OPTIMAL TUBERCULOSIS CASE-FINDING METHODOLOGIES FOR FIELD TRIALS OF NEW TUBERCULOSIS VACCINES IN YOUNG CHILDREN

SIZULU MOYO
MJMSIZ001

MBCHB, MPH

Thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

In the School of Child and Adolescent Health

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date 10 May 2013

Supervisors:

Professor G. Hussey

Associate Professor M. Hatherill

Dr S. Verver

Declarations

I, Sizulu Moyo, hereby declare this thesis is my original work based on studies conducted at the South African Tuberculosis Vaccine Initiative trial site. I further declare that neither this work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

This work was supervised by Professor G. Hussey, Associate Professor M. Hatherill and Dr S. Verver. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Date: 10 May 2013

Signature: Signed by candidate

Signature removed

Abstract

Background: There is paucity of evidence to guide case-finding strategies in field trials of new tuberculosis vaccines conducted in young children.

Aim: To investigate case-finding and case detection methods for tuberculosis in tuberculosis field trials conducted in young children.

Methods: Age-specific tuberculosis incidence was calculated among children less than 5 years old. In a second study, Bacille Calmette-Guérin vaccinated infants were randomised to tuberculosis case finding through regular home visits and record surveillance (Group 1) or record surveillance only (Group 2), and followed up for at least 2 years. TB suspects were evaluated for tuberculosis using standardized tests including chest radiographs. A sample of children was also tested using the QuantiFERON TB-Gold-In-Tube assay. Hospital admission and mortality data in children enrolled in two tuberculosis vaccine studies were analysed.

Results: The incidence of tuberculosis peaked at a rate of 1.21% in the 12-23 month age group, with rates remaining high beyond 24 months (1% in the 24-35 month age group). The case-finding rate was significantly greater in Group 1 case-finding:-2.2/100 py compared to:-0.8/100 py in Group 2 case-finding; case-finding rate ratio 2.6 (95%CI 1.8–4.0, $p < 0.001$). Agreement between the tuberculin skin test and the QuantiFERON assay was excellent (94%, κ =0.79, 95%CI 0.69–0.89). Isolated hilar lymphadenopathy and parenchymal consolidation were the most frequent chest radiographic findings in children with suspected tuberculosis. TB accounted for 4% and 0.3%, and 6% and 1% of admissions and deaths respectively in the two tuberculosis vaccine studies analysed.

Conclusions: Infant tuberculosis vaccine trials can increase case accrual by extending participant follow-up beyond 24 months. Tuberculosis case finding that incorporates regular home visits maximises case detection, yielding more cases than record surveillance with a study close-out visit. The tuberculin skin test remains a useful diagnostic aid for tuberculosis in young children in our setting. Active case finding in young children detects uncomplicated primary complex TB with a small proportion of uncontained parenchymal disease. Growth failure is the clinical hallmark of uncontained pulmonary TB in young children in this setting. Although the overall proportions were low, hospital admissions and deaths due to TB are significant contributors to serious adverse events in TB vaccine trials.

Table of Contents

List of abbreviations:

ACF	Active case finding
BCG	Bacille Calmette Guérin
CI	Confidence interval
CXR	Chest radiograph
DP	Definite and probable
DOTS	Directly Observed Treatment Short course
ECF	Enhanced case finding
GW	Gastric washing
HIV	Human immunodeficiency virus
IFN-γ	Interferon- gamma
IGRA	Interferon gamma release assay
IPT	Isoniazid preventive therapy
IS	Induced sputum
LTBI	Latent tuberculosis infection
LTFU	Lost to follow-up
MDR	Multidrug resistant
MGIT	Microscopic growth indicator tube
M.tb	Mycobacterium tuberculosis
NTM	Non tuberculous mycobacteria
PAL	Practical Approach to Lung Health
PCF	Passive case finding
PHC	Primary Health Care
QFT	QuantiFERON
SATVI	South African Tuberculosis Vaccine Initiative
SD	Standard deviation
TB	Tuberculosis
TST	Tuberculin skin test
WHO	World Health Organisation

Acknowledgements:

This work is dedicated to my wonderful family. I am grateful for their support during this journey. I love you; you are the centre of my world. To my husband, Professor P. Moyo thank for listening to my ideas, for your wise advice and for holding the home-front while I did this work. To my children Keren and Timothy, thank you for understanding when "I had to go to the office".

The work presented in this thesis had taken place over a long period, with support from many people whose input I have highly appreciated. I am especially grateful to my supervisors, Professor Hussey (founder of the South African Tuberculosis Vaccine Initiative (SATVI)), Associate Professor Hatherill and Dr Verver. Thank you, Professor Hussey, for giving my first position in research, for your support during my Master's degree, for planting the first thoughts about pursuing a PhD in this field, and for supervising this work. You are a brilliant teacher and a true mentor. Dr Verver, thank you for taking me through this journey from the time we first meet in 2004. Thank you for the wonderful discussions we have had as you guided me, and encouraged my independent thinking and ideas. You are a remarkable epidemiologist, researcher, mentor, colleague and friend. Professor Hatherill, thank you for introducing me to a different way of looking at things, for encouraging me to think about the difficult questions and seek possible answers. You are a true academic, brilliant researcher and a great mentor.

I was privileged to work with Dr Hawkridge, whom I hold in high esteem. I am grateful for his many valuable insights and for "teaching me to be a researcher". Thank you also to Professor Hanekom, present Director of SATVI, who challenged my thinking and whose critique, insight and role at SATVI, has been very valuable and pivotal to this work. I am also grateful to Dr H. Mahomed, previously co-director at SATVI, whose insights and guidance were crucial to this work. I also thank Dr L. Geiter for his wise encouragement when I began my journey in research. Thank you for your input into this work and into my career.

I would also like to thank all the SATVI staff members, partners and collaborators (past and present), who were part of the studies reported in this thesis. The different roles played by all staff members, ranging from fieldwork, laboratory work, administrative functions and scientific support is highly appreciated:-these studies would not have been successful without

your input. Particular mention goes to Dr M. Tameris, Dr H. Geldenhuys, Professor F. Little, Mr W. Msemburi, Professor M. Pai, and Ms L. Matizirofa. Thank you also to the brilliant scientists at the KNCV TB Foundation, the Netherlands, for hosting my visits during the period of this work. I enjoyed presenting my work at your meetings and value the comments and critique that you gave.

I am also grateful to the Aeras Global Tuberculosis Vaccine Foundation for funding the bulk of this work. Thanks also go to Aeras staff, Ms M. Snowden, Dr L. Barker, Dr B. McLain, and others, for the discussions and insights shared during the many meetings we had over the course of this work.

I also thank the many parents whose children participated in the studies presented in this thesis. Thank you for your "faith" in science and for helping us move forward in our quest to deal with a disease that is undoubtedly one of the worst scourges of our time. We value and treasure your partnership with us. Lastly I thank and praise God for taking me through this journey.

Expanded Summary

The re-emergence of tuberculosis (TB) as a significant cause of morbidity and mortality, particularly in developing countries, has increased efforts to develop and test new and more efficacious vaccines against the disease. The existing vaccine against TB, the Bacille Calmette-Guérin (BCG) vaccine, which was first introduced about 90 years ago, confers protection against severe forms of TB in young children [1-3]. However protection against pulmonary TB the commonest form of the disease is variable and largely poor, hence the large burden of TB even where BCG vaccination coverage is high [4-8]. An example is South Africa, which has BCG vaccine coverage rates of up to 98%, and yet is one of the 22 high TB burden countries[4, 5]. In 2000 the case rate among children less than 15 years old was 237/100 000 children and 502/100 000 population [6]. In the Western Cape Province, in 2007, the incidence of childhood TB was estimated at 620/100 000/year [9]. In 2005, the cumulative incidence of notified TB in children below the age of five years in three sub-districts in the Cape Winelands district where most of the studies described in this thesis were conducted was 2.4% (95%CI 2.2–2.6) [10]. Furthermore, the BCG vaccine does not confer life-long protection against TB and revaccination has not demonstrated improved protection [11-13].

Infants and young children are a special target group for new vaccines against TB, because they have a greater risk of developing TB disease and progressing to severe morbidity [14, 15], and because it would be cost-effective and logistically easier to integrate new vaccines into existing childhood immunization programmes. However, the assessment of the efficacy of new TB vaccines in children is significantly hampered by the absence of a biological correlate of protective immunity against TB [16], which means that the current end-point for TB vaccine trials is pulmonary TB disease. Howbeit, the detection of pulmonary TB in young children is challenging because the symptoms and signs are non-specific and overlap with those of other childhood illnesses. Bacteriological confirmation of the disease, the diagnostic gold standard, is limited by the paucibacillary nature of the disease in young children even in the advent of new diagnostic modalities [17-20]. Furthermore, modern TB vaccine clinical trials are being conducted in an era where isoniazid preventive therapy (IPT) is recommended for children (and adults) with known exposure *M.tb* after exclusion of TB disease [21]. IPT impacts on the natural progression of TB and its manifestation [14] making a definitive diagnosis more difficult, in contrast to when BCG first entered human clinical trials in the 20th century [22-26]. In addition widespread use of IPT poses risk of development of *M.tb* strains that are resistant

to isoniazid a key compound in the treatment of TB [27]. However in children the risk of drug resistance from IPT is unlikely because microbial load is generally low since disease is paucibacillary and drug penetration good [28, 29].

The conduct of TB vaccine trials, specifically phase II and phase III trials in young children therefore, requires robust, efficient and accurate case-finding and case-detection methodologies to ensure that vaccine safety and efficacy are determined accurately. Weaknesses in case detection and case ascertainment introduce the risk of misclassification bias which can dilute or obscure the true efficacy of the vaccine being tested.

This PhD thesis explores methodologies for active TB case-finding, and TB case detection to inform the selection of case-finding strategies for clinical trials of TB vaccines conducted in young children, in high TB burden settings, in the 21st century.

We calculated age-specific TB incidence in children less than 5 years of age in a high TB burden setting and investigated the effect of the categorization of TB case definitions on the measurement of the incidence of TB in these children. This work showed the highest incidence of TB to be in children aged one and two years old, with high rates maintained beyond the age of 24 months. This suggests that clinical trials of new TB vaccines given at birth could increase case accrual by extending follow-up and TB case-finding for at least three years. However most studies have limited participant follow-up until the age of two years because the risk of developing TB disease in children is greatest in those below two years of age [14], and because of resource constraints.

We also investigated the impact of two active-case finding (ACF) strategies for TB on case yield and disease phenotype in children between birth and two years of age. Studies in adults have shown that ACF detects more cases and that they are less symptomatic than those detected passively [30-36]. However, few studies on ACF have been conducted in children, and no studies had yet compared different active case-finding strategies in young children [36-38]. It has therefore been unclear which case-finding strategies should be adopted in infant TB vaccine trials. We compared regular home visits for symptom screening for TB combined with medical record review (Group 1), with medical record review and a single screening visit at the end of the follow-up period (Group 2). Our hypotheses were that TB cases remaining undetected by the less intensive strategy (Group 2) would be found at a close-out

visit at two years of age, resulting in a similar TB case yield in the two groups, and that the TB cases would differ in clinical, radiological and bacteriological features, due to an early, less severe disease phenotype detected by the more intensive strategy (Group 1).

This study showed that regular home visits combined with record surveillance detected significantly more TB cases at a younger age than record surveillance alone with a single study end visit. However there was no significant difference in TB symptoms, signs, radiological and bacteriological profiles of TB cases in the two groups, and in both groups bacteriologically confirmed cases were few. We concluded that more intensive screening for TB, incorporating symptom based screening will maximise case detection in TB vaccine trials conducted in young children in similar settings. However, since bacteriologically confirmed cases were few, diagnostic algorithms based on symptoms, signs and other investigations should be highly specific to minimise misclassification bias which can dilute or obscure true vaccine efficacy.

We also compared the utility of an interferon gamma release assay with the tuberculin skin test (TST) for detecting *M.tb* infection in children less than 3 years old who were suspected of having TB disease. These children were tested using the QuantiFERON-Gold-in-tube (QFT) assay and the TST in addition to other standard investigations for TB disease. We found excellent concordance between TST and QFT in this population. Both tests had much lower sensitivity than has been reported in older age groups [39-41]. This finding suggests that the TST remains a useful test for detecting *M.tb* infection in populations and settings similar to ours.

Given the likelihood of the adoption of ACF strategies in infant TB vaccine trials, the low likelihood of bacteriological confirmation of *M.tb* among TB suspects, and the role of chest radiography in the diagnosis of TB in children [42], we analysed the radiographic features of children with suspected TB who were identified through ACF. This analysis showed that ACF in young children in a high TB burden setting mainly detects isolated hilar lymphadenopathy, compatible with uncomplicated primary complex TB, rather than progressive pulmonary disease or severe TB as commonly reported in these settings [37-43]. Growth failure was associated with lymphadenopathy and consolidation occurring in combination, suggesting that growth failure is a useful sentinel sign for detecting TB in ACF settings. There was low agreement between radiologists (weighted κ 0.27) for the detection of

radiologic abnormalities, highlighting the diagnostic difficulties associated with diagnosing TB in young children in infant TB vaccine trials.

Modern TB vaccine field trials are being conducted in developing countries where the burden of childhood morbidity and mortality is high. Therefore we analysed the main causes of hospital admissions and mortality at a vaccine trial site in a rural setting with aim of detecting TB morbidity and mortality occurring among trial participants. We found a wide spectrum of morbidity and mortality that was however dominated by respiratory tract infections (RTIs). This suggests that without improved diagnostics for TB, it may be difficult to determine the true proportion of undiagnosed TB as it has been shown that pulmonary TB may be undetected in children with RTIs in high TB burden settings due to overlap between symptoms [44-46].

The findings of this thesis provide significant contributions to the subject of TB case-finding in TB vaccine field trials and other TB preventive studies conducted in infants and young children in high TB burden settings. Our findings show that;

i) TB incidence in children is high throughout the first five years of life: - therefore clinical trials of new TB vaccines given at birth could increase case accrual by extending participant follow-up and TB case-finding for at least the first three years of life.
ii) Intensive active case-finding strategies will maximise TB case detection in infant TB vaccine trials.
iii) The TST remains a useful adjunct test for the detection of *M.tb* infection among young children with TB disease in high TB burden settings where the incidence of HIV is low.
iv) Active TB case-finding in a high TB burden setting largely detects a mild chest radiographic TB phenotype, rather than the classical phenotype of severe disease commonly reported in high TB burden countries. This mild radiographic disease phenotype may reflect radiological changes associated only with *M.tb*, and not TB disease. Growth failure, rather than persistent cough, appears to be the clinical hallmark of uncontained pulmonary TB in young children. Therefore infant TB vaccine trials adopting active case-finding should prioritize growth failure as a sentinel clinical feature for early detection of childhood TB in high TB burden settings.
v) The spectrum of morbidity and mortality among study participants at a TB vaccine trial site was dominated by respiratory tract infections and diarrhoeal disease. Although the

overall proportions were low, hospital admissions and deaths due to TB were significant contributors to serious adverse events in these trials. Despite extensive follow-up and data collection the ability to detect TB among trial participants who are hospitalised and those who may die is limited by the unavailability of better performing diagnostic tools for childhood TB.

The studies reported in this thesis were conducted before molecular TB diagnostics were available, hence we cannot comment on their performance in infant TB vaccine trial settings. However paediatric studies on the Xpert/RIF in hospital settings have shown greater sensitivity and specificity compared to specimen culture (using mycobacterial growth indicator and the Lowenstein-Jensen methods), suggesting that these would be valuable in infant TB vaccine trials [19, 47, 48]. Evaluation of these tests in these settings is essential.

References

1. Rodrigues LC, Divan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol* 1993; 22: 1154–1158.
2. Colditz GA, Brewer TF, Berkey CS. Efficacy of the BCG vaccine in the prevention of tuberculosis. *JAMA* 1994; 271: 698-702.
3. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; 367: 1173–1180.
4. The BCG World Atlas: A Database of Global BCG Vaccination Policies and Practices. http://www.bcgatlas.org/ (Accessed on 2o November 2012)
5. Corrigal J, Coetzee D, Cameron N. Is the Western Cape at risk of an outbreak of preventable childhood diseases? Lessons from an evaluation of routine immunisation coverage. *S Afr Med J* 2008; 98: 41–45.
6. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. *Int J Tuberc Lung Dis* 2004; 8:636-47.
7. Donald P R, 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
8. World Health Organization. Global Tuberculosis Control. 2010. WHO/HTM/TB/2011.16 Geneva: World Health Organization; 2011. http://whqlibdoc.who.int/publications/2011/9789241564380_eng.pdf
9. Van Wyk S, Reid AJ, Mandalakas A, *et al* Operational challenges in managing Isoniazid Preventive Therapy in child contacts: A high-burden setting perspective. *BMC Public Health* 2011, 11:544.
10. 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; 2:149-154.
11. (CDC). Global routine vaccination coverage, 2009. *MMWR Morb Mortal Wkly Rep* 2010; 59:1367-1371.7.
12. Waning immunity Sterne JA, Rodrigues LC, Guedes IN: Does the efficacy of BCG decline with time since vaccination? *Int J Tuberc Lung Dis* 1998; 2:200-207.

13. Weir RE, Gorak-Stolinska P, Floyd S, et al. Persistence of the immune response induced by BCG vaccination. *BMC Infect Dis.* 2008; 8:9.
14. Rodrigues LC, Pereira SM, Cunha SS, *et al*. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet.* 20058; 366:1290-5.
15. Natural history Marais B J, Gie R P, Schaaf H S, *et al*. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004; 8: 392–402.
16. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Enarson DA, Beyers N. The spectrum of disease in children treated for tuberculosis in a highly endemic area. *Int J Tuberc Lung Dis.* 2006; 10:732-8.
17. Ottenhoff TH, Ellner JJ, Kaufmann SH. Ten challenges for TB biomarkers. *Tuberculosis* 2012; 92 Suppl 1:S17-20.
18. Zar H J, 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* 2005; 365: 130–134.
19. Nicol MP, Workman L, Isaacs W, *et al*. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. *Lancet Infect Dis* 2011; 11:819-24.
20. Connell TG, Zar HJ, Nicol MP. Advances in the diagnosis of pulmonary tuberculosis in HIV-infected and HIV-uninfected children. *J Infect Dis.* 2011; 204 Suppl 4:S1151-8. Review.
21. 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:S209-15.
22. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. WHO/HTM/TB/2006.371, WHO/FCH/CAH/2006.7. Geneva, Switzerland: WHO, 2006. http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf (Accessed on 10 June 2012).
23. Hatherill M, Verver S, Mahomed H; Taskforce on Clinical Research Issues, Stop TB Partnership Working Group on TB Vaccines. Consensus statement on diagnostic endpoints for infant tuberculosis vaccine trials. *Clin Infect Dis* 2012;54:493-501

24. 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:S199-208.
25. Aronson J. Protective vaccination against tuberculosis with special reference to BCG vaccination. *Am Rev Tuberc* 1948; 58:255-281.
26. Aronson J. Palmer C. Experience with BCG vaccine in the control of tuberculosis among North American Indians. *Public Health Rep* 1946; 61:802-850.
27. Balcells ME, Thomas SL, Godfrey-Faussett P, Grant AD, Isoniazid preventive therapy and risk for resistant tuberculosis *Emerg Infect Dis* 2006;5:744-751.
28. Smieja MJ, Marchetti CA, Cook DJ, *et al*. Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev 2000 ;(2): CD001363.
29. Marais BJ, Ayles H, Graham SM, Godfrey-Faussett P, Screening and preventive therapy for tuberculosis. *Clin Chest Med* 2009; 30:827–846.
30. Rosenthal SR, Leppman M. Tuberculosis control: BCG vaccination in a tuberculosis control program in infants, children and adults. *Trans Natl Tuberc Assoc* 1953;161-168
31. Golub J E, Mohan C I, Comstock G W, Chaisson R E. Active case finding of tuberculosis: historical perspective and future prospects. *Int J Tuberc Lung Dis* 2005; 9: 1183–1203.
32. Miller A C, Golub J E, Cavalcante S C, et al. Controlled trial of active tuberculosis case finding in a Brazilian *favela*. *Int J Tuberc Lung Dis* 2010; 14: 720–726.
33. Corbett E L, Bandason T, Duong T, *et al*. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster randomised trial. *Lancet* 2010; 376: 1244–1253.
34. Eang MT, Satha P, Yadav RP, *et al*. Early detection of tuberculosis through community-based active case finding in Cambodia. *BMC Public Health* 2012; 12:469
35. Story A, Aldridge R W, Abubakar I, *et al*. Active case finding for pulmonary tuberculosis using mobile digital chest radiography: an observational study. *Int J Tuberc Lung Dis* 2012; 16: 1461–1467.
36. Churchyard G J, Fielding K, Roux S, *et al*. Twelve-monthly versus six-monthly radiological screening for active case-finding of tuberculosis: a randomised controlled trial. *Thorax* 2011; 66: 134–139.

37. Den Boon S, Verver S, Lombard C J, *et al*. Comparison of symptoms and treatment outcomes between actively and passively detected tuberculosis cases: the additional value of active case finding. *Epidemiol Infect* 2008; 136: 1342–1349
38. Zachariah R, Spielmann M-P, Harries AD, *et al*. Passive versus active tuberculosis case finding and isoniazid preventive therapy among household contacts in a rural district of Malawi. *Int J Tuberc Lung Dis* 2003; 11: 1033–1039.
39. Ward H A, Marciniuk DD, Pahwa P, Hoeppner V H. Extent of pulmonary tuberculosis in patients diagnosed by active compared to passive case finding. *Int J Tuberc Lung Dis* 2004; 8: 593–597.
40. Dheda K, Smit R V, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. *Current Opinion Pulm Med* 2009; 15: 188–200.
41. Dogra S, Narang P, Mendiratta D K, et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. *J Infect* 2006; 54: 267–276.
42. Detjen A K, Keil T, Roll S, *et al*. Interferon-gamma release assay improve the diagnosis of tuberculosis and non-tuberculosis mycobacterial disease in children in a country with a low incidence of tuberculosis. *Clin Infect Dis* 2007; 45: 322–328.
43. Andronikou S, Wieselthaler N. Imaging for tuberculosis in children. In: Schaaf SH, Zumla AI, Tuberculosis–a comprehensive clinical reference. United Kingdom: Saunders Elsevier, 2009: 262-296.
44. Salazar G E, Schmitz T L, Cama R, *et al*. Pulmonary tuberculosis in children in a developing country. *Pediatrics* 2001; 108: 448–453.
45. Chintu C, Mudenda V, Lucas S. *et al* Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; 360:985-90.
46. 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: a pneumococcal conjugate vaccine probe study. *Pediatr Infect Dis J.* 2010; 29:1099-04.
47. Sekadde MP, Wobudeya E, Joloba ML *et al*. Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. *BMC Infectious Diseases* 2013, 13:133.

48. Rachow APC, Saathoff E, Mtafya B, *et al*. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. *Clin Infect Dis* 2012, 10; 1388–1396.

Aims:

1. To conduct a review of active case-finding strategies for TB in adults and children. (Chapter 2)
2. To compare TB case yield and disease profile among Bacille Calmette-Guérin (BCG) vaccinated children from birth until two years of age using two active case-finding strategies. (Chapter 3).
3. To compare results from the tuberculin skin test (TST) and the QuantiFERON®-TB Gold In-Tube assay (QFT) in young children investigated for TB disease in a high TB burden and low HIV setting. (Chapter 4).
4. To describe chest radiographic features in young children with suspected pulmonary TB, in active case-finding in a high TB burden setting, and to identify the key clinical features associated with these radiographic phenotypes. (Chapter 5).
5. To determine the nature and rate of serious adverse events, defined as hospital admissions and deaths, and the proportion due to TB in young children enrolled in TB vaccine trials. (Chapter 6).

Chapter 1: General introduction

Tuberculosis transmission and risk of disease

Tuberculosis (TB) is caused by the *Mycobacterium tuberculosis* (*M.tb*) bacillus and is transmitted via droplet infection from individuals with active disease. Healthy individuals who inhale the bacilli may successfully resist infection, or become latently infected (latent tuberculosis infection, (LTBI)), whereby the bacilli are inactivated and lie dormant, or develop active TB disease (primary TB). Latently infected individuals may develop active TB (secondary TB) at a later stage. It is estimated that approximately 30% of the world population is latently infected with *M.tb* [1] The life time risk (among HIV negative individuals) of developing TB following infection with *M.tb* is estimated to be 10% [2-4], with about half of those who develop the disease doing so within the first five years post infection [5].

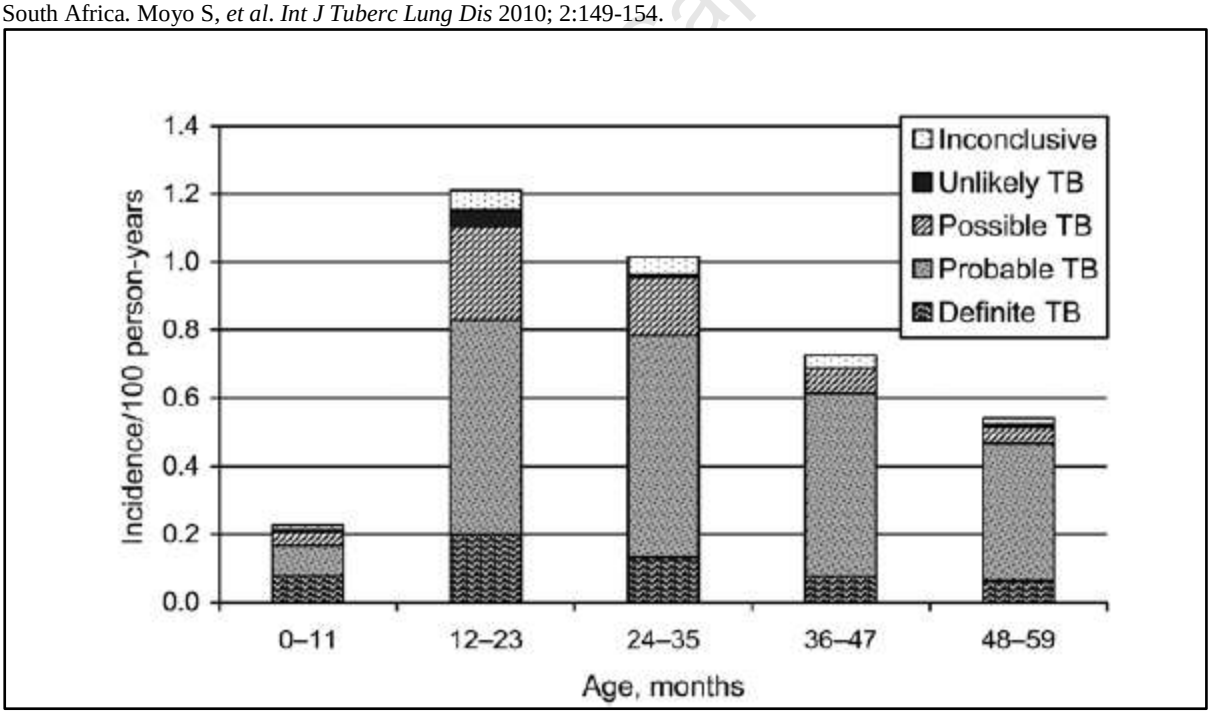
Young children have a high risk of developing active disease following infection with *M.tb* because children generally have 'immature' immune systems than compared to adults, and are therefore less able to mount adequate responses to resist infection and the development of disease [6-8]. This risk is greatest among the youngest children, with those who are less than 1 year old having up to 40% risk of developing primary TB disease following exposure to *M.tb* in the absence of any preventive measures [9]. In addition young children are at greatest risk of developing the severe forms of the disease namely miliary tuberculosis and tuberculous meningitis.

Population groups with compromised immune systems are also at high risk of developing TB. In individuals who are infected with the human immunodeficiency virus (HIV) the risk of developing TB is 20-37 times greater than in those who are uninfected, and TB is the leading cause of mortality among HIV infected individuals, including HIV infected children. Other conditions that weaken the immune system such as malnutrition, cancer, stress also increase the risk of developing TB. TB is also a disease associated with poverty. Poor social conditions such as overcrowding and poor ventilation that facilitate the transmission of *M.tb,* and are common in high TB burden settings increase the risk of TB.

Tuberculosis in children

Although the majority of cases of TB occur in adults, the large burden of childhood TB disease has been increasingly recognised over the past two decades. While young children with TB typically do not transmit disease and hence do not contribute to maintaining the TB epidemic, TB is a significant cause of morbidity and mortality in children [5, 10-13]. Of the 9 million annual TB cases in the world, it is estimated that about 1 million of these cases occur in children less than 15 years old [12]. In high burden countries childhood TB contributes up to 20% or more of the TB case load [12]. Since the youngest children are at the highest risk of developing disease, the disease is thus largely borne by those less than 5 years old in high burden countries [9]. In Cape Town, Moyo *et al*, found the highest incidence of TB in children aged one and two years old, in a cohort of children below the age of 5 years (**Figure1**) [14].

Figure1: TB incidence rate by age among children less than 5 years old in Cape Town (1999-2004) Age related tuberculosis incidence and severity in children under 5 years of age in Cape Town, South Africa. Moyo S, *et al*. *Int J Tuberc Lung Dis* 2010; 2:149-154.



The youngest children are also at greatest risk of developing the more severe forms of the disease: - miliary TB and tuberculous meningitis, which can cause long lasting sequelae if not diagnosed and treated timeously and appropriately. In the study mentioned above (Moyo *et*

al,) 11% of all children with probable and definite TB had disseminated disease, the largest proportion (6%) being miliary TB[14].

