leth F, Prins JM. TB diagnostic process management of patients in a referral hospital in Mozambique in comparison with the 2007 WHO recommendations for the diagnosis of smear-negative pulmonary TB and extrapulmonary TB. Int Health [Internet]. 2013 Dec [cited 2016 Jun 7];5(4):302–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24130098

37. García-Basteiro AL, López-Varela E, Respeito D, González R, Naniche D, Manhiça I, et al. High tuberculosis burden among people living with HIV in southern Mozambique. Eur Respir J [Internet]. 2014;04:09031936.00145714 – . Available from: http://erj.ersjournals.com/content/early/2014/11/13/09031936.00145714.extract

38. López-Varela E, Augusto OJ, Guerra L, Respeito D, Sacoor C, Sacarlal J, et al. Low paediatric tuberculosis case detection rate in Southern Mozambique. Eur Respir J [Internet]. 2015 Dec 23 [cited 2016 Jan 14];ERJ – 01454–2015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26699721

39. López-Varela E, Augusto OJ, Gondo K, García-Basteiro AL, Fraile O, Ira T, et al. Incidence of Tuberculosis Among Young Children in Rural Mozambique. Pediatr Infect Dis J [Internet]. 2015;34(7):686–92. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006454-201507000-00002

40. García-Basteiro AL, Respeito D, Augusto OJ, López-Varela E, Sacoor C, Sequera VG, et al. Poor tuberculosis treatment outcomes in Southern Mozambique (2011-2012). BMC Infect Dis [Internet]. 2016 [cited 2016 Jun 7];16(1):214. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27198545

41. González R, Augusto OJ, Munguambe K, Pierrat C, Pedro EN, Sacoor C, et al. HIV Incidence and Spatial Clustering in a Rural Area of Southern Mozambique. PLoS One [Internet]. 2015 [cited 2016 Jun 28];10(7):e0132053. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26147473

42. Ministério da Saúde de Moçamique. Resposta Global à SIDA. Relatório do Progresso. Maputo, Moçambique, 2016. Available from: http://www.unaids.org/sites/default/files/country/documents/MOZ_narrative_report_2016.pdf

43. Wikman-Jorgensen PE, Morales-Cartagena A, Llenas-García J, Pérez-Porcuna TM, Hobbins M, Ehmer J, et al. Implementation challenges of a TB programme in rural northern mozambique: evaluation of 2012-2013 outcomes. Pathog Glob Health [Internet]. 2015 Jul [cited 2016 Jun 8];109(5):221–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26239760

44. Cowan J, Michel C, Manhiça I, Monivo C, Saize D, Creswell J, et al. Implementing rapid testing for tuberculosis in Mozambique. Bull World Health Organ [Internet]. 2015 Feb 1 [cited 2016 Jun 8];93(2):125–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25883406

45. López-Varela E, García-Basteiro AL. World health organization guidelines for childhood tuberculosis management: successes achieved and challenges ahead. Pediatr Infect Dis J [Internet]. 2014 Dec [cited 2014 Nov 30];33(12):1310–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25386971

46. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med [Internet]. 2015 Jun [cited 2016 Jun 9];3(6):451–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25812968

47. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2014.

48. World Health Organization (WHO). Global Tuberculosis Report 2015. Geneva, Switzerland. WHO/HTM/TB/2015.08. 2015;

49. Donald PR. Childhood tuberculosis : the hidden epidemic. Int J Tuberc Lung Dis. 2004;8(5):627–9.

50. Seddon JA, Shingadia D. Epidemiology and disease burden of tuberculosis in children: a global perspective. Infect Drug Resist [Internet]. 2014 Jan [cited 2016 May 31];7:153–65. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4069045&tool=pmcentrez&rendertype=abstract

51. Stop TB Partnership. Stop TB Partnership and World Health Organization. Global Plan to Stop TB 2006– 2015. Geneva, Switzerland, 2006. WHO/HTM/STB/2006.35.

52. Starke JR. Transmission of mycobacterium tuberculosis to and from children and adolescents. Semin Pediatr Infect Dis. 2001;12(2):115–23.

