*The **K**ilifi **I**mproving **D**iagnosis &*

***S**urveillance of Childhood **TB** Study*

The KIDS TB Study

Andrew J Brent

Imperial College London

Department of Paediatrics

PhD Thesis

Declaration of Originality & Acknowledgements

The KIDS TB study presented in this thesis represents the fruits of my own efforts to improve understanding of the epidemiology and diagnosis of childhood TB in a low resource, high burden setting. The idea for the study was conceived while conducting a separate study of paediatric bacteraemia at the KEMRI-Wellcome Trust Research Programme in 2003. Struck by the difficulties paediatricians face in trying to diagnose TB I asked myself two questions: how can one better diagnose TB in children, and what is the true burden of childhood TB in this and similar settings? These questions then became the basis for the KIDS TB study and for my PhD. The study design, results, analyses, and interpretations presented in this thesis are all my own work.

While the work presented is my own, I nevertheless owe a huge debt of gratitude to several people without whose support and belief in me I would never have been able to accomplish the work. Firstly, I would like to thank my supervisors, Michael Levin, Anthony Scott, and Giovanni Montana, and my sponsor Kevin Marsh, for their strong moral and practical support. My thanks, too, to Hemed Twahir, Victor Bandika, Jay Berkley, Matron Otieno, and the Chief Administrator at Coast Provincial General Hospital (CPGH), without whose support we would not have been able to work at CPGH. I am particularly grateful for the very constructive assistance that both Hajara Elbusaidy and Meshack Mwangala afforded the study throughout as Provincial and District TB and Leprosy Coordinators, respectively.

Space precludes mentioning everyone that supported the study, but my special thanks also go to all those involved in screening and caring for patients in the study, especially Joyce Langat, Carole Mulunda, and Joshua Wambua; to Robert Musyimi, Daisy Mugo, and Agnes Mutiso whose hard work has been instrumental in creating a first class TB diagnostic laboratory; to Moses Mosobo, Salim Mwarumba and Susan Morpeth for their support of the laboratory work; to the KEMRI Outpatient Department team; to Benson Katana and Stephen Mangi for field follow ups; to Christopher Nyundo for coding the KHDSS residence status of all patients in the District TB Register; to John Ojal for answering my statistical questions; and to Jennifer Moisi for sharing with me her raster of travel time from KDH for my surveillance sensitivity analysis.

The majority of the work was supported by a training fellowship from the Wellcome Trust (081697). Comparison of the MODS, MGIT and Xpert MTB/RIF assays was supported by a grant from the PERCH Consortium of the Bill and Melinda Gates Foundation. I also received support via a Wellcome Trust Value in People (VIP) Award from Imperial College.

Above all I would like to thank my family: my parents for all the sacrifices they have made to provide their children with opportunities; and my wife, Bernadette, and my children, Joshua and Sophia, for their love, patience and support. The work is dedicated to them, and to all the patients and their families who made the study possible and give it meaning.

Copyright Declaration

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

Abstract

Improving diagnosis and surveillance of childhood TB are key research priorities. We established intensified case finding and state of the art TB diagnostics to investigate the performance of clinical and laboratory tools for childhood TB diagnosis at 2 hospitals in Kenya. We estimated the community incidence of childhood TB using a continuous demographic surveillance survey and detailed surveillance sensitivity analysis.

2041 children were investigated for suspected TB. 70 (3.4%) had bacteriologically confirmed TB, 63 (3.1%) had clinically highly probable TB, and a further 144 (7.1%) were treated for TB based on their clinical presenting features. 107/133 (80%) confirmed/highly probable TB (CHPTB) cases had pulmonary TB. CHPTB was associated with HIV infection (OR 2.1, 95% CI 1.3-3.2), malnutrition (1.5, 1.0-2.1) and close TB contact (5.7, 3.8-8.5). The population attributable fraction of a known close TB contact was 38.8-52.5%. The estimated community incidence of CHPTB locally and nationally was 46 and 83 per 100,000 per year, respectively.

The performance of published clinical diagnostic tools varied widely, but the accuracy of all was limited. We derived and independently validated a simple KIDS TB Score that ruled out TB in 2/3 suspects with 98.8% negative predictive value, stratifying other children into groups of increasing risk. Bacteriological yield was highest for the Mycobacterial Growth Inhibitor Tube (MGIT) method (sensitivity 34%, 29-39%, among CHPTB patient samples), and lower for the Microscopic Observation Drug Susceptibility (MODS) assay (30%, 24-35%) and Xpert MTB/RIF assay (29%, 24-34%).

The study provides the first comprehensive description from the region of the clinical spectrum of childhood TB, and the only prospective incidence estimates. It suggests up

to half of all cases are potentially preventable by implementing current recommendations for isoniazid chemoprophylaxis. The diagnostic performance of clinical and laboratory methods should inform development of future clinical guidelines and laboratory capacity.

Table of Contents

Declaration of Originality & Acknowledgements 2

Copyright Declaration 3

Abstract 4

1 INTRODUCTION 14
1.1 Challenges to global tuberculosis control 14
1.2 Children and the tuberculosis epidemic 14
1.3 Diagnosis of childhood TB 15
1.4 Available surveillance data and burden of disease estimates 17
1.5 Challenges, opportunities and strategies for the future 20
1.6 Aims of KIDS TB Study 22
1.7 Overview of the work presented in this thesis 23
1.8 A note about terminology 23

2 PATIENTS & METHODS 24
2.1 KIDS TB Study Sites 24
2.1.1 Kenya in context: the national tuberculosis profile 24
2.1.2 Site 1: Kilifi District Hospital (KDH) and the KHDSS 25
2.1.3 Coast Provincial General Hospital (CPGH), Mombasa 27
2.2 Establishing the platform for childhood TB research 27
2.3 Inclusion criteria 28
2.4 Sample size 28
2.5 Sampling Procedure 29
2.5.1 Sampling procedure at KDH 30
2.5.2 Sampling procedure at CPGH 31
2.6 Clinical Methods 33
2.6.1 Investigation of children with suspected TB 33
2.7 Laboratory methods 34
2.7.1 Specimen processing, AFB microscopy, and mycobacterial culture 35
2.7.2 Real-time mycobacterial PCR: Xpert MTB/RIF Assay 35
2.7.3 Interferon Gamma Release Assays (IGRA) 35
2.8 Case definitions, clinical management and follow up 36
2.9 Summary of recruitment strategy & analysis overview 38
2.10 Ethical approval 38

3 HOSPITAL BURDEN & SPECTRUM OF CHILDHOOD TUBERCULOSIS 40
3.1 Introduction 40
3.2 Methods 41
3.2.1 Recruitment and description of patient cohorts 41
3.2.2 Clinical spectrum of childhood tuberculosis 41
3.2.3 Inpatient burden of childhood TB 41
3.3 Results 43
3.3.1 Patient enrolment and follow up at each site 43
3.3.2 Cohort description and disease assignments 45
3.3.3 Clinical spectrum of childhood tuberculosis 45
3.3.4 Multi-drug resistant tuberculosis (MDR-TB) 51
3.3.5 Inpatient burden of childhood TB 54
3.4 Discussion 54
3.4.1 Clinical spectrum of childhood TB in the KIDS TB Study 54
3.4.2 Multi-drug resistant tuberculosis (MDR-TB) 55
3.4.3 Case definitions of childhood tuberculosis 56

3.4.4 Healthcare burden of childhood TB and the need for better diagnostic tools *57*

4 RISK FACTORS FOR CHILDHOOD TUBERCULOSIS **58**
4.1 Introduction 58
4.2 Methods 58
4.2.1 Selection of cases and controls for nested case control analyses *58*
4.2.2 Choice and definition of exposure variables *59*
4.2.3 Univariable analysis of risk factors for childhood TB *59*
4.2.4 Multivariable analysis of risk factors for childhood TB *60*
4.2.5 Attributable fractions *60*
4.3 Results 62
4.3.1 Comparison of confirmed and highly probable TB cases *62*
4.3.2 Referral source and socio-demographic and social variables *62*
4.3.3 Host factors: HIV infection, nutritional status and BCG vaccination status *62*
4.3.4 TB contact history *66*
4.3.5 Multivariable analysis of risk factors for TB *68*
4.3.6 Attributable Fractions *68*
4.4 Discussion 69

5 CLINICAL DIAGNOSIS OF CHILDHOOD TUBERCULOSIS **74**
5.1 Introduction 74
5.2 Methods 75
5.2.1 Choice of cases and controls *75*
5.2.2 Clinical definitions *76*
5.2.3 Univariable analysis of individual clinical predictors of childhood TB *77*
5.2.4 Assessment of the performance of published clinical diagnostic tools *77*
5.2.5 Derivation and validation of new clinical diagnostic tool for this setting *78*
5.3 Results 80
5.3.1 Univariable analysis of individual clinical predictors of childhood TB *80*
5.3.2 Assessment of the performance of published clinical diagnostic tools *91*
5.3.3 Derivation and validation of a new clinical diagnostic tool for this setting *97*
5.4 Discussion 105
5.4.1 Individual clinical and radiological features *105*
5.4.2 Clinical diagnostic tools *106*
5.4.3 The KIDS TB Score *108*
5.4.4 Conclusion *110*

6 MICROBIOLOGICAL DIAGNOSIS OF CHILDHOOD TUBERCULOSIS **111**
6.1 Introduction 111
6.2 Methods 113
6.2.1 Processing of specimens for TB microscopy and culture *113*
6.2.2 Microscopy for Acid Fast Bacilli (AFB) *114*
6.2.3 Automated liquid mycobacterial culture: BD MGIT 960 system *114*
6.2.4 Microscopic Observation Drug Susceptibility (MODS) assay *115*
6.2.5 Mycobacterial speciation & identification of M. tuberculosis complex (MTBC) *115*
6.2.6 Xpert MTB/RIF Assay *116*
6.2.7 Quality assurance *117*
6.2.8 Statistical Methods *117*
6.3 Results 118
6.3.1 Microscopy and culture yield *118*
6.3.2 Diagnostic performance of MGIT and MODS culture methods *118*
6.3.3 Diagnostic performance of the Xpert MTB/RIF assay *122*
6.3.4 Culture contamination *124*
6.3.5 Test sensitivity among samples from confirmed and highly probable TB cases *125*
6.4 Discussion 127

6.4.1 Current challenges, opportunities and evidence gaps ... *127*
6.4.2 Strengths of the KIDS TB Study for evaluation of laboratory diagnostic methods ... *127*
6.4.3 Smear microscopy and culture yield ... *128*
6.4.4 Comparison of MGIT and MODS culture for detection of MTBC ... *129*
6.4.5 Performance of the Xpert MTB/RIF assay ... *130*
6.4.6 Study limitations ... *131*
6.4.7 Conclusion ... *132*

7 COMMUNITY INCIDENCE OF CHILDHOOD TUBERCULOSIS ... **134**
7.1 Introduction ... 136
7.2 Methods ... 138
7.2.1 Crude estimation of the community incidence of childhood TB ... *138*
7.2.2 Application of clinical diagnostic tools to estimate incidence of childhood TB ... *139*
7.2.3 Estimating the case detection rate ... *139*
7.2.4 Using NTP notification data to estimate the case detection rate in the passive arm ... *142*
7.2.5 Using NTP notification data to estimate the case detection rate in the active arm ... *143*
7.2.6 Using hospital-based mortality surveillance to estimate the case detection rate ... *143*
7.2.7 Using verbal autopsy (VA) to estimate the case detection rate ... *144*
7.2.8 Final adjusted estimates of the incidence of childhood TB in Kilifi ... *145*
7.2.9 Estimating the national burden of childhood TB in Kenya ... *145*
7.3 Results ... 147
7.3.1 Crude incidence of childhood TB ... *147*
7.3.2 Application of clinical diagnostic tools to estimate incidence of childhood TB ... *150*
7.3.3 Using NTP notification data to estimate the case detection rate in the passive arm ... *150*
7.3.4 Using NTP notification data to estimate the case detection rate in the active arm ... *150*
7.3.5 Using hospital-based mortality surveillance to estimate the case detection rate ... *151*
7.3.6 Using verbal autopsy (VA) to estimate the case detection rate ... *151*
7.3.7 Adjusted incidence estimates for childhood TB in Kilifi ... *152*
7.3.8 Kenyan national burden of childhood TB ... *154*
7.4 Discussion ... 155
7.4.1 Crude incidence estimates & definitions of childhood TB ... *155*
7.4.2 Case detection rate ... *156*
7.4.3 Adjusted incidence estimates for childhood TB in Kilifi & nationally ... *159*
7.4.4 Public health implications & lessons for childhood TB surveillance in Kenya ... *160*

8 CONCLUDING REMARKS ... **162**
8.1.1 Clinical spectrum and risk factors of childhood TB ... *162*
8.1.2 Clinical diagnosis of childhood TB ... *162*
8.1.3 Microbiological diagnosis of childhood TB ... *163*
8.1.4 Community incidence of childhood TB ... *164*
8.1.5 Conclusion and directions for future research ... *164*

9 REFERENCES ... **165**

10 APPENDICES ... **178**
10.1 Statistical derivations ... 178
10.1.1 Derivation of the confidence interval for the sum of two rates ... *178*
10.1.2 Estimating 95% confidence intervals for the sensitivity of active case ascertainment 179
10.1.3 Estimating the national burden of childhood TB in Kenya ... *180*
10.2 Supplementary tables ... 181
10.2.1 Chapter 4 supplementary tables ... *181*
10.2.2 Chapter 5 supplementary tables and figures ... *184*

Index of Tables

Table 1.1 Estimated numbers of new cases, case rates in children, overall case rates (all ages), and the percentage of all TB estimated to occur among children in the 22 high-burden countries [table taken from reference [10]] **18**

Table 2.1 Key elements in establishing TB diagnostic laboratory to underpin the KIDS TB Study .. **27**

Table 2.2 Incidence estimates and 95% confidence intervals based on a range of scenarios for the number of cases of TB diagnosed over the 2 year study period. **29**

Table 2.4 Symptoms and signs of possible TB that triggered further investigation of child household TB contacts in the active case detection arm of the study. **34**

Table 2.5 Childhood TB case definitions **37**

Table 3.1 Definitions of the site of extra-pulmonary TB among children treated for possible TB who did not meet the stringent criteria for confirmed or highly probable TB (see **Table 2.5**) **42**

Table 3.2 Referral source and demographic characteristics of children investigated for TB **45**

Table 3.3 Clinical spectrum of TB among cases in each category of diagnostic certainty. [2] **47**

Table 3.4 Baseline characteristics of children by TB diagnostic category **48**

Table 3.5. Clinical spectrum of TB cases by age category and HIV infection status [1] **53**

Table 4.1 Association of socio-demographic variables with confirmed and highly probable TB **64**

Table 4.2 Associations of host factors with confirmed and highly probable TB in the passive and active case detection arms **65**

Table 4.3 Association of TB contact history with CHPTB (passive case detection arm) **67**

Table 4.4 Association of TB contact history with CHPTB (active case detection arm) **67**

Table 4.5 Adjusted odds ratios for risk factors included in the final models **68**

Table 4.6 Adjusted population attributable fraction for risk factors included in the final models . **69**

Table 5.1 Associations of clinical history variables with CHPTB **83**

Table 5.2 Associations of clinical examination findings with CHPTB **86**

Table 5.3 Associations of chest x-ray features with CHPTB **88**

Table 5.4 TST responses among cases and controls, overall and among subgroups defined by age and HIV/malnutrition status **90**

Table 5.5 Existing clinical diagnostic algorithms whose performance was assessed among children recruited to the KIDS TB study **94**

Table 5.6 Regression coefficients and adjusted odds ratios for variables in the final model **97**

Table 5.7 Area under ROC curves for the simplified TB score as a predictor of confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets **97**

Table 5.8 The KIDS TB SCORE **99**

Table 5.9 Area under ROC curves for the KIDS TB SCORE as a predictor of TB among subgroups defined by age category and HIV status in the testing (CPGH) dataset **100**

Table 5.10 Distribution of final TB diagnostic categories across risk strata defined by the KIDS TB SCORE among all children enrolled in the passive case detection arm of the study **100**