In endemic settings children also experience a wide spectrum of TB disease manifestations and complications related to pulmonary disease. These include pleural disease, pericarditis, tuberculomas, airway compression, and dissemination to other organs such as the bones and the joints [9, 15]. Children also experience significant TB mortality [10, 16]. An audit of deaths in children <13 years old found TB to be a leading cause of death among children in South African hospitals, with 7% of all audited deaths attributed to TB [16]. An autopsy study in young children dying from respiratory tract infections in Zambia, found TB to be a major cause of mortality [10].

Cases of childhood TB are important because they reflect recent infection and thus active disease transmission in the community since disease in children manifests relatively shortly after infection, commonly within one year of primary infection. Therefore, the incidence of childhood TB is a marker of TB control. An analysis estimating TB incidence rates in children <15 years old globally, revealed very high rates in 2000, with rates in five African countries exceeding 100/100 000 [11]. Although these estimates may now be outdated they nonetheless reflect sub-optimal control of the TB epidemic with on-going transmission of disease in high TB burden countries.

Children who are infected with *M.tb* are a reservoir for later disease, because latent bacteria can be reactivated resulting in secondary TB later on in life [9]. This has significant impact on the long-term control of the TB epidemic in the absence of a vaccine that can prevent reactivation of latent bacilli [17, 18].

TB control

The global strategy for TB control is the World Health Organisation's (WHO) STOP TB strategy [19]. The strategy has six main components which are:

i) Pursuing high-quality Directly Observed Treatment Short course (DOTS) expansion and enhancement;

ii) Addressing TB/HIV and multi-drug resistant (MDR)-TB and other special challenges;

iii) Contributing to health systems strengthening;

iv) Engaging all care providers;

v) Empowering people with TB, and communities;

vi) Enabling and promoting research.

In many high prevalence countries TB control has predominantly focused on the provision of DOTS and therefore has been targeted at the detection and treatment of infectious cases, largely adults who are the main transmitters of disease. In children, control strategies include vaccination with the Bacille Calmette-Guérin (BCG) vaccine, in countries with a high prevalence of TB, and contact tracing and prescription of isoniazid preventive therapy (IPT) to children less than five years old who have had close contact with an adult TB case, where appropriate evaluation has excluded TB disease [12, 20]. Until recently in many of these countries TB control in children has largely consisted of BCG vaccination only, with poor implementation of contact tracing and IPT provision [21, 22]. IPT is now also recommended for HIV infected individuals, including children, given the high risk of TB in HIV infection [23].

While IPT has been shown to be effective in preventing TB disease following exposure to *M.tb*, there are risks of adverse effects associated with its use[24-28]. A major concern is the development hepatotoxicity [26-28]. However children have demonstrated better tolerance for TB drugs than adults, hence adverse events in children have been rare [22, 25]. Secondly, although wide spread use of IPT creates risk of development of *M.tb* strains that are resistant to isoniazid, where TB disease has been excluded prior to initiation of therapy this risk is minimal [26]. In children the risk of drug resistance from IPT is particularly unlikely because the microbial load is low since disease is paucibacillary, and drug penetration is good in this population [22].

The BCG vaccine

The BCG vaccine is currently the only vaccine available for protection against TB. The WHO recommends administration of BCG at birth in countries with a high prevalence of TB. While studies have shown that BCG confers protection against the severe forms of childhood

TB [29, 30], protection against pulmonary TB is highly variable [29-32]. Early trials of BCG reported vaccine efficacy ranging from 80%, indicating substantial protection, to -56%, indicating detrimental vaccine effects [32-35]. A meta-analysis of clinical trials of BCG showed an overall protective effect of 51% [31].

Variability in the protective efficacy of BCG with the lowest protection observed in countries with the highest incidence of TB has been attributed to a number of factors. These include genetic and nutritional differences between populations, geographic latitude, climate, differences in the strains of the BCG preparation used in the manufacture of the vaccine, poor cold chain management, and differences in exposure to environmental mycobacteria [36-38]. It has also been postulated that BCG efficacy is reduced when given in the early neonatal period when the immune system is immature. Kagina *et al* showed improved BCG efficacy when vaccination was delayed to 10 weeks of age [39]. However early administration is preferred in high TB burden countries because children may not return for vaccination at a later date and because exposure to *M.tb* occurs from a very early age.

The current prevailing hypotheses for BCG failure are based on exposure to environmental mycobacteria, where it is postulated i) that exposure to environmental mycobacteria prevents any additional protective effect from BCG vaccination (the masking hypothesis) or ii) that pre-existing immune responses to antigens common to mycobacteria (induced by environmental mycobacteria) block the replication of BCG and therefore inhibit vaccine "take" [38].

Severe cellular immune deficiencies have been associated with systemic or disseminated BCG disease following vaccination with BCG [40]. This risk has also been demonstrated in HIV positive children and has been associated with a high rate of all-cause mortality [41, 42]. Therefore BCG vaccination is not recommended for HIV positive children. However, a systematic review by Azzopardi *et al*, found the studies on BCG dissemination in HIV infected children to be heterogeneous and showed that data on the risk of BCG vaccination in this population are limited [43]. This suggests that further studies and data on this topic are required.

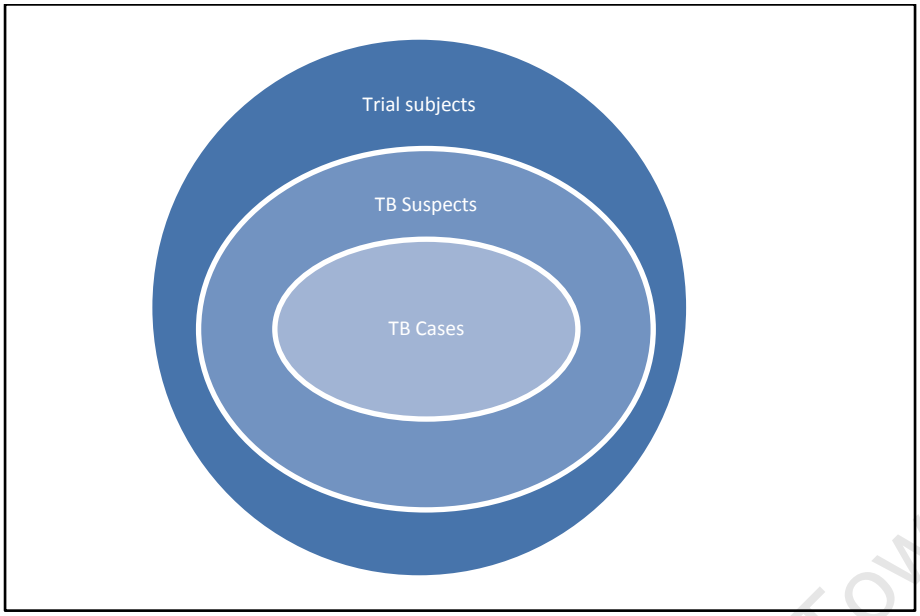
The sub-optimal protective efficacy of BCG and the safety concerns in HIV infected children in an environment of a massive TB disease burden have renewed interest in the development

and testing of more effective vaccines against TB. This forms part of wider efforts directed toward new and improved strategies for TB control and prevention, given the prevailing high disease burden, poor treatment outcomes, the emergence of drug resistant strains of *M.tb*, and the high risk of TB among HIV positive individuals. The new vaccines being developed to combat TB are targeted at different age groups including infants and young children. Infants and young children are a special target group for new TB vaccines because of their greater risk of developing TB disease and because it would be beneficial (cost-effective and logistically simpler) to integrate new vaccines into the already existing childhood immunization programmes.

TB case-finding for clinical trials of new TB vaccines in young children

As with trials of other therapeutic interventions, TB vaccine trials in infants and young children require sound and robust methodologies for randomization, blinding, subject follow-up, subject assessment, case detection and case ascertainment, and statistical analyses, to ensure accurate determination of vaccine safety and efficacy. In the context of conflicting results of early BCG vaccine trials, Clemens *et al* conducted a statistical and methodological appraisal of eight early community trials of the BCG vaccine [32]. He reported that poor precision and methodological flaws in the conduct of these trials resulted in various biases (susceptibility bias, detection bias, diagnostic bias, and diagnostic interpretation bias) that contributed to the conflicting data reported [32]. Bias in the detection of TB cases was cited as a major source of vulnerability [32]. Therefore, methodologies for detecting cases are a critical component of TB vaccine trials, particularly those conducted in infants and young children. **Figure 2** illustrates the relationships between trial subjects, TB suspects and TB cases in a vaccine trial.

Figure 2: Relationships between trial subjects, TB suspects and TB cases in a vaccine trial

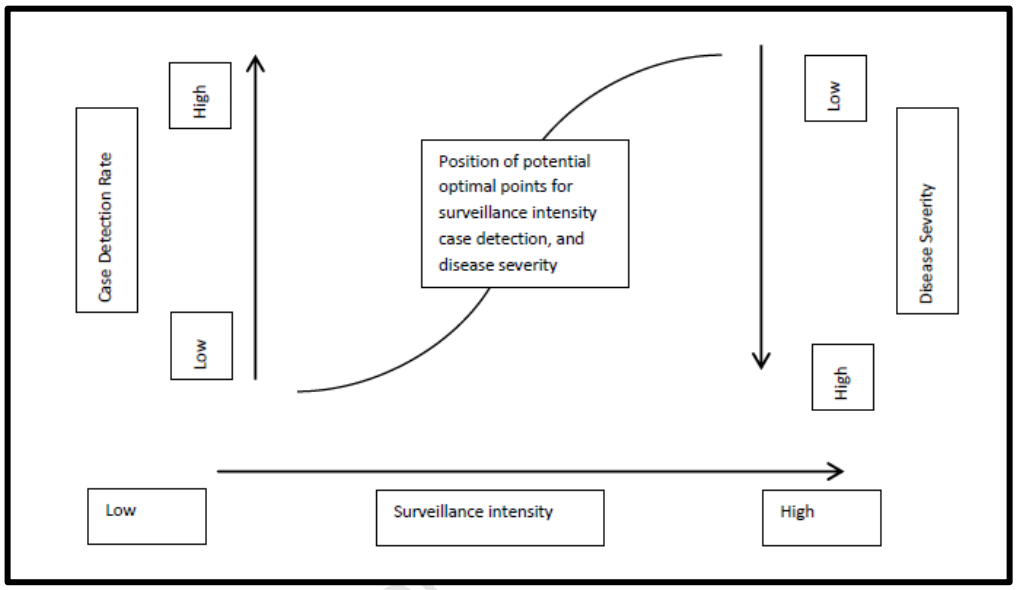


The detection of TB cases rests on the identification and evaluation of children suspected of having disease from the pool of trial subjects. However the signs and symptoms of pulmonary TB (PTB) in young children are non-specific making it difficult to identify suspects; hence the movement of trial subjects into the TB suspect and the TB case groups is critical. Secondly, in the context of vaccine trials, highly specific definitions for both suspects and cases are important to minimize disease misclassification which may dilute vaccine efficacy measurements. The current diagnostic gold standard (with high specificity) for TB disease is positive mycobacterial culture. However positive culture results are achieved in only 5–25% of children investigated for suspected TB [44-47]. Therefore diagnosis also has to rely on diagnostic algorithms incorporating symptoms, clinical signs, chest radiographic findings and other investigations such as the tuberculin skin test, or interferon gamma release assays. These algorithms however, have limitations and have been shown to perform differently in different settings [48-51]. Given the high costs of conducting clinical trials [52], it is imperative that surveillance methodologies detect TB suspects efficiently maximizing the likelihood of disease confirmation, the current TB vaccine trial endpoint.

Figure 3 shows the interplay between surveillance intensity, the TB case detection rate and TB disease severity. Intensive surveillance increases TB case detection. However, it detects mild cases, for which diagnosis and therefore bacteriological disease confirmation is most difficult. This factor is relevant for TB vaccine trials in infants, since TB case finding and

accurate case definition impact on the measurement of vaccine efficacy [53, 54]. It seems unlikely that the relationship between these factors is a simple linear one, because there are many factors that impact surveillance intensity, the TB case detection rate and TB disease severity individually and collectively [55]. These include the recognition and reporting of symptoms by parents, the duration of illness before reporting to a healthcare facility, and the recognition of TB symptoms and diagnosis by healthcare workers. The critical point for *optimal surveillance intensity, case detection rate and disease severity* therefore lies somewhere along the curve that is displayed in **Figure 3**.

Figure 3: Surveillance intensity, case detection rate and disease severity



Contributors

This chapter was written by Dr Sizulu Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey.

References

1. Raviglione MC, Snider DE Jr, Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. *JAMA* 1995; 273: 220–226.
2. Barry CE, III, Boshof HI, Dartois V, *et al*. The spectrum of latent tuberculosis, rethinking the biology and intervention strategies *Nat Rev Microbiol* 2009, 7: 845-855.
3. Bloom BR, Murray CJL. Tuberculosis: a commentary on a re-emergent killer. *Science* 1992; 457: 1055–64.
4. Broekmans JF. Control strategies and programme management. In: Porter JDH, McAdam KPWJ, eds. Tuberculosis. Back to the future. Chichester, England: John Wiley and Sons Ltd, 1994:171–88.
5. Harries AD, Dye C. Tuberculosis *Ann med Parasitol* 2006; 100:415-431.
6. Lewinsohn DA, Lewinsohn DM. Immunologic susceptibility of young children to Mycobacterium tuberculosis Pediatr Res. 2008; 63:115.
7. PrabhuDas M, Adkins B, Gans H, *et al*. Challenges in infant immunity: Implications for responses to infection and vaccines. *Nat Immunol* 2011; 12:189-194.
8. Lewinsohn DA, Gennaro ML, Scholvinck L, Lewinsohn DM. Tuberculosis immunology in children: diagnostic and therapeutic challenges and opportunities. *Int J Tuberc Lung Dis* 2004; 8:658–674.
9. Marais BJ, Gie RP, Schaaf HS, *et al*. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004; 8: 392–402.
10. Chintu C, Mudenda V, Lucas S. *et al* Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; 360:985-90.
11. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. *Int J Tuber Lung Dis* 2004; 85:636–647.
12. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. WHO/HTM/TB/2006.371, WHO/FCH/CAH/2006.7. Geneva, Switzerland: WHO, 2006. http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf. (Accessed on 10 June 2012).

13. van Rie A, Beyers N, Gie RP, Kunneke M, Zietsman L, Donald PR. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Arch Dis Child.* 1999 80:5; 433-7.
14. 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; 2:149-154.
15. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Enarson DA, Beyers N. The spectrum of disease in children treated for tuberculosis in a high endemic area. *Int J Tuber Lung* Dis 2006; 10: 732-738.
16. Stephen CR, Bamford LJ, Patrick ME, Wittenberg DF eds. Saving Children 2009: Five Years of Data A sixth survey of child healthcare in South Africa. Pretoria: Tshepesa Press, MRC, CDC; 2011 Accessed from http://www.childpip.org.za/documents/saving_children_2009.pdf (Accessed on 3 June 2012).
17. Paul-Henri Lambert P, Hawkridge T, Hanekom WA. New vaccines against tuberculosis. *Clin Chest Med* 2009 30; 811–826.
18. Kaufmann SHE, Hussey G, Lambert P. New vaccines for tuberculosis. *Lancet* 2010; 375: 2110–19.
19. The Stop TB Strategy. WHO, 2006. WHO/HTM/TB/2006.368. http://whqlibdoc.who.int/hq/2006/WHO_HTM_STB_2006.368_eng.pdf (Accessed on 3 April 2012).
20. 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.
21. Nyirenda M, Sinfield R, Haves S, Molyneux EM, Graham SM. Poor attendance at a child TB contact clinic in Malawi *Int J Tuberc Lung Dis* 2006; 10:585–587.
22. Marais BJ, Ayles H, Graham SM, Godfrey-Faussett P, Screening and preventive therapy for tuberculosis. *Clin Chest Med* 2009; 30:827–846.
23. WHO policy on collaborative TB/HIV activities. Guidelines for national programmes and other stakeholders. WHO, 2012. WWHO/HTM/TB/2012.1. http://whqlibdoc.who.int/publications/2012/9789241503006_eng.pdf (Accessed on 3 April 2012).
24. Hsu KH. Isoniazid in the prevention and treatment of tuberculosis. A 20-year study of the effectiveness in children. *JAMA* 1974; 229:528–33.

25. Hsu KH. Thirty years after isoniazid: its impact on tuberculosis in children and adolescents. *JAMA* 1984; 251:1283–5.
26. Smieja MJ, Marchetti CA, Cook DJ, *et al*. Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev 2000 ;(2): CD001363.
27. Cook PP, Maldonado RA, Yarnell CT, *et al*. Safety and completion rate of short-course therapy for treatment of latent tuberculosis infection. *Clin Infect Dis* 2006; 43: 271–5.
28. Lobato MN, Jereb JA, Starke JR. Unintended consequences: mandatory tuberculin skin testing and severe isoniazid hepatotoxicity. *Pediatrics* 2008; 121:e1732–3.
29. Rodrigues LC, Divan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol* 1993; 22: 1154–1158.
30. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; 367: 1173–1180.
31. Colditz GA, Brewer TF, Berkey CS. Efficacy of the BCG vaccine in the prevention of tuberculosis. *JAMA* 1994; 271: 698-702.
32. Clemens JD, Chuong JJ, Feinstein AR. The BCG controversy. A methodological and statistical reappraisal. *JAMA* 1983; 6: 2362-9.
33. Aronson JD. Protective vaccination against tuberculosis with special reference to BCG vaccination. *Am Rev Tuberc*. 1948; 58: 255-281.
34. Cormstock GW, Livesay VT, Woolpert SP. Evaluation of BCG vaccination among Puerto Rican children. *Am J Public Health*. 1974; 64: 283:291.
35. Anon. Fifteen year follow-up of trial of BCG vaccines in South India for tuberculosis prevention. Tuberculosis Research Centre (ICMR), Chennai Indian. *J. Med Res* 1999:110: 56-59.
36. Fine PEM, Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* 1995; 346:1339-45.
37. Fine PEM, Rodgrigues LC. Modern vaccines: mycobacterial diseases. *Lancet* 335:1016-20.
38. Anderson P, Doherty TM. The success and failure of BCG- implications for a novel tuberculosis vaccine. *Nat Rev Microbiol* 2005; 3:656-62.

39. Kagina BMN, Abel B, Bowmaker M *et al*. Delaying BCG vaccination from birth to four weeks may result in an enhanced memory CD4 T cell response. *Vaccine.* 2009; 40: 5488–5495.
40. Lotte A, Wasz-Hockert O, Poisson N, Dumitrescu N, Verron M, Couvert E. BCG complications: estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics. 1984 *Adv Tub Res*, 21:107–193.
41. Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated vaccination: case report and review. *Clin Infect Dis* 1997; 24: 1139–1146.
42. Hesseling AC, Cotton MF, von Reyn CF *et al*. Consensus statement on the revised World Health Organization recommendations for BCG vaccination in HIV-infected infants *Int J Tuber Lung Dis* 2008;121376–1379.
43. Azzopardi P, Bennett CM, Graham SM, Duke T. Bacille Calmette-Guérin vaccine-related disease in HIV-infected children: a systematic review. *Int J Tuberc Lung Dis.* 2009; 13:1331-44.
44. Coulter JBS. Diagnosis of pulmonary tuberculosis in young children. *Ann Trop Paediatr* 2008; 28: 3–12.
45. 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* 2005; 365: 130–4.
46. Hawkridge A, Hatherill M, Little F, *et al*. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. *BMJ* 2008; 337: a2052.
47. Moyo S, Verver S, Hawkridge A, *et al*. and the South African Tuberculosis Vaccine Initiative Neonatal Study Team. Tuberculosis case finding for vaccine trials in young children in high incidence settings: a randomised trial. *Int J Tuberc Lung Dis* 2012; 16: 185–191.
48. Starke JR. Pediatric tuberculosis: time for a new approach. *Tuberculosis* 2003; 83: 208–212.
49. Marais BJ, Obihara C, Gie RP, *et al*. The prevalence of symptoms associated with pulmonary tuberculosis in randomly selected children from a high burden community. *Arch Dis Child* 2005; 11:1166–1170.
50. Hatherill M, Hanslo M, Hawkridge T, et *al*. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. *Bull World Health Organ* 2010; 88:312–20.

51. Hesseling AC, 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* 2002; 6:1038–45.
52. Stop TB Partnership Working Group on New TB Vaccines. Strategic Plan of the Stop TB Partnership Working Group on New TB Vaccines 2006-2015 http://www.stoptb.org/wg/new_vaccines/assets/documents/Strat%20Plan%20New%20Vaccines.pdf (Accessed on 30 August 2012).
53. Orenstein WA, Bernier RH, Hinman A R. Assessing vaccine efficacy in the field: further observations. *Epidemiol Rev* 1988; 10: 212–241.
54. Hackshaw A. Types of outcome measures and understanding them. In A concise guide to clinical trials.1st ed. Chichester, UK: Wiley-Blackwell Publishing, 2009
55. Lonnroth K, Uplekar M, Ottmani S, Blanc L. Stop TB Partnership. An action framework for higher and earlier TB case detection. Background document for DOTS Expansion Working Group Meeting. http://www.stoptb.org/assets/documents/global/awards/tbreach/Achieving%20higher%20case%20detection.pdf (Accessed on 30 August 2012).

Chapter 2: Active case finding for tuberculosis: A review

Summary

Background: The persistently high burden of tuberculosis (TB) has increased interest in active case-finding for tuberculosis as a component of TB control, since it has been shown to increase case detection.

Objectives: To conduct a review of active case-finding strategies for TB in adults and children.

Methodology: We conducted a review of active case-finding methods for TB.

Results: Approaches to active case-finding for TB involve either direct contact with individuals for screening, or the provision of information about TB and encouragement of symptomatic individuals to present to healthcare centres for screening. Strategies include contact tracing, mass radiography, outpatient screening, enhanced case-finding, and intensified case-finding. The majority of studies and evaluations of the various active case-finding strategies have been conducted in adults. Studies in children have focused on contact tracing, which has been shown to be effective in detecting disease and latent TB infection in child and adult contacts when appropriately implemented.

Conclusion: Although active case-finding detects more cases than passive case finding, there is very little data to inform the selection of active case-finding strategies for infant TB vaccine trials.

Introduction

The World Health Organisation's (WHO) STOP TB strategy for TB control includes the Directly Observed Treatment Short Course (DOTS) program, where cases of TB are detected passively (passive case-finding (PCF)), with emphasis on the treatment of infectious cases to reduce disease transmission [1]. PCF is the detection of TB cases among symptomatic individuals who self-present to healthcare centres [2, 3]. It is patient initiated and thereafter relies on the ability of the health system to identify TB among those seeking care. However in PCF, TB cases tend to be detected at a late stage. The delay in the detection and subsequent treatment of cases leads to prolonged on-going transmission of *M.tb*, which has a significant negative impact on TB control efforts and epidemiological targets [1, 4].

The delay in presentation and initiation of treatment of infectious cases, the high prevalence of TB, the increased risk of TB in HIV positive individuals, poor adherence to treatment among TB patients, and the emergence of drug resistant strains of *M.tb*, have increased interest and focus on active case finding (ACF) for TB as a means of improving TB control through early detection and treatment [5, 6]. Early detection and treatment of cases interrupts disease transmission thereby decreasing the infectious pool of *M.tb* bacilli [2, 3, 7, 8.]

ACF relies on the initiative or special effort by the health system to identify and evaluate people who would otherwise not seek care on their own initiative. It is based on direct contact with individuals and evaluation for TB on-site or shortly thereafter [2, 9]. ACF has been shown to detect cases of TB earlier than occurs in PCF[2]. It is therefore beneficial in TB control efforts since the early detection and treatment of infectious cases can reduce the number of subsequent and secondary cases of TB [2, 3, 9, 10]. In children the distinction between *M.tb* infection and active TB disease can be difficult because making a definitive diagnosis of disease is more challenging due non-specific symptoms and signs and the paucibacillary nature of the disease in this population [11-13].

A review focusing on the strengths, limitations, and the history future and prospects of ACF has previously been published [2]. Presently, further work is being undertaken to update this review (*Personal communication Shapiro AE, John Hopkins Centre for TB Research*).

The objective of this chapter is to summarize ACF strategies for TB that have been used in adults and children.

Review of active case finding methods

Contact tracing

Contact tracing is the identification and evaluation for TB disease of individuals who have had close contact with a recently diagnosed infectious case of TB (mostly smear positive adults). Thereafter, appropriate treatment is given: - curative therapy for those with TB disease and preventive therapy for those with latent TB infection (LTBI). Contact tracing has been considered to be the first logical extension beyond PCF, since it relies on the diagnosis of TB in contacts of TB cases who would have self-presented for care [3]. While close contacts have traditionally been individuals living in the same household as the index TB case, it has however been shown that the definition of close contacts should also include non-house hold members who have had any prolonged or regular contact with the TB case, especially in high TB prevalence areas [14]. Secondly, recent data demonstrate a need to broaden the definition of "household", in the context of contact tracing to allow all possible cases at risk to be identified [15]. While in the majority of cases an adult is the index TB case, searching for contacts where a child is the index has also been shown to be valuable in detecting previously undiagnosed TB among adults [16, 17].

In developed countries contact tracing is routinely conducted on all contacts regardless of age, and has contributed to the decline and the maintenance of the low TB burden in these countries [18, 19]. In developing countries, until recently, contact tracing within national TB programmes has focused only on young children (less than 5 years old), because of the higher risk of developing TB disease following exposure to *M.tb* in this age group [13]. These countries have generally had limited resources for wider contact tracing, and provision of IPT beyond this age group. However, even among young children contact tracing is often not undertaken in many high TB prevalence settings, because of limited capacity for chest radiography and tuberculin skin testing which have traditionally been used for TB screening. Secondly, most national TB programmes have prioritized the management of infectious cases [20, 21] leading to the relative neglect of contact tracing. To increase contact tracing among children in resource limited settings symptom based screening of child contacts is now recommended where chest radiography and the tuberculin skin testing are not available [22].

Furthermore, in the context of the HIV epidemic and the benefits of IPT, current policies strongly advocate regular screening for TB among HIV positive individuals and their household members, and provision of IPT to HIV infected individuals of all ages. [7, 23-27].

Mass radiography

Mass radiography entails taking chest radiographs (generally miniature radiographs) in large population groups as a screening measure for TB. Before the advent of effective chemotherapy against TB, mass radiography was a fundamental component of TB control in developed countries [2, 28-33]. Entire communities and cities were surveyed [30, 32, 33]. In developing countries mass radiography was mainly conducted in India [34, 35]. Mass radiography was successful in detecting previously unknown cases of TB and diagnosing them earlier than would have occurred otherwise. The strategy was supported by specialised TB case management through dedicated TB units, and was effective in decreasing TB in developed countries. However, this could not be achieved in developing countries because of limited resources and infrastructure to support mass radiography and the accompanying specialised TB management units that existed in the developed world [36]. Thus, although population surveys using mass miniature radiography may detect up to 90% of prevalent cases of TB in participating populations, the resource and logistical arrangements make these surveys very costly, and cost-ineffective when compared to other active case finding approaches [37]. Mass radiography was therefore officially discouraged by the WHO Expert Committee on Tuberculosis in the 1960s and the 1970s [38].

Chest radiographs have however remained an integral part of diagnostic algorithms for TB, and mass radiography has continued to be used in targeted settings; - namely TB prevalence surveys, screening for TB in schools and occupational settings, and screening of high risk groups, such as immigrants from TB endemic countries entering low TB incidence countries, drug users, the homeless and prisoners [39-41]. In South Africa, 12 and 6-monthly radiographic screening were investigated and compared as ACF strategies among gold miners, a high risk group for TB [42]. Six monthly screening detected less extensive disease and showed tendency toward lower TB-specific mortality [42]. A recent TB prevalence survey in Kenya found a higher sensitivity for chest radiography than for symptoms alone, with the highest sensitivity being that for combined chest radiography and symptom screening [43]. In the United Kingdom, digital chest radiography had very high sensitivity, specificity, and positive predictive value in high risk populations (the homeless, drug users and asylum seekers)

[41].These findings underscore the value of chest radiography in these targeted settings [44]. There is however limited evidence of the value of mass radiography for tuberculosis in infants and young children. This could be because chest radiographic interpretation is difficult and has low inter and intra-observer agreement [45, 46], in this age group.

Enhanced case-finding

During the era of mass chest radiographic screening for TB, developing countries mainly those in Asia began to investigate other strategies for ACF since they could not meet the requirements for mass radiography as was it practiced in the developed countries [2, 47]. The focus was on detecting and screening symptomatic individuals only, rather than screening entire communities, a strategy termed enhanced case-finding (ECF),[2, 48]. By increasing the awareness of the population for TB symptoms and encouraging self-presentation to healthcare centres, ECF, has lower costs than mass radiography since only symptomatic individuals are seen and tested for TB. Awareness campaigns and educational activities about TB and TB symptoms in ECF have been undertaken through radio, television, newspapers, leaflets, and dramatization [3, 49]. In Zambia, TB awareness and educational activities targeting school children resulted in the information being successfully passed on to adults in the community [50].

ECF requires an alert health system that can detect TB among people presenting to health facilities as result of awareness campaigns and educational activities. However, it has been shown that screening for TB is not always conducted in eligible individuals seeking care at healthcare centres [2, 51]. The WHO therefore recommends the Practical Approach to Lung Health (PAL), a syndromic approach to the management of patients attending primary health care (PHC) services for respiratory symptoms, which has been shown to increase screening for TB in PHC settings [52, 53]. The standardization of diagnosis and treatment of respiratory conditions, and coordination among health workers at different levels of the health system in this syndromic approach, improves the quality of diagnosis for TB through the appropriate management of patients with respiratory symptoms [54].