53. Curtis AB, Ridzon R, Vogel R, McDonough S, Hargreaves J, Ferry J, et al. Extensive transmission of Mycobacterium tuberculosis from a child. N Engl J Med [Internet]. 1999 Nov 11 [cited 2016 Jun 9];341(20):1491–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10559449

54. Cruz AT, Starke JR. A current review of infection control for childhood tuberculosis. Tuberculosis (Edinb) [Internet]. 2011 Dec [cited 2016 Jun 9];91 Suppl 1:S11–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22079589

55. Marais BJ, Schaaf HS, Graham SM. Child health and tuberculosis. Lancet Respir Med [Internet]. Elsevier Ltd; 2014 Mar [cited 2014 Mar 25];2(4):254–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2213260014700098

56. Sandgren A, Cuevas LE, Dara M, Gie RP, Grzemska M, Hawkridge A, et al. Childhood tuberculosis: progress requires an advocacy strategy now. Eur Respir J [Internet]. 2012 Aug [cited 2016 Jun 9];40(2):294–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22337859

57. World Health Organization. Global Tuberculosis Report 2013. WHO/HTM/TB/2013.11; 2013.

58. Stop TB Partnership and World Health Organization. The Global Plan to Stop TB (2011-2015). [cited 2016 Jun 24]; Available from: http://www.stoptb.org/assets/documents/global/plan/tb_globalplantostoptb2011-2015.pdf

59. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med [Internet]. 2012 Jul 26 [cited 2013 Mar 3];367(4):348–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22830465

60. Kordy F, Richardson SE, Stephens D, Lam R, Jamieson F, Kitai I. Utility of Gastric Aspirates for Diagnosing Tuberculosis in Children in a Low Prevalence Area: Predictors of Positive Cultures and Significance of Non-Tuberculous Mycobacteria. Pediatr Infect Dis J [Internet]. 2014 Aug 6 [cited 2014 Oct 22]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25101762

61. Hatherill M, Hawkridge T, Whitelaw A, Tameris M, Mahomed H, Moyo S, et al. Isolation of non-tuberculous mycobacteria in children investigated for pulmonary tuberculosis. PLoS One [Internet]. 2006 Jan [cited 2013 Mar 5];1(1):e21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1762386&tool=pmcentrez&rendertype=abstract

62. Asiimwe BB, Bagyenzi GB, Ssengooba W, Mumbowa F, Mboowa G, Wajja A, et al.

Species and genotypic diversity of non-tuberculous mycobacteria isolated from children investigated for pulmonary tuberculosis in rural Uganda. BMC Infect Dis [Internet]. 2013 Jan [cited 2014 Dec 5];13:88. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3599115&tool=pmcentrez&rendertype=abstract

63. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. Paediatr Respir Rev [Internet]. 2011 Mar [cited 2013 Mar 3];12(1):16–21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3052970&tool=pmcentrez&rendertype=abstract

64. Shingadia D. The diagnosis of tuberculosis. Pediatr Infect Dis J [Internet]. 2012 Mar [cited 2013 Jun 24];31(3):302–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22330167

65. Cuevas LE, Browning R, Bossuyt P, Casenghi M, Cotton MF, Cruz AT, et al. Evaluation of tuberculosis diagnostics in children: 2. Methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for intrathoracic tuberculosis in children. Consensus from an expert panel. J Infect Dis [Internet]. 2012 May 15 [cited 2013 Feb 27];205 Suppl (Suppl 2):S209–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22476719

66. Marcy O, Ung V, Goyet S, Borand L, Msellati P, Tejiokem M, et al. Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children. Clin Infect Dis [Internet]. 2016 May 1 [cited 2016 Jun 9];62(9):1161–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26908804

67. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa. http://dx.doi.org/101056/NEJMoa1303657. Massachusetts Medical Society; 2014;

68. Reid MJA, Saito S, Fayorsey R, Carter RJ, Abrams EJ. Assessing capacity for diagnosing tuberculosis in children in sub-Saharan African HIV care settings. Int J Tuberc Lung Dis [Internet]. 2012 Jul [cited 2016 Jun 9];16(7):924–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22583761