Table 6.1 Reporting of AFB microscopy results **114**
Table 6.2 AFB microscopy and culture yield by specimen type **119**
Table 6.3 Identity of non-MTBC isolates from MGIT and MODS culture **120**
Table 6.4 Summary of MGIT & MODS MTBC culture positive results **120**
Table 6.5 Number of MTBC isolates from MGIT and MODS by quarter (2010 to 2011) **121**
Table 6.6 Breakdown of Xpert MTB/RIF results by mycobacterial culture result **122**
Table 6.7 Sensitivity of the Xpert MTB/RIF assay against the composite gold standard **123**
Table 6.8 Test sensitivity among specimens from confirmed and highly probable TB cases **126**
Table 7.1 Comparison of methods for estimating the sensitivity of hospital-based surveillance for childhood TB **141**
Table 7.2 TB diagnostic category of children enrolled during two years' comprehensive inpatient and outpatient, active and passive screening for childhood TB at KDH **147**
Table 7.3 Crude incidence of TB by diagnostic certainty and age category in the active and passive case detection arms and overall **148**
Table 7.4 Incidence of childhood TB derived by applying published clinical definitions to children enrolled in the passive case detection arm. Results presented in order of ascending incidence. Incidence based on KIDS TB Study definitions and KIDS TB Score also presented for comparison. **149**
Table 7.5 Estimated case detection rates by age category (mortality surveillance) **151**
Table 7.6 Estimated CDR for fatal illness associated with clinical features of suspected TB (by age category) **151**
Table 7.7 Adjusted incidence and projected annual caseload of childhood TB in the KHDSS **153**
Table 7.8 Estimated national case burden and incidence of childhood TB by age group **154**
Table 10.1 Effect of TB contact history on confirmed versus highly probable TB in the passive case detection arm of the study **181**
Table 10.2 Effect of TB contact history on confirmed TB (passive case detection arm) **182**
Table 10.3 Effect of TB contact history on highly probable TB (passive case detection arm) **182**
Table 10.4 Effect of TB contact history on confirmed TB (active case detection arm) **183**
Table 10.5 Effect of TB contact history on highly probable TB (active case detection arm) **183**
Table 10.6 Associations of clinical history variables with confirmed TB **184**
Table 10.7 Associations of clinical examination findings with confirmed TB **186**
Table 10.8 Associations of chest x-ray features with confirmed TB **188**
Table 10.9 Comparison of proportions of each clinical history variable among confirmed and highly probable TB cases. **190**
Table 10.10 Comparison of proportions of each clinical examination variable **192**
Table 10.11 Comparison of proportions of each radiological variable among confirmed and highly probable TB cases. **194**
Table 10.12 Associations of available microbiology results with CHPTB* **195**
Table 10.13 Associations of available microbiology results with confirmed TB* **196**
Table 10.14 Comparison of proportions of each microbiology variable among confirmed and highly probable TB cases. **197**

Table 10.15 Performance of existing clinical diagnostic tools in identifying children with CHPTB in this study 198
Table 10.16 Performance of existing clinical diagnostic tools in identifying children with confirmed TB in this study 199
Table 10.17 Variables included in generating a logistic regression model for TB and their categories and weightings. 201

Index of Figures

Figure 1.1 Causes of death among children under 5 years of age (data from [6]) **15**
Figure 2.1 TB case notification rates by province, 2007 (1, Nyanza; 2, Nairobi; 3, Coast Province). **25**
Figure 2.2 Map of Kilifi District and the KHDSS area showing administrative locations, Kilifi District Hospital (KDH), and other TB treatment facilities. **26**
Figure 2.3 Overview of patient enrolment, investigation and diagnostic classifications in each study arm **32**
Figure 2.4 Schematic overview of recruitment strategy and analyses **39**
Figure 3.1 Timeline of patient enrolment in each study arm **42**
Figure 3.2 Patient enrolment and final TB category assignments in each of the study recruitment arms **44**
Figure 3.3 Age distribution of TB suspects and cases **46**
Figure 3.4 Radiological spectrum of intra-thoracic childhood TB among children enrolled in the KIDS TB Study **49**
Figure 3.5 Age distribution of pulmonary TB (PTB) and extra-pulmonary TB (EPTB) **52**
Figure 3.6 Age distribution of sites of extra-pulmonary tuberculosis among cases of confirmed and highly probable TB (including among PTB cases): number (a) and percent (b) with miliary TB and TBM; number (c) and percent (d) with other forms of extrapulmonary TB. **52**
Figure 5.1 Receiver operator characteristic (ROC) curves for six core symptoms showing the sensitivity and specificity of different cutoffs of symptom duration (in weeks) as predictors of CHPTB. Arrows indicate the chosen cutoffs for inclusion in the mulitivariable model. **85**
Figure 5.2 Receiver operator characteristics curves showing the performance of each clinical tool for diagnosis of (A) CHPTB and (B) CTB **92**
Figure 5.3 Receiver operator characteristic (ROC) curves for numerical diagnostic scores showing the sensitivity and specificity of different score cutoffs for a diagnosis of CHPTB. Arrows indicate the recommended cutoffs used in the published scores. **95**
Figure 5.4 Receiver operator characteristic (ROC) curves for numerical diagnostic scores showing the sensitivity and specificity of different score cutoffs for a diagnosis of Confirmed TB. Arrows indicate the recommended cutoffs used in the published scores. **96**
Figure 5.5 Receiver operator characteristics curves for the simplified TB score as a predictor of Confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets. **101**
Figure 5.6 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets. **102**
Figure 5.7 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB and CHPTB in the testing (CPGH) dataset, by age category. **103**
Figure 5.8 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB & CHPTB in **104**
Figure 6.1 Characteristic cord-like growth of *M. tuberculosis* **112**
Figure 6.2 Median time to detection of MTBC positive MODS cultures, ... **122**

Figure 6.3 Monthly MGIT contamination rate as a percentage of samples processed (2009-2011) **124**
Figure 7.2 Schematic of infrastructure and resources required for accurate estimation of community childhood TB incidence **137**
Figure 7.3 Potential healthcare seeking behaviour of children resident in the KHDSS. A, presents to KDH; B, presents to another healthcare facility within the KHDSS area; C, presents to another healthcare facility outside the KHDSS area but within Kilifi district; D, presents to another healthcare facility outside Kilifi district; E, does not present to a healthcare facility. **140**
Figure 7.4 Case detection rate estimates and 95% confidence intervals derived by each method **152**
Figure 10.1 Receiver operator characteristic (ROC) curves for six core symptoms showing the sensitivity and specificity of different cutoffs of symptom duration (in weeks) as predictors of confirmed TB **200**
Figure 10.2 Log odds of TB plotted across categories of ordinal categorical variables included in generation of the model **203**

1 INTRODUCTION

1.1 Challenges to global tuberculosis control

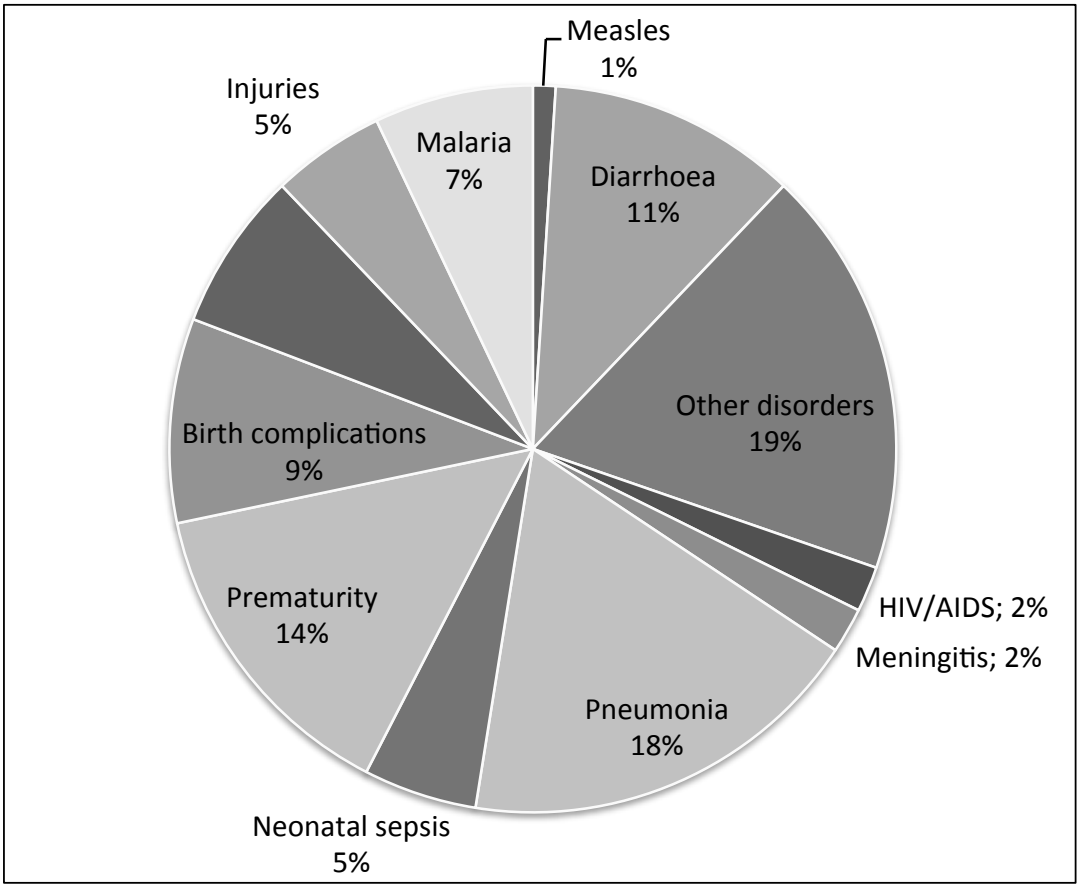
Three main challenges currently hamper efforts to control tuberculosis (TB) globally. Firstly, the ability to identify and diagnose cases remains sub-optimal, and is particularly poor among children and human immunodeficiency virus (HIV) infected individuals, with direct implications for the availability and quality of surveillance data in these groups. Secondly, there is a need for better (particularly shorter) treatment regimens to improve individual case management and to prevent and control the emergence and spread of drug resistant strains. Thirdly, the search is still on for better tuberculosis vaccines to provide more complete and longer lasting protection against active TB. Success in all three areas is likely to be required to achieve the Stop TB goal to eliminate TB as a public health problem by 2050.[1, 2] This thesis addresses the first of these challenges, focusing on improving diagnosis and surveillance of childhood TB in a low resource, high burden setting.

1.2 Children and the tuberculosis epidemic

The World Health Organization estimated there were 8.8 million cases of TB worldwide in 2010, equivalent to an annual global incidence of 137 per 100,000 population.[3] In the same year there were 1.1 million deaths attributed to TB among HIV negative people and a further 0.35 million from HIV-associated TB.[3] TB remains second only to HIV as a leading infectious cause of death globally.[4] Global estimates of the causes of death among children have also been derived,[5-7] and estimates based on data from 2008 are illustrated in Figure 1.1.[7] Given the overwhelming burden of tuberculosis among the population as a whole, and the well documented vulnerability of young children to active TB including the severe and fatal forms of the disease,[8] it is perhaps surprising that TB does not feature among the leading causes of death in children. Protection afforded by Bacille Calmette Guérin (BCG) vaccination among infants and young children, particularly against disseminated TB and TB meningitis, provides one important explanation for this apparent discrepancy.[9] However even in this group the protective efficacy of BCG is sub-optimal, and the best available estimates suggest a high global burden of childhood TB despite wide scale neonatal BCG vaccination within the Expanded Programme of Immunization.[10, 11] The contribution of TB to childhood

mortality may simply be dwarfed by the large mortality burden due to other common childhood illnesses, but it may also be 'hidden' within other categories such as pneumonia, HIV/AIDS, meningitis or 'other disorders'. Properly to reconcile these data requires a clear understanding of their limitations and the underlying challenges of TB diagnosis in children.

Figure 1.1 Causes of death among children under 5 years of age (data from [6])



1.3 Diagnosis of childhood TB

The pathophysiology, clinical presentation and investigation of TB in children differs from that in adults due to a combination of immunological, anatomical and epidemiological factors, all of which make diagnosis considerably more challenging.[12] Young children are at much higher risk of progression to active TB following infection.[8] Larger inocula due to prolonged close contact with adults in the household from whom they frequently acquire their infection might contribute to this higher risk,[13, 14] but age-

related differences in both innate and adaptive immune responses to TB are thought to be the most important determinants of their increased vulnerability.[15] Although poorly understood, a less robust immune response is also likely to underlie the paucity of lung cavitations in young children with TB, in stark contrast to the classic picture of adult pulmonary TB in which large numbers of bacilli may 'spill' from the cavities into the airways. Whatever the exact mechanisms, important consequences of these differences in host immunity include relatively fewer bacilli, particularly in clinical specimens ('paucibacillary' disease), and an increased propensity for disseminated, extra-pulmonary disease in young children.[12]

This in turn has implications for diagnosis since specimen collection is often more difficult from extra-pulmonary sites, especially in low resource settings, and low numbers of bacilli greatly reduce the sensitivity of both smear microscopy and mycobacterial culture. Anatomical differences limit access to appropriate respiratory specimens in childhood pulmonary TB, too, since smaller airways and less tussive force limit a child's ability to expectorate sputum, necessitating more invasive and labour intensive methods of specimen collection such as sputum induction or gastric aspiration.[16] Immune based diagnosis using the tuberculin skin test (TST) or newer interferon gamma release assays (IGRA) is limited by poor sensitivity and specificity.[17] Furthermore, although they are recommended components of the TB diagnostic workup for children where available, in practice TST, gastric lavage and sputum induction are often not available in high burden settings due to limited training, a lack of the basic equipment and consumables required,[18, 19] very low yield in the absence of mycobacterial culture facilities,[16] and cost.[20, 21] IGRA remain far too expensive and complex for use at the point of care in low resource settings and are not recommended for routine use in childhood TB diagnosis or contact tracing.[22]

In the absence of a reliable gold standard, and lacking resources for further investigations in most settings, diagnosis therefore usually has to rely on clinical case definitions. To compound the problem, clinical diagnosis is also more challenging in children, since the clinical and radiological features of childhood TB overlap those of other common childhood diseases, particularly where HIV and malnutrition - two of the most important risk factors - are endemic.[23] Several clinical algorithms have been proposed but although they continue to be widely promoted, most have not been

validated in the settings in which they are used.[24] The net result is frequent disease misclassification that severely limits the quality of both clinical care and surveillance data.

1.4 Available surveillance data and burden of disease estimates

The main source of global surveillance data for childhood TB comes from notification data collected by National Tuberculosis Programmes (NTPs) under the WHO DOTS strategy.[25] However both the quantity and the quality of DOTS notification data relating to childhood TB are limited. Until recently data from children were aggregated in a single category (defined by the WHO for the purposes of TB reporting as <15 years),[26] ignoring important age-related differences in disease incidence between pre-school, school age and adolescent children. This reflected the lower priority traditionally afforded to children by the DOTS strategy, which understandably prioritizes detection and treatment of smear positive pulmonary cases that contribute most to propagation of the epidemic but occur predominantly in adults. One consequence is a lack of data on age-related trends among children that might help refine burden of disease estimates or afford additional insights into the effects of the HIV epidemic or other epidemiological risk factors on disease presentation or transmission patterns. The WHO recommendation in 2006 for NTPs to report aggregated data for children in at least two categories (0-4 years and 5-14 years) does improve notification based surveillance of childhood TB.[21] Nevertheless severe limitations still exist in the quality of available TB notification data for children due to frequent misclassification arising from the very real diagnostic challenges highlighted above, a lack of resources for active case finding through contact tracing in most settings, as well as limitations in the notification process itself. A recent study from South Africa demonstrated significant under-notification of TB cases,[27] and even in Europe 1 in 5 children with TB are not notified,[28] so the situation is likely to be considerably worse in less well resourced settings. Perhaps reflecting all these problems with the available data, WHO estimates of the global TB burden do not currently include a breakdown of the disease burden in children.[3]

To date the best available published estimates of the global burden of childhood TB have been derived by combining DOTS notification data for smear positive TB with age-specific estimates of the proportion of the total number of cases that are smear positive.[10, 29] These estimates suggest there were approximately 900,000 new cases of

TB in children in 2000, which represented about 11% of the total global tuberculosis burden. National incidence estimates for childhood TB in the 22 highest burden TB countries in 2000 are show in Table 1.1.[10] These are crude estimates, however, due to the inherent limitations of the data on which they are based, including assumptions about the proportion of childhood TB cases that one might expect to be smear positive. In the absence of a reliable gold standard for diagnosis the latter is not straightforward to ascertain, since it requires knowledge of the total number of childhood cases in a particular setting; furthermore, this proportion may vary geographically or temporally with the prevalence of HIV infection, malnutrition or other risk factors. Recently a more conservative global estimate of half a million TB cases among children in 2000 has been attributed to the WHO,[11] but no formal WHO estimate has yet been published.