Out-patient screening

This form of ACF involves screening for symptoms and signs of TB among people attending outpatient departments of healthcare centres. This differs from ECF in that it need not be linked to TB awareness campaigns or educational activities. It is based on the premise that

people attending hospital are willing to seek care, but may not recognize that their symptoms are indicative of TB, and that these cases may not be detected by healthcare workers unless they are adequately trained and are aware of TB [55-59]. In Kenya more than 80% of TB cases detected through a door-to-door survey or interview of local leaders reported that they had attended a health unit while symptomatic but had not been diagnosed [59]. Outpatient screening has been used in various out-patient settings including primary health care centres, district hospitals and antenatal clinics, and has detected a high burden of TB that would have otherwise been undetected [58, 60-63].

Intensified case-finding

Intensified case-finding, is a form of ACF that is being widely practiced in the context of human immunodeficiency virus (HIV) infection [6, 8, 10]. It is a major component of key interventions that are aimed at decreasing the impact of TB among people living with HIV as part of the 3 I's (Intensified TB case finding, Isoniazid preventive therapy, Infection control) strategy for HIV/TB [6, 8, 10]. It is defined as the "regular screening of all people with HIV infection, or at high risk for HIV infection, or in congregate settings (such as mines, prisons, military barracks) for symptoms and signs of TB followed promptly with diagnosis and treatment" [6]. Among HIV infected individuals it is recommended that this screening be conducted at every contact with a healthcare worker. In addition all household contacts of those diagnosed with TB must also be screened for TB [6].

Comparison of ACF strategies

Studies that have evaluated the impact of ACF, for TB have largely been conducted in adults, because adults have been the focus of TB control programmes as they aimed to decrease transmission by identifying and treating infectious cases [3, 49, 61-68].These studies showed that ACF increases the yield of cases, and that cases are detected earlier. In Brazil, door-to-door ACF using symptom screening and sputum collection detected more prevalent TB cases (9.3/1000py) and resulted in more people presenting for care than ECF that delivered a televised pamphlet to households (6.04/1000 py), rate ratio 1.55; 95% CI (1.10-1.99) [49]. The leaflet described TB symptoms, advertised free TB services, and encouraged those with symptoms to visit the local clinic for evaluation. In a cluster randomised trial in Zimbabwe,

using a mobile van to publicise educational leaflets and TB screening services detected significantly more smear-positive TB than door-to-door visits (mean cumulative TB yield 4.22/1000 and 2.46/1000 adults per cluster respectively; OR 1.48; 95% CI (1.11-1.96)) [69]. Among South African gold miners, 6 monthly radiological screening for TB detected less extensive disease and showed tendency toward lower TB-specific mortality than 12 monthly screening [42]. A Cape Town study that linked ACF to a mobile HIV service found a high TB case yield and had high treatment success:-the smear-positive TB prevalence was 2% among individual who provided sputum samples, with an overall treatment success rate of 81.0% [70].

ACF has generally been noted to be more costly than PCF since it requires more resources to detect cases outside the health system. A historical review of ACF strategies noted that most studies had not included an assessment of the cost-effectiveness of these strategies [2]. Recent studies on ACF have investigated and compared the costs of various ACF models [2, 66, 70, 71]. In the study that linked ACF to a mobile HIV service, although the overall treatment success rate was high, the cost per case treated was triple the cost per case treated under PCF [70]. In Cambodia, ACF targeting contacts of smear positive cases was found to be cost-effective in detecting cases in poor vulnerable communities [66]. Dodd *et al* demonstrated that periodic ACF could improve control and save medium-term health care costs in high TB and HIV burden settings, and where the majority of cases are due to recent *M.tb* infection [71].

Active case finding in children

Apart from contact tracing, few studies have evaluated other forms of ACF in young children. In Malawi, Zachariah *et al* compared ACF and PCF, and the uptake of isoniazid preventive therapy (IPT) among household contacts in a study that included both adults and children [21]. PCF was defined as the prevailing local standard of care in the Malawi TB programme at the time. Symptomatic individuals self-referred to healthcare centres, and index cases were informed that all household contacts should attend hospital for evaluation and appropriate management. In the ACF cohort, the evaluation of household contacts was actively facilitated through the collection of sputum specimens at home, and the provision of referral slips for chest radiography for child contacts. ACF detected significantly more cases than PCF (1.74% and 0.19% respectively, p =0.01). In the PCF cohort, none of the child contacts identified

underwent chest radiography, (17%) received IPT, and none were diagnosed with TB. In the ACF cohort, 43% of child contacts identified underwent chest radiography, 25% received IPT and 4% were diagnosed with active TB and received curative TB therapy.

Ward *et al*, reviewed routine TB data in Canada and compared cases detected passively through self-presentation, with those identified through contact tracing and high risk population screening surveys [64]. This included preschool and school screening in communities with a high incidence of TB. There was no difference in age among the cases detected actively or passively; median age three years and two years respectively, p=0.3. Among those less than 19 years old, the cases detected by PCF were significantly more likely to report symptoms (cough, fever, weight loss, haemoptysis) than those detected by ACF.

In a study that included adults and children, in Peru, ACF was conducted through visits to the homes of TB index cases, in comparison to household contacts being identified and advised to report to the local health centre if they experienced cough for more than two weeks (PCF) [72]. No TB was diagnosed in children less than 15 years old in both groups. This was attributed to the fact that children could not produce sputum specimens required for diagnostic purposes, since in this study diagnosis was only based on microscopy and culture results.

Conclusion

ACF has been shown to increase the detection of TB cases. Various strategies for ACF have been evaluated in adults. The strategies include door-to-door household visits, ECF, out-patient screening, and mass radiographic screening. Periodic ACF could offer medium term healthcare cost savings in high TB and HIV prevalence settings. Besides contact tracing which has been shown to be effective in detecting TB in children when appropriately implemented, there has been limited investigation of other ACF strategies in this population. There is therefore limited evidence to guide the selection of ACF strategies suited for young children in the context of TB vaccine settings.

Contributors

This chapter was written by Dr Sizulu Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey.

References

1. WHO. The StopTB strategy. Building on and enhancing DOTS to meet the TB-related Millennium Development Goals http://whqlibdoc.who.int/hq/2006/WHO_HTM_STB_2006.368_eng.pdf (Accessed on 3 June 2012).
2. Golub J E, Mohan C I, Comstock G W, Chaisson R E. Active case finding of tuberculosis: historical perspective and future prospects. *Int J Tuberc Lung Dis* 2005; 9: 1183–1203.
3. Lonnroth K, Uplekar M, Ottmani S, Blanc L. Stop TB Partnership. An action framework for higher and earlier TB case detection. Background document for DOTS Expansion Working Group Meeting**.** http://www.stoptb.org/assets/documents/global/awards/tbreach/Achieving%20higher%20case%20detection.pdf (Accessed on 30 August 2012).
4. Dye C, Garnett GP, Sleeman K, Williams BG. Prospects for worldwide tuberculosis control under the WHO DOTS strategy. Directly Observed Short Course therapy. *Lancet* 1998; 352:1886-91.
5. Getahun H, Raviglione M (2010) Active case-finding for TB in the community: time to act. *Lancet* 2010; 376: 1205–1206.
6. WHO policy on collaborative TB/HIV activities. Guidelines for national programmes and other stakeholders. WHO/HTM/TB/2012.1. WHO/HIV/2012.1 http://whqlibdoc.who.int/publications/2012/9789241503006_eng.pdf (Accessed on 30 August 2012).
7. Rothman JR. Modern epidemiology, 2nd Edn. Boston: Little, Brown and Company.
8. Lonnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sc Med* 2009; 68: 2240–6.
9. National Collaborating Centre for Chronic conditions (UK). Tuberculosis: Clinical Diagnosis and Management of Tuberculosis, and Measures for Its Prevention and Control. NICE Clinical guidelines, No 33. http://www.ncbi.nlm.nih.gov/books/NBK45801/ (Accessed on 29 August 2012).
10. WHO. STOP TB Department. Scoping meeting for the development of guidelines on screening for active TB. http://www.who.int/tb/TBscreeningmeetingreport2011.pdf (Accessed on 10 September 2012).

11. Starke J R. Diagnosis of tuberculosis in children. Pediatr *Infect Dis J 2000;* 19: 1095–1096.
12. Rigouts L. Clinical practice: diagnosis of childhood tuberculosis. *Eur J Pediatr* 2009; 168: 1285–1290.
13. Natural history Marais B J, Gie R P, Schaaf H S, *et al*. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the prechemotherapy era. *Int J Tuberc Lung Dis* 2004; 8: 392–402.
14. Schaaf H S, Michaelis I A, Richardson M, *et al*. Adult-to-child transmission of tuberculosis: household or community contact? *Int J Tuberc Lung Dis* 2003; 7: 426–431.
15. Van Wyk SS, Mandalakas AM, Enarson DA, Gie RP, Beyers N, Hesseling AC. Tuberculosis contact investigation in a high-burden setting: house or household? *Int J of Tuberc Lung Dis* 2012; 16:157-142.
16. Lobato MN, Royce SE, Mohle-Boetani JC. Yield of source-case and contact investigations in identifying previously undiagnosed childhood tuberculosis**.** *Int J Tuberc Lung Dis* 2003; 7S391-6.
17. Paranjothy S, Eisenhut M, Lilley M, *et al.* Extensive transmission of Mycobacterium tuberculosis from 9 year old child with pulmonary tuberculosis and negative sputum smear. *BMJ* 2008; 337: a1184.
18. Bothamley GH, Ditiu L, Migliori GB, *et al*. Active case finding of tuberculosis in Europe: a Tuberculosis Network European Trials Group (TBNET) survey. *Eur Respir J* 2008; 32:1023–1030.
19. Centers for Disease Control. Guidelines for the Investigation of Contacts of Persons with Infectious Tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recommendations and reports .*MMWR* 2005; 54:1-47. http://www.cdc.gov/mmwr/pdf/rr/rr5415.pdf (Accessed on 10 October 2012).
20. Nyirenda M, Sinfield R, Haves S, Molyneux EM, Graham SM. Poor attendance at a child TB contact clinic in Malawi *Int J Tuberc Lung Dis* 2006; 10:585–587.
21. Zachariah R, Spielmann MP, Harries AD, *et al*. Passive versus active tuberculosis case finding and isoniazid preventive therapy among household contacts in a rural district of Malawi. *Int J Tuberc Lung Dis* 2003; 7: 1033–1039.
22. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. WHO/HTM/TB/2006.371,

WHO/FCH/CAH/2006.7. Geneva, Switzerland: WHO, 2006. http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf (Accessed on 10 June 2012).

23. Nuwaha F. Chemoprophylaxis for tuberculosis in HIV-infected individuals in sub-Saharan Africa. *East Afr Med J* 1998; 75:520-7. Review.
24. Bucher HC, Griffith LE, Guyatt GH, *et al* Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials. *AIDS* 1999; 13:501-7.
25. Churchyard GJ, Fielding K, Charalambous S, *et al* Efficacy of secondary isoniazid preventive therapy among HIV-infected Southern Africans: time to change policy? *AIDS.* 2003; 17:2063-70.
26. Akolo C, Adetifa I, Shepperd S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. *Cochrane Database Syst Rev* 2010; 20:CD000171.
27. Laserson KF, Wells CD. Reaching the targets for tuberculosis control: the impact of HIV. *Bull World Health Organ* 2007; 85: 377-386.
28. Plunkett RE. Case-finding: An evaluation of various techniques. *Am Rev Tuberc* 1939; 39: 256–265.
29. Anderson RJ. Community-wide chest X-ray survey: rationale and results. *Public Health Service Publication* No 222. Washington, DC: USPHS, 1952: 1–19.
30. Davies R. The effect on tuberculosis morbidity of a complete community survey with hospitalization of all active cases. *Am Rev Tuberc* 1946; 54: 254–260
31. Davies R, Hedberg A, Fischer M. A complete community survey for tuberculosis: a second report on effectiveness of the procedure as a method of tuberculosis control. *Am Rev Tuberc* 1948; 58: 77–85.
32. Davies R, Hedberg G A, Fischer M. The St. Louis County tuberculosis survey. *Am Rev Tuberc* 1946; 53: 240–245.
33. Horwitz O. Principles and effects of mass screening: Danish experience in tuberculosis screening. *Public Health Rep* 1976; 97: 146–153.
34. Mass radiography in India. *Tubercle* 1946; 27: 25–30.
35. Frimodt-Moller J. A community-wide tuberculosis study in South Indian rural population, 1950–1955. *Bull World Health Organ* 1960; 22: 61–170.
36. Raviglione M C, Pio A. Evolution of WHO policies for tuberculosis control, 1948–2001. *Lancet* 2002; 359: 775–780.

37. Borgdorff MW, Floyd K, Broekmans JF. Interventions to reduce tuberculosis mortality and transmission in low- and middle-income countries. *Bull World Health Organ* 2002; 80: 217-27.
38. World Health Organization. WHO expert Committee on Tuberculosis. Ninth report. WHO Technical series, No 552. Geneva: World Health Organisation, 1974.
39. Rieder HL, Zellweger JP, Raviglione MC, Keizer ST, Migliori GB. Tuberculosis control in Europe and international migration. *Eur Respir J* 1994; 7: 1545–1553,
40. George SA, Ko CA, Kirchner HL, Starke JR, Dragga TA, Mandalakas AM. Role of chest radiographs and tuberculin skin tests in tuberculosis screening of internationally adopted children. *Pediatr Infect Dis J* 2011; 30:387-91.
41. Story A, Aldridge RW, Abubakar I, *et al*. Active case finding for pulmonary tuberculosis using mobile digital chest radiography: an observational study. *Int J Tuberc Lung Dis* 2012; 16: 1461–1467.
42. Churchyard GJ, Fielding K, Roux S, *et al*. Twelve-monthly versus six-monthly radiological screening for active case-finding of tuberculosis: a randomised controlled trial. *Thorax* 2011; 66: 134–139.
43. van't Hoog AH, Meme HK, Laserson KF, *et al*. Screening Strategies for Tuberculosis Prevalence Surveys: The value of chest and symptoms. PLoS ONE 2012; 7: e38691.
44. Iademarco, MF, O'Grady J Knut L. Chest radiography for tuberculosis screening is back on the agenda [Editorial] *Int J Tuberc Lung Dis* 2012; 16: 1421–1422.
45. Andronikou S, Wieselthaler N. Imaging for tuberculosis in children. In: Schaaf SH, Zumla AI, Tuberculosis–a comprehensive clinical reference. United Kingdom: Saunders Elsevier, 2009: 262-296.
46. Du Toit G, Swingler G, IIoni K. Observer variation in detecting lymphadenopathy on chest radiography. *Int J Tuberc Lung Dis* 2002; 9: 814-817.
47. Nagpaul D R, Vishwanath M K, Dwarakanath G. A socioepidemiological study of out-patients attending a city tuberculosis clinic in India to judge the place of specialized centres in a tuberculosis control programme. *Bull World Health Organ* 1970; 43: 17–34.
48. Banerji D, Anderson S. A sociological study of awareness of symptoms among persons with pulmonary tuberculosis. *Bull World Health Organ* 1963; 29: 665–683.
49. Miller AC, Golub JE, Cavalcante SC, *et al*. Controlled trial of active tuberculosis case finding in a Brazilian *favela. Int J Tuberc Lung Dis* 2010; 14: 720–726.
50. Bond V, Chilikwela L, Simwinga M, *et al* Children's role in enhanced case finding in Zambia. *Int J Tuberc Lung Dis*. 2010; 14:1280-7.

51. Me'emary F, Ottmani SE, Pio A, *et al*. Results of the feasibility test of the Practical Approach to Lung Health in the Syrian Arab Republic. *East Mediterr Health J* 2009; 15:504-15.
52. World Health Organisation. Strategic framework to decrease the burden of TB/HIV. WHO/CDS/TB/2002.296.Geneva: WHO, 2002. http://whqlibdoc.who.int/hq/2002/WHO_CDS_TB_2002.296.pdf (Accessed on 15 September 2012).
53. Fairall LR, Zwarenstein M, Bateman ET, *et* al. Effect of educational outreach to nurses on tuberculosis case detection and primary care of respiratory illness: pragmatic cluster randomized controlled trial. *BMJ* 2005; 331:750-754.
54. World Health Organisation. Practical Approach to Lung Health. Manual on initiating PAL implementation. WHO/HTM/TB/2008.410. http://whqlibdoc.who.int/hq/2008/WHO_HTM_TB_2008.410_eng.pdf WHO/HTM/TB/2008.410 (Accessed on 15 September 2012).
55. Baily GVJ, Savic D, Gothi G D, Naidu V B, Nair SS. Potential yield of pulmonary tuberculosis cases by direct microscopy of sputum in a district of South India. *Bull World Health Organ* 1967; 37: 875–892.
56. Aluoch JA, Edwards EA, Stott H, Fox W, Sutherland I. A fourth study of case-finding methods for pulmonary tuberculosis in Kenya. *Trans R Soc Trop Med Hyg* 1982; 76: 679–6.
57. Aluoch JA, Swai OB, Edwards EA, *et al*. Study of case-finding for pulmonary tuberculosis in outpatients complaining of a chronic cough at a district hospital in Kenya. *Am Rev Respir Dis* 1984; 129: 915–920.
58. Aluoch JA, Swai OB, Edwards EA, *et al*. Studies of case-finding for pulmonary tuberculosis in outpatients at 4 district hospitals in Kenya. *Tubercle* 1985; 66: 237–249.
59. Aluoch JA, Edwards EA, Stott H, Fox W, Sutherland I. A fourth study of case-finding methods for pulmonary tuberculosis in Kenya. *Trans R Soc Trop Med Hyg* 1982; 76: 679–691.
60. Gounder CR, Wada NI, Kensler C, *et al*. Active tuberculosis case-finding among pregnant women presenting to antenatal clinics in Soweto, South Africa. *J Acquir Immune Defic Syndr* 2011; 57:e77-8.
61. Nachenga J, Coetzee J, Adendorff T, *et al*. Tuberculosis active case-finding in a mother-to-child transmission prevention programme in Soweto, South Africa. *AIDS* 2003, 17; 1398-400.

62. Kail KB, Gray GE, Violari A, Chaisson RE, McIntry JA, Martinson NA. Combining PMTCT with active case finding for tuberculosis. *JAIDS* 2006; 42:379-81.
63. Sanchez-Perez HJ, Hernan MA, Hernandez-Diaz S, Jansa J M, Halperin D, Ascherio A. Detection of pulmonary tuberculosis in Chiapas, Mexico. *Ann Epidemiol* 2002; 12: 166–172.
64. Ward HA, Marciniuk DD, Pahwa P, Hoeppner V H. Extent of pulmonary tuberculosis in patients diagnosed by active compared to passive case finding. *Int J Tuberc Lung Dis* 2004; 8:593–597.
65. Den Boon S, Verver S, Lombard CJ, *et al*. Comparison of symptoms and treatment outcomes between actively and passively detected tuberculosis cases: the additional value of active case finding. *Epidemiol Infect* 2008; 136: 1342–1349.
66. Eang MT, Satha P, Yadav RP, *et al*. Early detection of tuberculosis through community-based active case finding in Cambodia. *BMC Public Health* 2012; 12:469.
67. Yimer S, Holm-Hansen C, Yimaldu T Bjune G. Evaluating an active case-finding strategy to identify smear-positive tuberculosis in rural Ethiopia. *Int J Tuberc Lung Dis* 13:1399–1404.
68. Pronyk PM, Joshi B, Hargreaves J R, *et al*. Active case finding: understanding the burden of tuberculosis in rural South Africa. *Int J Tuberc Lung Dis* 2001; 5: 611–618.
69. Corbett EL, Bandason T, Duong T, *et al*. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster randomised trial. *Lancet* 2010; 376: 1244–1253.
70. Kranzer K, Lawn SD, Meyer-Rath G, *et al*. Feasibility, Yield, and Cost of Active Tuberculosis Case Finding Linked to a Mobile HIV Service in Cape Town, South Africa: A Cross-sectional Study. *PLoS Med* 2012; 9: e1001281.
71. Dodd PJ, White RG, Corbett EL. Periodic active case finding for TB: when to look? *PLoS One*. 2011; 6:e29130.
72. Becerra MC, Pachao-Torreblancha IF, Bayona J, *et al*. Expanding tuberculosis case detection by screening household contacts. *Public Health Rep* 2005; 120: 271–277.

Chapter 3: Comparison of two active case finding methods in young children in a high TB incidence setting: Implications for case finding in TB vaccine trials in young children

Summary

Setting: A tuberculosis (TB) vaccine trial site in a high TB burden rural area in South Africa.

Objectives: To compare TB case yield and disease profile among Bacille Calmette-Guérin (BCG) vaccinated children from birth until two years of age using two active case-finding strategies.

Methodology: BCG-vaccinated infants were enrolled within 2 weeks of birth and randomised to 3-monthly home visits for questionnaire-based TB screening plus record surveillance of TB registers, hospital admission and X-ray lists at health facilities, for TB suspects and cases, (Group 1), or record surveillance (as above) only, (Group 2). Both groups received a close-out visit after 2 years. Participants were evaluated for suspected TB disease using standardised investigations.

Results: A total of 4786 infants were enrolled: 2392 were randomised to Group 1 and 2394 to Group 2. The case-finding rate was significantly greater in Group 1(2.2/100 py) than in Group 2 (0.8/100 py), with a case finding rate ratio of 2.6 (95%CI 1.8–4.0, $P < 0.001$). Although the proportion of cases with bacteriological confirmation was lower in Group 1, this difference did not reach statistical significance. There was also no significant difference in the proportions with TB symptoms and signs.

Conclusion: Home visits combined with record surveillance detected significantly more TB cases, at a younger age, than record surveillance alone with a single study end visit. There was no significant difference in the TB symptoms, signs, radiologic and bacteriologic profile of TB cases in the two groups. Regular screening for TB combined with record surveillance will maximise case detection in paediatric TB vaccine trials conducted in similar settings.

Background

It has been established that active case-finding (ACF) for tuberculosis (TB) increases case detection and detects cases that are less symptomatic than those detected passively [1-4]. More recent studies have compared various ACF methods, and some have evaluated the costs of different ACF methods [5-9]. The high case yield from ACF makes it likely to be the preferred case-finding strategy in clinical trials of new TB vaccines as well as in other TB preventive studies conducted in young children. The greater number of cases that would be detected in ACF increases study efficiency by reducing sample size requirements (in comparison to PCF), while maintaining the power of these studies [10].

In young children, case finding studies have largely focused on the yield of contact tracing (which can be considered a form of ACF), in households of smear positive adults [11-13]. Few studies have investigated or compared other forms of ACF with PCF in children [3, 4]. In Malawi, ACF detected more cases in all ages (adults and children less than 6 years old), than PCF. More children in the ACF group were screened and prescribed preventive and curative therapy than in the PCF group [3].

Since there are limited data on ACF strategies in young children, it is not clear what ACF strategies should be adopted in TB vaccine trials conducted in young children. Differences in adult and childhood TB restrict the direct application of some of the ACF strategies that have been successful in adult studies. Firstly, TB symptoms in children are non-specific and overlap with those of other childhood conditions especially in the very young children, hence where symptom screening is adopted for ACF in children, this must be designed to have high specificity in comparison to that used in adults [14-17]. Secondly while adults can fairly easily produce sputum samples for microbiological testing for *M.tb*, this is more difficult to achieve in children, hence household sputum sample collection is not feasible in this population. Furthermore, since TB in children is paucibacillary, gastric washing (GW) samples or sputum samples collected by the more effective induction of sputum (IS) techniques yield very few bacteriologically confirmed cases. It is also worth noting that sputum induction techniques require investment into training of staff and the purchase of equipment [18-20].

The study described in this chapter aimed to compare the case yield of two active TB case-finding strategies in young children over a two year period. We tested the hypotheses that i)

TB cases remaining undetected by the less intensive strategy would be found at a close-out visit at two years of age, resulting in similar TB case yields in the two groups, and that ii) the TB case profile would differ in clinical, radiological, and bacteriologic features, due to an early, less severe disease phenotype, in the more intensive strategy.

Study Design

Setting

The study was conducted between 2005 and 2008, in the Cape Winelands district (~100km from Cape Town) of South Africa. The population in the study area during 2007-2008 was estimated at 327,822 [21]. The annual birth cohort is estimated at 7000 births. The region is well serviced by clinics and hospitals including a regional specialist TB hospital. The overall TB incidence was 1442/100000 and antenatal HIV prevalence 12.8% in 2007 [21].

Study population

Healthy infants vaccinated with Bacille Calmette-Guérin (BCG) vaccine (intradermal Danish strain 1331, Statens Serum Institut, Denmark), within 72 hours of birth, were enrolled within two weeks of birth. Enrolment took place at birthing units (clinics or hospitals) or at home. Infants were randomized in a 1:1 ratio to Group 1 or Group 2 case-finding (described below) using simple random allocation. After obtaining consent from a parent or legal guardian, field workers telephoned the study administrator for the infant's randomization group and study number. These were assigned from a pre-generated randomization list. Follow-up was scheduled for at least two years. Recruitment took place between 2005 and 2006 and follow-up was completed in 2008.

Case finding strategies

Group 1 - Home visits and record surveillance

The ACF strategy in this group entailed

i) home visits every three months for questionnaire based screening for TB symptoms and contacts. Participants were considered TB suspects if they reported close contact with an adult TB source case or at least one of the following symptoms for a period greater than 2 weeks: fever, cough, weight loss or loss of appetite without another plausible aetiology ,

ii) surveillance of TB registers in all clinics in the study area for adult TB cases in contact with study participants (as shown by the same home address), and for participants diagnosed with TB,

iii) surveillance of hospital admission name lists in all hospitals in the study area for study participants seen or admitted and diagnosed with TB or TB related conditions such as respiratory tract infections,

iv) surveillance of clinic and hospital x-ray department name lists to identify participants who had undergone chest radiography (CXR) during health centre attendance.

TB suspects and cases identified from the records described in ii-iv, above were visited at home (in addition to the regular three monthly visits) to verify these findings. All suspects and cases were referred to a study research ward for further evaluation and investigations for TB disease. Investigations were prioritized for those who had already been diagnosed with TB at local clinics or hospitals.

Group 2 - Record surveillance

ACF in this group involved all aspects of record surveillance as described for Group 1, but excluded the three monthly home visits. Suspects and cases were referred to the research ward for further evaluation and investigations for TB disease as for Group 1.

Both groups

In both groups, parents or guardians could contact the study team directly if they suspected their child had TB symptoms or a TB contact. Healthcare workers in the area could also refer participants (identifiable by study stickers attached to the Road-To-Health-Card at enrolment) suspected of TB to the study team for arrangements for investigations in the study research ward.

Close-out visit

Attempts were made to contact each participant to administer a TB symptom and contact screening questionnaire at a close-out visit scheduled at two years of age. Four attempts were made to contact each participant. Admission into the research ward for investigations for TB disease was extended to the age of 26 months to allow sufficient time for all close-out visit attempts and to accommodate the time lag between the close-out visit and admission to the ward.

Evaluation and investigations for TB disease

Participants with suspected TB disease during follow-up, or at the close-out visit, were admitted to a research ward for investigations over three days. A medical history and examination were performed by a clinician and the following investigations conducted: CXR (anterolateral and antero-posterior films), tuberculin skin test (TST) by the Mantoux method, Human Immunodeficiency Virus (HIV) testing (with parental/legal guardian consent), paired early morning GW and IS specimen collection on two consecutive days. Smear microscopy (auramine fluorescent microscopy) and culture for *Mycobacterium tuberculosis* (*M.tb)* (Mycobacterial Growth Indicator Tube (MGIT) 960) were performed on all GW and IS specimens. Cultures were incubated for at least 6 weeks. Positive cultures underwent speciation to exclude non-tuberculous mycobacteria (NTM). The TST was read 48-72 hours after administration.

All children who were admitted into the research ward were managed using results available during admission and at the time of discharge, by the research ward clinician. The clinical management of these children was independent of the study processes. Those diagnosed with TB disease by the research ward clinician were started on curative TB therapy. Those diagnosed with latent tuberculosis infection (LTBI) were started on preventive TB therapy. Upon discharge, participants were referred to the regular healthcare system for further management. Sputum microscopy and culture results that arrived after discharge were forwarded to the appropriate public health clinics to inform continuing patient management. Study follow-up continued until the end of study (close-out visit) for all participants including those who had been had been evaluated for TB in the research ward.