69. Mukherjee A, Singh S, Lodha R, Singh V, Hesseling a C, Grewal HMS, et al. Ambulatory gastric lavages provide better yields of Mycobacterium tuberculosis than induced sputum in children with intrathoracic tuberculosis. Pediatr Infect Dis J [Internet]. 2013;32(12):1313–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23958816

70. Hatherill M, Hawkridge T, Zar HJ, Whitelaw A, Tameris M, Workman L, et al. Induced sputum or gastric lavage for community-based diagnosis of childhood pulmonary tuberculosis? Arch Dis Child [Internet]. 2009 Mar [cited 2015 Feb 18];94(3):195–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18829621

71. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet [Internet]. 2005;365(9454):130–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15639294

72. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis [Internet]. 2006 Apr 15 [cited 2015 Feb 18];42(8):e69–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16575719

73. Seddon JA, Jenkins HE, Liu L, Cohen T, Black RE, Vos T, et al. Counting children with tuberculosis: why numbers matter. Int J Tuberc Lung Dis [Internet]. 2015;19 Suppl 1(0 1):9–16. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4708268&tool=pmcentrez &rendertype=abstract

74. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Lombard C, Enarson D a, et al. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics [Internet]. 2006 Nov [cited 2013 Mar 5];118(5):e1350–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17079536

75. Graham SM, Sismanidis C, Menzies HJ, Marais BJ, Detjen AK, Black RE. Importance of tuberculosis control to address child survival. Lancet [Internet]. Elsevier Ltd; 2014 Mar [cited 2014 Mar 26];6736(14):5–7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673614604207

76. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. Clin Infect Dis. 2015;61(Suppl 3):S179–87.

77. Oliwa JN, Karumbi JM, Marais BJ, Madhi SA, Graham SM, Walker C, et al. Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review. Lancet Respir Med [Internet]. Elsevier; 2015 Mar [cited 2016 Jun 30];3(3):235–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25648115

78. Chisti MJ, Ahmed T, Pietroni MAC, Faruque ASG, Ashraf H, Bardhan PK, et al. Pulmonary tuberculosis in severely-malnourished or HIV-infected children with pneumonia: a review. J Health Popul Nutr [Internet]. BioMed Central; 2013 Sep [cited 2016 Jun 30];31(3):308–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24288943

79. Moore DP, Klugman KP, Madhi SA. Role of Streptococcus pneumoniae in Hospitalization for Acute Community-acquired Pneumonia Associated With Culture-confirmed Mycobacterium tuberculosis in Children. Pediatr Infect Dis J [Internet]. 2010 Dec [cited 2016 Jul 11];29(12):1099–104. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=0000645 4-201012000-00009

80. Swingler GH, du Toit G, Andronikou S, van der Merwe L, Zar HJ. Diagnostic accuracy of chest radiography in detecting mediastinal lymphadenopathy in suspected pulmonary tuberculosis. Arch Dis Child [Internet]. 2005 Nov [cited 2014 Jan 22];90(11):1153–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1720188&tool=pmcentrez &rendertype=abstract

81. Pinto LM, Dheda K, Theron G, Allwood B, Calligaro G, van Zyl-Smit R, et al. Development of a simple reliable radiographic scoring system to aid the diagnosis of pulmonary tuberculosis. PLoS One. 2013 Jan;8(1):e54235.

82. Menzies D, Nahid P. Update in tuberculosis and nontuberculous mycobacterial disease 2012. Am J Respir Crit Care Med [Internet]. 2013 Oct 15 [cited 2014 Jan 21];188(8):923–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24127799

83. Jaganath D, Mupere E. Childhood tuberculosis and malnutrition. J Infect Dis [Internet]. 2012 Dec 15 [cited 2014 Jan 21];206(12):1809–15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3502375&tool=pmcentrez &rendertype=abstract

84. Hatherill M, Hanslo M, Hawkridge T, Little F, Workman L, Mahomed H, et al. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. Bull World Health Organ [Internet]. 2010 Apr [cited 2013 Mar 4];88(4):312–20. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2855594&tool=pmcentrez&rendertype=abstract