Table 1.1 Estimated numbers of new cases, case rates in children, overall case rates (all ages), and the percentage of all TB estimated to occur among children in the 22 high-burden countries [table taken from reference [10]]

Country	Children aged 0–14 years	% of TB occurring in children	Estimated TB case rates <15 years of age*	Estimated case rate†
Afghanistan	17 540	25.3	189	324
Bangladesh	33 166	10.2	61	236
Brazil	23 520	20.7	47	66
Cambodia	3 966	5.3	70	571
China	86 978	5.3	27	129
Democratic Republic of Congo	24 052	16.1	106	306
Ethiopia	28 675	16.1	95	272
India	185 233	10.2	53	179
Indonesia	15 691	2.7	23	263
Kenya	22 124	16.1	167	450
Mozambique	7 703	16.1	98	268
Myanmar	8 007	10.2	51	165
Nigeria	32 310	12.4	63	228
Pakistan	61 905	25.3	103	172
Philippines	12 167	5.3	43	304
Russian Federation	7 778	4.2	30	126
South Africa	35 449	16.1	237	501
Thailand	2 317	2.7	15	141
Uganda	12 099	16.1	103	320
United Republic of Tanzania	18 890	16.1	118	337
Vietnam	7 559	5.3	29	183
Zimbabwe	12 267	16.1	221	603
Total for the 22 high-burden countries	659 397	9.6		

* Case rates per 100 000 children. United Nations population estimates used for denominator.
† Case rates per 100 000 population.
TB = tuberculosis.

Better quality surveillance data are available from many industrialized, low burden settings,[30, 31] where they are underpinned by stronger health systems with better diagnostic facilities and active case finding through routine contact tracing. Burden of disease estimates from some high burden communities are also available, and suggest that children may account for approximately 15-20% of the total caseload in such settings.[32] In one frequently cited study based on official notification data from two urban settings in Cape Town, South Africa, children accounted for almost 40% of the total caseload over a 10 year period.[33] In the absence of a standardized case definition in this study it is unclear whether this reflects the true potential burden of childhood TB in settings where deteriorating socio-economic conditions drive high TB transmission rates, or a tendency towards over-diagnosis by clinicians aware of the high TB incidence combined with the poor performance of available diagnostic tools and the potentially devastating consequences of not treating a child with TB. A prospective observational study of childhood TB in a very similar urban setting in Cape Town suggested children accounted for about 15% of all TB cases.[34] However the number of published studies is small, and they derive from a minority of countries that may not be representative of the overall picture. Importantly, the proportion of the total TB caseload that occurs among children depends critically on local demography, which also varies widely: a higher proportion of paediatric cases would be expected in countries with a wide based population pyramid than in those where children contribute a smaller proportion of the total population, and vice versa.

The need for better quality surveillance data from different communities and underpinned by improved case definitions was therefore recognized as among the leading research priorities for childhood TB in the WHO's *Research Agenda for Childhood Tuberculosis*.[32] There has been very limited progress in this area, however. A PubMed literature search performed on 14 October 2011 using the MeSH and text search terms "tuberculosis" or "TB", plus "child" or "children" and "incidence" or "prevalence" identified only 15 studies presenting population based data on the disease burden of childhood TB since the report's publication 5 years previously: 5, 8 and 2 from high,[30, 31, 35-37] middle[38-45] and low[46, 47] income countries, respectively. There were no studies from any low/middle income or high TB burden countries[25] that met the basic criteria for prospective community incidence studies set out in the WHO report.[32]

In the absence of good quality surveillance data, other data sources may also be used to inform burden of disease estimates. Historical data from Europe correlating the incidence of childhood TB disease with the annual rate of tuberculosis infection (ARTI, derived from tuberculin surveys), has been extrapolated to estimate childhood TB incidence in modern communities in whom the population structure and ARTI is known.[48] Interesting data also come from pneumonia aetiology studies among children, some of which have found active TB in 8-15% of children with acute pneumonia.[49, 50] This may be particularly pertinent to the question of hidden childhood TB mortality, since pneumonia is responsible for the largest proportion of deaths in children under 5 years (figure 1). Autopsy data are scarce, but a study in Zambia prior to the availability of antiretroviral therapy or widespread use of co-trimoxazole prophylaxis found active TB in 26% HIV-negative and 18% HIV positive children who died from pneumonia.[51] These findings call for similar studies in other settings to investigate their generalizability, and further emphasize the need for better quality paediatric TB surveillance data.

1.5 Challenges, opportunities and strategies for the future

Better estimates of childhood TB incidence and its contribution to childhood mortality will depend critically on improved surveillance in a wide range of settings. Rigorous implementation of existing guidelines for contact investigation among children[21] would improve active case detection as well as providing real clinical benefit through identification or earlier diagnosis and treatment of many cases. Diagnosis remains the fundamental challenge, but recent progress in this area provides opportunities to improve case ascertainment and reduce disease misclassification. Standardized case definitions are key, so the publication by the WHO of guidance on the investigation and diagnosis of childhood TB including case definitions for smear positive and negative pulmonary disease is an important step.[21] Further research to build on these case definitions and to incorporate them into validated diagnostic algorithm(s) based on high quality evidence from a range of settings – as has been done with some success for TB in HIV-positive adults[52] – would offer benefits for both surveillance and clinical care.

Ideally diagnosis should be supported by the best available methods for specimen collection and microbiological confirmation of *M. tuberculosis*. Indeed, despite the very real challenges already outlined, some experts contend that where adequate resources

for diagnosis do exist an accurate diagnosis is possible in most cases.[53] While this is unlikely to be feasible in most low resource, rural settings, research gains slowly being translated into clinical practice do provide an opportunity to develop heightened surveillance in some centres. Ideally this should be linked to existing TB prevalence surveys or studies of TB incidence among adults, in order to place in context and maximize the scientific and public health benefits of the epidemiological data they generate.

The same sites could be used to assess and monitor the local performance of more basic clinical case definitions and diagnostic algorithms. Sputum induction has been shown to be well tolerated, safe, and more sensitive than gastric aspiration for diagnosis of pulmonary TB in children, and has the added advantage that it can be performed at a single outpatient visit.[16] Although uptake of the method has been slow in high burden settings, it is now being adopted in some urban centres. Mycobacterial culture facilities are also required if the full benefit of improved specimen collection is to be realized, but progress is also being made on this front by the Stop TB Partnership Global Laboratory Initiative (www.stoptb.org/wg/gli).[54, 55] Although these are likely in the medium term to remain restricted to a few urban referral centres in many developing countries, other developments in laboratory diagnosis might yet offer the possibility of expanding access to high quality microbiological diagnosis in low resource, high TB burden settings.

Microcolony culture techniques such as the Microscopic Observation Drug Susceptibility (MODS) assay provide culture and drug susceptibility results at a fraction of the cost of commercial liquid culture systems and do not require expensive biosafety level 3 laboratory facilities, so may have a role in expanding childhood TB surveillance in low resource settings. However they are relatively labour intensive, and require significant training.[56]

More recently the Xpert MTB/RIF automated real time PCR assay, which requires minimal training and infrastructure but is more expensive, has demonstrated modest sensitivity for diagnosis of culture positive TB in children.[57] All require further research to assess their performance and cost effectiveness for diagnosis of childhood TB in a range of settings.

The benefits of improved childhood TB surveillance are likely to be many. At a public health level, good quality regional and global data are critical to understand the causes

of childhood illness and death, and thereby to inform policy decisions and priorities. Disease visibility is key to ensure appropriate investment in prevention and control strategies, and will also be important to monitor the impact of new TB vaccines in the future. In addition, since TB in a child must by definition reflect recent infection (within the lifetime of the child), and in most cases occurs within a year following infection,[8] the incidence of TB in children and their *M. tuberculosis* isolates provide a useful window on current transmission dynamics within a community, including transmission of drug resistance strains. Benefits to clinical care might be expected due to improved case finding, standardization of case definitions, local validation and/or adaptation of diagnostic algorithms utilizing data from sentinel sites with access to more advance diagnostic facilities, and a greater awareness of the burden and diagnostic algorithms among clinicians. Children remain among the most vulnerable to this common and all too often devastating disease. A better understanding of the size of the problem will be essential to any public health strategy to control childhood TB.

1.6 Aims of KIDS TB Study

The aims of the **K**ilifi **I**mproving **D**iagnosis & **S**urveillance of childhood **TB** (**KIDS TB**) study presented here closely reflect the research priorities outlined above, and focus on improving diagnosis and surveillance of childhood TB in both a rural and an urban setting in Kenya. Utilizing heightened clinical surveillance, state of the art TB diagnostics and careful follow up - a combination usually only possible in a few high resource, industrialized settings - the study carefully characterized children with and without active TB in order to achieve the following specific aims:

(1) To describe the hospital burden and clinical spectrum of childhood TB.

(2) To explore risk factors for childhood TB in this setting.

(3) To assess the utility of clinical features and the performance of clinical algorithms for identifying children with TB in Kenya.

(4) To assess the performance of laboratory tests in identifying children with TB.

(5) To determine the burden of childhood TB in Kilifi District, Kenya.

1.7 Overview of the work presented in this thesis

In this thesis I present and discuss results of aims 1 to 5 of the KIDS TB study above. The study sites and the clinical and laboratory methods used to recruit and phenotype children with and without active TB are described in chapter 2, *Patients & Methods*. Patient recruitment, diagnostic assignments, and the hospital burden and spectrum of childhood TB are presented and discussed in chapter 3, *Hospital Burden and Spectrum of Childhood Tuberculosis*. These well-characterized cohorts of children with and without TB then provide the platform to explore risk factors for childhood TB in this setting (chapter 4, *Risk Factors of Childhood Tuberculosis*); and for analysis of the performance of clinical tools (chapter 5, *Clinical Diagnosis of Childhood Tuberculosis*) and available laboratory methods (chapter 6, *Microbiological Diagnosis of Childhood Tuberculosis*) for diagnosis of childhood TB in the study population. In the final section (chapter 7, *Community Incidence of Childhood Tuberculosis*) we estimate the incidence of childhood TB among Kenyan children, and interpret our findings in the light of the preceding chapters. Details of the analytical methods used are presented in the relevant chapters. The results of each of these elements are finally drawn together in the *Concluding Remarks*. Additional supporting information is provided in the Appendices and referenced in the relevant sections of the main text.

1.8 A note about terminology

Most people who are *infected* with TB do not go on to develop TB disease because their immune response controls the infection. They are therefore said to have "latent TB infection" (LTBI). Over a lifetime some of these individuals will go on to develop active TB through reactivation of their LTBI, usually due to deteriorating immune function (e.g. due to advanced age, HIV or immunosuppressive treatment). Throughout this thesis the terms "tuberculosis" and "TB" refer to active TB disease, and "LTBI" refers to latent tuberculosis infection.

2 PATIENTS & METHODS

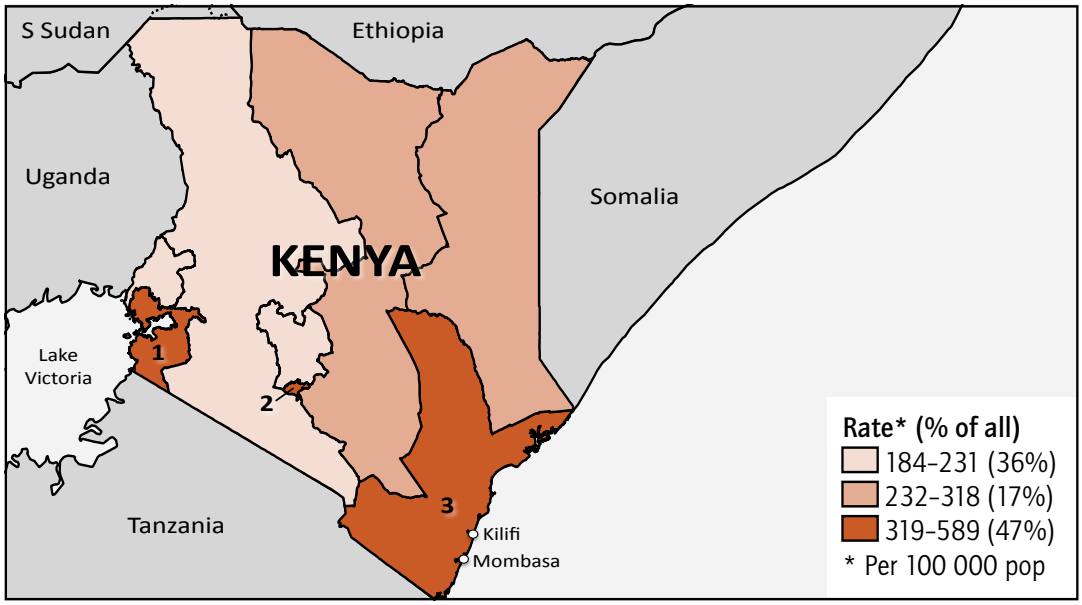
2.1 KIDS TB Study Sites

2.1.1 Kenya in context: the national tuberculosis profile

The study was based in Coast Province, Kenya. Kenya is among the 22 highest burden TB countries which together account for 82% of the global burden of tuberculosis, and in 2010 had a national TB incidence of 298 per 100,000 population, the seventh highest in the world.[3] In the same year 91% TB patients were tested for HIV nationally, of whom 41% were HIV co-infected. Children accounted for 6% of new notified cases.[58] However the true burden of childhood TB is difficult to estimate due to very limited access to diagnostic facilities for childhood TB by most healthcare providers in Kenya, and the very real challenges of paediatric TB diagnosis and surveillance already discussed. The best published estimates of the 'true' burden of childhood TB in Kenya were derived using DOTS notification data from 2000 and suggested an annual tuberculosis incidence of 167 per 100,000 among children under the age of 15 years (in the context of an overall national TB incidence of 450 per 100,000 in the same year), with children accounting for 16% of the national TB caseload (Table 1.1).[10] Together these data suggest a high burden of TB among Kenyan children, but good quality surveillance data for childhood TB in Kenya are still lacking.

Within Kenya the tuberculosis burden is not uniformly distributed, reflecting wider heterogeneity in demographic and health indices.[59] The three provinces with the highest burden of disease are illustrated in Figure 2.1. These are Nyanza, where the epidemic has been driven by the high HIV prevalence in this area around Lake Victoria;[60] Nairobi, the capital city, where poverty and overcrowding exacerbated by increasing urbanization have fuelled the epidemic; and Coast Province, where urban conditions in Kenya's second largest city, Mombasa, also drive up the burden of disease.[61] In collaboration with the Provincial and District TB & Leprosy Coordinators and Kenyan National Leprosy and Tuberculosis Programme (NLTP), the KIDS TB Study enrolled patients at two sites within Coast Province, which together span the demographic spectrum of communities in Kenya: one district hospital at Kilifi, with a rural and semi-urban catchment population; and one large urban hospital in Mombasa (Figure 2.1).

Figure 2.1 TB case notification rates by province, 2007 (1, Nyanza; 2, Nairobi; 3, Coast Province).