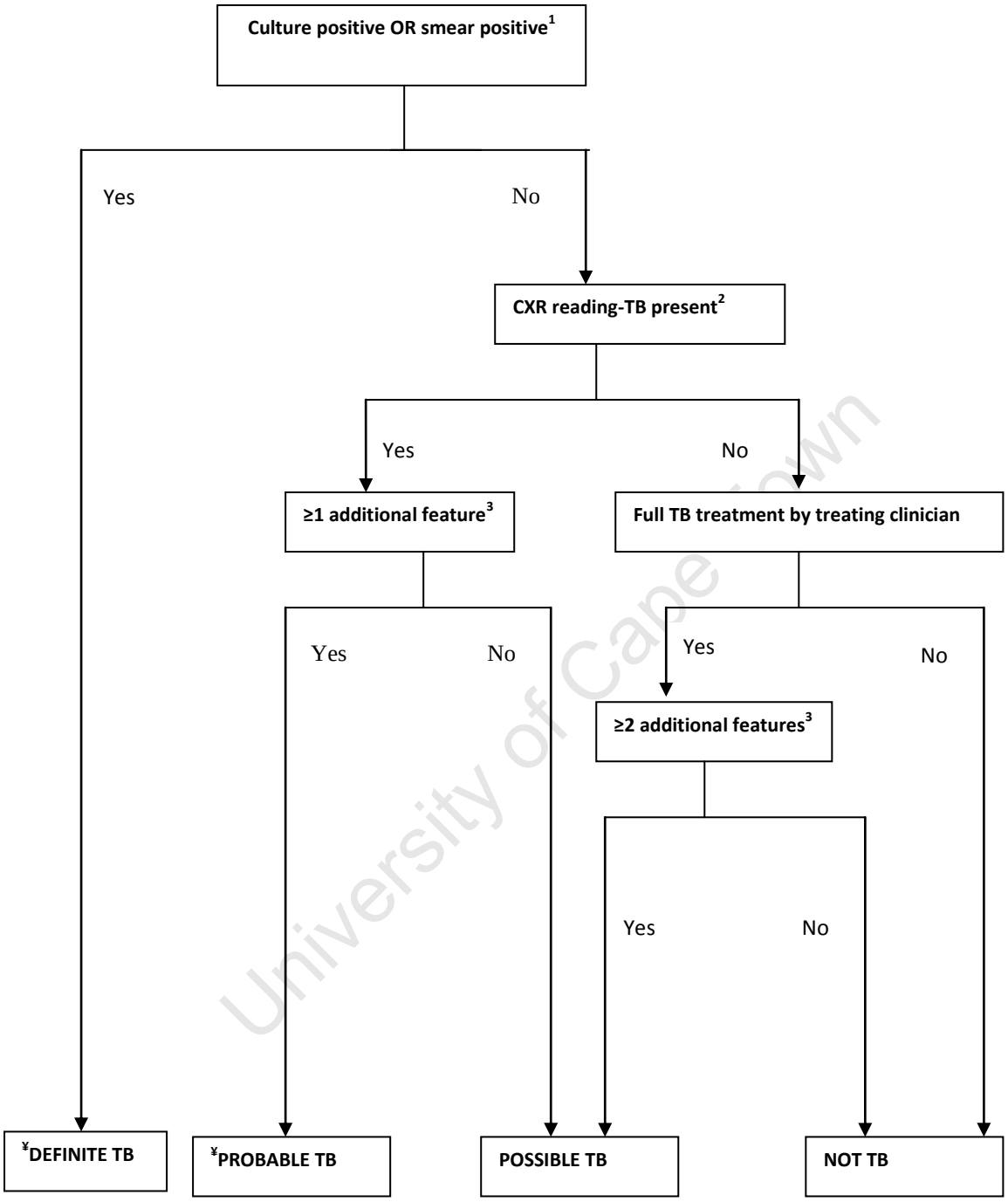
For the purposes of this study, TB diagnosis was classified into, “Definite TB”, “Probable, TB”, “Possible TB”, or “not TB”, using a diagnostic algorithm based on history, examination and the results of investigations conducted, as shown in **Figure 1**. For this classification, CXRs were reviewed independently by a panel of three paediatric radiologists who were blinded to the clinical information. Readings were based on and recorded on a predesigned CXR reading and recording form (Appendix 2). CXR findings were categorised as; “TB-Present” (CXR findings consistent with TB); “TB-Not present” (no CXR findings consistent with TB, or findings for and against TB equivocal, or CXR not readable). Airway compression or displacement, definitive soft tissue masses at the hilar, para-tracheal and para-

cardiac regions, and miliary nodules were considered definitive evidence of TB. A final CXR reading for each suspect was determined by concordance between two of the three radiologists.

Ethics approval

The study was approved by the University Of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (Cape Town, South Africa), and the Chesapeake Research Review Board (Columbia, MD, USA).

Figure 1: Diagnostic algorithm for classification of TB cases



[1]Excludes scanty and 1+ smear positive,
[2]X-ray reading based on paediatric radiologist panel final chest x-ray finding,
[3]Additional features: Mantoux ≥10mm (5mm if HIV infected), Cough >2 weeks, Failure to thrive (defined as persistent inadequate weight gain or persistent weight loss determined by the research ward clinician on review of the Road to Health Card), TB contact
¥Only cases classified as definite and probable TB were considered as TB cases

Statistical considerations and analysis

Sample size

We calculated that 2620 infants in each group would detect a 1% absolute difference in rates between the groups (Group 1 disease detection estimated at 2% and Group 2 at 1%), at 80% power and $\alpha = 0.05$. The sample was reduced to 2400 in each group because of financial and logistical considerations that included the duration of recruitment and the length of participant follow-up.

Data analysis

Data were analysed using STATA version 10.0 (Stata Corp College Station, Texas, USA). Rate ratios were calculated for the primary analysis. Odds ratios were calculated to compare TB clinical features in suspects and cases between the groups; 95% confidence intervals and p values were calculated. The t-test was used to compare continuous variables, since the sample size was fairly large. The case finding rate was calculated as cases detected over person-years of follow-up. Person-years were calculated from date of birth until the date of TB diagnosis, death or the close-out visit, whichever was first. For participants lost to follow-up (LTFU), person-years were calculated as midway between the last contact date and date of the next expected visit (3 monthly or close-out), and for those who withdrew, from the date of birth to date of withdrawal. Sensitivity analyses under various assumptions about length of follow-up and the TB status of participants LTFU were also performed.

For those participants with multiple admissions to the research ward, the admission with the more severe TB classification was analysed; otherwise, the first admission was used. Only definite and probable (DP) cases of TB were included in this analysis as they were considered as the most robust case definitions. Study participants who were diagnosed with TB outside the research ward were not included in the primary analysis, since the diagnostic methods differed from those in the health system. Sputum induction was not conducted, GW was not always done, and the results were not always available.

Results

Participants

A total of 4786 infants were enrolled: 2392 were randomized to Group 1 and 2394 to Group 2. **Table 1** summarizes the baseline characteristics of participants in the two groups.

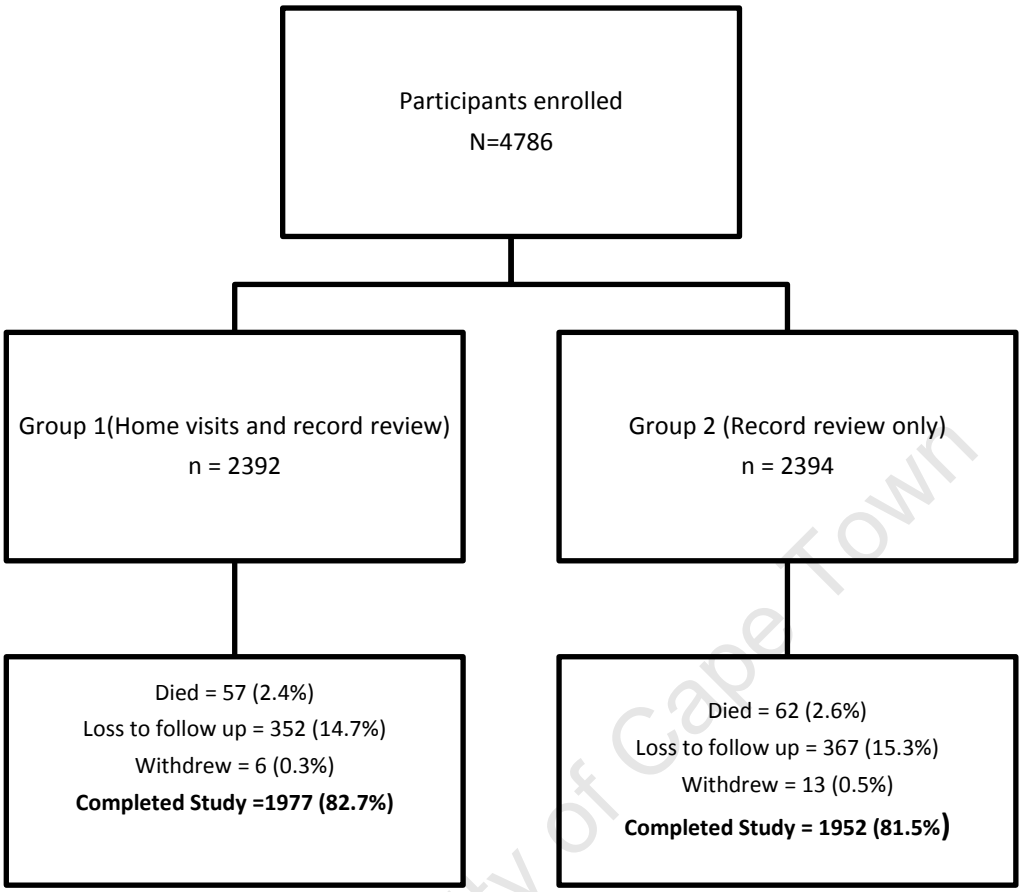
Table 1: Baseline characteristics of all participants enrolled (N=4786)

Parameter	[‡]**Group 1 n=2392 (%)**	[ᵖ]**Group 2 n=2394 (%)**
Males	1205 (50.4)	1199 (50.1)
Low birth weight (<2.5kg)	437 (18.3)	430 (18.0)
Mean maternal age at infant's birth (years)	26.5 (SD= 6.7)	26.3 (SD= 6.7)
Mother level of education (Primary level and lower)	631 (26.4)	610 (25.5)
Peri urban residential area[§]	399(16.7)	384(16.0)
Informal housing	306 (12.8)	288 (12.0)

[‡]Home visits and record review, [ᵖ]Record review only, [§]Informal settlements situated at periphery of formal urban housing, SD-standard deviation

The proportion of deaths, withdrawals, and LTFU were similar in the two groups, as shown in **Figure 2**.

Figure 2: Participant status at the end of the study



Case finding rates

Twenty-three participants (11 Group 1; 12 Group 2) were diagnosed with TB outside the research ward and were excluded from the primary analysis because of insufficient information to apply the study diagnostic algorithm. Mean follow-up time was 20.7 months in Group 1 (standard deviation (SD) 8.2) and 22.0 months in Group 2 (SD 6.8). Eighteen percent of participants (527 Group 1; 353 Group 2) were admitted to the research ward. Among these 880 children admitted to the research ward, eight (0.9%) children (5 Group 1; 3 Group 2) were HIV positive.

The overall case finding rate was significantly higher in Group 1, (case finding rate ratio 2.6 (95% CI 1.8-4.0), p< 0.001) as shown in **Table 2A**. There was no significant difference in the case finding rate in the youngest (0-6 months) and the oldest age categories (18-26 months).

Table 2A: Case finding rates by randomisation group (N=4763)[#]

	[‡]Group 1 n=2381			[ϮGroup 2 n=2382			Case finding rate ratio (95% CI)	p value
Age category (months)	No. of TB cases Total (definite; probable)	Pys	Case finding rate/100pys (95% CI)	No. of TB cases Total (definite, probable)	Pys	Case finding rate /100pys (95% CI)		
0-6	11 (2; 9)	1133	1.0 (0.5-1.7)	5 (2;3)	1168	0.4 (0.1-1.0)	2.3 (0.7-8.3)	0.06
6-12	**28 (5 ;23)**	**1008**	**2.8 (1.9-4.0)**	**7 (4;3)**	**1120**	**0.6 (0.3-1.3)**	***4.4 (1.9-12.1)**	***<0.001**
12-18	**24 (1;23)**	**905**	**2.7 (1.7-3.9)**	**4 (0;4)**	**935**	**0.4 (0.1-1.1)**	***6.2 (2.1-24.6)**	***<0.001**
18-26	26 (2;24)	1063	2.4 (1.6-3.6)	20 (2;18)	1149	1.7 (1.1-2.7)	1.4 (0.8-2.7)	0.13
0-26	**89 (10;79)**	**4109**	**2.2 (1.7-2.7)**	**36 (8;28)**	**4372**	**0.8 (0.6-1.1)**	***2.6 (1.8-4.0)**	***<0.001**

[#]23 children diagnosed with TB outside the study research ward were excluded due to insufficient clinical details, [‡]Home visits and record review, [Ϯ]Record review only, *statistically significant, pys- personyears, CI-confidence interval. All participants lost to follow-up are assumed not to have TB disease.

Sensitivity analyses

Sensitivity analyses taking into account different scenarios regarding loss to follow-up and disease status were also conducted. This analysis covered the entire study period 0-26 months. The results were similar to those found in the primary analyses (above) and are shown in Table 2B; where the more intensive strategy (Group 1) detected significantly more cases of TB.

Table 2B: Sensitivity analyses: Case finding rates by randomisation group (0-26 months)

	‡Group 1			ᵖGroup 2			Case finding rate ratio (95% CI)	p value
Assumptions	No. of TB cases	Pys	Case finding rate/100pys (95% CI)	No. of TB cases	Pys	Case finding rate/100pys (95% CI)		
TB cases diagnosed outside the research ward excluded	**n=2381**			**n=2382**				
Participants lost to follow up do not have TB disease, and are censored at the date of last contact	89	4072	2.2 (1.8-2.7)	36	4120	0.9 (0.6-1.2)	*2.5 (1.7-3.8)	*<0.001
Participants lost to follow up do not have TB disease, and have completed the study	89	4445	2.0 (1.6-2.5)	36	4621	0.8 (0.5-1.1)	*2.6 (1.7-3.9)	*<0.001
Participants lost to follow up have TB disease, and length of follow-up as in the primary analysis	441	4109	10.7(9.8-11.7)	403	4372	9.2 (8.4-10.1)	1.2 (1.0-1.3)	*0.01
Participants lost to follow up have TB disease, and are censored at the date of last contact	441	4072	10.8 (9.9-11.8)	403	4120	9.8 (8.9-10.7)	1.1 (1.0-1.3)	0.07
Participants lost to follow up have TB disease, and have completed the study	441	4445	9.9 (9.1-10.8)	403	4621	8.7 (7.9-9.6)	1.1 (1.0-1.3)	* 0.03
TB cases diagnosed outside the research ward included	**n=2392**			**n=2394**				
Cases diagnosed outside the research ward included, participants lost to follow up do not have TB disease and length of follow-up as in the primary analysis	100	4123	2.4 (2.0-2.9)	48	4383	1.1 (0.8-1.4)	*2.2 (1.6-3.2)	* <0.001
Cases diagnosed outside the research ward included, participants lost to follow up have TB disease and length of follow-up as in the primary analysis	452	4123	11.0 (10.0-12.0)	415	4383	9.5 (8.6-10.4)	1.2 (1.0-1.3)	*0.02

Home visits and record review, ᵖRecord review only, *statistically significant, pys- personyears, CI- confidence interval.

Comparison of clinical features

We compared clinical features between TB suspects and cases in the two groups. The results are shown in **Table 3.** Significantly more TB suspects in Group 1 had CXR abnormalities consistent with TB (OR 1.77 (95%CI 1.17-2.69)), p= 0.01 (**Table 3**). Although, the

proportion of bacteriologically positive cases was lower in Group I, there was no significant difference in the proportions with TB symptoms, clinical and radiological signs, and positive bacteriology between the groups. Age at TB diagnosis was 13.2 months (SD 6.3) in Group 1, and 16.6 months (SD 8.4) in Group 2; difference between means 3.4 months (95% CI 0.3-6.5).

Table 3: Clinical profile of suspects and cases

	TB suspects				TB cases			
Variable	‡Group 1 n= 527 (%)	ᵖGroup 2 n= 353 (%)	OR (95% CI)	p value	‡Group 1 n= 89 (%)	ᵖGroup 2 n= 36 (%)	OR (95% CI)	p value
Cough>2/52 Y	204 (39)	119(34)	**1. 24 (0.94-1.65)**	0.13	33 (37)	15 (42)	**0.83 (0.38-1.80)**	0.63
N	323 (61)	234 (66)			56 (63)	21 (58)		
Failure to thrive Y	189 (36)	124 (35)	**1. 02 (0.77-1.36)**	0.86	36 (40)	18 (50)	**0.72 (0.33-1.56)**	0.41
N	330(63)	222 (63)			50 (56)	18 (50)		
UNK	8 (2)	7 (2)			3 (3)	0		
TB contact Y	339 (64)	230 (65)	**0.96 (0.73-1.28)**	0.80	64 (72)	29 (81)	**0. 62 (0.25-1.56)**	0.32
N	188 (36)	123 (35)			25 (28)	7 (19)		
TST +ve (≥10mm) Y	120 (23)	87 (25)	**0.90 (0.66-1.24)**	0.52	43 (48)	18 (50)	**0.93 (0.43-2.01)**	0.86
N	407 (77)	266 (75)			46 (52)	18 (50)		
CXR abnormalities Y	ᴬ87 (17)	35 (10)	***1. 77 (1.17-2.69)**	*0.01	83 (93)	29 (81)	**2.29 (0.62-8.48)**	0.23
N	431 (82)	308 (87)			5 (6)	4 (11		
UNK	9 (2)	10 (3)			1 (1)	3 (8)		
ᵀᴴBacteriology				0.71				0.13
positive	ᴬ10 (2)	8 (2)	**0.84 (0.34-2.08)**		ᴬ10 (11)	8 (22)	**0.46 (0.17-1.25)**	
negative	514 (98)	345 (98)			76 (85)	28 (78)		
UNK	3 (1)	0			3 (3)	0		

‡Home visits and record review, ᵖRecord review only,*statistically significant, OR- odds ratio, +ve- positive, CI-confidence interval, CXR- chest x-ray, Y- yes, N- no, UNK- unknown/missing, Failure to thrive was defined as persistent inadequate weight gain or persistent weight loss determined by the research ward clinician on review of the Road to Health Card, ᵀᴴ*Mycobacterium tuberculosis* culture or smear positive, excluding scanty and 1+ smear positive, ᴬPercentages do not add up to 100% because of rounding off errors. The N (no) category is the reference group for each odds ratio.

Preventive TB therapy

Six percent and 9% of suspects in Group 1 and 2, respectively, were on preventive or curative TB therapy at the time of evaluation in the research ward (p=0.06). A significantly greater proportion of participants in Group 1 were prescribed curative TB therapy (11% Group 1 vs 7% Group 2, (p< 0.001)) on discharge from the ward.

Suspect detection methods

Table 4 shows the number and percentage of suspects and cases by method of detection within the two strategies. Three monthly home visits detected most of the cases (56%) in Group 1, while in Group 2, the close –out visit detected just over a third of the cases. The various forms of record surveillance combined (TB register review, hospitalisation record review, X-ray list review) detected 53% of cases in Group 2.

Table 4: TB suspect and case detection by individual methods (N=4763)[#]

Detection method	[‡]Group 1		[ᵖ]Group 2	
	Suspects identified n=527 (%)	Cases identified Definite and probable TB n=89 (%)	Suspects identified n=353 (%)	Cases identified Definite and probable TB n=36 (%)
Home visit	303 (58)	50 (56)	N/A	N/A
Close out visit	33 (6)	4 (4)	98 (28)	13 (36)
TB register review	56 (11)	12 (13)	95 (27)	10 (28)
Hospitalisation record review	56 (11)	7 (8)	63(18)	5 (14)
X-ray list review	24 (5)	6 (7)	43 (12)	4 (11)
Clinic referral	23 (4)	3 (3)	30 (9)	4 (11)
[^]Self-reporting	18 (3)	4 (4)	13 (4)	0 (0)
Combination of methods	12(2)	3 (3)	3 (0.9)	0 (0)
Other/Method not recorded	2 (0.4)	0 (0)	8 (2)	0 (0)

[#]23 children diagnosed with TB outside the study research ward were excluded due to insufficient clinical details, [‡]Home visits and record review, [ᵖ]Record review only, [^]Self-reporting to the study team

Non-tuberculous mycobacteria

NTM were detected in 6% (32/527) children who were investigated for tuberculosis. None of these children had *M.tb*. Eight strains of NTM were detected. These are shown in **Table 5** below. In 18 children, the NTM could not be identified. However BCG disease was excluded in all cases.

Table 5: Non-tuberculous mycobacteria isolates obtained from gastric lave or induced sputum specimens in children investigated for tuberculosis (n=527)

Non-Tuberculous mycobacteria species	n (%)
M intracellulare	6(1.1)
M gordonae	3 (0.6)
M peregrinum	3 (0.6)
M asiaticum	1 (0.2)
M chelonae	1(0.2)
M kansasii	1(0.2)
M scofulaceum	1(0.2)
Unknown	18 (3.4)

Mortality

Both groups reported a similar proportion of deaths (2.4% in Group 1 and 2.6% in Group 2). Pneumonia, gastroenteritis and septicaemia were the leading causes of mortality in both groups also reported in similar proportions in the two groups. For the purposes of this study causes of deaths were assigned based on clinical records, a verbal autopsy interview and a complete (unabridged) death certificate, where these were available. Accurate cause of death information based on the documents mentioned above was not available in 26% of deaths in Group 1 and 52% of deaths in Group 2 (p= 0.005).

Discussion

Home visits combined with record surveillance detected significantly more TB cases and at a younger age than record surveillance alone, even when a close-out visit allowed detection of cases that were potentially missed during the period of follow-up. Sensitivity analyses also found a significantly higher case yield from home visits combined with record surveillance. The case finding rate in the 18–26 month age category, which included TB cases found at the close-out visit, was similar between the two groups. Although the proportion of cases with bacteriologic confirmation was lower in Group 1, the difference did not reach statistical significance. There was also no significant difference in the proportion of participants with TB symptoms and signs in both case finding strategies.

Our study shows the value of regular screening for TB through home visits in detecting TB cases that may have been missed by record surveillance. Although the close-out visit detected

a large number of cases in Group 2, the overall case finding rate in Group 2 was significantly surpassed by that in Group 1. A Phase IV infant trial conducted in the same community and used record surveillance similar to that for Group 2, reported a cumulative DP TB incidence rate of 3% over two years [22]. This rate is lower than that in Group 1 (4% over 26 months), and higher than in Group 2 (2% over 26 months). Together with our finding that 36% of cases in Group 2 were detected at the close-out visit, this demonstrates the value of home visits in detecting additional cases of TB, which is important in trials of TB vaccines.

Although home visits detected most cases, our findings show that to maximise case detection, an important requirement in TB vaccine trials [23-25], record surveillance is also required. Eighteen percent of cases in Group 1 self-referred to a healthcare centre (clinic referral, hospitalisation, and x-ray list review), (**Table 4**), despite regular contact with study staff, demonstrating the usefulness of record surveillance. These cases may have been undetected in the absence of record surveillance.

We had hypothesized that cases detected at an earlier age, by the more intensive case finding strategy, would present with milder clinical and radiological disease. Instead, we found significantly more suspects with radiologic abnormalities consistent with TB in Group 1, although this difference was not statistically significant among cases. This difference between suspects in the two groups might be due to transient radiological evidence of the primary TB complex that self-cures without intervention. This is consistent with the natural history of TB infection and disease in young children, wherein children recently exposed to *M.tb* may develop transient radiological changes [26-30]. Nonetheless, these data show that a case finding strategy that includes regular home visits identifies children who are exposed to *M.tb* much earlier than would occur otherwise. This is important because children less than 2 years old are at greatest risk of progressing to severe or disseminated TB disease following exposure to *M.tb* [26]. No cases of severe or disseminated TB were detected in both groups, indicating that these are unlikely to be observed in vaccine trials utilising similar case finding strategies [22].

Despite using IS in addition to GWs to collect specimens for microscopy and culture for *M.tb*, we only had a few bacteriologically confirmed cases [18]. Although this could be partly because some participants had started TB therapy at the time of evaluation, these were few (7%) and evaluations were conducted within a few days of initiation of treatment. This finding suggests that case definitions in TB vaccine trials in young children should include

history, clinical and radiologic features, as bacteriologically confirmed cases are likely to be few [22]. However, there are challenges and limitations in using clinically based definitions in research settings, and these have been well described in the literature [27, 31-35]. These include poor specificity and varying performance of the algorithms in different settings [31, 32]. While these diagnostic algorithms may be appropriate in clinical settings within specific environments, in TB vaccine trials they introduce the risk of disease misclassification which could have significant bearing on the measures of efficacy that are calculated. Differential misclassification can either under or over-estimate efficacy (depending on its direction) while non-differential misclassification can mask a moderate effect [10, 23, 36]. Recent publications from expert groups have attempted to build consensus and standardize TB case definitions in research settings to increase specificity and reduce the risk of disease misclassification in TB vaccine trials and other TB research studies [34, 35].

The mortality profile in this study reflects that found in the same community in a previous study [37]. Mortality was dominated by pneumonia and diarrhoeal disease. It is notable that in the less intensive strategy, there were more cases without accurate cause of death information, despite the extensive follow-up methods used in the study. It is possible that some of these deaths could have been due to TB or could have happened in children with TB, and this could therefore represent undetected cases. While both groups were cases affected, it is likely that proportionally more of these cases would have been in the less intensive case-finding group where contact with participants was minimal.

The 6% crude NTM yield is similar to what was found in previous study in the same community [38]. However there was slight difference in the species detected.

This study had some limitations. Not all children were seen at the end of study; therefore, some TB cases or deaths may have been missed. However, since the overall findings remained unchanged in sensitivity analyses and the proportion of deaths did not differ between groups, the impact was probably the same and minor for both groups. The 23 children missed by our case-finding strategies and were diagnosed with TB outside the research ward may have had more severe disease. These participants self-reported to healthcare centres and were diagnosed with TB. However review of their clinical records revealed insufficient information to apply the study diagnostic algorithm. Seven percent of participants were already on preventive or curative TB therapy at the time of evaluation, and

five percent of participants evaluated in the research ward were prescribed preventive therapy on discharge. Both factors could have reduced the number of cases in both groups. This study was limited to two years of follow-up, while we have previously shown that TB incidence is still high in the third year of life [25]. Additional follow-up might have provided additional cases and additional data on the profile of cases and disease severity. There was a small number of TB cases (89 cases in Group 1 and 36 cases in Group 2) detected and this may have been inadequate to detect differences in the profile of cases in the two groups. Extended follow-up or a larger sample size would be required to investigate this further. A larger sample size would have also detected more bacteriologically confirmed disease.

Conclusion

Home visits combined with record surveillance detected significantly more TB cases, at a younger age, than record surveillance alone with a single study end visit. There was no significant difference in the TB symptoms, signs, radiologic and bacteriologic profile of TB cases in the two groups. Regular screening for TB combined with record surveillance will maximise case detection in paediatric TB vaccine trials conducted in similar settings.

Contributors

This chapter was written by Dr S. Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. S.Moyo managed the study and analysed data under the supervision of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. The study was designed by S. Moyo, G. Hussey, S. Verver, L. Geiter and A. Hawkridge. L. Workman and H. Mulenga managed the study database. Professor F. Little and W. Msemburi assisted with data analysis. M. Tameris, H. Geldenhuys and C. Ontong participated in data collection.

References

1. Golub J E, Mohan C I, Comstock G W, Chaisson R E. Active case finding of tuberculosis: historical perspective and future prospects. *Int J Tuberc Lung Dis* 2005; 9: 1183–1203.
2. Zachariah R, Spielmann M-P, Harries AD, *et al*. Passive versus active tuberculosis case finding and isoniazid preventive therapy among household contacts in a rural district of Malawi. *Int J Tuberc Lung Dis* 2003; 11: 1033–1039.
3. Ward H A, Marciniuk DD, Pahwa P, Hoeppner VH. Extent of pulmonary tuberculosis in patients diagnosed by active compared to passive case finding. *Int J Tuberc Lung Dis* 2004; 8: 593–597.
4. Den Boon S, Verver S, Lombard C J, *et al*. Comparison of symptoms and treatment outcomes between actively and passively detected tuberculosis cases: the additional value of active case finding. *Epidemiol Infect* 2008; 136: 1342–1349.
5. Miller A C, Golub J E, Cavalcante S C, *et al*. Controlled trial of active tuberculosis case finding in a Brazilian *favela*. *Int J Tuberc Lung Dis* 2010; 14: 720–726.
6. Corbett E L, Bandason T, Duong T, *et al*. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster randomised trial. *Lancet* 2010; 376: 1244–1253.
7. Churchyard G J, Fielding K, Roux S, *et al*. Twelve-monthly versus six-monthly radiological screening for active case-finding of tuberculosis: a randomised controlled trial. *Thorax* 2011; 66: 134–139.
8. Kranzer K, Lawn SD, Meyer-Rath G, Vassall A, Raditlhalo E, *et al*. Feasibility, Yield, and Cost of Active Tuberculosis Case Finding Linked to a Mobile HIV Service in Cape Town, South Africa: A Cross-sectional Study. *PLoS Med* 2012; 9(8): e1001281.
9. Dodd PJ, White RG, Corbett EL. Periodic active case finding for TB: when to look? *PLoS One*. 2011; 6(12):e29130.
10. Rothman JR. Modern epidemiology, 2nd Edn. Boston: Little, Brown and Company.
11. Becerra M C, Pachao-Torreblancha I F, Bayona J, *et al*. Expanding tuberculosis case detection by screening household contacts. *Public Health Rep* 2005; 120: 271–277.