85. Edwards DJ, Kitetele F, Van Rie A. Agreement between clinical scoring systems used for the diagnosis of pediatric tuberculosis in the HIV era. Int J Tuberc Lung Dis [Internet]. 2007 Mar [cited 2016 Jun 25];11(3):263–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17352090

86. Hesseling a C, Schaaf HS, Gie RP, Starke JR, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. Int J Tuberc Lung Dis [Internet]. 2002 Dec;6(12):1038–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12546110

87. Pearce EC, Woodward JF, Nyandiko WM, Vreeman RC, Ayaya SO. A systematic review of clinical diagnostic systems used in the diagnosis of tuberculosis in children. AIDS Res Treat [Internet]. 2012 [cited 2016 Jun 30];2012:401896. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22848799

88. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS [Internet]. Wolters Kluwer Health; 2015 Sep 24 [cited 2016 Jun 30];29(15):1987–2002. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26266773

89. Stop TB Partnership. Key populations brief: children. Geneva, Switzerland, 2016. [cited 2016 Jun 27]; Available from: http://www.stoptb.org/assets/documents/resources/publications/acsm/KP_Children_Spreads.pdf

90. Bjerrum S, Rose M V, Bygbjerg IC, Mfinanga SG, Tersboel BP, Ravn P. Primary health care staff's perceptions of childhood tuberculosis: a qualitative study from Tanzania. BMC Health Serv Res [Internet]. BioMed Central; 2012 [cited 2016 Jul 9];12:6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22229965

91. Seddon JA. Knowing how many children to find is the first step in finding them. Public Heal action [Internet]. 2016 Jun 21 [cited 2016 Jul 1];6(2):52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27358794

92. Acosta CD, Rusovich V, Harries AD, Ahmedov S, van den Boom M, Dara M. A new roadmap for childhood tuberculosis. The Lancet Global Health. 2014.

93. Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. Clin Infect Dis. 2010 May;50 Suppl 3(Suppl 3):S184–94.

94. Graham SM, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis [Internet]. 2012 May 15 [cited 2013 Mar 5];205 Suppl (Suppl 2):S199–208. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22448023

95. Holm LL, Rose MV, Ravn P. Perspectives in implementing standardized case definitions for tuberculosis research involving children in a low-income, high-burden setting. J Infect Dis [Internet]. 2013 Mar 1 [cited 2016 Jun 9];207(5):870–1. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/23242543

96. Foster N, Vassall A, Cleary S, Cunnama L, Churchyard G, Sinanovic E. The economic burden of TB diagnosis and treatment in South Africa. Soc Sci Med. 2015;130:42–50.

97. World Health Organization. The importance of caregiver-child interactions for the survival of child and healthy development of young children. A review. 2004.

98. Cremers AL, de Laat MM, Kapata N, Gerrets R, Klipstein-Grobusch K, Grobusch MP, et al. Assessing the Consequences of Stigma for Tuberculosis Patients in Urban Zambia. Kumar A, editor. PLoS One [Internet]. Public Library of Science; 2015 Mar 25 [cited 2016 Jul 1];10(3):e0119861. Available from: http://dx.plos.org/10.1371/journal.pone.0119861

99. Preez K Du, Schaaf HS, Dunbar R, Swartz A, Bissell K, Enarson DA, et al. Incomplete registration and reporting of culture-confirmed childhood tuberculosis diagnosed in hospital. Public Heal Action [Internet]. The International Union Against Tuberculosis and Lung Disease; 2011 Sep 21 [cited 2016 Jul 1];1(1):19–24. Available from: http://openurl.ingenta.com/content/xref?genre=article&issn=2220-8372&volume=1&issue=1&spage=19

100. Coghlan R, Gardiner E, Amanullah F, Ihekweazu C, Triasih R, Grzemska M, et al. Understanding Market Size and Reporting Gaps for Paediatric TB in Indonesia, Nigeria and Pakistan: Supporting Improved Treatment of Childhood TB in the Advent of New Medicines. Esposito S, editor. PLoS One [Internet]. Public Library of Science; 2015 Oct 13 [cited 2016 Jul 1];10(10):e0138323. Available from: http://dx.plos.org/10.1371/journal.pone.0138323