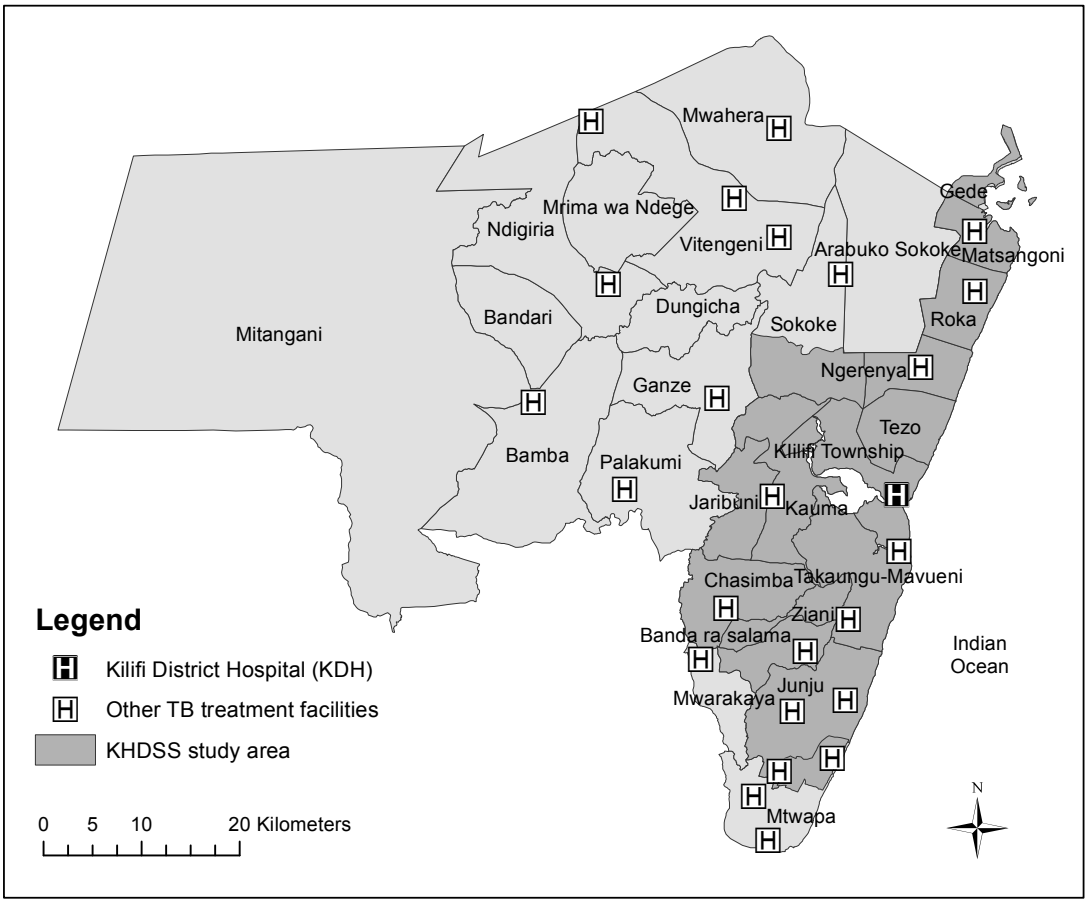
2.1.2 Site 1: Kilifi District Hospital (KDH) and the KHDSS

The study was based at the KEMRI-Wellcome Trust (KEMRI-WT) Research Programme in Kilifi (Figure 2.1). Kilifi District Hospital (KDH) shares a site and strong clinical and research links with the KEMRI-WT Programme, and was the first study site for patient recruitment. The hospital is located in Kilifi town, provides primary care to the surrounding population, and also serves as a first-level referral centre for the district. It is nested within the Kilifi Health and Demographic Surveillance System (KDHSS, Figure 2.2), which since 2001 has monitored the resident population in an area of 891 km^2 immediately surrounding the hospital through 2 to 3 census rounds each year.[62] The KDHSS comprises 40 administrative sub-locations in 15 administrative locations, which in March 2011 were home to 261,919 residents in 29,970 households, and from which two thirds of paediatric admissions to KDH were derived during the study period. It is a predominantly rural area: 12% KDHSS residents live in Kilifi town, with the vast majority living in rural homesteads.[62]

In addition to KDH, there are 10 other government health facilities and 1 private clinic within the KHDSS that are officially designated TB treatment centres; of these, 3 (KDH, Vipingo health centre and Chumani private clinic) provide quality assured smear microscopy for TB diagnosis (Figure 2.2). TB suspects who present to each of the other clinics are referred by the clinician to one of these 3 laboratories for sputum smear

microscopy. Those who are referred to the KDH laboratory for sputum microscopy and are found to be sputum smear positive for acid fast bacilli (AFB) are referred directly to the KDH TB clinic for initiation of treatment. Importantly, the National TB Programme also requires all patients with suspected smear negative TB (including children) to be referred to the TB clinic at KDH for clinical review, chest x-ray and treatment initiation if indicated.

Figure 2.2 Map of Kilifi District and the KHDSS area showing administrative locations, Kilifi District Hospital (KDH), and other TB treatment facilities.



All children who are admitted to KDH, and all study participants seen in the outpatient department, are matched to the KHDSS population register by a unique personal identification (PID) number. This linkage of detailed demographic and residence data to clinical episodes allows the combination of numerator data from clinical studies with denominator population data to estimate disease incidence with greater precision, as well as facilitating patient follow up.

2.1.3 Coast Provincial General Hospital (CPGH), Mombasa

Coast Provincial General Hospital (CPGH) in Mombasa is Kenya's second largest hospital. It provides primary and secondary level care to the predominantly urban surrounding population but also acts as a regional referral hospital for Coast Province. There are approximately 10,000 admissions per year to the paediatric ward.

2.2 Establishing the platform for childhood TB research

Central to achieving the aims of the KIDS TB Study were high quality clinical and laboratory resources for paediatric TB diagnosis. In collaboration with the National Tuberculosis Programme and clinical staff at each of the hospital sites we therefore established the following platform for paediatric TB diagnosis and surveillance:

i. Enhanced screening for childhood TB among inpatients;
ii. Dedicated paediatric outpatient clinics for children with suspected TB;
iii. Outpatient referral systems from Maternal & Child Health and paediatric HIV clinics;
iv. Contact tracing of child household contacts of pulmonary TB cases (KDH site only);
v. Detailed clinical and radiological assessment of children with suspected TB;
vi. Appropriate specimen collection for mycobacteriology (incl. sputum induction);
vii. A comprehensive biosafety level 3 (BSL3) TB laboratory service (see below).

Mycobacterial culture is a prerequisite for high quality paediatric TB diagnosis. Following an intensive mycobacteriology apprenticeship in Cape Town, I therefore set up a TB diagnostic laboratory in Kilifi. By May 2009 we had established all the necessary systems and processes for the safe and effective running of a category 3 TB diagnostic laboratory service to Good Clinical Laboratory Practice (GCLP) standards (Table 2.1). Formal GCLP accreditation was granted to the laboratory in April 2010.

Table 2.1 Key elements in establishing TB diagnostic laboratory to underpin the KIDS TB Study

- Equipment procurement
- Development of Standard Operating Procedures for the safe operation and maintenance of the category 3 laboratory and equipment, and for all aspects of the laboratory work
- Recruitment and training of a core laboratory staff in all laboratory procedures
- Smear microscopy for Acid Fast Bacilli (white light and fluorescence microscopy)
- Automated liquid mycobacterial culture (BD MGIT system)
- Mycobacterial PCR (Hain GenoType Lineprobe Assays & GeneXpert MTB/RIF Assay)
- Interferon Gamma Release Assays (IGRA) for TB infection
- Development of paper and electronic systems for data capture to meet GCLP standards
- Internal and External Quality Assurance systems

2.3 Inclusion criteria

Children who presented to either KDH or CPGH and met all of the following inclusion criteria were eligible for inclusion in the study:

i. Age <15 years (consistent with the WHO definition of children for TB notification);
ii. Admitted to hospital; or referred for outpatient investigation of TB; or household contact <5 years old of a KHDSS resident with confirmed smear positive pulmonary TB;
iii. One or more features of "Suspected TB" present (see below);
iv. No established alternative diagnosis that explained all the clinical features;
v. Not already on TB treatment for >2 weeks at presentation;

Suspected TB was defined as the presence of any of the following:

- persistent cough for >2 weeks;
- pneumonia that, in the view of the clinician, fails to respond to first line antibiotics;
- unexplained fever for >2 weeks;
- unexplained progressive weight loss or failure to thrive for >4 weeks;
- living in the same household as, or in frequent contact with, a suspected or confirmed case of pulmonary TB;
- doctor's clinical suspicion of TB for any other reason.

These broad inclusion criteria are based closely on published literature and national and international guidelines.[63-70] They reflect a balance between the desire to capture as many children with TB as possible - acknowledging the non-specific nature of many clinical features of paediatric TB - and the practical constraints inherent in investigating a large number of children.

2.4 Sample size

The sample size at KDH was chosen to optimize precision in estimates of the incidence of childhood TB (KIDS TB Study aim 5) within the available time and resource constraints. Although comprehensive data on the risk factors and clinical features of childhood TB had not been collected prospectively at KDH prior to the KIDS TB Study, the available data suggested that about 400 paediatric inpatients merited investigation for suspected TB annually. In addition, between 2002 and 2008 there were an estimated 140 new adult cases of smear positive pulmonary TB in the KHDSS area each year, with

an average in the KHDSS of about 2 children under 5 years per household. Therefore in 2 years we expected to investigate about 800 paediatric inpatients and 500 child contacts of KHDSS resident adults with smear positive TB (allowing for loss to follow up and more than one adult case of TB in a single household). The best available published estimate of the incidence of paediatric TB in Kenya is 167 per 100,000 per year.[10] Table 2.2 summarizes incidence point estimates and 95% confidence intervals for a range of scenarios spanning this estimate, given a mid-study KHDSS population of ~120,000 children <15 years old. Even the most conservative incidence scenario of 8 per 100,000 per year gave reasonable confidence intervals of 5 to 13 cases per 100,000 per year.

Table 2.2 Incidence estimates and 95% confidence intervals based on a range of scenarios for the number of cases of TB diagnosed over the 2 year study period.

No. cases at KDH in 2 years	Incidence point estimate (per 100,000 / year)	95% confidence interval (per 100,000 / year)
20	8	5 to 13
50	21	15 to 27
100	42	34 to 51
150	63	53 to 73
200	83	72 to 96
250	104	92 to 118
300	125	111 to 140
350	146	131 to 162
400	167	151 to 184
450	188	171 to 206

By extending recruitment to CPGH we sought to recruit as many TB cases as possible within the logistic and financial constraints of the study, in order (a) to maximize power to demonstrate clinical and epidemiological associations with TB (study aims 2 and 3); and (b) to maximize the precision of sensitivity estimates for each diagnostic tool assessed (study aims 3 and 4).

2.5 Sampling Procedure

An overview of patient enrolment, investigation and diagnostic classifications in each of the study arms is shown schematically in Figure 2.3. At KDH children were identified for inclusion in the study by both active and passive case detection (Figure 2.3 A and B). Passive case detection was also used to identify children presenting to CPGH with features of suspected TB (Figure 2.3 C), but due to logistical constraints active case ascertainment among child household contacts was not possible at the CPGH site.

2.5.1 Sampling procedure at KDH

Passive Case Detection: Children admitted to the paediatric ward or referred to the paediatric TB outpatient clinic at KDH were first registered and electronically matched to the KHDSS population register using their PID number (if they carried this) or their name, date of birth, and detailed information about their residence location and household composition. Clinical data were then entered onto a bespoke clinical database, and linked via the PID to the child's record in the KHDSS population register. All children referred to the outpatient paediatric TB clinic (who *de facto* met the definition of suspected TB) were comprehensively investigated for TB. Paediatric inpatients meeting the definition of suspected TB were identified by a computer algorithm and flagged for a comprehensive TB work up. In addition clinicians working on the paediatric ward, and in the Maternal and Child Health and HIV outpatient clinics, were encouraged and regularly reminded to refer any children with suspected TB for appropriate investigation. All children registered and followed up at the hospital HIV clinic were formally screened for any features of suspected TB at enrolment using a standardized proforma. Through collaboration with the HIV clinic and the National Tuberculosis Programme we also actively engaged clinicians working at other heath centres within the KHDSS area, and encouraged them to refer children with suspected TB to KDH, to benefit from the paediatric diagnostic service we had founded.

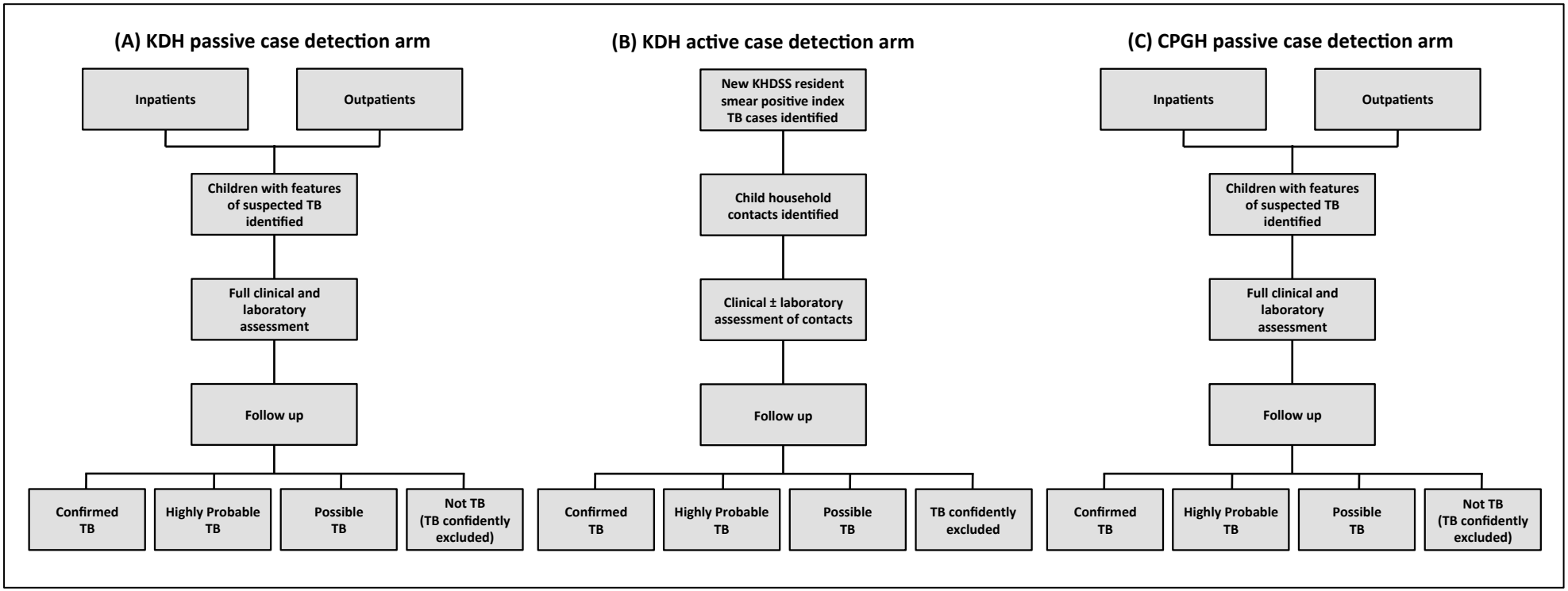
Active Case Detection: Although current Kenyan national guidelines recommend screening all child household contacts of smear positive pulmonary TB contacts,[71] in practice contact tracing rarely occurs due to limited resources and training. During the KIDS TB Study, new cases of smear positive pulmonary TB resident within the KHDSS were identified through the KDH TB clinic, and child household contacts of these index cases were identified on the KHDSS population register. For pragmatic reasons, and in keeping with Kenyan national guidelines, contact tracing focused on children under 5 years of age resident in the same household as a case of smear positive pulmonary TB, as smear positive cases are the most infectious and young children are most vulnerable to developing active TB following infection.[12] An 'index case' was therefore defined for the purposes of the study as a case of sputum smear positive pulmonary TB resident within the KHDSS area. Each index case was then invited to bring all children under 5 years in the household (symptomatic or asymptomatic) to the paediatric TB outpatient clinic for further assessment, and given a small amount of money sufficient to cover the

return fare to hospital.

2.5.2 Sampling procedure at CPGH

Demographic and clinical data from paediatric admissions are captured on a standardized Paediatric Admission Record (PAR) form, and only later double entered into an electronic database, so an electronic screening algorithm like that used at KDH was not possible. Instead ward clinicians and clinicians in the MCH and paediatric HIV clinics were encouraged and regularly reminded to refer to the study team any child in whom they suspected TB, and simple systems for referral were put in place. In addition a study clinician manually screened the written records of new paediatric admissions and attended the daily paediatric ward rounds to identify any other children with suspected TB eligible for inclusion in the study.