12. Topley J M, Maher D, Mbewe L N. Transmission of tuberculosis to contacts of sputum-positive adults in Malawi. *Arch Dis Child* 1996; 74: 140–143.
13. Beyers N, Gie R P, Schaaf H S, *et al*. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1997; 1: 38–43.
14. Marais B J, Gie R P, Hesseling A C, *et al*. Radiographic signs and symptoms in children treated for tuberculosis: possible implications for symptom-based screening in resource-limited settings. *Pediatr Infect Dis J* 2006; 25: 237–240.
15. Azzopardi P, Graham S. What are the most useful clinical indicators of tuberculosis in childhood? International Child Health Review Collaboration, 2008. http://www.ichrc.org/TBclin.html. (Accessed November 2009).
16. Marais B J, Gie R P, Hesseling A C, *et al*. A refined symptom based approach to diagnose pulmonary TB in children. *Pediatrics* 2006; 5: 1350–1359.
17. 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.
18. 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*. 2005; 365:130–134.
19. Moore HA, Apolles P, de Villiers PJ, Zar HJ. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. *Int J Tuberc Lung Dis*. 2011; 15: 1185-90.
20. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. *Paediatr Respir Rev* 2011; 12:16–21.
21. English R, Information Management Office, Boland/Overberg Regional Office. Boland/Overberg Region annual health status report 2007/2008. Worcester, South Africa: Boland/Overberg Regional Department of Health, 2009.
22. Hawkridge A, Hatherill M, Little F, *et al*. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. *BMJ* 2008; 337: a2052.
23. Orenstein W A, Bernier R H, Hinman A R. Assessing vaccine efficacy in the field: further observations. *Epidemiol Rev* 1988; 10: 212–241.
24. Hawkridge A. Clinical studies of TB vaccines. *Hum Vaccin* 2009; 5: 773–776.

25. Kaufmann S H E, Hussey G, Lambert P. New vaccines for tuberculosis. *Lancet* 2010; 375: 2110–2119.
26. Marais B J, Gie R P, Schaaf H S, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004; 8: 392–402.
27. Marais B J. Performing TB research in children: issues to consider. Indian Pediatr 2008; 45: 737–739.
28. Starke J R. Pediatric tuberculosis: time for a new approach. *Tuberculosis* 2003; 83: 208–212.
29. Coulter J B. Diagnosis of pulmonary tuberculosis in young children. *Ann Trop Paediatr* 2008; 28: 3–12.
30. Starke J R. Diagnosis of tuberculosis in children. *Pediatr Infect Dis J* 2000; 19: 1095–1096.
31. Hatherill M, Hanslo M, Hawkridge T, *et al*. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. *Bull World Health Organ* 2010; 88:312–20.
32. Hesseling AC, 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* 2002; 6:1038–45.
33. Mulenga H. Moyo S, Workman L, *et al.* Phenotypic variability in childhood TB: Implications for diagnostic endpoints in tuberculosis vaccine trials. *Vaccine* 2011; 29:4316-4321.
34. Hatherill M, Verver S, Mahomed H; Taskforce on Clinical Research Issues, Stop TB Partnership Working Group on TB Vaccines. Consensus statement on diagnostic endpoints for infant tuberculosis vaccine trials. *Clin Infect Dis*.2012; 54:493-501.
35. 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:S199-208.
36. Hackshaw A. Types of outcome measures and understanding them. In A concise guide to clinical trials. 1st ed. Chichester, UK: Wiley-Blackwell Publishing, 2009.
37. Moyo S, Hawkridge T, Mahomed H, *et al*. Determining causes of mortality in children enrolled in a vaccine field trial in a rural area in the Western Cape Province of South Africa. *J Paediatr Child Health* 2007; 43: 178–183.

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

Chapter 4: Tuberculin skin test and QuantiFERON assay in young children investigated for tuberculosis in South Africa: a comparison

Summary

Setting: A tuberculosis (TB) vaccine trial site in high TB burden rural area in South Africa.

Objectives: To compare results from the tuberculin skin test (TST) and the QuantiFERON-TB Gold In-Tube assay (QFT) in young children investigated for TB disease in a high TB burden setting.

Methodology: TB suspects were evaluated by medical history and examination, TST, QFT, chest radiography, induced sputum and gastric washings for smear and culture for *Mycobacterium tuberculosis (M.tb).*

Results: Four hundred children were enrolled. Among 397 children with both test results, 68 (17%) were QFT result positive and 72 (18%) were TST result positive (≥10 mm). Agreement between the tests was excellent (94%; κ = 0.79 (95% CI 0.69-0.89)). TB disease was diagnosed in 52/397 (13%) participants: 3 definite, 35 probable, and 14 possible TB. QFT sensitivity and specificity for *M.tb* infection in children diagnosed with TB disease were 38% and 81% respectively. TST sensitivity and specificity were 35% and 84% respectively.

Conclusion: While TST and QFT had excellent concordance in this population, both tests had much lower sensitivity than has been reported for other age groups. Our results suggested equivalent performance of the QFT and the TST in detecting *M.tb* infection in children with pulmonary TB disease in a high burden setting.

Introduction

Establishing a definitive diagnosis of pulmonary TB in young children is challenging because disease is pauci-bacillary and specimen collection more difficult compared to adults [1, 2]. Although there have been advances in techniques (such as sputum induction) to collect sputum specimens, and in techniques to detect *Mycobacterium tuberculosis* (*M.tb)* in these specimens, disease confirmation by positive *M.tb* culture remains a challenge where TB suspects are not severely ill such as in research settings undertaking active case-finding for TB [3, 4]. In the absence of *M.tb* culture confirmation, the diagnosis of pulmonary TB in children continues to rely on diagnostic algorithms that include history of exposure to *M.tb*, the tuberculin skin test (TST), or interferon gamma release assays (IGRAs), clinical, and radiological features [1, 2, 5, 6]. However clinical features for the diagnosis of TB in children are unreliable [1, 2, 3, 5].

Although a positive TST result is only indicative of infection with *M.*tb, and therefore indicates latent TB infection (LTBI), it is a useful adjunct test for TB disease since a positive result indicates recent infection which in young children is strongly associated with a high risk of progression to TB disease [7]. The interpretation of the TST result is however confounded by infection with environmental mycobacteria, Bacille Calmette-Guérin (BCG) vaccination, the immune status, and by the techniques used to administer the test and to read results. IGRAs which are largely unaffected by previous exposure to environmental mycobacteria and BCG have now been developed for the diagnosis of LTBI. These assays have increased specificity for detecting infection with *M.tb*. This is important in TB vaccine trials as it has potential to minimise disease misclassification (false positivity), that could result from the use of TST results in clinically based TB suspect and case definitions.

Initial testing of IGRAs was largely been conducted in adults [8-27], with relatively limited literature on IGRAs among very young children in high TB settings [9, 27, 28].

The main objectives of the study described in this chapter were to compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the TST and the QuantiFERON-TB Gold In-Tube assay (QFT) in children less than 3 years old, in a high TB incidence setting, who were evaluated for TB disease. A secondary objective was to

compare the absolute interferon gamma (IFN-γ) levels in children with various risk factors for TB.

Methods

Setting

Children included in this study had been enrolled in a study that compared two active case-finding (ACF) strategies on the incidence of TB in children aged 0-2 years old. (*This study is described in Chapter 3*). The study was conducted between 2005 and 2008, in a rural district of the Western Cape Province, about 100 km from Cape Town, South Africa. The population in the study area during 2007-2008 was estimated at 327,822 [29]. The overall TB incidence was 1442/100000 and antenatal HIV prevalence 12.8% in 2007 [29]. South Africa has a policy of universal BCG vaccination at birth, with high coverage of up to 99% in the Western Cape [30].

Study participants

Convenience systematic sampling was used select the study participants, because funding was limited. Among the 4786 children enrolled in the ACF study, 400 children sequentially investigated for TB disease in the study research ward between July 2007 and September 2008, were included in the study. The participants had been identified for TB investigation by either of the case detection strategies described in Chapter 3.

Investigations

Investigations for TB were undertaken in a dedicated research ward as described in Chapter 3. However for this study in addition to all other investigations, a blood sample was also drawn for the QFT assay. Chest radiographs (CXRs) were reviewed independently by three paediatric radiologists, blinded to clinical information and classified as described in Chapter 3.

QFT assay

The qualitative QFT was performed in accordance with manufacturer's instructions – this delivered a positive, negative or indeterminate result. The cut-off for positivity is an IFN-γ

response of ≥0.35 IU/mL for TB antigens, after subtracting values for the negative control. While the assay read-out provides absolute levels of IFN-γ, high values above 4IU/mL, the highest standard provided cannot be precisely measured [31]. Therefore, we stored QFT supernatants at -20°C, and later determined the quantitative IFN-γ levels in all participants again, using serial plasma dilutions. The optimal dilution determined in preliminary experiments was 1:25. Samples with indeterminate results were not repeated because a limited number of kits were available.

Definitions

Children were categorized as having "TB disease" or "No TB" based on an algorithm that included history of close household contact with a TB source case (household TB contact), clinical features suggestive of TB on medical examination, and CXR findings consistent with TB. The clinical features suggestive of TB were defined as cough for a period greater than 2 weeks or failure to thrive. Failure to thrive was defined as persistent inadequate weight gain or persistent weight loss determined by the research ward clinician on review of the Road to Health Card. TB disease included cases classified as having "Definite TB", "Probable TB", and "Possible TB" which were defined as follows;

- definite TB:- bacteriologically confirmed cases (culture or smear positive);
- probable TB:- CXR findings consistent with TB and ≥1 clinical features suggestive of TB, or a household TB contact ;
- possible TB: - CXR findings consistent with TB only, or ≥2 clinical features suggestive of TB only or 1 clinical feature suggestive of TB and a household TB contact in children prescribed standard TB therapy by the treating clinician.

(*CXR findings are described in detail in Chapter 5*). Children who could not be classified into definite, probable or possible TB were categorized as "No TB". TST and QFT results were not included in the definition of TB disease to prevent incorporation bias. A positive TST result was defined as an induration ≥10mm.

Data analysis

Data were entered into a Microsoft Access database and were analysed using STATA version10.0 (STATA Corp, College Station, TX, USA). Chi-square tests were used to

compare proportions. The Mann-Whitney test was used to assess differences between groups when results from continuous variables were not normally distributed.

Ethics approval and participant follow-up

The study protocol was approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee. Informed consent for study participation was obtained from parents or guardian of each child. The research ward clinician managed participants utilising the clinical findings and CXR interpretation available at the time of admission and discharge. Children diagnosed with TB disease were started on standard TB therapy and those with LTBI (defined as those with a household TB contact, TST ≥10mm and no other findings) on preventive TB therapy. QFT results were not used for clinical management as they were not routine investigations for TB in our setting.

Results

Participants

TST and QFT results were available for 397/400 (99%) of children, who were included in the final analysis for this study. **Table 1** shows their demographic and clinical characteristics, and the TB disease status according to the study algorithm. TST indurations were <5mm in 317(80%) of participants, while (72)18% had a TST result ≥10mm. The QFT was positive in (68)17% of the participants. Just over a third of the participants reported close contact with an adult TB source case. Thirteen children (3.3%) were diagnosed as having TB; and among these only one had bacteriologically confirmed disease.

Table 1: Demographic and clinical profile of participants evaluated (N=397)

Variable		n (%)
Age median (range), months		23 (9-34)
Gender n (%)	Male	206 (52)
	Female	191 (48)
Tuberculin skin test(mm)	0-<5	317 (80)
	5-<10	8 (2)
	10-<15	8 (2)
	≥15	64 (16)
QFT	Positive	68 (17)
	Negative	308 (78)
	Indeterminate	21 (5)
Household TB contact	Yes	145 (37)
	No	252 (63)
Cough > 2weeks	Yes	128 (32)
	No	269 (68)
Failure to thrive[a]	Yes	152 (38)
	No	239 (60)
	Unknown/ Missing	6 (2)
Fever	Yes	34 (9)
	No	363 (91)
Wheeze	Yes	125 (31)
	No	272 (69)
CXR findings consistent with TB	Yes	51 (13)
	No	337 (85)
	Unknown / Missing	9 (2)
***M.tb.* positive(Culture)**[b]	Yes	3 (1)
	No	394 (99)
TB Disease[c]	Yes	52 (13)
	No	345 (87)

QFT: QuantiFERON-TB Gold In-Tube; CXR: Chest x-ray; *M.tb*: Mycobacterium tuberculosis Failure to thrive; [a]persistent inadequate weight gain or persistent weight loss determined by the research ward clinician on review of the Road to Health Card; *M.tb.* positive (Culture); [b]There were no smear positive cases TB Disease; [c]Definite, probable and possible TB

Comparison of QFT and TST

Sixty eight (17%) children had positive QFT results and 72 (18%) had positive TST results (p=0.6). QFT results were indeterminate in 21 (5%) children. Children with indeterminate

QFT results were younger than those with positive or negative results ($p<0.05$). There was excellent agreement between the TST and QFT test results (94%, kappa = 0.79 (95% confidence interval (CI) 0.69-0.89); children with indeterminate QFT results excluded) (**Table 2).** There was no difference in age and in the proportions with TB related features (cough, household TB contact, failure to thrive) between children with discordant and those with concordant test results. Amongst the 145 children with a household TB contact the proportion with a positive QFT test result was similar to that with a positive TST result (38/145, (26%) in both groups).

Table 2: Comparison of TST and QFT results in participants evaluated and by TB disease status (N= 397)

	TST result n (%)		**TB Disease Status n (%)**	
	Positive n=72	**Negative n=325**	**TB Disease[c] n=52**	**No TB Disease n=345**
QFT result positive (IFN-γ ≥ 0.35/mL)	57 (79%)	11 (3%)	20 (38%)	48 (14%)
QFT result negative	13 (18%)	295 (91%)	29 (56%)	279 (81%)
QFT result Indeterminate	2 (3%)	19 (6%)	3(6%)	18(5%)

TB Disease[c]: Definite, probable and possible TB; TST: Tuberculin skin test; QFT: QuantiFERON-TB Gold In-Tube

Sensitivity and Specificity

The sensitivity and specificity for detecting *M.tb* infection in children with TB disease (definite, probable or possible TB) were similar for both tests **(Table 3**). Exclusion of children with indeterminate QFT results gave a slight increase in both the sensitivity and specificity of QFT. TST sensitivity and specificity were similar regardless of whether a 10 or 15 mm cut-off was used. The NPVs of TST and QFT results, in all analyses, were consistently around 90%. The PPVs of the two tests were low (29% for QFT, 25% for TST). This is shown in **Table 3**. Using a stricter definition of TB disease (definite and probable TB only) the sensitivity, specificity and NPVs of both tests remained largely unchanged.

Table 3: Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of QFT and TST for detecting *M.tb* infection in children diagnosed with TB disease

Diagnostic test accuracy N=397	Definite/Probable/Possible TB n=52			Definite/Probable TB n=38		
	QFT (95% CI)	TST ≥10mm (95% CI)	TST ≥15mm (95% CI)	QFT (95% CI)	TST ≥10mm (95% CI)	TST ≥15mm (95% CI)
Sensitivity (%)	38 (25-53) 41 (27-56)[d]	35 (22-49)	31 (19-45)	39 (24-57) 42 (26-59)[d]	35 (22-49)	32 (17-49)
Specificity (%)	81 (76-85) 85 (81-99)[d]	84 (80-88)	86 (82-90)	80 (75-84) 84 (80-88)[d]	84 (79-87)	86 (81-89)
PPV (%)	29 (19-42)	25 (16-37)	25 (15-37)	22 (13-34)	18 (1-29)	19 (10-30)
NPV (%)	91(87-94)	90 (86-93)	89 (85-92)	94 (90-96)	92 (89-95)	92 (89-95)

[d]After excluding QFT indeterminates (denominator n=376); PPV: Positive predictive value; NPV: negative predictive value; TST: Tuberculin skin test; QFT: QuantiFERON-TB Gold In-Tube; CI: confidence interval

Factors associated with a positive QFT or TST result

In univariate and multivariate analyses, children with a household TB contact or with CXR abnormalities were more likely to have a positive QFT or TST test result (Tables 4A and 4B). Age, gender and the most common symptoms (cough, failure to thrive, fever and wheeze) were not significantly associated with either QFT or TST result positivity.

Table 4A: Association between QFT and TST results and other diagnostic features of TB disease: Univariate analysis (Participants with indeterminate QFT results were excluded from this analysis)

Clinical Feature		Total n (%)	QFT positive n (%)	QFT positive Unadjusted Odds Ratio (95% CI)	TST positive n (%)	TST positive Unadjusted Odds Ratio (95% CI)
n		376	68 (18)		70 (19)	
	Age					
	9-11 months	9 (3)	0 (0)	na	0 (0)	na
	12-23 months	185 (49)	34 (18)	1.0 (0.6-1.7)	35 (19)	1.0 (0.6-1.7)
	24-34 months	182 (48)	34 (19)	1	33 (17)	1
Gender	Male	198 (53)	31 (16)	1.1 (0.6-1.8)	37(19)	1.0 (0.6-1.7)
	Female	178 (47)	37 (21)	1	33 (19)	1
HHC	Yes					
	No	138 (34)	38 (25)	***2.6 (1.5-4.5)***	38 (26)	***2.4(1.4-4.1)***
		238 (63)	30 (13)	1	32 (13)	1
Cough >2weeks	Yes	122 (86)	22 (18)	1.0 (0.6-1.7)	20 (16)	0.8 (0.5-1.4)
	No	254 (68)	46 (18)	1	50 (20)	1
Failure to thrive	Yes	145 (39)	19 (13)	0.6 (0.3-1.0)	22 (15)	0.7 (0.4-1.2)
	No	231 (61)	49 (21)	1	48 (21)	1
Fever	Yes	33 (9)	4 (12)	0.6 (0.2- 1.7)	5 (15)	0.8 (0.3-2.0)
	No	343 (91)	64 (19)	1	65 (19)	1
Wheeze	Yes	118 (31)	21 (18)	1.0 (0.6-1.7)	19 (16)	0.8 (0.4-1.4)
	No	258 (69)	47 (18)	1	51 (20)	1
CXR abnormalities	Yes	48 (13)	20 (42)	***4.2 (2.2-8.1)***	18 (38)	**3.2 (1.7-6.2)**
	No	319 (85)	46 (14)	1	50 (16)	1
	Unknown/Missing	9 (2)	2 (22)		2 (22)	

QFT: QuantiFERON –TB-Gold in-Tube; TST: Tuberculin skin test; HHC: Household TB contact; CXR: chest-x-ray;
*****statistically significant

Table 4B: Association between QFT and TST results and other diagnostic features of TB disease:-Multivariate analysis (Participants with an indeterminate QFT result were excluded from this analysis)

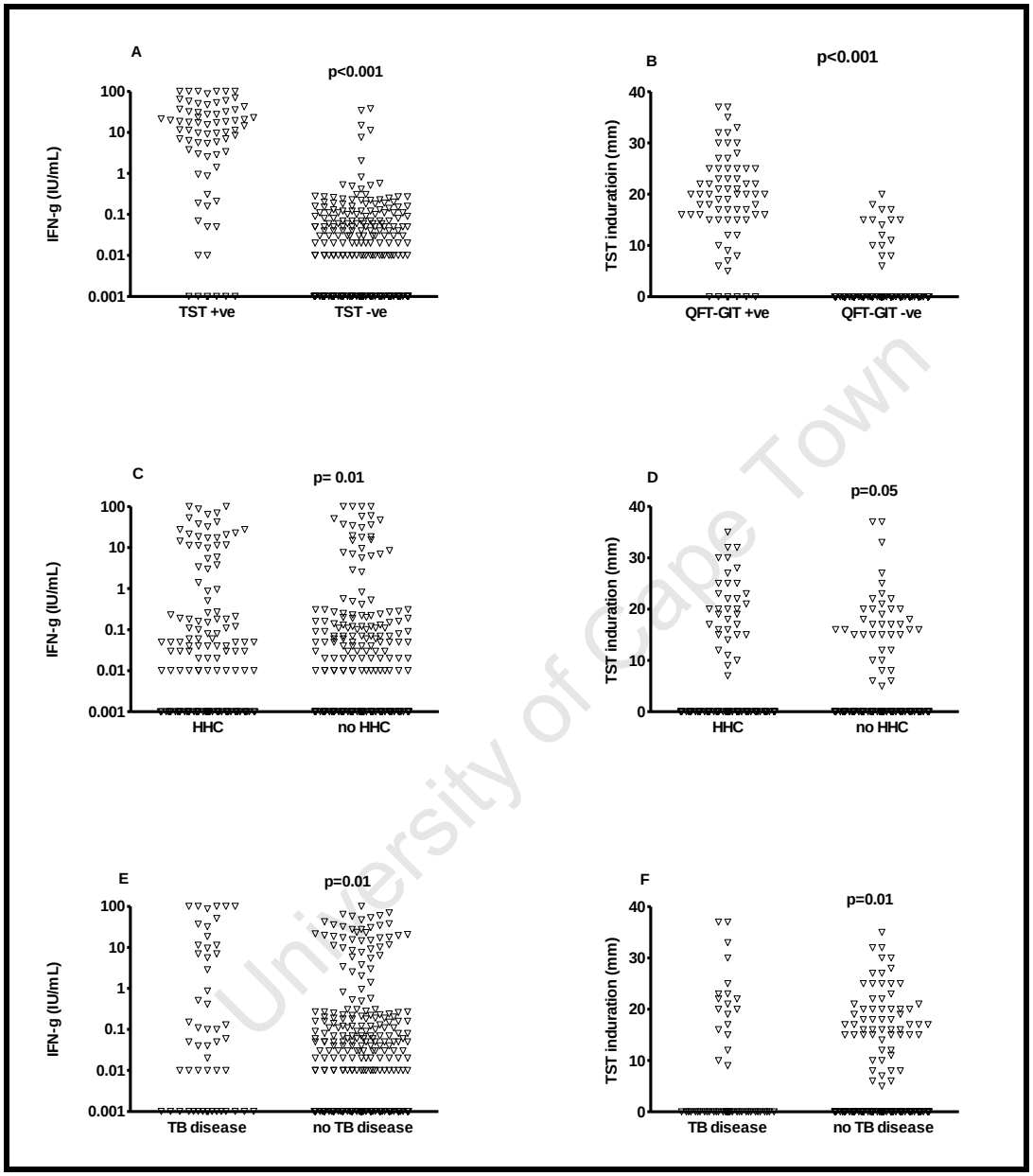
Clinical Feature	QFT positive Adjusted Odds Ratio[§] (95% CI)	TST positive Adjusted Odds Ratio (95% CI)
Age	1 (0.6-1.8)	1.0 (0.6-1.7)
Gender	1.1 (0.6-1.9)	1.0 (0.6-1.7)
Household TB Contact	***2.6(1.5-4.6)**	***2.3 (1.3-4.0)**
Cough >2weeks	1.5(0.6-3.7)	1.2(0.5-2.9)
Failure to thrive	0.6 (0.3-1.1)	0.7 (0.4-1.3)
Fever	0.5 (0.1-1.7)	0.8 (0.3-2.4)
Wheeze	0.8 (0.3-1.9)	0.7 (0.3-1.7)
CXR abnormalities	***4.5(2.2-9.0)**	***3.4 (1.7-6.8)**

QFT: QuantiFERON –TB-Gold in-Tube; CXR: chest-x-ray; *statistically significant;

Quantitative results from the QFT and TST

Fifty-four children (14%) had IFN-γ levels of QFT that were above the highest standard provided for the assay [31]. Plasma dilutions were therefore necessary, and were then included in the analysis of data from all participants. Children with a positive TST result, a household TB contact or with TB disease had significantly higher IFN-γ levels, compared with children without these features ($p<0.001$, $p=0.01$, $p=0.01$ respectively. Figure 1, Panels A, C, E). Similarly, those with a positive QFT result or with TB disease had significantly higher TST indurations compared with children without these features ($p<0.001$, $p=0.01$ respectively. Figure 1; Panels B, F). TST indurations were similar between those with and without a household TB contact (Figure 1, Panel D).

Figure 4: Relationship between absolute IFN- γ levels, measured in plasma from the QFT after appropriate dilution: ***with a positive TST (A), presence of a household contact (C) and presence of TB disease (E). The relationship between absolute TST induration and a positive QFT (B), presence of a household contact (D), and presence of TB disease (F) are also shown.***



HHC- Household TB contact; QFT-GIT: QFT.

Median bars have been excluded because for some median = 0

The Mann Whitney test was used to assess differences between groups.

Discussion

We found similar proportions of children less than 3 years old with suspected TB disease who had positive TST and QFT results. Agreement between the tests was excellent. Children with indeterminate QFT results were younger than those with positive or negative results. The sensitivities and specificities of both tests for detecting *M.tb* infection in children diagnosed with TB disease were similar, and both tests had a low PPV, while the NPV was high. As expected absolute QFT IFN-γ levels and TST indurations were significantly higher in children with a household TB contact or TB disease.

The level of agreement (kappa = 0.79) between the TST and QFT in our study was comparable to that observed in studies conducted in children in India and South Africa, which reported kappa scores of 0.73 and 0.78 respectively [15,22]. Other studies in children mainly from low TB incidence countries have reported lower levels of agreement between QFT and TST (kappa range 0.5-0.56) [11, 24, 25, 33-36]. Lower kappa scores have also been reported in studies conducted in TB endemic countries such as Cambodia (0.63) [19], Gambia (0.52)[21] and South Africa (0.56) [25]. The literature thus shows variable performance of IGRAs in different populations, and TB incidence settings [5, 7]. Different levels of concordance between the TST and QFT could be due to the inclusion of children of different ages in the various studies, different TST cut offs used in the comparisons, differences in the TB disease status of the children analysed, variation in BCG vaccination status, and different exposures to *M.tb* and environmental mycobacteria. In our study all children were from the same area, all were less than 3 years old, and all had been vaccinated with BCG. The level of agreement that we found suggests that TST cross reactivity with BCG in this population may be low, as was found in Botswana where TST responses ≥ 10mm were attributed to exposure to *M.tb* and not to BCG cross reactivity [36]. This is significant for the use of TST in TB vaccine trials and research studies conducted in settings similar to ours.

Although very few children in our study were HIV positive, QFT and TST sensitivity for *M.tb* infection (38% and 35%, respectively) was lower than observed in other paediatric studies on IGRAs [15-17, 34- 36] from both high and low TB incidence countries. These studies included older children, and had IGRA sensitivities ranging between 40% and 94%, while TST sensitivities ranged between 52% and 100% [15-17, 34-36]. In our study sensitivity could

have been reduced by over-diagnosis of TB since very few cases were bacteriologically confirmed. Our observed QFT and TST specificities (82% and 84% respectively) differ from those reported by other authors in low and high TB prevalence settings. Detjen *et al* [35] reported specificities of 100% and 58% respectively, while Bianchi *et al* [33] reported QFT and TST specificities of 86% and 87% at (5mm TST cut off). A more recent study conducted in Tanzania reported QFT and TST specifities of 90% and 98% respectively in children with TB disease (microbiologically confirmed and those with probable TB) [28].

In a study conducted in the same community as ours, T.SPOT *TB* sensitivity among children <5 years old with TB (definite and probable TB) was 40%, and specificity 84% [16], comparable to our findings. In KwaZulu-Natal, South Africa, Liebesheutz *et al* [14] found a higher sensitivity of 83% for the ELISPOT assay, compared with 63% for TST, among children <14 years old with TB disease. Other studies in children, mainly from low TB incidence countries have also reported QFT sensitivities better than that of the TST [33, 34, 36]. Overall, the literature suggests that IGRAs have a lower sensitivity and specificity for *M.tb* infection in high compared to low TB incidence settings [8, 10].The low sensitivity that we observed supports reports that IGRAs may perform differently in younger children compared with older children, in agreement with reported findings of lower IFN-γ production in younger children and a positive correlation between age and IFN-γ production [37, 38]. The younger age of children with indeterminate QFT results is also consistent with findings that QFT may deliver indeterminate results in young children [39].

Kampmann *et al* [35] and Bamford *et al* [11] showed that combining TST and IGRA results improves sensitivity in individuals with TB disease. This approach would not have been useful in our population, as only 2 of the 49 TB cases (excluding 3 cases with indeterminate QFT) had discordant results.

It was not surprising that both the TST and QFT were significantly associated with a household TB contact, as both indicate exposure to *M.tb*. The equally strong association of both tests with CXR abnormalities suggests that the QFT does not provide additional advantage above the TST as a diagnostic aid for TB disease in young children in this setting. Age was not associated with either test, probably because we had a limited variation in age groups (all children were less than three years old). Both tests were not associated with any of

the TB related clinical features investigated, probably because these clinical features are not specific to TB disease in young children.

Quantitative analysis

We found a large proportion of participants with IFN-γ levels above the highest standard results. This may indicate that if quantitative levels are to be used in a study setting, plasma dilutions are required for reliable results in similar settings. This has been confirmed in dilution studies conducted on blood samples from adolescents (South African tuberculosis Vaccine Initiative (SATVI), unpublished data).

Strengthens and limitations

This study conducted intensive investigations for TB disease on a large number of children and used well defined diagnostic criteria to classify TB cases. Serial dilutions were performed to precisely estimate quantitative IFN- γ levels. However there were some limitations. A small proportion of children had active TB (13%) and an even smaller proportion had bacteriologically confirmed disease (1%). Therefore, test sensitivities were estimated with relatively poor precision. There were no data on duration of exposure or smear status of adult contacts; hence we could not analyze household exposure in detail. Despite rigorous diagnostic criteria, use of radiological and clinical features to determine TB diagnosis could have resulted in over-diagnosis of TB (probable and possible TB) and underestimation of the sensitivity of both tests. However, since sensitivity remained largely unchanged with a stricter TB definition (definite and probable TB only), over-diagnosis is unlikely to have influenced results markedly. The predictive values should be interpreted with caution because they are affected by prior probabilities which were not determined in this study. Follow-up data on all children are unavailable, since no follow-up testing was done. Only 2 children were HIV positive, therefore we could not assess the impact of HIV positivity on test results.