101. World Health Organization (WHO). Global Tuberculosis Report 2012. Geneva, Switzerland. WHO/HTM/TB/2012.06. [cited 2016 Jul 1]; Available from: http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf

102. Jenkins HE, Tolman AW, Yuen CM, Parr JB, Keshavjee S, Pérez-Vélez CM, et al. Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. Lancet [Internet]. 2014 Mar [cited 2014 Mar 25];6736(14):1–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673614601951

103. Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Glob Heal [Internet]. 2014 Jul [cited 2014 Jul 10];2(8):e453–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25103518

104. World Health Organization. Global Tuberculosis Report 2014. Geneva, Switzerland: WHO/HTM/TB/2014.08; 2014.

105. Oeltmann JE, Chengeta B, Mboya JJ, Wells CD, Kilmarx PH, Samandari T, et al. Reported childhood tuberculosis treatment outcomes, Gaborone and Francistown, Botswana, 1998-2002. Int J Tuberc Lung Dis [Internet]. 2008 Feb [cited 2016 Jun 25];12(2):186–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18230252

106. Harries a D, Hargreaves NJ, Graham SM, Mwansambo C, Kazembe P, Broadhead RL, et al. Childhood tuberculosis in Malawi: nationwide case-finding and treatment outcomes. Int J Tuberc Lung Dis [Internet]. 2002 May;6(5):424–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12019918

107. Adejumo OA, Daniel OJ, Adebayo BI, Adejumo EN, Jaiyesimi EO, Akang G, et al. Treatment Outcomes of Childhood TB in Lagos, Nigeria. J Trop Pediatr [Internet]. 2016 Apr [cited 2016 Jun 25];62(2):131–8. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/26705331

108. Hailu D, Abegaz WE, Belay M. Childhood tuberculosis and its treatment outcomes in Addis Ababa : a 5-years retrospective study [Internet]. BMC Pediatrics. BMC Pediatrics; 2014. p. 1–7. Available from: BMC Pediatrics

109. Khawaja H. Treatment Outcomes in Children with Tuberculosis: A Systematic Review and Meta-analysis. BSc Dissertation in Reproductive and Developmental Sciences, Imperial College London. 2014.

110. Vassall A. Tuberculosis perspective paper: Benefits and costs of the education targets for the post-2015 development agenda. Copenhagen Consensus Center; 2014. Available from: http://www.copenhagenconsensus.com/sites/default/files/health_perspective_tb_-_vassall.pdf

111. Seddon JA, Graham SM. Childhood TB: can the End TB Strategy deliver? Trans R Soc Trop Med Hyg [Internet]. Oxford University Press; 2016 Mar [cited 2016 Jun 29];110(3):155–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26884493

112. Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, et al. Profile: Manhica Health Research Centre (Manhica HDSS). Int J Epidemiol [Internet]. 2013 Oct [cited 2013 Nov 8];42(5):1309–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24159076

113. World Health Organization (WHO). World Health Organization (WHO).Vaccine Preventable Disease Monitoring System: 2014 Global Summary. Geneva, Switzerland, 2006. 2014; Available from: http://apps.who.int/immunization_monitoring/globalsummary

114. Lanaspa M, Balcells R, Sacoor C, Nhama A, Aponte JJ, Bassat Q. The performance of the expanded programme on immunization in a rural area of Mozambique. Acta Trop [Internet]. 2015 Sep [cited 2016 Mar 9];149:262–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26095045

115. Ministério da Saúde de Moçamique. Manual de Tuberculose Infantil em Moçambique. 2013. [cited 2016 Jul 10]; Available from: http://www.ensinoadistancia.edu.mz/sites/default/files/Manual TB Pediatrica2013.pdf

116. Lienhardt C, Cook S V., Burgos M, Yorke-Edwards V, Rigouts L, Anyo G, et al. Efficacy and Safety of a 4-Drug Fixed-Dose Combination Regimen Compared With Separate Drugs for Treatment of Pulmonary Tuberculosis. JAMA [Internet]. American Medical Association; 2011 Apr 13 [cited 2016 Jul 10];305(14):1415. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2011.436