Figure 2.3 Overview of patient enrolment, investigation and diagnostic classifications in each study arm



2.6 Clinical Methods

2.6.1 Investigation of children with suspected TB

Baseline investigations performed on all children admitted to KDH include a full blood count (FBC; Beckman/Coulter, Fullerton, UK), thick and thin blood films for malaria parasites (malaria parasite slide, MPS), and a blood culture (BACTEC PedsPlus, Becton Dickinson, CA, USA). Blood tests were not performed routinely on inpatients at CPGH, nor on outpatients at either site, however FBC and MPS were routinely available if clinically indicated. Other blood tests and more specialist investigations (e.g. imaging) were performed at the discretion of the clinical team caring for the child.

As part of the clinical standard of care established at both hospitals, all children with suspected TB identified in the passive case detection arm of the study were weighed and underwent a structured history and examination and a posterior-anterior (PA) or anterior-posterior (AP) chest x-ray (CXR). Lateral CXRs were performed at the discretion of the clinician after reviewing the PA/AP CXR, for example to assess further any suspected hilar lymphadenopathy. The study clinician read all CXRs entered data onto a standardized CXR reporting form closely based on published radiological classifications.[72-74] Tuberculin skin testing (TST) was performed using the Mantoux method and 2 tuberculin units (TU) of tuberculin PPD RT23.[21] A positive TST was defined as a diameter of induration ≥10mm, or ≥5mm in HIV infected or severely malnourished children, in keeping with WHO guidelines for childhood TB.[21] Severe malnutrition was defined as a weight for age z-score (WAZ) of < -3, or the presence of nutritional oedema.[75] Other clinical definitions including definitions of very severe, severe and non-severe pneumonia also conformed to WHO guidelines.[75] Provider initiated testing and counseling (PiTC) for HIV was performed according to Kenyan national guidelines, which recommend testing for all inpatients and for all patients investigated for TB, on an opt-out basis.[71, 76]

Initial assessment of child household contacts of index TB cases identified in the active case detection arm of the study also included a structured history and examination, anthropometry, CXR, and a TST. Those with symptoms or signs of possible TB (Table 2.3), an abnormal CXR, or a positive TST were further investigated for suspected TB as described below.

Table 2.3 Symptoms and signs of possible TB that triggered further investigation of child household TB contacts in the active case detection arm of the study.

Symptoms of possible TB	*Signs of possible TB*
Any of the following: • fever, night sweats, weight loss, lethargy, or failure to thrive; • cough or dyspnoea; • chest infection not responding to appropriate first line antibiotics; • abdominal pain or swelling; • change in temperament or conscious level, or convulsions; • new or progressive spinal or joint deformity.	Any of the following: • fever, wasting, lymphadenopathy; • cough, tachypnoea, signs of respiratory distress; • focal chest signs (e.g. bronchial breathing, crackles, wheeze, pleural rub, signs of pleural effusion); • signs of pericardial effusion and/or congestive cardiac failure; • abdominal mass, hepatomegaly, splenomegaly or ascites; • lethargy, decreased conscious level, signs of meningism (photophobia, neck stiffness, Kernig's sign) or convulsions; • spinal gibbus or enlarged non-tender joint; • signs of tuberculin hypersensitivity (e.g. erythema nodosum, phlyctenular conjunctivitis).

Appropriate clinical specimens were collected for AFB microscopy and mycobacterial culture from all children with suspected TB (except well contacts of index TB cases who were identified in the active case detection arm of the study but had no evidence of TB - see above). Children who were able to expectorate provided three spontaneous sputum samples. Sputum induction was performed on the remainder. To maximize mycobacterial culture yield within the logistical and budgetary constraints of the study, sputum induction was performed twice on each patient and the specimens pooled for analysis to halve laboratory costs. If sputum induction was contraindicated (e.g. due to severe respiratory distress), gastric aspiration was performed. Standard operating procedures (SOPs) for both sputum induction and gastric aspiration were based closely on international guidance from the WHO.[21] Further investigations including fine needle aspiration (FNA) of lymph nodes, mycobacterial culture of CSF, urine, pleural/ascitic/joint fluid, or biopsy material, or repeat sampling, were performed at the discretion of the clinical team caring for the patient according to clinical indications in individual cases. A sample of venous blood was also collected for interferon gamma release assay (IGRA, sodium heparin tube, 1ml) following separate informed consent.

2.7 Laboratory methods

Laboratory methods for TB diagnosis are summarized below. Each method is described in more detail in chapter 6.

2.7.1 Specimen processing, AFB microscopy, and mycobacterial culture

All specimens for TB culture were routinely decontaminated using the modified Petroff's method,[77] with the exception of specimens from normally sterile sites. Larger volume fluid specimens were pre-concentrated by centrifugation to optimize culture yield. Decontaminated specimens were centrifuged and re-suspended in phosphate buffer for preparation of smears, inoculation of culture media, and real-time mycobacterial PCR.

Smears were prepared from each specimen and stained using the Ziehl Neelsen (ZN) method. The entirety of each smear was then examined for acid fast bacilli (AFB) under the x100 objective and results reported based on WHO guidelines.[78]

Automated liquid mycobacterial culture was performed using the Mycobacterial Growth Inhibitor Tube (MGIT) 960 system (BD Diagnostics, Sparks, MD, USA) and standard protocols.[77] Following a study protocol amendment in April 2010, a proportion of the re-suspended, decontaminated sediment was also cultured by the Microscopic Observation Drug Susceptibility (MODS) assay using standard protocols.[79, 80] Positive cultures were identified as *M. tuberculosis* complex (MTBC) or non-MTBC by the BD MGIT TBc Identification test (TBc ID, Becton Dickinson, Sparks, MD, USA) according to the manufacturer's instructions. They were then further speciated and probed for isoniazid and rifampicin resistance mutations by PCR, using the Hain Genotype® line probe assay platform (Hain Lifescience Gmbh, Nehren, Germany).

2.7.2 Real-time mycobacterial PCR: Xpert MTB/RIF Assay

Frozen aliquots of the decontaminated and re-suspended sediments of selected clinical specimens were assayed for the presence of MTBC DNA using the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions. To mitigate against reduced diagnostic yield due to mycobacterial culture contamination, the Xpert MTB/RIF assay was performed on all specimens whose MGIT culture was contaminated, as well as on all culture positive and a selection of culture negative specimens to compare the performance of the Xpert MTB/RIF assay with mycobacterial culture in this cohort (see section 6.2.6 for details).

2.7.3 Interferon Gamma Release Assays (IGRA)

TB infection/sensitization status was determined by an in house whole blood interferon gamma (IFN-γ) release assay (IGRA). This assay utilized a recombinant fusion protein of

Early Secretory Antigen-6 and Culture Filtrate Protein-10 that has been shown to provide similar sensitivity to peptides used in commercial IGRAs.[81] Heparinized whole blood (0.2ml) was diluted 1:10 in RPMI-1640 and 180µl aliquots stimulated in triplicate on a 96-well plate with TB antigen (TBAg), phytohaemaglutinin (PHA, positive control) and RPMI (negative control). Plates were cultured at 37°C for 7 days and IFN-γ levels in the supernatants determined by sandwich ELISA.[82] A positive response to PHA or TBAg was defined as a concentration of IFN-γ >100pg/ml ([IFN-γ]>100pg/ml) and more than 3 times the [IFN-γ] in the RPMI well. A positive IGRA result was defined as a positive response to both PHA and TBAg. Tests without a positive response to PHA were defined as indeterminate. Healthy control samples were included in each assay.

2.8 Case definitions, clinical management and follow up

In the absence of a reliable gold standard for TB diagnosis in children, and in keeping with other studies of paediatric TB, a hierarchy of diagnostic categories was used to reflect the inevitable range of diagnostic certainty in individual cases (Table 2.4). Categories were defined *a priori* and based closely on published definitions, modified slightly to include information provided by IGRA results and careful follow up.[70, 83]

All children with 'Confirmed TB' (CTB) or 'Highly Probable TB' (HPTB) were referred for TB treatment; children with 'Possible TB' could also be started on TB treatment at the discretion of the study clinician and clinician responsible for the patient. All treatment regimens including isoniazid chemoprophylaxis followed Kenyan national guidelines.[84]

Children treated for TB were followed up at 2 and 8 weeks after commencement of TB treatment, and then monthly until completion of treatment at 6 months, in keeping with national guidelines.[84] Children with 'Possible TB' for whom immediate empiric TB treatment was not thought to be justified were followed up at 1, 3 and 6 months, or until TB could be confidently excluded. In Kilifi children classified as 'Not TB' at initial presentation were also followed up at 3 months to ensure the accuracy of our diagnostic assignment. For logistical reasons follow up of this group of children was not possible in Mombasa. Children who did not present for a follow up appointment were contacted by telephone or visited at home and reminded to bring their child for review. Travel fares were reimbursed for all follow up appointments. In order to identify 'new converters', children with a negative IGRA at enrolment had a repeat IGRA after 3 months following separate informed consent. Final diagnoses were revised in the light of follow up data.

Table 2.4 Childhood TB case definitions

Confirmed TB (CTB)

- *Disease at any site*: definitive identification of *M. tuberculosis* complex (MTBC) from clinical specimens in the appropriate clinical context.

Highly Probable TB (HPTB)

- *Disease at any site*: demonstration of acid fast bacilli (AFB) on microscopy of clinical specimens in the appropriate clinical context; or
- *Disease at any site*: histology of biopsy tissue showing caseating granulomata;
- *Intra-thoracic TB*: CXR appearances highly suggestive of active TB
  - non-pyogenic pleural effusion with no evidence of alternative cause
  - cavitation associated with subacute/chronic pneumonia and no other identified cause of cavitation (e.g. Klebsiella or Staphylococcal sepsis)
  - hilar/mediastinal lymph nodes plus a positive TST and no other identified cause;
- *Miliary TB*: miliary shadowing on CXR in an HIV un-infected child;
- *TB Meningitis (TBM)*: clinical features of meningitis with CSF changes consistent with TBM (predominantly lymphocytic cellular infiltrate, protein concentration >0.8g/l, glucose concentration <2.2mmol/l, and no established alternative diagnosis);
- *TB Lymphadenitis*: cervical lymphadenopathy plus sinus formation or a positive TST;
- *Abdominal TB*: abdominal mass or ascites, with abdominal lymphadenopathy on ultrasound;
- *Spinal TB*: spinal gibbus in the absence of another obvious cause;
- *Hypersensitivity reactions*: erythema nodosum or phlyctenular conjunctivitis with chest radiograph evidence of primary TB;
- *Disease at any site*: positive TST and IGRA (at initial assessment and/or at follow up), no definitive alternative diagnosis to explain clinical features of suspected TB, and favourable response to TB therapy;
- *Disease at any site*: HIV positive children with no definitive alternative diagnosis to explain clinical features of suspected TB, plus development of a positive TST and/or IGRA on TB therapy.

Possible TB

- Children who did not meet case definitions for 'Confirmed TB', 'Highly Probable TB' or 'Not TB'; some children in this group received empiric treatment for TB.

All TB cases

- Children with 'Confirmed TB' or 'Highly Probable TB', plus children with 'Possible TB' who received empiric TB treatment.

Not TB (TB confidently excluded)

- All clinical features explained by a definitive alternative diagnosis, making TB highly unlikely; and/or insufficient clinical indication for a trial of TB treatment and no clinical deterioration during follow up in the absence of TB therapy.

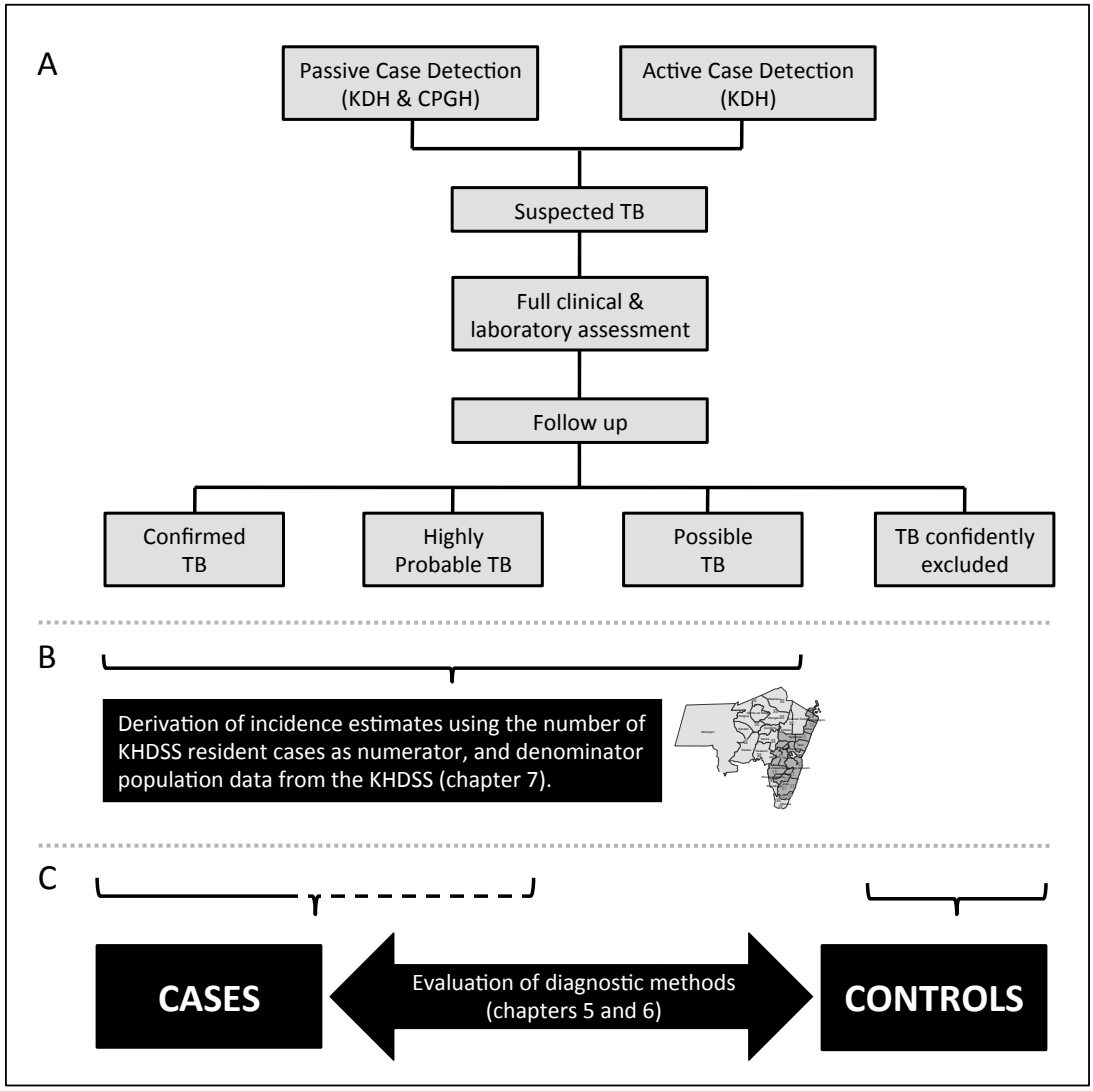
2.9 Summary of recruitment strategy & analysis overview

Following the thorough clinical and laboratory assessment and careful follow up described above, children with suspected TB identified through the active and passive case detection arms were thus assigned to one of the four TB diagnostic categories as shown schematically in Figure 2.4A. These carefully characterized diagnostic groups were than used to investigate each of the study's main aims: the spectrum of TB among the cases is described in chapter 3; in Kilifi the number of TB cases provided the numerator for estimates of the incidence of childhood TB in the KHDSS (Figure 2.4B, chapter 7); and children with and without TB served as the cases and controls in nested case control studies to assess risk factors and the performance of clinical and laboratory tools for childhood TB diagnosis in this setting (Figure 2.4C, chapters 4 to 6). All analyses were performed using Stata version 11 (StataCorp, USA) and the results were presented in keeping with STROBE[85] and STARD[86] guidelines for reporting of observational and diagnostic studies, respectively.

2.10 Ethical approval

The study protocol was approved by the KEMRI National Research Ethics Committee in Kenya, and by the Imperial College Research Ethics Committee. Written informed consent was provided by a parent or guardian.