Conclusion

This study showed excellent agreement between the TST and the QFT in this population of BCG vaccinated children in a high TB incidence setting. Both tests had low sensitivity, a reasonably high specificity for *M.tb* infection, and a high NPV. Comparable rates of test result positivity suggests that in HIV negative children younger than 3 years old, in high TB incidence settings, both the QFT and TST provide similar information and therefore either

could be used as a diagnostic aid in TB vaccine trials conducted in young children in similar settings.

Contributors

This chapter was written by Dr S. Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. S. Moyo, G. Hussey, M. Pai, W Hanekom, S. Gelderbloem, and A. Hawkridge designed the study. F. Isaacs conducted the QuantiFERON testing. S. Moyo managed the study and led the data analysis under the supervision of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. L. Workman managed the study database. M. Tameris and H. Geldenhuys participated in data collection.

References

1. Starke JR. Diagnosis of tuberculosis in children. *Pediatr Infect Dis J* 2000; 19: 1095–1096.
2. Rigouts L. Clinical practice: diagnosis of childhood tuberculosis. *Eur J Pediatr* 2009; 168: 1285–1290.
3. Moyo S, Verver S, Hawkridge A, *et al*. Tuberculosis case finding for vaccine trials in young children in high incidence settings: a randomised trial. *Int J Tuberc Lung Dis* 2012; 16: 185–191.
4. Mulenga H. Moyo S, Workman L, *et al.* Phenotypic variability in childhood TB: Implications for diagnostic endpoints in tuberculosis vaccine trials. *Vaccine* 2011; 29: 4316-4321.
5. Graham SM. Research into tuberculosis diagnosis in children. *Lancet Infect Dis* 2010; 10: 581-2.
6. Hesseling AC, Schaaf HS, Gie RP, Starke J R, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. *Int J Tuberc Lung Dis* 2002; 6:1038–1045.
7. Morán-Mendoza O, Marion SA, Elwood K, Patrick DM, FitzGerald JM. Tuberculin skin test size and risk of tuberculosis development: a large population-based study in contacts. *Int J Tuberc Lung Dis* 2007; 11: 1014–1020.
8. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008; 149: 177–184.
9. Lewinsohn DA, Lobato MN, Jereb JA. Interferon-[gamma] release assays: new diagnostic tests for *Mycobacterium tuberculosis* infection, and their use in children. *Curr Opin Pediatr* 2010; 22: 71–76.
10. Dheda K, Smit R, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. *Current Opinion in Pulm Med* 2009; 15: 188–200.
11. Bamford AR, Crook AM, Clark JE, *et al*. Comparison of interferon-gamma release assays and tuberculin skin test in predicting active tuberculosis (TB) in children in the UK: a Paediatric TB Network Study. *Arch Dis Child* 2010; 95: 180–186.
12. Herrmann JL, Belloy M, Porcher R, *et al*. Temporal dynamics of interferon gamma responses in children evaluated for tuberculosis. *PLoS One* 2009; 4: e4130.

13. Grare M, Derelle J, Dailloux M, Laurain C. QuantiFERON TB Gold In-Tube as help for the diagnosis of tuberculosis in a French paediatric hospital. *Diagn Microbiol Infect Dis* 2010; 66: 366–372.
14. Lucas M, Nicol P, McKinnon E, *et al*. A prospective large-scale study of methods for detection of latent *Mycobacterium tuberculosis* infection in refugee children. *Thorax* 2010; 65: 442–448.
15. Dogra S, Narang P, Mendiratta DK, *et al*. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. *J Infect* 2006; 54: 267–276.
16. Nicol MP, Davies MA, Wood K, *et al*. Comparison of T-SPOT. *TB* assay and tuberculin skin test for the evaluation of young children at high risk of tuberculosis in a community setting. *Pediatrics* 2009; 123: 38–43.
17. Liebesheutz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. *Lancet* 2004; 364: 2196–2203.
18. Hill PC, Brookes RH, Adetifa IMO, *et al*. Comparison of enzyme-linked immunospot assay and the tuberculin skin test in healthy children exposed to *Mycobacterium tuberculosis*. *Pediatrics* 2006; 117: 1542–1548.
19. Okada K, Mao TE, Mori T, *et al*. Performance of an interferon gamma release assay for diagnosing latent tuberculosis infection in children. *Epidemiol Infect* 2008; 136: 1179–1187.
20. Mandalakas AM, Hesseling AC, Chegou NN, *et al*. High level of discordant IGRA results in HIV-infected adults and children. *Int J Tuberc Lung Dis* 2008; 12: 417–423.
21. Adetifa I M O, Ota M O C, Jeffries D J, *et al*. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the Gambia. *Pediatr Infect Dis* 2010; 29: 439–443.
22. Hesseling AC, Mandalakas AM, Kirchner HL, *et al*. Highly discordant T-cell responses in individuals with recent exposure to household tuberculosis. *Thorax* 2009; 64: 840–846.
23. Nakaoka H, Lawson L, Squire SB, *et al*. Risk for tuberculosis among children. *Emerg Infect Dis* 2006; 12: 1383–1388.
24. Lighter J, Rigaud M, Eduardo R, Peng C, Pollack H. Latent tuberculosis diagnosis in children using the QuantiFERON-TB Gold In-Tube test. *Pediatrics* 2009; 123: 30–37.

25. Tsiouris SJ, Austin J, Toro P, *et al*. Results of a tuberculosis specific c IFN-γ assay in children at high risk of tuberculosis infection. *Int J Tuberc Lung Dis* 2006; 10: 939–941.
26. Lange C, Pai M, Drobniewski F, Migliori GB. Interferon-γ release assays for the diagnosis of active tuberculosis: sensible or silly? *Eur Respir J* 2009; 33: 1250–1253.
27. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K; IGRA Expert Committee; Centers for Disease Control andPrevention (CDC). Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection. *MMWR Recomm Rep* 2010; 59: 1–25.
28. Rose MV, Kimaro G, Nissen TN, *et al*. QuantiFERON-TB Gold In-Tube Performance for Diagnosing Active Tuberculosis in Children and Adults in a High Burden Setting. *PLoS ONE* 2012; 7: e37851.
29. English R, Information Management Office, Boland/Overberg Regional Office. Boland/Overberg Region annual health status report 2007/2008. Worcester, South Africa: Boland/Overberg Regional Department of Health, 2009.
30. Corrigal J, Coetzee D, Cameron N. Is the Western Cape at risk of an outbreak of preventable childhood diseases? Lessons from an evaluation of routine immunisation coverage. *S Afr Med J* 2008; 98: 41–45.
31. Cellestis. QuantiFERON® Gold (In-Tube Method) package insert doc. no. CA05990301A. Carnegie, VIC, Australia: Cellestis, 2006. http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1171 (Accessed 11 June 2011).
32. Connell TG, Ritz N, Paxton GA, Buttery JP, Curtis N, Ranganathan SCA threeway comparison of tuberculin skin testing, QuantiFERON-TB Gold and T-SPOT.*TB* in children. *PLoS One* 2008; 3: e2624.
33. Bianchi L, Galli L, Moriondo M, *et al*. Interferon-γ release assay improves that diagnosis of tuberculosis in children. Pediatr Infec Dis 2009; 28: 510–514.
34. Kampmann B, Whittaker E, Williams A, *et al*. Interferon gamma release assays do not identify more children with active tuberculosis than the tuberculin skin test. *Eur Respir J* 2009; 33: 1374–1382.
35. Detjen A K, Keil T, Roll S, *et al*. Interferon-gamma release assay improve the diagnosis of tuberculosis and non-tuberculosis mycobacterial disease in children in a country with a low incidence of tuberculosis. *Clin Infect Dis* 2007; 45: 322–328.

36. Centers for Disease Control and Prevention. Tuberculin skin test survey in a pediatric population with high BCG vaccination coverage: Botswana, 1996. *MMWR Morb Mortal Wkly Rep* 1997; 46: 846–851.
37. Kampmann B, Tena-Coki G, Anderson S. Blood tests for diagnosis of tuberculosis. *Lancet* 2006; 368: 282–283.
38. Connell TG, Curtis N, Ranganathan SC, Buttery JP. Performance of a whole blood interferon gamma assay for detecting latent infection with *Mycobacterium tuberculosis* in children. *Thorax* 2006; 61: 616–620.
39. Haustein T, Ridout DA, Hartley J C, *et al*. The likelihood of an indeterminate test result from a whole-blood interferon gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis* 2009; 28: 669–673.

Chapter 5: Radiographic abnormalities among young children detected through active TB case-finding who are investigated for pulmonary tuberculosis in a high TB burden setting

Summary

Background: Chest radiographic findings described among children with tuberculosis (TB) in high burden countries reflect severe, uncontained pulmonary disease. Our hypothesis was that this radiographic phenotype is related to passive case detection and late presentation.

Objectives: To describe chest radiographic features in young children with suspected pulmonary TB, in active case-finding in a high TB burden setting, and to identify the key clinical features associated with these radiographic phenotypes.

Methodology: Chest radiographic abnormalities in young children with suspected pulmonary TB, in an active TB case-finding study were analysed. TB suspects based on contact history or compatible symptoms underwent standardized clinical, radiographic, and microbiological testing. The radiographs were independently reviewed for pre-defined abnormalities by three paediatric radiologists who were blinded to clinical information.

Results: Nine hundred and forty-seven chest radiographs were reviewed. Hilar lymphadenopathy and parenchymal consolidation were isolated radiographic findings in 51 (5.4%) and 44 (4.6%) radiographs, respectively, and occurred together in 17 (1.8%). Cavitation, miliary nodules, and pleural disease were not demonstrated. Lymphadenopathy and consolidation, occurring in combination, was associated with failure to thrive (OR 3.74; 95%CI 1.33-10.48) and a positive culture for *Mycobacterium tuberculosis (M.tb)* (OR 10.33; 95% CI 1.78-60.0).

Conclusion: The radiographic features demonstrated by active TB case-finding in this population largely reflect uncomplicated primary complex TB with a small proportion of uncontained parenchymal disease. This mild radiographic disease phenotype may reflect radiological changes associated only with *M.tb* infection, and not TB disease. Growth failure, rather than persistent cough, was the clinical hallmark of uncontained pulmonary TB in young children in this setting. Infant TB vaccine trials, other preventive trials, and TB control programmes adopting active case-finding should prioritize growth failure as a sentinel clinical feature for early detection of childhood TB in high burden settings.

Introduction

Chest radiographs (CXRs) play a definitive role in the diagnosis of childhood pulmonary tuberculosis (TB), since bacterial confirmation occurs in less than 40% of cases in children [1-6]. Diagnosis is therefore usually based on a combination of history of contact with an infectious source case, compatible symptoms, a positive tuberculin skin test (TST) or *Mycobacterium tuberculosis (M.tb)* specific interferon gamma release assay (IGRA), and specific changes on CXR [1-8]. Although the interpretation of CXR findings in children with pulmonary TB is variable, intrathoracic lymph node, parenchymal, and extra-thoracic radiographic abnormalities compatible with TB have been well-described [5, 9, 10]. Published descriptions of CXR findings among children with pulmonary TB in high burden countries have generally reflected severe disease, which is characterized by complicated intrathoracic lymphadenopathy and progressive, uncontained parenchymal disease [9].

Our hypothesis was that this radiographic profile is a function of passive case detection, with delayed presentation in older children, and that active case-finding (ACF) would result in a different profile. This is based on studies that have shown that in ACF settings TB disease tends to be mild, is detected earlier, in younger children, and that the rate of bacteriologic confirmation is extremely [11-15].

There is limited data on the chest radiographic phenotype in ACF settings in young children in developing countries. This is because TB cases including those in children have largely been detected passively in these countries [16, 17]. The description and understanding of CXR findings in ACF in young children in high TB burden countries is essential to inform the development of case definitions for research settings, since ACF is likely to be integral to clinical trials of new TB vaccines, drugs, and other interventions against TB conducted in these settings.

The objectives of this study were to describe CXR abnormalities in young children with suspected pulmonary TB, detected through ACF in a high TB incidence setting, and to identify the key clinical features associated with these radiographic phenotypes.

Methods

Setting and participants

The CXRs analysed in this study were obtained from participants enrolled in a study comparing two ACF strategies that is described in **Chapter 3**. CXRs were taken shortly after determining that a child had suspected TB, and hence a temporal association with symptomatology was assumed.

Reading of chest radiographs

The original CXR films were scanned using a high-resolution flatbed scanner (Epson E1680-PRO), saved as jpeg images, and reviewed independently by three paediatric radiologists experienced in childhood TB. The radiologists were blinded to clinical information. Findings were recorded on a standardized reading and recording form (Appendix 2). The reviewers reported on whether any of the following abnormalities were "definitely present", "definitely absent", "possibly present", or "not visible" (due to technical reasons);

- intrathoracic lymphadenopathy (hilar or paratracheal),
- parenchymal consolidation,
- pleural abnormalities (pleural thickening or pleural effusion),
- miliary disease,
- cavitation

Radiographs in which no single feature was visible due to technical factors were regarded as "unreadable".

Data analysis

Data were captured in a Microsoft Access database and analysed using STATA version 10.0 (Stata Corp College Station, Texas, USA). Abnormalities were regarded as "present" when recorded as "definitely present" by at least two of the three reviewers. The Pearson Chi-square test and the Fisher's test (1-sided) were used to compare clinical features between children with and without CXR abnormalities in univariate analysis. The Mann-Whitney and the Kruskal-Wallis tests were used to compare groups with continuous data that were not normally distributed. Forward, nested logistic regression was used in multivariate analysis of clinical and radiographic variables. Statistical significance was determined at $p<0.05$, or if the

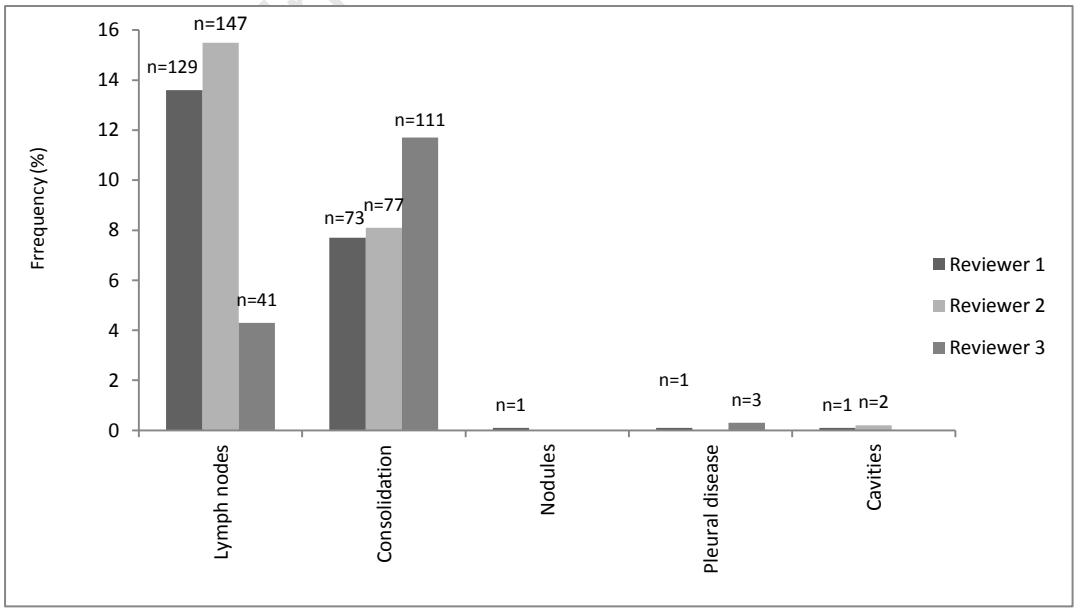
confidence interval (CI) did not cross 1. Sensitivities, specificities and predictive values of TB clinical features were calculated for CXR abnormalities of interest.

Results

Nine hundred and seventy (970) CXRs were obtained from 880 children identified as TB suspects and investigated for TB, from a cohort of 4,786 children under active surveillance for pulmonary TB [17]. Twenty-three radiographs that were not reviewed by at least two reviewers were excluded from further analysis. The remaining 947 radiographs, obtained from 862 children, were analysed. Eighty-three children were evaluated for TB on more than one occasion during the study follow-up period, therefore 81 children had two CXRs and two children had three CXRs each. The median age at the time of chest radiography was 13.2 months (IQR 7.8–21.0 months).

Lymphadenopathy and parenchymal consolidation were the most frequent CXR abnormalities detected by all reviewers (**Figure 1**). Lung tissue nodules, miliary TB, pleural disease, and cavities were detected in a very small number of radiographs by individual reviewers (less than 1%, **Figure 1**), with no two reviewers identifying these features on the same CXR.

Figure 1: Radiographic findings in 947 chest radiographs from child TB suspects identified through active case-finding for TB



Using the two-out-of-three reviewer agreement criteria for classification of radiographic features as definitely present, abnormalities were detected in a total of 112 (11.8%) radiographs. Lymphadenopathy was detected in 68 (7.5%) and parenchymal consolidation in 61 (6.4%). Isolated lymphadenopathy was detected in 51(5.4%), isolated parenchymal consolidation in 44 (4.6%), and lymphadenopathy and parenchymal consolidation occurred together in 17 (1.8 %) radiographs. There was no difference in ages between children who presented with isolated lymphadenopathy, isolated parenchymal consolidation, or lymphadenopathy and parenchymal consolidation in combination (p=0.82). Six children (0.7%) were HIV infected, of whom five had normal CXRs, and one had isolated lymphadenopathy.

Table 1 shows the clinical, epidemiological and microbiological features of TB in children with or without radiographic abnormalities. There was no significant difference in the proportion of symptoms, signs, and positive culture for *M.tb* in the two groups, except that children with CXR abnormalities more often reported history of contact with a TB source case, or were more likely to have a positive TST. There was no difference in age between the two groups (p=0.08).

Table 1: Association between clinical, epidemiological and microbiological features of TB disease and chest radiographic abnormalities (N= 947 chest radiographs)

Clinical feature	CXR abnormalities present n= 112 (%)	No CXR abnormalities n= 835 (%)	§OR 95% CI	p value
Cough >2 weeks Y	43 (38)	304 (36)	1.09(0.73-1.63)	0.68
N	69	531		
FTT Y	48 (43)	279 (33)	1.49(0.98-2.17)	0.05
N	64	556		
TB contact Y	68 (61)	286 (34)	2.97(1.98-4.44)	*<0.001
N	44	549		
TST ≥10mm Y	42 (38)	167 (20)	2.4 (1.58-3.64)	*<0.001
N	70	668		
[a]Positive *M.tb* culture				
Y	4 (4)	10 (1)	3.06 (1.00-9.39)	0.07
N	108	825		Fisher's test

CXR-Chest radiograph, Y-Yes, N-No, FTT-failure to thrive, TST-tuberculin skin test, *M.tb-Mycobacterium tuberculosis*, OR-odds ratio, CI-confidence interval, [a]There were no smear positive cases, *statistically significant

Among the 68 radiographs with lymphadenopathy, 8 (12%) showed compression of the airway. Four (50%) of the 8 children with radiological evidence of airway compression reported a history of wheezing for more than two weeks. Three of these four children also reported cough for more than two weeks.

Of the 947 radiographs analysed, 73(8%) were deemed unreadable by Reviewer 1; five (0.5%) deemed unreadable by Reviewer 2; and three (0.3%) were deemed unreadable by Reviewer 3. For radiographs that were read, agreement between reviewer pairs for radiographic findings ranged from 82%-90%; and weighted kappa scores for positive detection of lymphadenopathy or consolidation ranged between 0.17-0.32 (**Table 2**). The average weighted kappa scores for the detection of lymphadenopathy and consolidation were 0.27 and 0.28, respectively.

Table 2: Detection of lymphadenopathy and parenchymal consolidation: Agreement between reviewer pairs

	Lymphadenopathy			Parenchymal consolidation		
	Reviewer 1	**Reviewer 2**	**Reviewer 3**	**Reviewer 1**	**Reviewer 2**	**Reviewer 3**
Reviewer 1		82%	91%		94%	96%
Reviewer 2	0.25		84%	0.17		95%
Reviewer 3	0.32	0.23		0.32	0.27	

Percentage agreement between reviewer pairs is presented above the diagonal spaces for each category; weighted kappa scores are presented below the diagonal spaces for each category.

Table 3A shows the association between clinical, epidemiological and microbiological features of TB with radiographic phenotypes in univariate analysis. History of a contact with a TB source was associated with isolated parenchymal consolidation (a negative association). A positive TST was associated with isolated lymphadenopathy. Failure to thrive, and a positive culture for *M.tb*, was associated with lymphadenopathy and parenchymal consolidation occurring in combination, as was the combined presence of a positive TST, cough for more than 2 weeks, and failure to thrive. Associations between individual clinical features and radiographic abnormalities that were statistically significant in univariate analysis were also found to be significant in multivariate analysis (**Table 3B**).

Table 3A: Association between clinical, epidemiological and microbiological features of TB disease with individual or combined chest radiograph abnormalities (N= 947 chest radiographs): Univariate analysis

Clinical features	Radiographic Pathology											
	Isolated lymphadenopathy				Isolated parenchymal consolidation				Lymphadenopathy and Parenchymal consolidation			
	Yes N=51 (%)	No N=896 (%)	OR 95% CI	p value	Yes N=44 (%)	No N=903 (%)	§OR 95% CI	p value	Yes N= 17 (%)	No N=930 (%)	§OR 95% CI	p value
Cough >2 weeks			0.94	0.84			1.09	0.78			1.55	0.37
Y	18 (35)	329 (37)	0.52-1.68		17 (39)	330 (37)	0.59-2.02		8 (47)	339 (36)	0.61-3.93	
N	33	567			27	573			9	591		
FTT Y	20 (34)	307 (34)	1.24	0.47	17 (39)	310 (33)	1.20	0.56	11 (65)	316 (34)	*3.56	*0.008
N	31	589	0.70-2.19		27 (61)	593	0.65-2.23		6	614	1.35-9.37	
TB contact Y	37(73)	580 (65)	1.44	0.25	22 (50)	595 (66)	*0.52	*0.03	9 (53)	608 (65)	0.60	0.29
N	14	316	0.77-2.68		22	308	0.28-0.94		8	322	0.24-1.51	
TST ≥ 10mm Y	27 (53)	182 (20)	4.41	*<0.001	8 (18)	201(22)	0.78		7 (41)	202 (22)	2.52	0.06
N	24	714	*2.50-7.78		36	702	0.36-1.67	0.52	10	728	0.98-6.49	
[a]Positive *M. tb* culture Y	1 (2)	13(1)	1.36	0.54	1 (2)	13 (1)	1.59	0.49	2 (12)	12 (1)	10.2	*0.02
N	50	883	0.00-8.32	Fisher's test	43	890	0.00-9.79		15	918	0.00-44.81	Fisher's test
TST ≥10mm and asymptomatic Y	13 (25)	82(9)	*3.40	<0.001	5(11)	90(10)	1.16	0.76	0(0)	95 (10)	-	-
N	38	814	1.76-6.51		39	813	0.40-2.92		17	835		
Cough >2 weeks, FTT and TST<10mm Y	6 (12)	105 (12)	1.00	0.99	7 (16)	104 (12)	1.44	0.39	0 (0)	111 (12)	-	-
N	45	791	0.43-2.36		37	791	0.64-3.25		17	819		
TST ≥10mm, cough >2 weeks and FTT Y	3 (6)	26 (3)	2.09	0.20	0 (0)	29 (3)	-	-	4(24)	25(3)	11.13	*0.001
N	48	870	0.65-6.74	Fisher's test	44	874			13	905	3.58-35.0	Fisher's test

Y-Yes, N-No, FTT-failure to thrive, TST-tuberculin skin test, OR-odds ratio, CI-confidence interval, TST ≥10mm, *M.tb-Mycobacterium tuberculosis*, [a]there were no smear positive cases, *statistically significant

Table 3B: Association between clinical, epidemiological and microbiological features of TB disease with individual or combined chest radiograph abnormalities (N= 947 chest radiographs): Multivariate analysis

Clinical features	Radiographic Pathology					
	Isolated lymphadenopathy		Isolated parenchymal consolidation		Lymphadenopathy and Parenchymal consolidation	
	Adjusted OR 95% CI	p value	Adjusted OR 95% CI	p value	Adjusted OR 95% CI	p value
Cough >2 weeks	0.99 (0.54-1.81)	0.97	1.06 (0.57-1.99)	0.85	1.60 (0.59-4.33)	0.35
FTT	1.21 (0.67-2.19)	0.52	1.16 (0.62-2.18)	0.64	3.74 (1.33-10.48)	0.12
[b]TB contact	-	-	0.51 (0.28-0.95)	0.03	-	-
[c]TST ≥ 10mm	4.46 (2.50-7.96)	<0.001	-	-	2.02 (0.71-5.76)	0.19
[a]Positive *M. tb* culture	0.68 (0.08-5.44)	0.71	2.05 (0.26-16.35)	0.50	10.33(1.78-60.0)	*0.009

FTT-failure to thrive, TST-tuberculin skin test, OR-Odds Ratio, *M.tb-Mycobacterium tuberculosis*, [a]there were no smear positive cases, [b]The variables TB contact and TST were not included together in the models because of collinearity; TB contact was included in the model testing associations with parenchymal consolidation since it showed a significant association in univariate analysis; the [c]TST was included in the other two models. *statistically significant

Individual TB symptoms and signs had low positive predictive values (PPVs), high negative predictive values (NPVs), low sensitivities and high specificities for each of the radiographic phenotypes, despite the statistically significant associations noted above (**Table 4**). History of contact with a TB source case demonstrated 73% sensitivity for isolated lymphadenopathy, and failure to thrive 65% sensitivity for combined radiographic pathology. The combined presence of a positive TST, cough for more than two weeks, and failure to thrive, had low sensitivity, high specificity, a low PPV and a high NPV for the three radiographic phenotypes.

Table 4: Sensitivity, specificity and predictive values of clinical, epidemiological and microbiological features of TB disease for prediction of chest radiograph pathology

Clinical features	Radiographic Pathology											
	Isolated lymphadenopathy				Isolated parenchymal consolidation				Lymphadenopathy and parenchymal consolidation			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cough >2 weeks	35	63	5	95	39	63	5	96	47	64	2	99
FTT	34	66	3	91	39	64	5	96	65	66	3	99
TB contact	73	35	6	96	50	34	4	93	53	35	1	98
TST ≥ 10mm	53	80	13	97	18	22	4	95	41	78	3	99
Positive M.tb culture[‡]	1	99	7	95	2	99	7	95	12	99	14	98
TST ≥10mm and asymptomatic	25	91	14	96	11	90	5	95	-	90	-	98
Cough >2 weeks, FTT and TST<10mm	12	90	5	95	16	88	6	97	-	80	-	98
TST ≥10mm, cough >2 weeks and FTT	6	97	15	95	-	97	-	95	24	97	14	99

TST-Tuberculin skin test, M.tb-Mycobacterium tuberculosis, FTT-failure to thrive, [a]*M.tb-Mycobacterium tuberculosis*, [‡]there were no smear positive cases

Discussion

Uncomplicated intrathoracic lymphadenopathy and parenchymal consolidation were the most frequent chest radiographic findings among very young children with suspected TB, in ACF in a high TB burden setting. Isolated hilar or paratracheal lymphadenopathy, which are consistent with early or contained primary complex TB, occurred three times more often than the combination of intrathoracic lymphadenopathy and parenchymal consolidation, which are suggestive of progressive or uncontained pulmonary TB. Radiographic evidence of pleural TB, cavitatory TB, or disseminated miliary disease, was not demonstrated.

A positive TST was significantly associated with isolated lymphadenopathy, as might be expected, and failure to thrive and a positive culture for *M.tb* were each significantly associated with lymphadenopathy and parenchymal consolidation occurring in combination. However, persistent cough, a hallmark of childhood TB diagnosis among older children, was not associated with radiographic evidence of intrathoracic lymphadenopathy or parenchymal consolidation, alone or in combination, in this age group and study setting. Persistent wheeze was reported in a small number of children who had radiographic evidence of intrathoracic airway compression.

The most common radiographic phenotype in this analysis, isolated intrathoracic lymphadenopathy, is frequently the only radiographic finding in children identified for evaluation for TB through contact tracing and screening [13]. We found isolated lymphadenopathy and isolated parenchymal consolidation in similar proportions, whereas a study of actively detected child contacts of adult TB cases in Cape Town, did not report isolated parenchymal consolidation, but a greater proportion (2.5% compared to 1.8% in our study) of the combination of lymphadenopathy and consolidation [13]. This difference could be due to the relatively broad screening criteria that we used to identify TB suspects. This might have also identified children with other respiratory infections apart from TB for investigation. This is consistent with our observed negative association of TB contact history and isolated parenchymal consolidation, suggesting that this radiographic feature is frequently unrelated to TB disease in this age group and study setting. Conversely, since we conducted intensive case-finding, it is also likely that many children in our study were investigated while they were in the early stages of *M.tb* infection and disease, before development of uncontained, progressive parenchymal changes. This may explain the lower proportion of chest radiographs with both lymphadenopathy and parenchymal consolidation in our study.

A positive TST was the only individual feature significantly associated with isolated lymphadenopathy. This is consistent with early radiographic changes after infection with *M.tb*. However, it is not possible to determine for any individual child whether isolated intrathoracic lymphadenopathy is a manifestation of early TB disease that has not yet progressed into the parenchyma, static lymphadenopathy that will remain contained, or transient primary complex TB that will spontaneously resolve, as has been described in the natural course of childhood *M.tb* infection and disease [10].