117. Diacon AH, van der Merwe L, Barnard M, von Groote-Bidlingmaier F, Lange C, García-Basteiro AL, et al. β-Lactams against Tuberculosis--New Trick for an Old Dog? N Engl J Med [Internet]. 2016 Jul 28 [cited 2016 Sep 12];375(4):393–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27433841

118. Tameris M, Hokey DA, Nduba V, Sacarlal J, Laher F, Kiringa G, et al. A double-blind, randomised, placebo-controlled, dose-finding trial of the novel tuberculosis vaccine AERAS-402, an adenovirus-vectored fusion protein, in healthy, BCG-vaccinated infants. Vaccine. 2015;33(25):2944–54.

119. Holm LL, Rose MV, Ravn P. Perspectives in implementing standardized case definitions for tuberculosis research involving children in a low-income, high-burden setting. J Infect Dis [Internet]. Oxford University Press; 2013 Mar 1 [cited 2016 Jul

11];207(5):870–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23242543

120. Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: A NTM-NET collaborative study. Eur Respir J Off J Eur Soc Clin Respir Physiol [Internet]. 2013 Apr 18 [cited 2013 Jun 27]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/23598956

Agradecimientos

H. AGRADECIMIENTOS

A mis directores de tesis por haber hecho posible que realice este proyecto. A Pedro, por la inspiración, la confianza y el apoyo durante los últimos ocho años, por invitarme a formar parte del equipo y por darme la oportunidad de tener el trabajo de mi vida. A Denise, por ser una gran mentora y amiga, por su capacidad de estimular y enseñar, por su visión de la ciencia y de las posibilidades de colaboración, por su disponibilidad incondicional y su inteligencia creativa (sus "comas"). Los últimos años trabajando bajo su supervisión han sido formidables, ojalá pueda continuar aprendiendo de ella mucho más tiempo.

A la dirección del CISM, Eusebio, Delino e Inacio, por abrirme las puertas del CISM. A Quique por su apoyo y amistad. Muchísimas gracias a los numerosos colegas y amigos del CISM y de ISGLOBAL que, de una manera u otra, han sido una parte importante de mi vida durante este tiempo. Son tantos que no es posible nombrarlos a todos, pero cada uno de ellos ha contribuido a que Manhiça fuera un hogar para mí durante estos años, los mejores de mi vida profesional.

A los compañeros del área de Tuberculosis y VIH de ISGLOBAL y del CISM. Especialmente a Orvalho y Alberto, mis compañeros de ITACA, camaradas en los momentos de grandes alegrías y logros, pero también en los sufrimientos. He aprendido mucho de ellos y a ellos debo gran parte de esta tesis. Gracias a Laura y a la nueva familia TESFAM, por ser el mejor equipo y por traerme tanta ilusión.

Gracias a todo el personal del hospital distrital de Manhiça el lugar de mayor aprendizaje y de mayor sufrimiento, las dos caras que han dado sentido a mi trabajo. A los compañeros médicos, al personal de enfermería y auxiliares, consejeras, personal de farmacia, estudiantes y rotantes y personal administrativo. Gracias a todos los niños y a sus padres, a tantas madres y abuelas que me han enseñado tanto; y un sentido agradecimiento a aquellos que hemos perdido en el camino.

A los grandes profesionales que he conocido en el camino gracias a este proyecto y que se han convertido en mentores y fuentes de inspiración; en especial a Steve Graham y a Paula Vaz que además han contribuido a evaluar esta tesis.

Gracias también a todos los amigos que me han acompañado en Mozambique: a los antiguos, que están a mi lado, incondicionalmente, a pesar de la distancia y a los nuevos. A ellos, porque su amistad ha sido uno de los mejores regalos de estos años.

Gracias a mi familia, mis padres y mi hermana Julia, por ser mis mejores consejeros y mi ejemplo.

Y gracias a Jaime, por ser mi compañero de vida y por hacer que a su lado, día tras día, todo sea sencillo y deseable.

Elisa