Figure 2.4 Schematic overview of recruitment strategy and analyses



3 HOSPITAL BURDEN & SPECTRUM OF CHILDHOOD TUBERCULOSIS

3.1 Introduction

Classic descriptions of the epidemiology, clinical presentation and natural history of childhood TB derive from the pre-chemotherapy era between about 1920 and 1950, when chest radiography allowed detailed characterization of the disease spectrum but effective anti-tuberculosis chemotherapy was not yet available.[8, 87] Unsurprisingly *infection* risk increases cumulatively with age - particularly among toddlers, around the age of school entry and with increased social mobility in adolescence.[12, 87] Nevertheless the picture of *disease* risk that emerges is bimodal, with the highest risk among young children and adolescents, and a lower risk among school age children (the so-called "safe school years").[8, 88] The reasons for these age related differences in disease risk following infection are not completely understood,[12] but the high incidence of active TB following infection in infants and young children is thought to reflect immature immune responses.[89-91]

The clinical spectrum of childhood TB also reflects differences in the host-pathogen balance, with both diminished and exaggerated immune responses contributing to pathogenesis. [12] Thus the clinical course following infection ranges from asymptomatic LTBI or transient symptoms accompanied by radiological changes, through various forms of intra-thoracic TB,[92] to severe disseminated disease.[8, 87, 93] Both age and immunodeficiency appear to have an important role in determining the outcome of infection. Thus while an exuberant immune response in immunocompetent adolescents tends to cause tuberculous hypersensitivity with pleural effusions or adult-type cavitating disease,[94] in young children and/or HIV co-infection poor cell mediated immunity is thought to allow unrestrained proliferation of bacilli[95] and dissemination.[96] Although dissemination can occur to almost any site, one of the commonest sites of extrapulmonary disease is TB meningitis (TBM),[97] which carries a high risk of devastating neurological sequelae and death.[98] Anatomical differences also modify the presentation of TB in young children, whose smaller airways are more susceptible to compression by enlarging lymph nodes or endo-bronchial TB, causing lobar collapse or hyperinflation.[8, 93]

Recruitment and careful characterization of children with and without active TB is a pre-requisite for assessment of each of the diagnostic methods we investigated, and for accurate estimation of the burden of childhood TB. It also provides an opportunity to describe the clinical spectrum of childhood tuberculosis in our setting.

3.2 Methods

3.2.1 Recruitment and description of patient cohorts

Children with and without active TB were identified and carefully characterized as described in detail in chapter 2. Baseline demographic and clinical features were summarized overall and by hospital site and final TB diagnostic category.

3.2.2 Clinical spectrum of childhood tuberculosis

Clinical syndromes reflecting the site of TB disease were summarized for all TB cases. We used standard definitions of sputum smear positive and negative pulmonary TB (PTB).[21] In keeping with WHO guidelines we reported the proportions with PTB and extra-pulmonary TB (EPTB) such that children with both PTB and EPTB were classified as PTB.[21] In addition to the disease site-specific definitions of confirmed and highly probable TB (CHPTB, see Table 2.4, page 37), we used the disease site-specific definitions of EPTB shown in Table 3.1 for treated cases of possible TB who did not meet these stringent criteria. The standard definition of multi-drug resistant tuberculosis (MDR-TB) was used, namely isolation from a patient with clinical TB of *M. tuberculosis* resistant to both isoniazid and rifampicin.

3.2.3 Inpatient burden of childhood TB

We estimated the inpatient burden of childhood TB as the proportion of the presenting inpatient caseload with a diagnosis of confirmed or highly probable TB (CHPTB).

Table 3.1 Definitions of the site of extra-pulmonary TB among children treated for possible TB who did not meet the stringent criteria for confirmed or highly probable TB (see **Table 2.4**)

Miliary TB: defined in the presence of HIV co-infection as miliary shadowing on CXR plus evidence of TB disease at another anatomical site. [1]

TB meningitis (TBM): clinical and/or CT/MRI features consistent with TBM (hydrocephalus and/or basal meningeal enhancement) and either

- CSF evidence of meningitis but not the classic changes of TBM (see Table 2.4); or
- LP contraindicated due to signs of raised intracranial pressure so unable to rule out other causes.

Pericardial TB: echocardiographic evidence of pericarditis / pericardial effusion in a child with other clinical features of TB.[2]

TB lymphadenitis: bulky cervical lymphadenopathy in an HIV negative child with other clinical features of TB.[2]

Abdominal TB: abdominal mass or ascites with other clinical features suggestive of TB,[2] but abdominal ultrasound imaging not diagnostic or not available.

Osteo-articular TB: chronic bone or joint infection with systemic features of TB, no evidence of another bacterial cause, and unresponsive to conventional antibiotics.

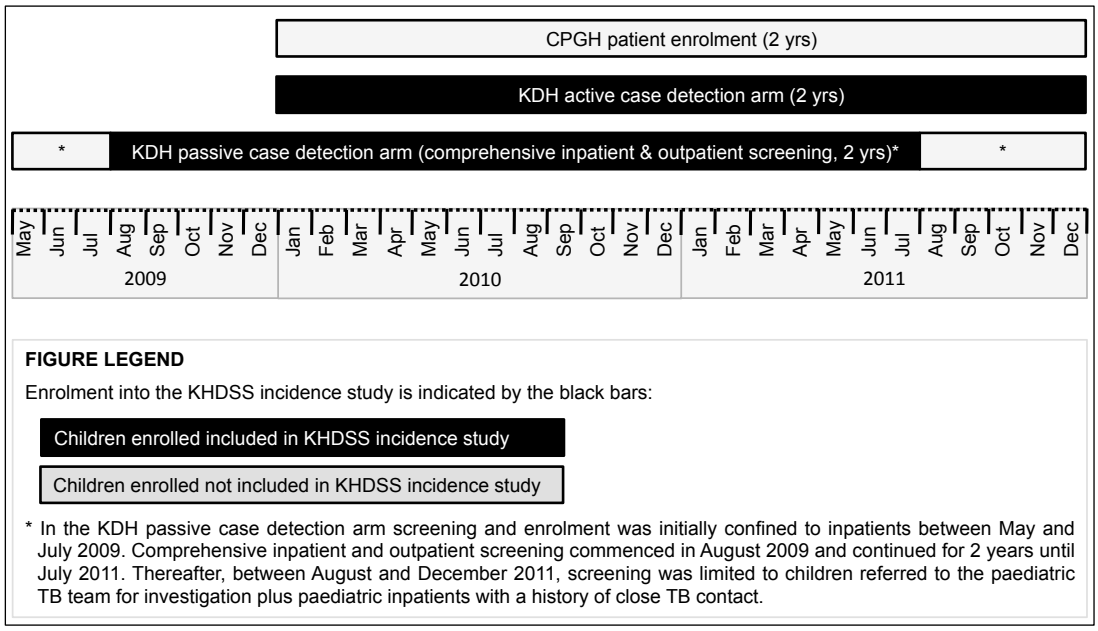
Pleural TB: Pleural effusion and other clinical features of TB[1] but other bacterial cause of effusion could not be excluded.

No focus identified: No clear clinical focus of TB but presence of ≥1 systemic clinical features (fever, night sweats, weight loss, and/or failure to thrive) warranted empiric TB treatment.

[1] Note that, due to poorer specificity of the radiological appearances in HIV infected children, a child had to be HIV un-infected for miliary shadowing on CXR to meet the criteria for Highly Probable TB.

[2] Other clinical features of TB include one or more of fever, night sweats, weight loss, failure to thrive and/or evidence of TB disease at another anatomical site.

Figure 3.1 Timeline of patient enrolment in each study arm



3.3 Results

3.3.1 Patient enrolment and follow up at each site

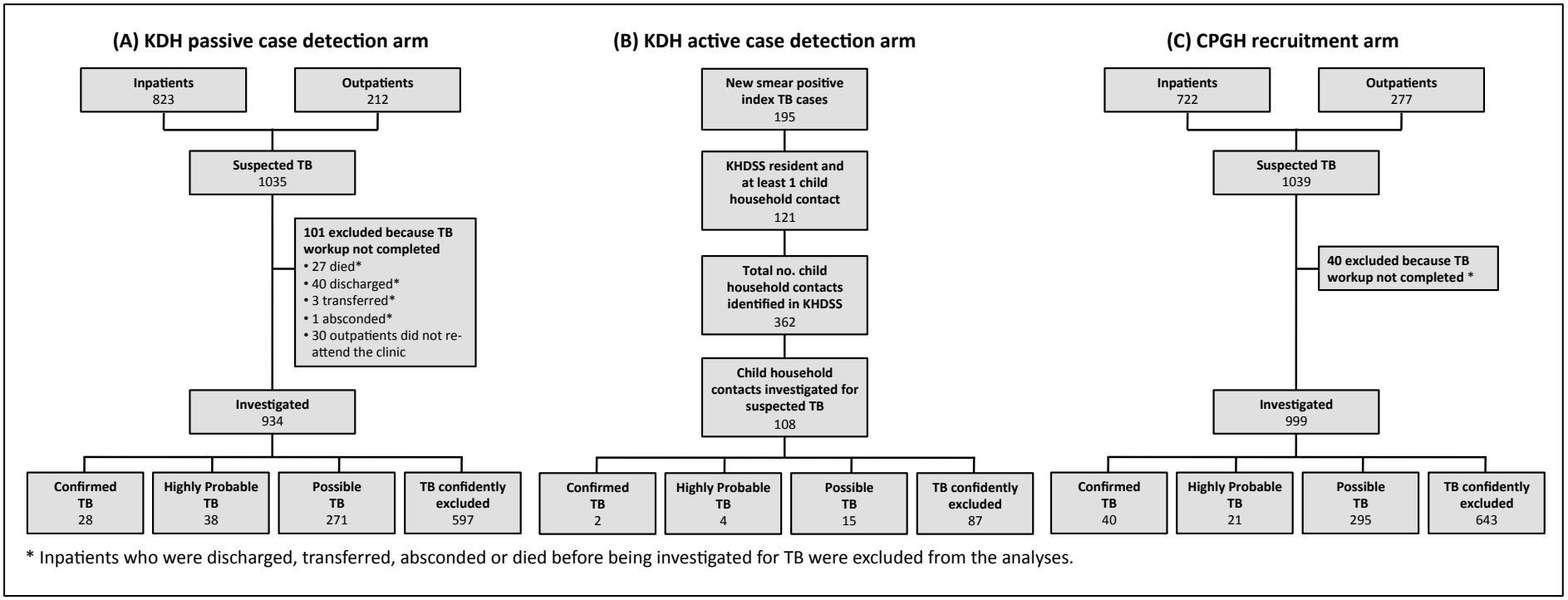
The timeline for patient enrolment in each study arm is summarized in Figure 3.1. Clinical recruitment began among inpatients at KDH in May 2009, and was extended to KDH outpatients in August 2009 with establishment of a paediatric TB outpatient clinic. Therefore although inpatient enrolment began earlier, comprehensive screening for the passive case detection arm of the KHDSS incidence analysis began on 1 August 2009 and continued for 2 years until 31 July 2011. Enrolment of child TB suspects for the diagnostic studies continued beyond this 2 year period of intense childhood TB surveillance until December 2011, but between August and December 2011 was limited to children referred to the paediatric TB team for investigation and children with a history of close TB contact.

Enrolment into the active case detection (contact tracing) arm of the study at KDH required protracted negotiations with the hospital and strengthening of the main KDH outpatient TB service, so did not begin until January 2010, and continued for 2 years, until December 2011. Patient enrolment at CPGH also began in January 2010 and continued until December 2011.

The numbers of children with suspected TB identified and investigated for TB in each recruitment arm of the study are summarized in Figure 3.2. Median follow up among children recruited at KDH was 6 months. 203 (19%) children did not complete follow up per protocol; of these, 39 (19%) died during their index admission and 55 (27%) died before the end of follow up. Data on the length of follow up of patients recruited at CPGH is not yet available for analysis at the time of writing.

101 children with features of suspected TB in the KDH passive case detection arm, and 40 children in the CPGH recruitment arm, were excluded because they were discharged, transferred, absconded or died before being investigated for TB, or did not re-attend the paediatric TB outpatient clinic to complete their investigations. There were no differences in age, sex or KHDSS residence status between children excluded and those included in the analyses ($p>0.5$ in each case).

Figure 3.2 Patient enrolment and final TB category assignments in each of the study recruitment arms



* Inpatients who were discharged, transferred, absconded or died before being investigated for TB were excluded from the analyses.

3.3.2 Cohort description and disease assignments

Baseline characteristics of children investigated for TB at KDH, CPGH and overall are summarized in Table 3.2. The proportions of inpatients and boys were very similar at the two hospitals, but both inpatients and outpatients recruited at CPGH tended to be younger with a higher proportion of infants. This reflected differences in the overall age distribution of admissions at the two sites (data not shown). The age distribution of child TB suspects at the two sites combined is shown in Figure 3.3(a).

The proportion of children with severe malnutrition was higher among those enrolled at KDH, reflecting the systematic application for all paediatric admissions of broad screening criteria, which included a history of weight loss or failure to thrive. However the proportion of children with HIV co-infection was similar at the two sites.

Table 3.2 Referral source and demographic characteristics of children investigated for TB

	Both study sites (total)	KDH	CPGH	
	(n = 2041)	(n = 1042)	(n = 999)	p value
Referral source				
Inpatient screening and referrals	1474 (72%)	752 (72%)	722 (72%)	0.958
All outpatient referrals	459 (22%)	182 (17%)	277 (28%)	
Contact tracing	108 (5%)	108 (10%)	-	-
Median age (months) [IQR[1]]				
All children investigated	17 [10-45][2]	23 [12-53]	14 [8-33][2]	<0.001
Inpatients	15 [9-36][2]	18 [10-41]	13 [8-26]	<0.001
Outpatients	31 [13-57][2]	43 [20-66]	19 [10-45]	<0.001
Age category				
< 1 year	707 (35%)	268 (26%)	439 (44%)	<0.001
1 to 4 years	963 (47%)	556 (53%)	407 (41%)	
5 to 9 years	261 (13%)	163 (16%)	101 (10%)	
10 to 14 years	110 (5%)	55 (5%)	56 (5%)	
Male	1071 (54%)	548 (53%)	545 (55%)	0.386
HIV infected	352 (18%)	179 (19%)	173 (18%)	0.823
Severely malnourished	744 (36%)	450 (43%)	294 (29%)	<0.001
KHDSS resident	-	686 (66%)	-	-

[1] IQR, interquartile range;

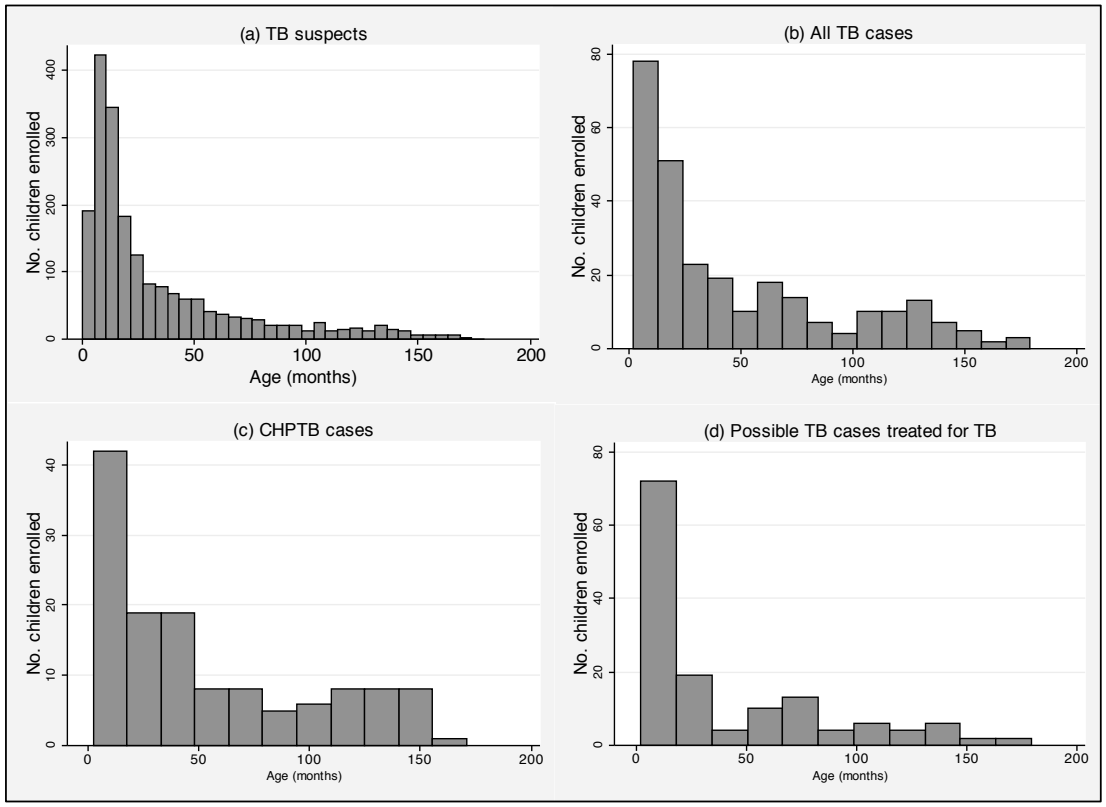
3.3.3 Clinical spectrum of childhood tuberculosis

Final TB categories for children in each of the study recruitment arms are shown in Figure 3.2. In addition to 133 children with CTB (70) and HPTB (63), a further 144 children with possible TB who did not meet the stringent definitions of highly probable

TB were treated for suspected TB on clinical grounds (61 from KDH and 83 from CPGH). Thus the total number of All TB cases was 277. The clinical spectra of TB among cases in each category of diagnostic certainty are shown in **Table 3.3**.