Our observed association between a positive culture for *M.tb* and failure to thrive with lymphadenopathy and parenchymal consolidation occurring in combination suggests that growth failure should be prioritized in efforts to detect TB cases early. The observed lack of an association between persistent cough and combined lymphadenopathy and parenchymal consolidation suggests that persistent cough could be a non-specific sentinel symptom for radiographic TB disease in this age group. However, it is possible that a more detailed characterisation of the nature of the cough could have yielded different results, since a persistent *non-remitting* cough for longer than two weeks is a sensitive indicator of TB disease in older children [7].

The sensitivities and PPVs of TB symptoms and signs for CXR pathology were low to moderate. However, a positive culture for *M.tb* and the combined presence of a positive TST, cough for greater two weeks, and failure to thrive, had ≥97% specificity for all three radiographic phenotypes. This suggests that diagnostic criteria incorporating this combination of clinical features might be useful in excluding TB in young children with respiratory or constitutional illnesses other than TB, who are identified as suspects in ACF settings.

The frequency of detecting radiographic features of TB varied considerably between expert reviewers, as has been shown previously [18]. The average weighted kappa score of 0.27 for the detection of lymphadenopathy was lower than that reported among hospitalized children investigated for pulmonary TB, where the average weighted kappa was 0.33 [18]. The lower level of agreement may be due to the fact that our study was community based, and that we conducted intensive surveillance for TB suspects. Therefore, we would expect that a large proportion of children with TB would have had early, mild disease, and hence, less clearly defined pathologic features on CXR. However, both scores are categorised as "fair" agreement [19].

Although this study had a large sample size, which allows evaluation of uncommon events, there were some limitations. First, follow-up CXRs were not performed; hence, we are unable to assess the evolution of radiographic features or response to TB treatment in children who received preventive or curative TB therapy on discharge from the research ward. Secondly, the CXR films were scanned and read as digital jpeg images, which might have affected image quality, although we do not regard this as significant. Thirdly, we detected few children with bacteriologically confirmed TB, which is not surprising, given the disease

spectrum, but this limited our ability to examine associations with *M.tb* culture positivity. Lastly, the criteria used to define the presence of CXR abnormalities (agreement by two-out-of-three reviewers) might be considered overly stringent, given the variability of CXR interpretation [5, 18]. However, we believe that this specific approach is appropriate, given the pivotal role of CXR in the diagnosis of childhood TB in a research setting.

Conclusion

The radiographic features demonstrated by active TB case-finding in this study population largely reflect uncomplicated primary complex TB with a small proportion of uncontained parenchymal disease. This mild radiographic disease phenotype may reflect radiological changes associated only with *M.tb* infection, and not TB disease. Growth failure, rather than persistent cough, appears to be the clinical hallmark of uncontained pulmonary TB in young children in this setting. Infant TB vaccine trials, other preventive trials, and TB control programmes adopting active case-finding should prioritize growth failure as a sentinel clinical feature for early detection of childhood TB in high burden settings.

Contributors

This chapter was written by Dr S. Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. S. Moyo managed the study and analysed data under the supervision of Associate Professor M. Hatherill, Dr S. Verver and Professor G. Hussey. The study was designed by S. Moyo, G. Hussey, S. Verver, L. Geiter and A. Hawkridge. L. Workman and H. Mulenga managed the study database. M. Tameris, H. Geldenhuys and C. Ontong participated in data collection.

References

1. Guidance for national tuberculosis programmes on the management of tuberculosis in children. World Health Organization. WHO/HTM/TB/2006.371. Available at http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf (Accessed 13 June 2011).
2. Coulter JBS. Diagnosis of pulmonary tuberculosis in young children. *Ann Trop Paediatr* 2008; 28: 3–12.
3. Rigouts L. Clinical practice: Diagnosis of childhood tuberculosis. *Eur J Pediatr* 2009; 168: 1285–1290.
4. Marais BJ, Gie RP, Schaaf HS *et al*. Childhood pulmonary tuberculosis: old wisdom and new challenges. *AM J Crit Care Med* 2006; 173: 1078-1090.
5. Andronikou S, Wieselthaler N. Imaging for tuberculosis in children. In: Schaaf SH, Zumla AI, Tuberculosis–a comprehensive clinical reference. United Kingdom: Saunders Elsevier, 2009: 262-296.
6. Starke JR. Paediatric tuberculosis: time for a new approach. *Tuberculosis* 2003; 368 83:208-12.
7. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Lombard C, Enarson DA, *et al*. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. *Pediatrics* 2006;118: e1350-9.
8. Mazurek GH, Jereb J, Vernon A, *et al*. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. *MMWR Recomm Rep* 2010; 59(RR-5):1-25.
9. Marais BJ, Gie RP, Schaaf HS *et al*. A proposed radiological classification of intrathoracic tuberculosis. *Pediatr Radiol* 2004; 34: 886–894.
10. 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.
11. Moyo S, Verver S, Hatherill M, *et al*. Tuberculosis active case for vaccine trials in young children in high incidence settings: a randomised trial. *Int J Tuberc Lung Dis*2012; 16:185-191.

12. Hawkridge A, Hatherill M, Little F, *et al*. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomized trial. *BMJ* 2008; 337:a2052.
13. Marais BJ, Gie RP, Hesseling AC, Schaaf SH, Donald EA, Beyers N. Radiographic signs in children treated for tuberculosis- Possible implications for symptom-based screening in resource limited settings. *Pediatr Infect Dis J* 2006; 25:237-240.
14. Mulenga H. Moyo S, Workman L, *et al.* Phenotypic variability in childhood TB: Implications for diagnostic endpoints in tuberculosis vaccine trials. *Vaccine* 2011; 29:4316-4321.
15. Hatherill M, Hawkridge T, Zar HJ, *et al*. Induced sputum or gastric lavage for community-based diagnosis of childhood pulmonary tuberculosis? *Arch Dis Child* 2009; 94: 195-201.
16. The Stop TB Strategy: building on and enhancing DOTS to meet the TB related Millennium Development Goals. Geneva, World Health Organization, 2006 (WHO/HTM/TB/2006.368). Available at http://www.ghdonline.org/uploads/WHO_HTM_STB_2006.368_eng.pdf (Accessed 28 June 2011).
17. Nyirenda M, Sinfield R, Haves S, Molyneux EM, Graham SM. Poor attendance at a child TB contact clinic in Malawi *Int J Tuberc Lung Dis* 2006; 10:585–587.
18. Du Toit G, Swingler G, IIoni K. Observer variation in detecting lymphadenopathy on chest radiography. *Int J Tuberc Lung Dis* 2002; 9: 814-817.
19. Altman D G. Practical statistics for medical research. 2nd ed. London, UK: Chapman & Hall, 1991: pp 403–409.

Chapter 6: Serious adverse events in young children enrolled in TB vaccine trials

Summary

Setting: A tuberculosis (TB) vaccine trial site in a rural area in South Africa.

Objectives: To determine the nature and rate of serious adverse events, defined as hospital admissions and deaths, and the proportion due to TB in young children enrolled in TB vaccine trials.

Methodology: Hospital admission and mortality data from two TB vaccine trials (Cohort 1 (2001-2006) and Cohort 2 (2005-2008)), that were conducted in children aged 0-2 years were analysed.

Results: In Cohort 1, 2895 hospital admissions were recorded from a cohort of 11680 children enrolled, giving a 19% hospitalisation rate over two years. In Cohort 2, 718 admissions were recorded from a cohort of 4786 infants enrolled, giving a hospitalisation rate of 15% over two years, (p=0.008). There was a broad spectrum of morbidity which was dominated by respiratory tract infections (33% Cohort 1, 31% Cohort 2), and diarrhoeal disease (32% Cohort 1; 24% Cohort 2). TB accounted for 4% and 6% of admissions in Cohorts 1 and 2 respectively (p=0.04). There were few HIV related illnesses and these were associated with TB (Cohort 1 OR 4.13, p< 0.05; Cohort 2 OR 84, p<0.0001). Mortality was also predominantly due to respiratory tract infections and diarrhoeal disease, with a small proportion of deaths due to TB, 0.3% in Cohort 1 and 1% in Cohort 2. Specific causes of mortality could not be assigned to 18% and 51% of deaths in Cohorts 1 and 2 respectively.

Conclusion: Serious adverse events in these trials were dominated by respiratory tract infections and diarrhoeal disease. Although the overall proportions were low, hospital admissions and deaths due to TB were significant contributors to serious adverse events in these trials. Specific causes of mortality could not be determined for a significant proportion of deaths.

Introduction

Clinical trials that evaluate new products are required to identify and report on the occurrence of trial endpoints and serious adverse events (SAEs) occurring among trial participants[1, 2, 3, 4]. A serious adverse event is defined as "any untoward or unfavourable medical occurrence in a clinical trial participant that is life threatening, results in death, hospitalisation, disability, congenital anomaly, or requirement of an intervention to prevent permanent impairment or damage" [3, 4]. The reporting of serious adverse events is essential in the assessment of product safety and efficacy[3, 4, 5].

Hospital admissions and deaths among trial participants are important serious adverse event in clinical trials of TB new vaccines that are conducted in young children in developing countries. In these settings the burden of morbidity and mortality in this age group is generally high [6-9]. Respiratory tract infections (RTIs), diarrhoea and malnutrition are major contributors to this burden [6-9]. This is significant because RTIs commonly occur together with TB and because TB may be undetected in the children diagnosed with RTIs in high TB burden settings, while malnutrition increases the risk of developing TB [10-15]. The detection of all TB cases is important in TB vaccine trials because TB disease is the current endpoint for these trials. Secondly, hospitalisation and mortality data are important for detecting events related to or caused by the investigational vaccine.

Causes of mortality among children enrolled in TB vaccine trials at a rural TB vaccine trial site in South Africa have previously been reported [16, 17]. Pneumonia, gastroenteritis and septicaemia were the main causes of mortality[16, 17] (**Figure 1**).

Figure 1: Immediate causes of death assigned by the record review method (CR/VA) and by death certificates (DC) Moyo S *et al*, Determining cause of mortality in children enrolled in a vaccine trial in a rural area in the Western Cape province of South Africa. *Journal of Pediatrics and Child health* 2007; 43; 178-183.

Table 3 Immediate causes of death assigned by the record review method (CR/VA) and by death certificates (DC)

Death certificate	Pneumonia	Gastroenteritis	Septicaemia	Natural causes	Tuberculosis	Trauma†	Other	Subtotal	Unknown	Total
Record review and VA										
Pneumonia	29	5	1	5	2		7	49	1	50
Gastroenteritis	1	26		5			2	34	4	38
Septicaemia	2	1	12	2	1		7	25	3	28
Sudden Unexplained causes	1	1		12			2	16	1	17
Tuberculosis					3		1	4	1	5
Trauma†						4		4		4
Ill-defined	1			7			1	9	1	10
Other	2		1	2			12	17	1	18
Subtotal	36	33	14	33	6	4	32	158	12	170
Unknown				1			3	4		4
Total	36	33	14	34	6	4	35	162	12	174

†Trauma (motor vehicle accident). Shaded boxes indicate agreement between CR/VA and DC. Data are expressed as number of deaths.

In this chapter our objectives were to determine the nature and rate of serious adverse events, (defined as hospital admissions and mortality), and the proportion due to TB in young children enrolled in TB vaccine trials at a vaccine trial site in South Africa. This analysis also provides baseline data for comparison with findings in future trials of novel TB vaccines to be conducted at the same site.

Methods

We analysed hospital admission and mortality data from two trials (Cohort 1 and Cohort 2) that were conducted at the South African Tuberculosis Vaccine Initiative (SATVI) trial site in the Cape Winelands district of South Africa over the period 2001 to 2008. The first trial (Cohort 1) was conducted between 2001 and 2006, while the second (Cohort 2) was conducted between 2005 and 2008. In 2007, the estimated population in the Cape Winelands district was 327 822 people [18]. The district is among the 20% least deprived districts in South Africa with a poverty rate of 21% in 2007 [19]. The 2007 infant mortality and under 5 mortality rates were 23/1000 and 32/1000 respectively [18].

Cohort 1- Efficacy of percutaneous versus intradermal Bacille Calmette Guérin (BCG) in the prevention of tuberculosis in South African infants: randomised trial (2001-2006)

This was a randomised controlled trial comparing the efficacy of the percutaneous and intradermal routes of administering the Bacille Calmette Guérin (BCG) vaccine in preventing TB in children aged 0-2 years [20]. Infants were randomized at birth to percutaneous or intradermal administration of BCG. Serious adverse events were detected by an extensive surveillance system developed at the trial site. Surveillance entailed i) a study visit at age 3 months where symptom screening for TB was conducted, ii) review of hospital admission, radiology department, and clinic attendance records, and iii) review of death certificates and conducting verbal autopsy interviews in case of deaths (where consent was granted by the family). The surveillance system also detected children with suspected TB. TB suspects underwent standardized evaluation for TB disease in a dedicated study research ward. These investigations are as described in Chapter 3. [A medical history and examination were performed by a clinician and the following investigations conducted: CXR (anterolateral and antero-posterior films), tuberculin skin test (TST) by the Mantoux method, Human Immunodeficiency Virus (HIV) testing (with parental/legal guardian consent), paired early morning gastric washing (GW) and induced sputum (IS) specimen collection on two consecutive days. Smear microscopy (auramine fluorescent microscopy) and culture for *M.tb* (Mycobacterial Growth Indicator Tube (MGIT) 960) were performed on all GW and IS specimens. Cultures were incubated for at least 6 weeks. Positive cultures underwent speciation to exclude non-tuberculous mycobacteria. The TST was read 48-72 hours after administration].

Personal identifiers:-names, date of birth and residential address, were used to identify study participants from hospital admission and clinic attendance lists in the four hospitals and 23 clinics in the study area. These lists were perused manually once every week to identify new entries. Medical records of participants who had been hospitalised were then obtained. Mortality records at the regional Health Office were also perused weekly to identify study participants who might have died, and attempts were then made to obtain all available medical records including death certificates. Where consent was given, verbal autopsy interviews were conducted with the family members of the deceased participant, to obtain additional information to aid determination of causes of death. All medical records, death

certificates and verbal autopsy interview records were reviewed by medical officers to determine and document the discharge diagnoses and causes of death.

Cohort 2- Tuberculosis case finding for vaccine trials in young children in high-incidence settings: a randomised trial (2005-2008)

This was a randomised trial that compared two active case-finding (ACF) methods on TB case accrual and disease severity in BCG vaccinated children aged 0-2 years [17]. (This study is described in Chapter 3). Participants were randomised to three monthly home visits for TB symptom screening and clinic and hospital admission record surveillance, or to clinic and hospital admission record surveillance only. Study participants were identified from hospital, clinic attendance lists, and from mortality records as was described for Cohort 1. All medical records, death certificates and verbal autopsy interview records were reviewed by medical officers as was described for Cohort 1. Children with suspected TB were also investigated as for Cohort 1.

Data were captured into a Microsoft access database, and analysed using STATA version 11. In both studies hospital admissions and mortality up to the age of 24 months were analysed. Diagnoses and causes of death were grouped into main disease categories that are common in young children in South Africa and other developing countries [6-9].

Results

Hospital admissions

In Cohort 1, 2895 hospital admissions were recorded for 2186 children from a cohort of 11680 children who were enrolled, giving a 19% hospitalisation rate over two years. The median age at hospitalisation was 9 months (IQR 5-15 months). In Cohort 2, 718 admissions were recorded for 604 children from a cohort of 4786 infants, giving a hospitalisation rate of 15% over two years, which was significantly lower than that for Cohort 1 (p=0.008). The median age at hospitalisation of 9 months (IQR 4-16 months) in Cohort 2 was similar to that observed for Cohort 1.

Table 1 shows the number of admissions per child and the total number of hospital admissions in each study. The majority of children were hospitalised once. In Cohort 1, 23%

of children were hospitalised more than once while in Cohort 2 this proportion was much smaller (14%) , ($p< 0.0001$).

Table 1: Hospital admissions

	Cohort 1 (N=11680) Period 2001-2006			Cohort 2 (N=4786) Period 2005-2008		
Number of hospital admissions per child	**Number of children hospitalised n=2186**	**Proportion of children hospitalised (%)**	**Total number of hospital admissions n= 2895**	**Number of children hospitalised n=604**	**Proportion of children hospitalised (%)**	**Total number of hospital admissions n=718**
1	1691	77	1691	510	84	510
2	354	16	708	78	13	156
3	98	5	294	13	2	39
4	27	1	108	2	0.3	8
5	11	0.5	55	1	0.2	5
6	1	0.05	6			
7	1	0.05	7			
8	1	0.05	8			
9	2	0.09	18			

In both cohorts the hospitalisation rate peaked in the 6-12 month age category before declining in the older ager categories (**Table 2)**. The hospitalisation rate was significantly greater in Cohort 2 in all age categories beyond the first month of life.

Table 2: Hospital admissions by age category

	Cohort 1				Cohort 2				
Age category (months)	**Persons at risk**	**Number of Hospitalisations**	**Number of Deaths**	**Hospitalisation Rate (%)**	**Persons at risk**	**Number of Hospitalisations**	**Number of Deaths**	**Hospitalisation Rate (%)**	**P value**
<1	11680	205	39	1.8%	4786	80	17	1.7%	0.7
1-<6	11641	718	74	6.2%	4769	165	44	3.5%	<0.0001*
6-<12	11567	868	37	7.5%	4725	181	34	3.8%	<0.0001*
12-<18	11530	627	23	5.4%	4691	159	19	3.4%	<0.0001*
18-<24	11507	414	13	3.6%	4672	133	5	2.8%	0.02*

Morbidity profile

The morbidity profile was broad and generally similar in the two cohorts, as shown in **Table 3** (Statistical comparison of all the variables in **Table 3** are presented in Appendix 1). In both cohorts RTIs (33% Cohort 1; 31% Cohort 2) and diarrhoeal disease (32% Cohort 1; 24% Cohort 2), were the most frequent conditions, and were followed by admissions for pulmonary TB. There were only a few admissions for tuberculous meningitis; two (0.07%) in Cohort 1 and three (0.4%) in Cohort 2.

Table 3: Hospital admissions morbidity profile

	Cohort 1	N= 2895	Cohort 2	N=718
Diagnosis	**n**	**%**	**n**	**%**
Respiratory tract infections (infections of the upper and lower respiratory tract), excludes pulmonary tuberculosis	954	33.0	223	31.1
Diarrhoeal disease (includes the term gastroenteritis, and diarrhoea and vomiting)	926	32.0	175	24.4
TB (pulmonary tuberculosis)	128	4.4	45	6.3
Malnutrition (includes marasmus and kwashiorkor)	88	3.0	11	1.5
Jaundice	60	2.1	16	2.2
Meningitis (viral, bacterial, unspecified, includes tuberculous meningitis)	53	1.8	9	1.3
Perinatal conditions and prematurity	49	1.7	7	1.0
Convulsions (includes, epilepsy)	49	1.7	6	0.8
Injuries (includes fractures, head injuries and injuries due to motor vehicle accidents	47	1.6	18	2.5
Febrile convulsions and pyrexia if unknown origin	40	1.4	12.0	1.7
Renal conditions (includes infections)	35	1.2	6	0.8
Wheezing (unspecified)	35	1.2	0	0.0
Burns (hot water and fire burns)	33	1.1	6	0.8
HIV related illnesses (included cases with confirmed HIV infection)	32	1.1	6	0.8
Congenital conditions	31	1.1	0	0.00
Otitis media	32	1.1	7	1.0
Sepsis (generalised bacterial infection including meningococcemia)	31	1.1	3	0.4
Poisoning (ingestion of hazardous material such including paraffin)	30	1.0	10	1.4
Abscess(includes cellulitis and empyema)	25	0.9	8	1.1
Dermatological conditions	20	0.7	6	0.8
Minor surgical procedures (includes circumcision)	15	0.5	10	1.4
Asthma	10	0.3	16	2.2
Eye conditions (includes infections)	9	0.3	3	0.4
Other (condition or symptoms and signs not included in the conditions above)	51	1.8	36	5.0
Unknown/ not specified	112	3.9	79	11.0

Table 4 compares the proportions of hospital admissions for selected conditions in the two cohorts. The proportion of admissions for diarrhoeal disease was significantly lower in Cohort 2 compared to Cohort 1. There were significantly more admissions for TB in Cohort 2. In Cohort 1, there were 32 (1.1%) hospitalisations for HIV related illnesses, while there were six (0.8%) in Cohort 2. Eleven percent of admissions Cohort 2 could not be assigned a specific diagnosis. This was significantly greater than the corresponding proportion (3.9%) observed for Cohort 1 (p=0.003).

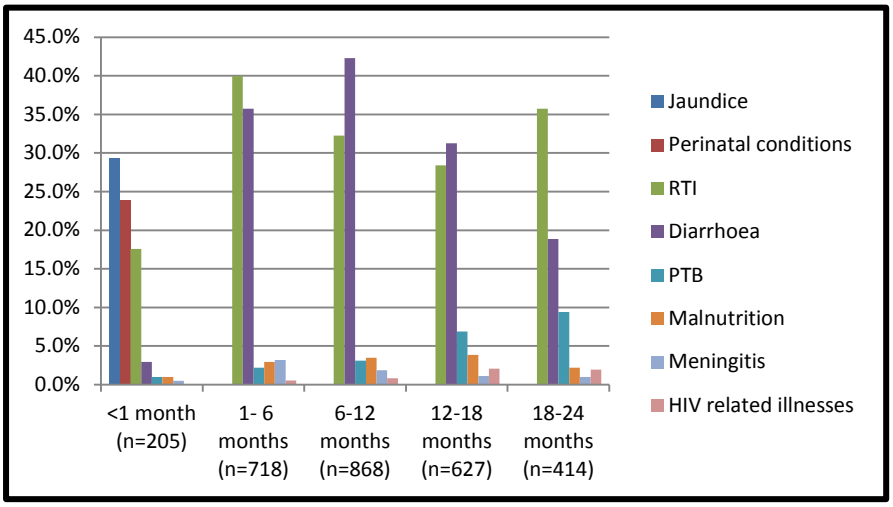
Table 4: Comparison of proportions of hospital admissions for selected conditions

	Cohort 1	N=2895	Cohort 2	N=718	p value
Diagnosis	n	%	n	%	
Respiratory tract infections (infections of the upper and lower respiratory tract), excludes pulmonary tuberculosis	954	33.0	223	31.1	0.33
Diarrhoeal disease (includes the term gastroenteritis, and diarrhoea and vomiting)	926	32.0	175	24.4	0.0001*
TB (pulmonary tuberculosis)	128	4.4	45	6.3	0.03*
Malnutrition (includes marasmus and kwashiorkor)	88	3.0	11	1.5	0.03*
Jaundice	60	2.1	16	2.2	0.79
Meningitis (viral, bacterial, unspecified, includes tuberculous meningitis	53	1.8	9	1.3	0.29
Perinatal conditions and prematurity	49	1.7	7	1.0	0.16
HIV related illnesses (included cases with confirmed HIV infection)	32	1.1	6	0.8	0.48
Other (condition or symptoms and signs not included in the conditions above)	51	1.8	36	5.0	<0.0001*

Hospital admissions by age category

The age specific morbidity profile was similar in the two cohorts (**Figure 2 and Figure 3**). In both cohorts, in the first month of life, jaundice (29.3% Cohort 1; 20.0% Cohort 2; p= 0.1) and perinatal conditions (23.9% Cohort 1; 8.8% Cohort 2; p=0.004) were the predominant serious adverse events. In the oldest age category, RTIs (35.7% Cohort 1; 22.6% Cohort 2; p=0.001) and diarrhoeal disease (18.8% Cohort 1; 17.3% Cohort 2; p= 0.7) were the most frequent conditions closely followed by pulmonary TB (9.4%. Cohort 1; 6.8% Cohort 2; p= 0.3). The proportion of admissions for pulmonary TB increased with increasing age in both cohorts.

Figure 2: Admissions for selected conditions by age category: Cohort 1



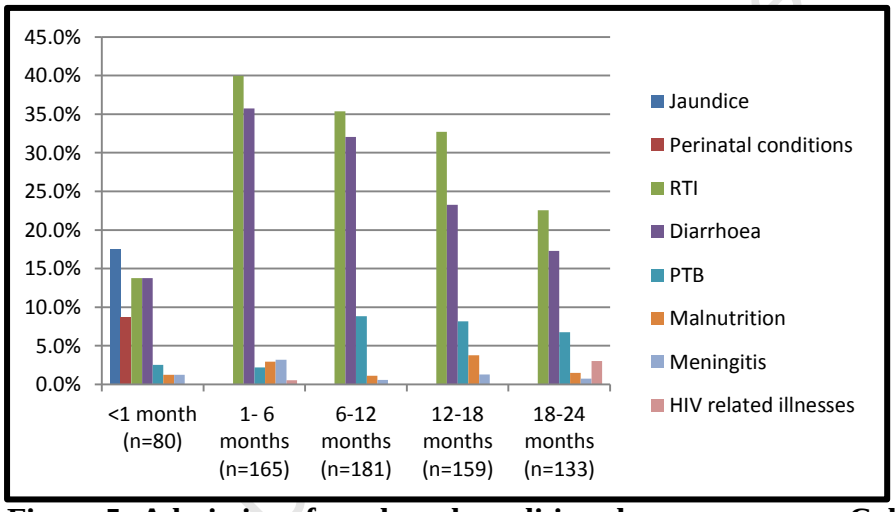


Figure 5: Admissions for selected conditions by age category: Cohort 2

RTI- respiratory tract infections
PTB- pulmonary tuberculosis

Mortality

Causes of mortality in these trials have previously been reported [16, 17]. The overall mortality rate in Cohort 1 was 1.6% over 2 years, and was 2.5% over 2 years in Cohort 2 ($p<0.0001$). The causes of mortality are shown in **Table 5**. RTIs and diarrhoea were the leading causes of mortality in both cohorts. The proportion of TB deaths was very small, and there was no significant difference in proportions in the two cohorts. In Cohort 1, 18% of deaths could not

be assigned specific causes; the corresponding proportion was much greater in Cohort 2, 51% (p=<0.0001).

Table 5: Mortality profile

Cause of death	Cohort 1 n (%)	Cohort 2 n (%)	P value
Respiratory tract infections	50 (26.9)	16 (13.4)	0.005*
Diarrhoeal disease	38 (20.4	14 (11.8)	0.05
Septicaemia	28 (15.1)	6 (5)	0.006*
Tuberculosis	5 (2.7)	1 (0.8)	0.2
Prematurity	4 (2.2)	4 (3.3)	0.6
Malnutrition	0	4 (3.3)	0
Meningitis	0	2 (0.02)	0
HIV related illnesses	2(1.1)	1 (0.8)	0.8
Trauma	6 (3.2)	5 (4.2)	0.6
Unknown/ ill-defined	35 (18.8)	61 (51.3)	<0.0001*
Other	18 (9.7)	5 (4.2)	0.08
Total	**186**	**119**	

Discussion

The morbidity and mortality profile at this trial site was dominated by RTIs and diarrhoeal disease. Although the overall proportions were relatively low, hospitalisations for pulmonary TB were among the most frequent serious adverse events. A significant proportion of admissions were for unknown or unspecified conditions in Cohort 2. Admissions peaked in the 6-12 month age category. The proportion of deaths due to TB was very low. In both cohorts a large proportion of deaths could not be assigned specific causes of death (18% Cohort 1; 51% in Cohort 2).

The morbidity and mortality burden and profile in both studies reflects that observed in the broader study area, and that which is commonly observed in similar settings in developing countries [6-9, 18]. RTIs and diarrhoeal disease were the leading causes of mortality among audited deaths in South African hospitals, in children aged 0-18 years between 2005 and 2009 [21]. The majority of these deaths occurred in children less than 5 years old [21]. Other vaccine trials conducted in Africa have also reported morbidity and mortality profiles consistent with those observed in our studies. In a pneumococcal conjugate vaccine trial

conducted in Soweto, 66% of deaths were due to pneumonia and bronchiolitis, and 9% due to gastroenteritis [22]. In another pneumococcal conjugate vaccine trial conducted in rural Gambia, pneumonia was a leading cause of hospitalisations [23]. Therefore our findings demonstrate and confirm the profiles that are likely to be observed in clinical trials of new TB vaccines conducted in developing country settings even when only healthy children are selected for enrolment[16, 17].

The proportion of hospital admissions for pulmonary TB was relatively low (4% in Cohort 1 and 6% in Cohort 2). This suggests that there were few cases of severe TB that required hospitalisation among trial participants. This could reflect the success of the surveillance system in detecting TB suspects for evaluation and management in the study research ward. (However, it is noted that in Cohort 2, 23 participants were missed by the surveillance system and diagnosed with TB outside the research ward, (Chapter 3)). It also demonstrates the impact of ACF on the severity of TB disease; ACF has been shown to detect TB cases early before progression to severe disease manifestations [16, 17, 24].

Although RTIs are common in young children in settings similar to ours, it is however possible that some children who were diagnosed with RTIs could have had pulmonary TB occurring together with an RTI or in isolation. Studies have demonstrated that TB can be missed where it occurs together with an RTI in young children[10-12].This is partly because the symptoms and signs of the two conditions overlap, and because making a definitive diagnosis of pulmonary TB in young children is not always possible. Only a few cases of pulmonary TB in young children are bacteriologically confirmed since the disease is paucibacillary and specimen collection difficult in this age group. The possibility of undetected TB raises the likelihood of detection bias in TB vaccine trials. This would underestimate the burden of TB disease (the trial endpoint) and inflate vaccine efficacy. This highlights the need for improved diagnostics for pulmonary TB in this age group.