Baseline characteristics of children in each category of TB diagnostic certainty are shown in Table 3.4. Cases of CTB tended to be older than HPTB cases and treated cases of possible TB (χ^2 test for trend, p=0.004 and p=0.020, respectively; Table 3.4). The age distribution of TB cases is shown in Figure 3.3 (b-d). HIV test results were available for 272/277 (98%) TB cases, of whom 74 (27%) were HIV infected.

Figure 3.3 Age distribution of TB suspects and cases



Pulmonary tuberculosis (PTB)

The most common clinical presentation was PTB, which accounted for 215/277 (78%) cases overall, and 107/133 (80%) of confirmed and highly probable (CHPTB) cases. Only 8/107 (7.5%) of CHPTB cases were smear positive. Interestingly, children with a BCG scar were less likely to have smear positive PTB than children with a scar (OR 0.20, 95% CI 0.05 to 0.85). There was no association between HIV or nutritional status and

having a positive sputum smear. Chest radiological features encompassed the full spectrum of intra-thoracic childhood TB, including segmental, lobar, expansile and cavitatory pneumonia, hilar/mediastinal lymphadenopathy, airway compression from endobronchial ("lymphobronchial") disease, tuberculous pleural effusions, miliary tuberculosis, and tuberculous pericarditis (Figure 3.4). The frequencies among TB cases of common radiological features are presented in chapter 5 (Table 5.3, page 88).

Table 3.3 Clinical spectrum of TB among cases in each category of diagnostic certainty. [2]

	CTB		HPTB		Possible TB (treated)		CHPTB[1]		All TB	
	(n = 70)		(n = 63)		(n = 144)		(n = 133)		(n = 277)	
Pulmonary TB										
Smear positive	6	(11)	2	(4)	0	(0)	8	(7)	8	(4)
Smear negative	49	(89)	50	(96)	108	(100)	99	(93)	207	(96)
Total [2]	**55**	**(79)**	**52**	**(83)**	**108**	**(75)**	**107**	**(80)**	**215**	**(78)**
Extra-pulmonary TB [3]										
Miliary TB	10	(67)	6	(55)	5	(14)	16	(62)	21	(34)
TB meningitis	5	(33)	1	(9)	8	(22)	6	(23)	14	(23)
Pleural TB	2	(13)	0	(0)	3	(8)	2	(8)	5	(8)
TB lymphadenitis	2	(13)	2	(18)	4	(11)	4	(16)	8	(13)
Osteo-articular TB	1	(7)	2	(18)	1	(3)	3	(12)	4	(6)
Abdominal TB	2	(14)	0	(0)	9	(25)	2	(8)	11	(18)
PUO without a focus	0	(0)	0	(0)	13	(36)	0	(0)	13	(21)
Total [2,5]	**15**	**(21)**	**11**	**(17)**	**36**	**(25)**	**26**	**(20)**	**62**	**(22)**
Extra-pulmonary TB among PTB cases										
TB meningitis	2	(4)	1	(2)	3	(3)	3	(2)	6	(2)
Pleural TB [4]	5	(9)	2	(4)	4	(4)	7	(6)	11	(5)
TB lymphadenitis	4	(7)	4	(8)	2	(2)	8	(7)	10	(5)
Osteo-articular TB	1	(2)	1	(2)	0	(0)	2	(2)	2	(1)
Pericardial TB	3	(5)	1	(2)	1	(1)	4	(4)	5	(2)
Abdominal TB	5	(9)	2	(4)	0	(0)	9	(8)	9	(4)
Total among PTB cases [5]	17	(32)	9	(17)	8	(7)	27	(25)	35	(16)

[1] CHPTB = confirmed and highly probable TB cases.

[2] Percentages are shown in parenthesis. Those in bold refer to proportions of the total number of cases in each diagnostic category (columns). Other percentages refer to the proportions of children in each subgroup of PTB or EPTB within that diagnostic category.

[3] 7 children with miliary TB also had TB meningitis, of whom 1 also grew *M. tuberculosis* in urine; 1 child had TB meningitis and musculoskeletal TB; and 1 child had musculoskeletal TB and TB lymphadenitis.

[4] 4 children with pulmonary and pleural TB also had another focus of TB disease: 2 had pericardial TB, 1 had TB meningitis, and 1 had TB lymphadenitis.

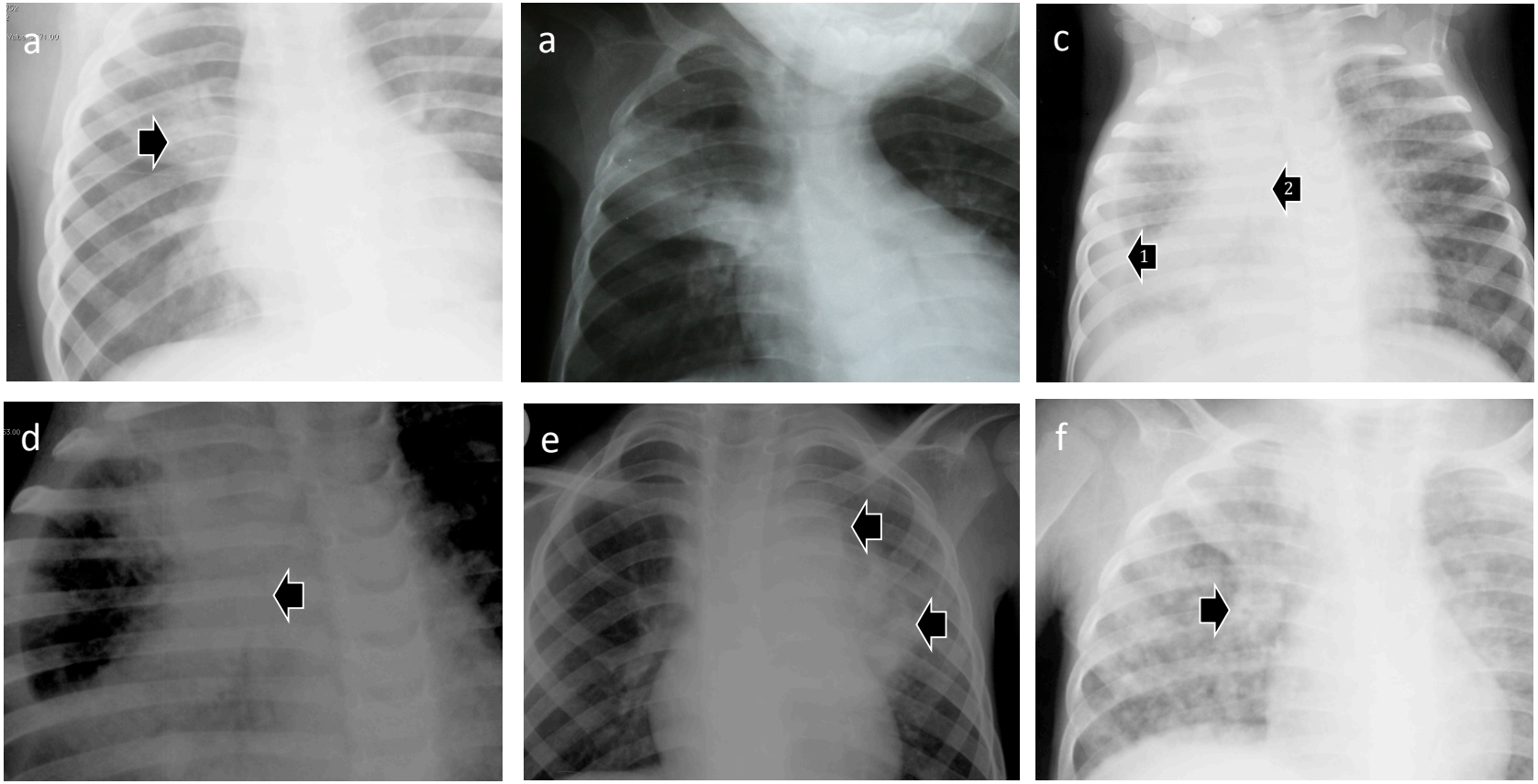
[5] Note sites of extra-pulmonary TB are not mutually exclusive (see notes 3 and 4 above). The total number of EPTB cases in each case represents the number of children with evidence of extra-pulmonary TB, not the total number of sites of extra-pulmonary TB.

Table 3.4 Baseline characteristics of children by TB diagnostic category

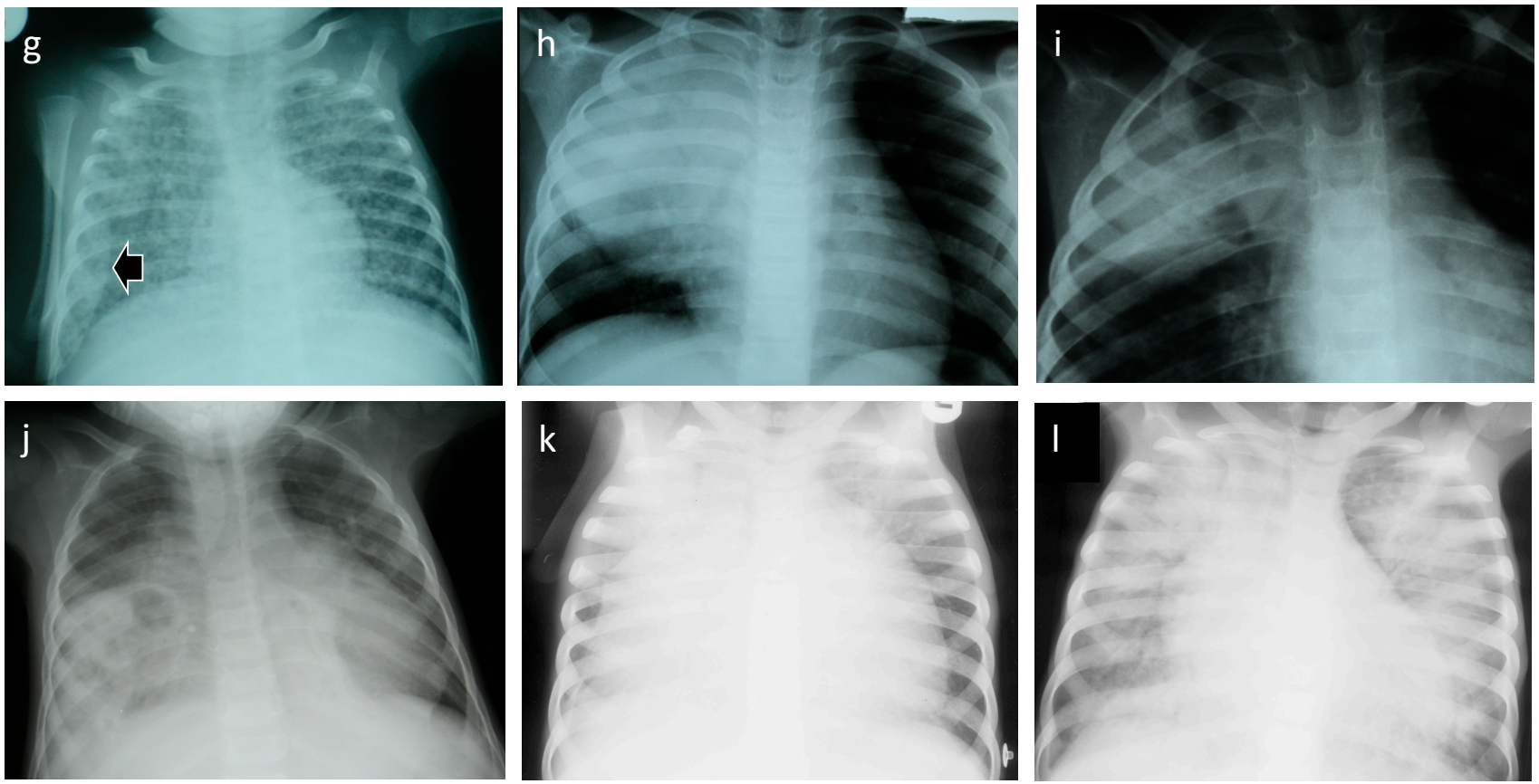
	Confirmed TB (CTB)	Highly Probable TB (HPTB)	Possible TB cases treated for TB	Possible TB cases not treated for TB	Not TB (TB excluded)	CHPTB	All TB cases
	(n = 70)	(n = 63)	(n = 144)	(n = 437)	(n = 1327)	(n = 133)	(n = 277)
Referral source							
Inpatient	55 (79%)	39 (62%)	113 (78%)	311 (71%)	956 (72%)	94 (70%)	207 (75%)
Outpatient	13 (18%)	20 (32%)	31 (22%)	102 (23%)	258 (19%)	33 (25%)	57 (21%)
Contact tracing	2 (3%)	4 (6%)	0 (0%)	15 (3%)	87 (7%)	6 (5%)	6 (3%)
Median age (months) [IQR[1]]	53 [16-115]	28 [13-46]	17 [10-68]	17 [10-47]	17 [10-40]	37 [15-87]	26 [12-71]
Age category							
< 1 year	11 (16%)	13 (21%)	46 (32%)	164 (38%)	472 (36%)	24 (19%)	70 (25%)
1 to 4 years	25 (36%)	36 (57%)	53 (37%)	189 (43%)	662 (50%)	61 (47%)	114 (41%)
5 to 9 years	19 (27%)	10 (16%)	31 (22%)	61 (14%)	141 (11%)	26 (20%)	60 (22%)
10 to 14 years	15 (21%)	4 (6%)	14 (10%)	23 (5%)	52 (4%)	18 (14%)	33 (12%)
Male sex	38 (54%)	33 (52%)	74 (51%)	235 (54%)	713 (54%)	71 (55%)	145 (52%)
HIV infected	17 (25%)	14 (22%)	45 (32%)	114 (28%)	162 (13%)	31 (23%)	76 (27%)
Severely malnourished	30 (43%)	31 (49%)	57 (40%)	164 (38%)	462 (35%)	61 (46%)	118 (43%)

[1] IQR, interquartile range;

Figure 3.4 Radiological spectrum of intra-thoracic childhood TB among children enrolled in the KIDS TB Study



(a) Right hilar lymphadenopathy; (b) peripheral consolidation with associated hilar lymphadenopathy (Ghon complex); (c) right upper and middle lobe consolidation with right hilar lymphadenopathy, right pleural effusion (arrow 1), and compression of the right main bronchus(arrow 2); (d) the same child, showing more clearly the bronchial compression by the hilar lymph nodes with a distal air bronchogram; (e) left hilar and paratracheal lymphadenopathy; (f) right hilar lymphadenopathy (arrow) and possible right paratracheal lymphadenopathy, with extensive bilateral miliary infiltrates;



(g) miliary tuberculosis with a right pleural effusion (arrow); (h) expansile pneumonia of the right upper lobe due to tuberculosis; (i) right upper lobe tuberculous pneumonia in another patient, this time with with marked cavitation; (j) cavitatory pneumonia of the right middle lobe due to TB with left lower lobe consolidation; (k) massive globular cardiomegally due to TB pericarditis with tamponade in a child with HIV co-infection; (l) widespread bilateral consolidation due to TB IRIS in the same patient a few weeks after pericardiocentesis and initiation of TB treatment followed by antiretroviral therapy.