Nearly 10% (7% in Cohort 1 and 11% in Cohort 2) of all admissions occurred in children less than 1 month old even though both studies only enrolled healthy children. This demonstrates the general morbidity burden that vaccine trials enrolling neonates in similar settings are likely to face. Mortality in the first month of life was higher in Cohort 1 were infants were enrolled at birth. This suggests that deferring enrolment beyond the early neonatal period

could reduce mortality and therefore serious adverse events, by excluding children with early neonatal problems [6-9].

In both cohorts, a significant proportion of deaths could not be assigned to specific causes of death, despite attempts to review clinical records including death certificates and information from verbal autopsy interviews. This is significant since it could not be determined if any of the deaths were due to TB. Unknown causes of deaths among trial participants in TB vaccine trials would impact on the assessment of vaccine safety and efficacy. This highlights a need for close follow-up of all trial participants and an investigation of other approaches for the accurate determination of causes of mortality among trial participants in settings similar to ours [16].

The overall morbidity and mortality profile was the same over the eight year period during which these studies were conducted. This was against a background of largely unchanged health policies in the area. The introduction of the vaccines against *rotavirus* and *Streptococcus pneumoniae* important causative agents of diarrhoea and pneumonia in young children in South Africa occurred after both studies had completed enrolment and follow-up [25]. Therefore the burden of morbidity and mortality due to RTIs and diarrhoeal disease at this site is likely to be different in future studies. However, similar profiles would be expected for other conditions and in trials that are conducted in similar settings.

The reason for a significantly higher proportion of admissions for unknown or unspecified conditions in Cohort 2 is not entirely clear, given that there was no difference in the participants enrolled in both cohorts. A possible explanation could be that participants in the three monthly home visits group in Cohort 2 had a high probability of being referred to healthcare centres on the basis of unclear or very mild symptoms of illness. A further explanation could relate to delays or difficulties in obtaining clinical records of children in the less intensive case finding group in Cohort 2 resulting in the reasons for these admissions being categorised as unknown or unspecified. This is consistent with the data on causes of mortality.

This analysis had several limitations. Diagnoses were based on record review, and could have been incorrect for some cases given the limitations associated with record review namely missing information and the inability to clarify or verify information. However our findings

are consistent with the regional and national childhood mortality profile suggesting that any error is insignificant. Data on disease severity were not recorded; therefore it is not possible to comment on disease severity in these cohorts. Thirdly only data on the main diagnosis or condition at admission were analysed, hence we cannot report on comorbidities an important factor in hospital admissions and mortality in young children in our setting.

Conclusion

Serious adverse events in these TB vaccine trials were dominated by RTIs and diarrhoeal disease. Hospital admissions and deaths due to TB were significant contributors to the morbidity and mortality burden in these trials, even though their overall proportions were low. Specific causes of mortality could not be determined for a significant proportion of deaths. Despite extensive follow-up and data collection the ability to detect TB among trial participants who are hospitalised and those who may die is limited by the unavailability of better performing diagnostic tools for childhood TB.

Contributors

This chapter was written by Dr S. Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. G. Hussey, A. Hawkridge, L. Geiter, S. Moyo, and S. Verver designed the studies. S. Moyo analysed the data under the supervision of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey. L. Workman managed the study database. M. Tameris and H. Geldenhuys participated in data collection.

References

1. Hawkridge A. Clinical studies of TB vaccines. *Human Vaccines* 2009, 5:11, 773-776.
2. Kaufmann SHE, Hussey G, Lambert P. New vaccines for tuberculosis. *Lancet* 2010; 375: 2110–19
3. USA, Food and Drug Administration. Safety. http://www.fda.gov/safety/medwatch/howtoreport/ucm053087.htm (Accessed on 3 October 2012).
4. National Institutes of Health. Clinical Research Study Investigator's Toolbox. http://www.nia.nih.gov/research/dgcg/clinical-research-study-investigators-toolbox/adverse-events. (Accessed on 4 October 2012).
5. Hackshaw A. A concise guide to clinical trials. Chapter 2: types of outcome measures and understanding them. 1st ed. Chichester, UK: Wiley-Blackwell Publishing, 2009.
6. Black RE, Cousens S, Johnson HL, et *al*, Global and national causes of child mortality in 2008: a systematic analysis. , *Lancet* 2010; 375: 1969–87
7. Liu L, Johnson HL PhD, Cousens S *et al* Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since *Lancet* 2012. 379; 9832: 2151-2161.
8. Mathers C, Ma Fat D, Inoue M, Lopez AD. Counting the dead and what they died of: as assessment of global status of cause of death data. *Bull WHO Health Organ*2005; 83:171-117.
9. Bradshaw D, Bourne D, Nannan N. What are the causes of death among South African children? Medical Research Council, South Africa, Policy Brief, November 2003. http:/www.mrc.ac.za/policybriefs/child mortality.pdf. (Accessed on 4 October 2012).
10. McNally LM, Jeena PM, Gajee K, *et al.* The effect of polymicrobial disease, maternal HIV status on treatment response and cause of pneumonia in South African children: a prospective descriptive study. *Lancet* 2007; 369:1440-51.
11. Chintu C, Mudenda V. Lucas S *et al.* Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; 360:985–90.
12. 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: a pneumococcal conjugate vaccine probe study. *Pediatr Infect Dis J* 2010; 29:1099-04.

13. Jaganath D, Mupere E. Childhood Tuberculosis and Malnutrition. *J Infect Dis* 2012; 206:1809-15.
14. Vijayakumar M, Bhaskaram P, Hemalatha P. Malnutrition and childhood tuberculosis. *J Trop Pediatr* 1990; 36:294-8.
15. Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S. Tuberculosis and nutrition. *Lung India* 2009; 26:9-16.
16. Moyo S, Hawkridge T, Mahomed H, *et al*. Determining causes of mortality in children enrolled in a vaccine field trial in a rural area in the Western Cape Province of South Africa. *J Paediatr Child Health* 2007; 43: 178–183.
17. Moyo S, Verver S, Hawkridge A, *et al* and the South African Tuberculosis Vaccine Initiative Neonatal Study Team. Tuberculosis case finding for vaccine trials in young children in high incidence settings: a randomised trial. *Int J Tuberc Lung Dis* 2012; 16: 185–191.
18. English R, Information Management Office, Boland/Overberg Regional Office. Boland/Overberg Region annual health status report 2007/2008. Worcester, South Africa: Boland/Overberg Regional Department of Health, 2009.
19. Barron P, Day C, Monticelli F, Eds. The District Health Barometer 2006/07. Durban: Health Systems Trust; December 2007.
20. Hawkridge A, Hatherill M, Little F, *et al*. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. *BMJ* 2008; 337: a2052.
21. Stephen CR, Bamford LJ, Patrick ME, Wittenberg DF eds. Saving Children 2009: Five Years of Data A sixth survey of child healthcare in South Africa. Pretoria: Tshepesa Press, MRC, CDC; 2011.
22. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. Atrial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003; 349:1341–8.
23. Cutts F, Zaman S, Enwere G, *et al*. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomized, double-blind, placebo controlled trial. *Lancet* 2005; 365(9465):1139–46.

24. Den Boon S, Verver S, Lombard C J, *et al*. Comparison of symptoms and treatment outcomes between actively and passively detected tuberculosis cases: the additional value of active case finding. *Epidemiol Infect* 2008; 136: 1342–1349.
25. National Department of Health. Strategic Plan for maternal, newborn, child and women's health (MNCWH) and Nutrition in South Africa, 2012–2016. http://www.doh.gov.za/list.php?type=Maternal and child health (Accessed on 4 October 2012)

Chapter 7: Discussion and General Conclusions

Despite the recent decline in the global tuberculosis (TB) incidence and mortality, the burden of the disease still remains high with an estimated 8.7 million incident cases (range, 8.3 million–9.0 million) globally reported in 2011 [1]. It is therefore recognised that a multi-pronged approach that includes the development of novel drugs, rapid and more effective diagnostics, and an effective vaccine is needed to accelerate the decline of TB cases worldwide and drive TB toward elimination [2].

While significant progress has been made in the development and testing of new TB vaccines, the conduct of clinical trials of new TB vaccines is still beset by challenges [2, 3]. A major challenge is the absence of a correlate or surrogate endpoint of protective immunity against TB [4-6]. Therefore the success of new TB vaccines cannot be easily predicted or identified in experimental animal models or early phase clinical trials. Efficacy can only be evaluated against a clinical endpoint: the development of TB disease [4, 7-10]. However, in infants and young children, who have been identified as a target group for new TB vaccines the detection and diagnosis of TB disease can be difficult. The signs and symptoms are non-specific, overlap with those of other childhood conditions, and there is a low rate of bacteriological disease confirmation [11-15]. Howbeit, the accurate determination of vaccine safety and efficacy rests on the detection of *all TB suspects and subsequently all TB cases* among trial participants in a TB vaccine trial. Inaccurate and incomplete detection of suspects and cases can result in the "dilution" and poor precision of vaccine efficacy measurements [16, 17]. Therefore TB case-finding is important in the conduct of TB vaccine trials.

Studies in adults have shown that active case-finding (ACF) for TB yields more TB cases than passive case-finding (PCF) [18]. However few studies have investigated ACF strategies in children. ACF strategies that have been tested in adults include door-to-door household visits, enhanced case-finding (ECF), out-patient screening, mass radiographic screening and contact tracing. Most of these strategies rely on the detection TB through the collection of spot sputum specimens for microbiological examination, or on the spot radiographic examination, or symptom assessment. Differences in adult and childhood TB restrict the direct application of these strategies to children. Young children cannot produce spot sputum specimens for microbiological examination, the interpretation of chest radiographs in childhood TB is

difficult, and symptoms of TB in children overlap with those of other childhood conditions [11, 19]. Contact tracing which has been successful in detecting TB in adults and children in low TB burden countries [20, 21], is not suited for the detection of cases in infant TB vaccine trials. This is because it relies on the detection of adult TB cases, and yet, adult cases especially in high TB burden settings do not always present to healthcare centres, and even when they do so, TB may not be correctly diagnosed [22, 23]. The child cases would therefore remain undetected.

There has therefore limited evidence to guide the selection of ACF strategies for clinical trials of new TB vaccines conducted in young children. This thesis has explored ACF in young children in a high TB burden setting with the aim of informing case-finding for clinical trials of TB vaccines conducted in this population in similar settings.

TB case yield from two ACF strategies in young children: Recommendations for ACF in infant TB vaccine trials

We compared two ACF strategies and have shown that a more intensive strategy that incorporates regular home visits maximises case detection, yielding more cases than a record surveillance system that only includes a study close-out visit [9]. Given the challenges of diagnosing pulmonary TB in young children it is possible that some of the TB cases detected by the more intensive strategy may have only exhibited transient features associated with recent infection with *M.tb*, and therefore may not have been "true" cases. However, such *cases* are significant since "transient primary TB reflects the desirable immune containment of *M.tb* which might actually be enhanced by an effective vaccine" [10, 24]. Therefore intensive case-finding incorporating regular contact for symptom based screening maximizes case detection and is recommended for clinical trials of new TB vaccines conducted in young children in high TB prevalence settings.

In both strategies the number of TB cases detected was low (89 and 36 cases of definite and probable TB in the more and less intensive groups respectively (Chapter 3)), and only a few cases were bacteriologically confirmed. This demonstrates that in the absence of biomarkers of protective immunity against TB, clinical trials of new TB vaccines in young children cannot solely rely on bacteriologically confirmed cases as endpoints, but will also have to

include clinically based case definitions [4-6]. Significant progress has been made in developing standardized and objective clinically based case definitions for pulmonary TB in young children, for research settings including TB vaccine trials [7, 8].

TB incidence by age in children less than 5 years old: Duration of subject follow-up in infant TB vaccine trials

Our analysis of age related incidence of TB in children under the age of 5 years, showed the incidence of TB peaking in the 12-23 month age category with high rates persisting beyond 24 months [25]. Given the relatively low case yield over a two year period as was shown in our ACF study, these findings suggest that extending participant follow-up time beyond 24 months could improve case accrual.

Comparison of the Tuberculin skin test and the QuantiFERON-TB-Gold–In-Tube assay: Detection of *M.tb* infection in young children with pulmonary TB

Our comparison of the tuberculin skin test (TST) and QuantiFERON-TB-Gold–In-Tube assay (QFT) among TB suspects showed equivalent performance of the two tests in detecting *M.tb* infection among young children with suspected TB disease in our setting [26]. Although interferon release assays have higher specificity for *M.tb* infection, given the logistical requirements for these assays (consumables, phlebotomy, adherence to strict times frames for laboratory processing), and the overall costs of conducting TB vaccine trials, our findings suggest that the TST remains a feasible and useful test to aid the diagnosis of TB disease (by detecting *M.tb* infection) when administered, read and interpreted appropriately. Our data supports the inclusion of the TST in the proposed case definitions for intrathoracic TB [7, 8]. A recent systematic review has also shown no difference in sensitivity of the TST and IGRAs for *M.tb*, while specificity was higher for the IGRAs [27]. Molecular tests with greater sensitivity and specificity for TB disease are ideal for TB vaccine settings as they increase diagnostic certainty [28-30]. (This work was done before these tests were available hence they were not evaluated in this setting).

Chest radiographic findings in ACF in young children:-Radiologic evidence of TB disease in infant TB vaccine trials

Chest radiographs (CXRs) remain an integral part of diagnosing TB in children [7, 8, 31, 32]. ACF in young children in a high TB burden setting detected uncomplicated primary complex TB with a small proportion of uncontained parenchymal disease. This mild radiographic disease phenotype may reflect radiological changes associated only with *M.tb* infection, and not TB disease. Growth failure appears to be the clinical hallmark of uncontained pulmonary TB in young children in this setting. Therefore, infant TB vaccine trials, adopting ACF should prioritize growth failure as a sentinel clinical feature for early detection of childhood TB in high burden settings.

Given the high inter observer variation in the interpretation of CXR findings [19], and the pivotal role of chest radiographs in the diagnosis of childhood TB, there is a need to standardize the reading and recording of CXR findings in TB vaccine trials and other research settings. In this regard, in 2010, a workshop to review and discuss the reading, interpretation and recording of paediatric CXR findings in infant TB vaccine trials was held at the University of Cape Town. The workshop brought together expert TB paediatricians, radiologists and clinicians from four TB vaccine trial sites in Africa (South Africa, Kenya, Uganda and Mozambique). A chest radiograph reading and recording form (Appendix 2) was developed as an output of this workshop. This form was used in the work presented in this thesis, and has now been proposed as one approach to ensure accurate and standardized reading and recording of CXR findings in young children in research settings [7]. There is however need for continual review of the harmonisation of CXR reading, interpretation and recording in TB vaccine trial sites.

Hospital admissions and mortality among TB vaccine trial participants: Detecting TB morbidity and mortality in infant TB vaccine trials

Our analysis of morbidity and mortality data from two TB vaccine trials showed a profile dominated by respiratory tract infections (RTIs) and diarrhoeal disease. Hospital admissions and deaths due to TB were significant contributors to the morbidity and mortality burden in these trials, even though their overall proportions were low. Up to 10% of participants were hospitalised for unknown or unspecified conditions, and up to 51% of deaths were due to unknown and unspecified conditions. Both these findings have a major impact on the assessment of vaccine safety and efficacy, since it is not possible to ascertain if any of these

cases had TB disease. Autopsies could add value in detecting cases of TB in vaccine trials where the proportion of unknown causes of mortality and that attributed to RTIs is high, as was found in our studies. Targeted post mortem biopsies have previously been conducted to investigate the presence TB in human immunodeficiency virus (HIV) infected individuals [33, 34]. However in many TB endemic countries the subject of autopsies is likely to be restricted by cultural, ethical and logistical reasons, as was found during community dialogues at the SATVI trial site. Our findings also demonstrate that the absence of better diagnostic tools for childhood TB limits the detection of cases among TB vaccine trial participants.

Relevance for TB control programmes

These findings from this thesis are also relevant for TB control programmes. We have shown that ACF beyond contact tracing can be beneficial in children. As in adults, disease is also detected at an earlier stage and is therefore mild. This minimizes the occurrence of severe and disseminated forms of TB which were very limited in our studies. In our ACF study, 18% of cases were detected through hospitalisation and health facility radiology department lists[9]. This demonstrates that a significant proportion of child TB suspects present to healthcare centres. A previous study showed a high prevalence of TB in hospitalised children [35]. Data have however indicated limited knowledge and skills in the diagnosis and management of childhood TB among healthcare workers [36, 37]. Therefore children with TB who present to health facilities may not be correctly diagnosed. Thus there is need to build, improve, and strengthen the capacity of clinic and hospital staff to diagnose TB in children since cases can easily be missed due to an clear pathopneumonic presentation in this age group, and overlap of symptoms and signs with those of other conditions.

Further research

In the absence of biomarkers of protective immunity against TB and in the absence of improved TB diagnostics, future studies should determine the costs of the different ACF strategies in children in TB vaccine trial settings and in programmatic settings. In addition efforts should also be made to monitor the harmonization of TB case definitions for research settings including the reading and interpretation of CXR findings in TB vaccine trial sites. Assessing vaccine efficacy using clinically based case definitions which have an inherent risk

of subjectivity and are therefore prone to a margin of error is a significant challenge in the conduct of TB vaccine trials in children and in the determination of the efficacy of these vaccines. Further work in the area of TB vaccine trials in infants should also continue to focus on the identification of biomarkers of protective immunity against TB.

Contributors

This chapter was written by Dr Sizulu Moyo under the guidance of Dr S. Verver, Associate Professor M. Hatherill and Professor G. Hussey.

References

1. World Health Organization. Global Tuberculosis Report 2012 http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf
2. STOPTB Partnership, The Global Plan to Stop TB 2011-2015 http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf (Accessed on 04 January 2012)
3. M.J. Brennan, J. Thole. Tuberculosis Vaccines: A Strategic Blueprint for the Next Decade. *Tuberculosis* 2012; 92Suppl 1:S6–S13.
4. Ottenhoff TH, Ellner JJ, Kaufmann SH. Ten challenges for TB biomarkers. *Tuberculosis* 2012; 92 Suppl 1:S17-20.
5. Wallis RS, Pai M, Menzies D. *et al*. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet.* 2010; 375:1920-37.
6. Kagina BM, Abel B, Scriba TJ, *et al*. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis, following BCG vaccination of newborns. *Am J Respir Crit Care Med*. 2010; 182(8):1073-9.
7. 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:S199-208.
8. Hatherill M, Verver S, Mahomed H; Taskforce on Clinical Research Issues, Stop TB Partnership Working Group on TB Vaccines. Consensus statement on diagnostic endpoints for infant tuberculosis vaccine trials. *Clin Infect Dis* 2012; 54:493-501.
9. Moyo S, Verver S, Hawkridge A, *et al.* Tuberculosis case finding for vaccine trials in young children in high incidence settings: a randomised trial. Int J Tuberc Lung Dis 2012; 16: 185–191.
10. Schwartzman K. Needles, haystacks and grails: the challenges and promise of tuberculosis vaccine trials. *J Tuberc Lung Dis* 2012; 16: 143.
11. Marais B J, Obihara C, Gie R P, *et al*. The prevalence of symptoms associated with pulmonary tuberculosis in randomly selected children from a high burden community. *Arch Dis Child* 2005; 11: 1166–1170.
12. Mulenga H. Moyo S, Workman L, *et al.* Phenotypic variability in childhood TB: Implications for diagnostic endpoints in tuberculosis vaccine trials. *Vaccine* 2011; 29:4316-4321.

13. 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* 2005; 365:130–4.
14. 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 2009; 94:195–201.
15. Tuyisenge L, Ndimubanzi CP, Ndayisaba G, et al. Evaluation of latent class analysis and decision thresholds to guide the diagnosis of pediatric tuberculosis in a Rwandan reference hospital. Pediatr Infect Dis J 2010; 29:e11–8.
16. Orenstein W A, Bernier R H, Hinman A R. Assessing vaccine efficacy in the field: further observations. *Epidemiol Rev* 1988; 10: 212–241.
17. Hackshaw A. A concise guide to clinical trials. Chapter 2: types of outcome measures and understanding them. 1st ed. Chichester, UK: Wiley-Blackwell Publishing, 2009
18. Golub J E, Mohan C I, Comstock G W, Chaisson R E. Active case finding of tuberculosis: historical perspective and future prospects. *Int J Tuberc Lung Dis* 2005; 9: 1183–1203.
19. Du Toit G, Swingler G, IIoni K. Observer variation in detecting lymphadenopathy on chest radiography. *Int J Tuberc Lung Dis* 2002; 9: 814-817.
20. Erkens CG, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, *et al* Tuberculosis contact investigation in low prevalence countries: a European consensus. *Eur Resp J* 2010; 36: 925-949.
21. Centers for Disease Control. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recommendations and reports: *MMWR* 2005; 54: 1-47.
22. World Health Organisation. Assessment of the fraction of cases being missed by routine TB notification data based on the "Onion" model. http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/ie_apr09_fracion_missed_en.pdf (Accessed on 10 August 2012).
23. Lonnroth K, Uplekar M, Ottmani S, Blanc L. Stop TB Partnership. An action framework for higher and earlier TB case detection. Background document for DOTS Expansion Working Group Meeting. http://www.stoptb.org/assets/documents/global/awards/tbreach/Achieving%20higher%20case%20detection.pdf (Accessed on 10 August 2012).

24. Marais B J, Gie R P, Schaaf H S, *et al*. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004; 8: 392–402.
25. 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.
26. Moyo S, Isaacs F, Gelderbloem S, *et al* Tuberculin skin test and QuantiFERON assay in young children investigated for tuberculosis in South Africa. *Int J Tuberc Lung Dis* 2011; 15: 1176–1181.
27. Chiappini E, Accetta G, Bonsignori F, et *al* Interferon-γ release assays for the diagnosis of Mycobacterium tuberculosis infection in children: a systematic review and meta-analysis. *Int J Immunopathol Pharmacol*. 2012; 25:557-64. Review.
28. Nicol MP, Workman L, Isaacs W, *et al*. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. *Lancet Infect Dis* 2011; 11:819-24.
29. Sekadde MP, Wobudeya E, Joloba ML *et al*. Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. *BMC Infectious Diseases* 2013, 13:133.
30. Rachow APC, Saathoff E, Mtafya B, *et al*. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. *Clin Infect Dis* 2012, 10; 1388–1396.
31. Guidance for national tuberculosis programmes on the management of tuberculosis in children. World Health Organization. WHO/HTM/TB/2006.371. Available at http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf Accessed 13 June 2011.
32. Andronikou S, Wieselthaler N. Imaging for tuberculosis in children. In: Schaaf SH, Zumla AI, Tuberculosis–a comprehensive clinical reference. United Kingdom: Saunders Elsevier, **2009**: 262-296.
33. Rennert WP, Kilner D, Hale M, Stevens G, Stevens W, Crewe-Brown H. Tuberculosis in children dying with HIV-related lung disease: clinical-pathological correlations *Int J Tuberc Lung Dis* 2002; 6: 806–813
34. Jeena P A, Coovadia H M, Chrystal V. *Pneumocystis carinii* and cytomegalovirus infections in severely ill, HIV-infected African infants. *Ann Trop Pediatr* 1996; 16: 361–368.

35. Chintu C, Mudenda V. Lucas S *et al.* Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; 360:985–90.
36. Vellema SC, Durrheim DN, Smith JE. Diagnosing childhood tuberculosis in rural clinics in Mpumalanga Province, South Africa. *Curationis*. 2008; 31: 52-8.
37. Bjerrum S, Rose MV, Bygbjerg IC, Mfinanga SG, Tersboel BP, Ravn P. Primary health care staff's perceptions of childhood tuberculosis: a qualitative study from Tanzania *BMC Health Services Research* 2012; 12:6.

Appendices

Appendix 1: Hospital admission morbidity profile (Cohort 1 and Cohort 2)

Supplementary Table: Hospital admission morbidity profile: Cohort 1 and Cohort 2

	Cohort 1	N= 2895	Cohort 2	N=718	p value
Diagnosis	n	%	n	%	
Respiratory tract infections (infections of the upper and lower respiratory tract), excludes pulmonary tuberculosis	954	33.0	223	31.1	0.33
Diarrhoeal disease (includes the term gastroenteritis, and diarrhoea and vomiting)	926	32.0	175	24.4	0.0001*
TB (pulmonary tuberculosis)	128	4.4	45	6.3	0.03*
Malnutrition (includes marasmus and kwashiorkor)	88	3.0	11	1.5	0.03*
Jaundice	60	2.1	16	2.2	0.87
Meningitis (viral, bacterial, unspecified, includes tuberculous meningitis	53	1.8	9	1.3	0.35
Perinatal conditions and prematurity	49	1.7	7	1.0	0.18
Convulsions (includes, epilepsy)	49	1.7	6	0.8	0.08*
Injuries (includes fractures, head injuries and injuries due to motor vehicle accidents	47	1.6	18	2.5	0.10
Febrile convulsions and pyrexia if unknown origin	40	1.4	12.0	1.7	05
Renal conditions (includes infections)	35	1.2	6	0.8	0.36
Wheezing (unspecified)	35	1.2	0	0.0	
Burns (hot water and fire burns)	33	1.1	6	0.8	0.48
HIV related illnesses (included cases with confirmed HIV infection)	32	1.1	6	0.8	048
Congenital conditions	31	1.1	0	0.00	
Otitis media	32	1.1	7	1.0	0.82
Sepsis (generalised bacterial infection including meningococcemia)	31	1.1	3	0.4	0.09
Poisoning (ingestion of hazardous material such including paraffin)	30	1.0	10	1.4	0.35
Abscess(includes cellulitis and empyema)	25	0.9	8	1.1	0.62
Dermatological conditions	20	0.7	6	0.8	0.78
Minor surgical procedures (includes circumcision)	15	0.5	10	1.4	0.01*
Asthma	10	0.3	16	2.2	<0.001*
Eye conditions (includes infections)	9	0.3	3	0.4	067
Other (condition or symptoms and signs not included in the conditions above)	51	1.8	36	5.0	<0.001*
Unknown/ not specified	112	3.9	79	11.0	<0.001*

Appendix 2: Chest radiograph reading and recording form

INFORMATION:

- Reader code: .....
- Study code: .....
- Patient code: .....
- Date of read: .....

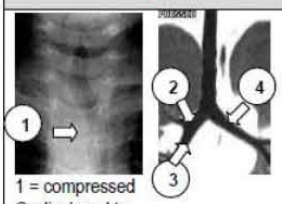
Instructions to tick-sheet:

A] Mark **only one** of the tick boxes for **each image**: Yes, No, Maybe, or Not visible
(Record **only the most positive grading** under each section. That means if there is one 'definite' node and 3 'possible' nodes, you must tick 'yes' and not 'maybe')
B] Please **also** cross any number of locations of disease **on** the appropriate **circled number**

Grading:
Yes = positive
Maybe
No = negative

Lymphadenopathy

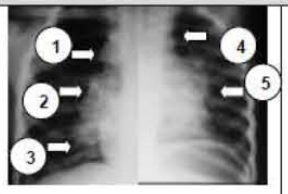
Airway compressed and tracheal displacement



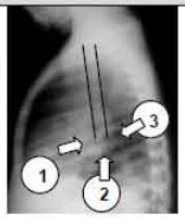
1 = compressed Or displaced to **left only**
2-4=compression

Yes No Maybe Not visible

Soft tissue density = nodal mass



Yes No Maybe Not visible



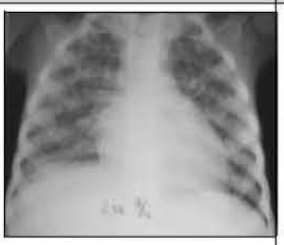
Lines indicate the trachea

Yes No Maybe Not visible

Post process: Overall

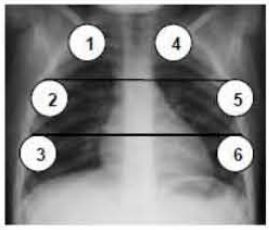
Lymphadenopathy
Yes
No
Maybe
Not visible

Nodular = Miliary or larger widespread and bilateral

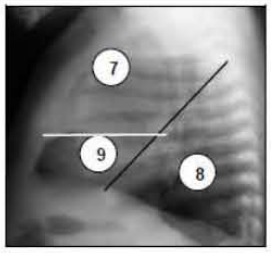


Yes No Maybe Not visible

Airspace consolidation



Yes No Maybe Not visible

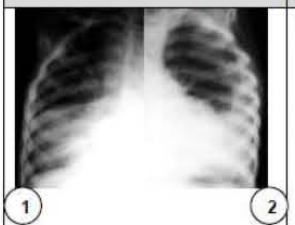


Yes No Maybe Not visible

Post process: Overall

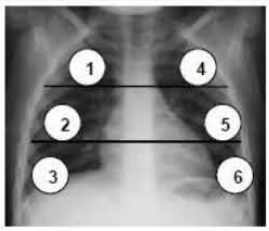
Lung disease
Yes
No
Maybe
Not visible

Pleural effusion/thickening

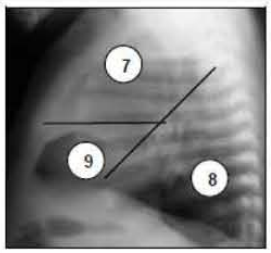


Yes No Maybe Not visible

Cavities



Yes No Maybe Not visible



Yes No Maybe Not visible

Post process: Overall

Pleura
Yes
No
Maybe
Not visible

TB Decision	☐ Lymphadenopathy or Miliary = **Yes**	☐ No lymphadenopathy/mill but positive = **Maybe**	☐ Normal = **NO**	☐ Bad quality = **Unreadable**