Extra-pulmonary TB (EPTB)

Sixty two (22%) of 277 cases treated for TB had a primary diagnosis of EPTB. A further 35 (16%) of the 215 children who presented with PTB had at least one extra-pulmonary focus (**Table 3.3**). In all 97/277 (35%) cases, and 52/133 (39%) CHPTB cases, had evidence of extra-pulmonary disease (with or without PTB). The proportion of cases with EPTB did not differ between the two study sites (34/133 cases at KDH compared with 28/144 at CPGH, p=0.222).

Figure 3.5 shows the age distributions of PTB and EPTB among all TB cases, CHPTB cases, and among children with and without HIV co-infection. The age distributions of individual clinical syndromes are shown in more detail for children with and without HIV co-infection in Table 3.5. There were no significant differences between PTB and EPTB cases in age distribution (χ^2 test for trend, p=0.161), nor in the proportions with HIV co-infection, severe malnutrition, or a visible BCG scar; this remained the case when the analysis was confined to CHPTB cases (p>0.05 in each case; data not shown). Among 47 HIV-uninfected children with EPTB 20 (43%) had severe malnutrition.

As expected, the majority of cases of miliary TB and TBM occurred among children <5 years old (Figure 3.6[a]). There were 4 cases of miliary TB among older children, of whom 2 were HIV co-infected and 1 had severe malnutrition. Together miliary TB and TBM accounted for 9/13 (69%) of EPTB cases among children with HIV co-infection. There were no cases of pleural TB among our HIV co-infected TB patients, but pleural TB was more common among those without HIV. Pleural disease complicated a small proportion of PTB cases among younger children, but the proportion of TB cases with pleural TB was much higher among young adolescents (Figure 3.6[c] and [d]), among whom larger pleural effusions due to tuberculous hypersensitivity were more common.

3.3.4 Multi-drug resistant tuberculosis (MDR-TB)

Among 70 CTB cases, only one child had confirmed MDR TB - a 2 years old boy with pulmonary tuberculosis who had not previously received TB treatment - giving a prevalence of MDR-TB in this group of 1.4% (0.04 to 7.7%)

Figure 3.5 Age distribution of pulmonary TB (PTB) and extra-pulmonary TB (EPTB)

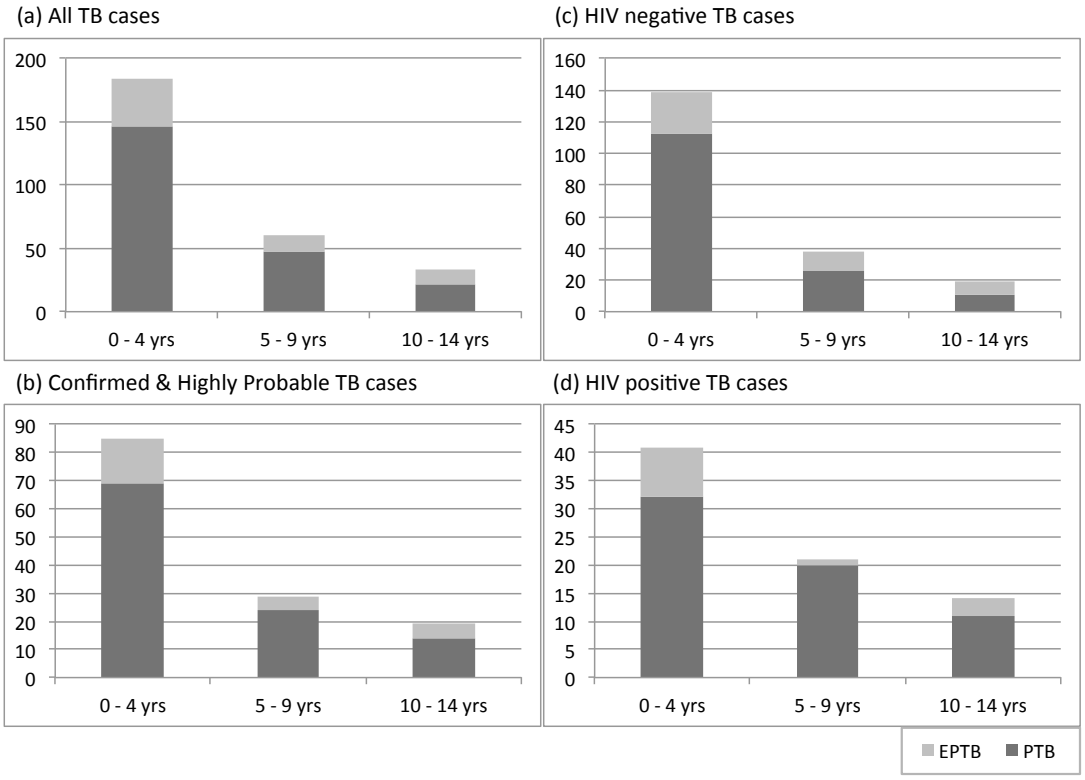


Figure 3.6 Age distribution of sites of extra-pulmonary tuberculosis among cases of confirmed and highly probable TB (including among PTB cases): number (a) and percent (b) with miliary TB and TBM; number (c) and percent (d) with other forms of extrapulmonary TB.

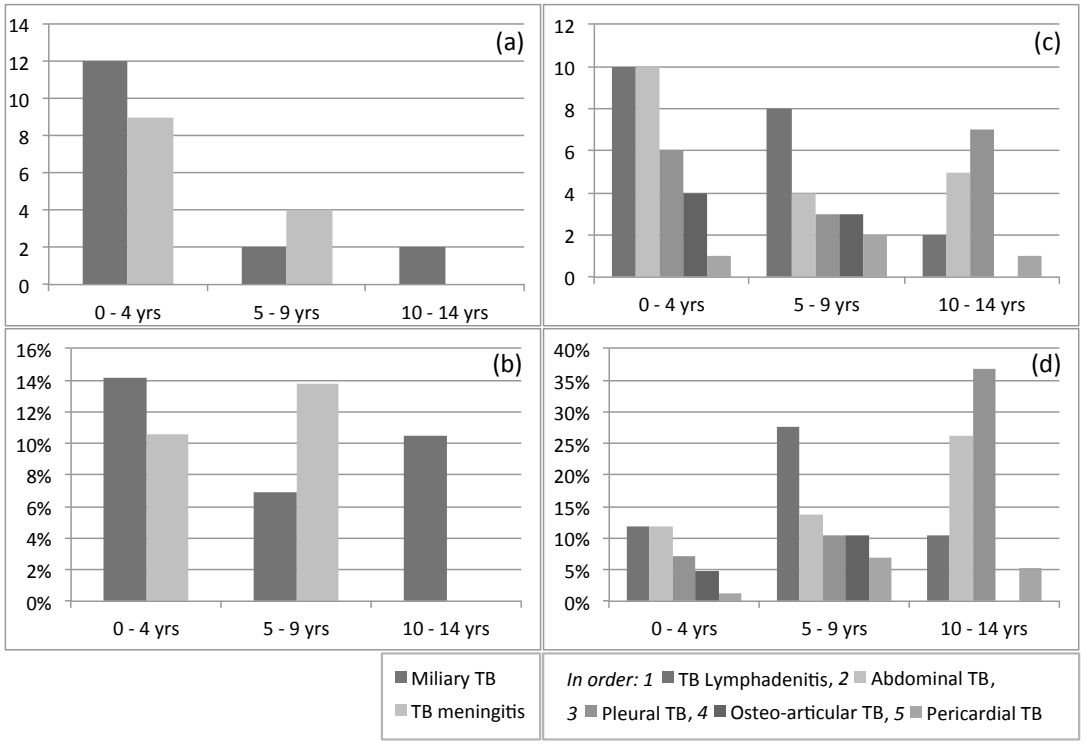


Table 3.5 Clinical spectrum of TB cases by age category and HIV infection status [1]

	HIV un-infected children (HIV -ve) [1]								HIV co-infected children (HIV +ve) [1]							
	0 - 4 yrs		**5 - 9 yrs**		**10 - 14 yrs**		**All HIV -ve**		**0 - 4 yrs**		**5 - 9 yrs**		**10 - 14 yrs**		**All HIV +ve**	
Pulmonary TB																
Smear positive	3	(3)	0	(0)	1	(9)	4	(3)	1	(3)	3	(15)	0	(0)	4	(6)
Smear negative	109	(97)	26	(100)	10	(91)	145	(97)	31	(97)	17	(85)	10	(100)	58	(94)
Total [2]	**112**	**(81)**	**26**	**(68)**	**11**	**(58)**	**149**	**(76)**	**32**	**(78)**	**20**	**(95)**	**11**	**(79)**	**63**	**(83)**
Extra-pulmonary TB																
Miliary TB	10	(37)	2	(17)	1	(13)	13	(28)	6	(75)	1	(100)	1	(33)	8	(67)
TB meningitis	5	(19)	6	(50)	1	(13)	12	(26)	3	(38)	0	(0)	0	(0)	3	(25)
Pleural TB	1	(4)	2	(17)	1	(13)	4	(9)	0	(0)	0	(0)	0	(0)	0	(0)
TB lymphadenitis	2	(7)	2	(17)	2	(25)	6	(13)	0	(0)	0	(0)	1	(33)	0	(0)
Osteo-articular TB	2	(7)	2	(17)	0	(0)	4	(9)	0	(0)	0	(0)	0	(0)	0	(0)
Abdominal TB	2	(7)	2	(17)	4	(50)	8	(17)	0	(0)	0	(0)	1	(33)	1	(8)
PUO without a focus	9	(33)	0	(0)	0	(0)	9	(19)	1	(13)	0	(0)	0	(0)	1	(8)
Total [2,3]	**27**	**(19)**	**12**	**(32)**	**8**	**(42)**	**47**	**(24)**	**9**	**(22)**	**1**	**(5)**	**3**	**(21)**	**13**	**(17)**
EPTB among PTB cases																
TB meningitis	6	(35)	0	(0)	0	(0)	6	(22)	0	(0)	0	(0)	0	(0)	0	(0)
Pericardial TB	1	(6)	2	(29)	0	(0)	3	(11)	0	(0)	1	(33)	1	(33)	2	(33)
Pleural TB	4	(24)	3	(43)	2	(67)	9	(33)	0	(0)	1	(33)	1	(33)	2	(33)
TB lymphadenitis	4	(24)	3	(43)	0	(0)	7	(26)	0	(0)	1	(33)	1	(33)	2	(33)
Osteo-articular TB	1	(6)	1	(14)	0	(0)	2	(7)	0	(0)	0	(0)	0	(0)	0	(0)
Abdominal TB	5	(29)	1	(14)	1	(33)	7	(26)	0	(0)	1	(33)	1	(33)	2	(33)
Total [2,3]	17	(15)	7	(27)	3	(27)	27	(18)	0	(0)	3	(15)	3	(27)	6	(10)
All EPTB (incl. in PTB cases) [2,3]	**44**	**(32)**	**19**	**(50)**	**11**	**(58)**	**74**	**(38)**	**8**	**(20)**	**4**	**(19)**	**6**	**(46)**	**18**	**(24)**
All TB cases [2]	**139**	**(100)**	**38**	**(100)**	**19**	**(100)**	**196**	**(100)**	**41**	**(100)**	**21**	**(100)**	**14**	**(100)**	**76**	**(100)**

[1] The HIV status of 5 (2%) cases was not known: three of these children had PTB (aged 4 months, 2 years and 5 years); one 4 month old had suspected abdominal TB; and one child aged 4 months was empiric TB treatment for a chronic febrile illness without a clear anatomical focus.

[2] Percentages are shown in parenthesis. Those in bold refer to proportions of the total number of cases in each diagnostic category (columns). Other percentages refer to the proportions of children in each subgroup of PTB or EPTB within that diagnostic category.

[3] Note sites of extra-pulmonary TB are not mutually exclusive. The total number of EPTB cases in each case represents the number of children with evidence of extra-pulmonary TB, not the total number of sites of extra-pulmonary TB.

3.3.5 Inpatient burden of childhood TB

There were 8,826 children admitted to KDH during the two years of comprehensive screening and enrolment between 1 August 2009 and 31 July 2011, of whom 547 (6%) were investigated for suspected TB and 80 (0.9%) were diagnosed or treated presumptively for TB. At CPGH 999 of 9,917 (10%) paediatric inpatients were investigated for TB during the study period from 1 January 2010 to 31 December 2011 but the proportion of TB cases was similar: 110 of 9,917 children admitted (1.1%).

3.4 Discussion

Descriptions of the clinical spectrum of childhood TB have changed very little since those of classic studies of European and North American children in the pre-chemotherapy era.[8, 12, 53, 93, 99] This is perhaps surprising, since there are several potential factors one might expect to modify the clinical presentation of disease in the highest burden countries today, including population differences in host genetics, HIV co-infection, and wide scale neonatal BCG vaccination. The scale and detailed longitudinal descriptions of some of these early studies do provide invaluable insights into the natural history of childhood TB in the absence of treatment. However continued reliance on these accounts also reflects a paucity of high quality studies describing in detail the contemporary clinical spectrum of childhood TB.

3.4.1 Clinical spectrum of childhood TB in the KIDS TB Study

Broad recruitment criteria and prospective collection of detailed clinical data on paediatric TB cases in the KIDS TB Study allowed a careful description of the spectrum of childhood tuberculosis among Kenyan children presenting to a rural and an urban hospital. The high number of TB suspects and cases among young children <5 years old, with relatively few cases among children aged 5 to 9 years (the 'safe school years'), is consistent with classic descriptions. However we did not observe the expected rise in the number of cases in early adolescence, probably at least in part due to the small number of children investigated in this age group (best illustrated by Figure 3.3). Case ascertainment was particularly difficult among young adolescents, as quantified and discussed in Chapter 7. Interestingly, the age distribution of our cases was not dissimilar to a contemporary study in Cape Town, South Africa.[99] Plausible explanations include

similarities in healthcare seeking behavior among young adolescents in the two settings; a much higher burden of TB in very young children in Cape Town[33] (so that adolescents contribute a smaller proportion of total caseload); and/or real differences in the age of presentation compared with the pre-chemotherapy, pre-HIV literature.

The importance of HIV as a risk factor for TB is well documented,[100-104] and is explored and discussed further in chapter 4. The proportion of HIV co-infected TB cases in our study (27%) is below the national average of 41% for all TB cases in Kenya, but probably in keeping with a lower prevalence of HIV among women aged 15-49 in Coast Province (4.4% compared with the national average of 8.0%).[59] Similar proportions of PTB and EPTB among children with and without HIV co-infection are consistent with other reports of HIV associated paediatric TB suggesting a similar clinical spectrum of disease.[103-106] Nevertheless it is interesting to note the high proportion of miliary TB and TBM cases among children with EPTB and HIV co-infection in our cohort, and the absence of any cases of pleural TB (except in association with PTB). Although small numbers preclude drawing any strong conclusion from these findings regarding the overall case mix, the observations are consistent with declining cell mediated immunity in HIV, leading to mycobacterial dissemination and impaired tuberculous hypersensitivity reactions.

While diminished host immune responses promote active TB in HIV infected children, BCG vaccination has been shown to provide good protection against severe forms of TB disease in both HIV infected and uninfected children.[107, 108] Despite a high BCG vaccination coverage among our study population that limits analysis of BCG protective efficacy (see chapter 4), we did observe an association between smear positive PTB and absence of a BCG scar. This might be due to vaccine protection against severe PTB; it might also plausibly be due to impaired host immunity curbing the response to both vaccine (resulting in the lack of a BCG scar) and wild type infection in some children.

3.4.2 Multi-drug resistant tuberculosis (MDR-TB)

The low proportion of MDR-TB cases in our study is in keeping with similarly low national estimates. In Kenya in 2010, MDR strains were estimated to account for <0.01% of all new TB cases.[58] Although our estimate is based on a relatively small number of culture positive cases, and therefore lacks precision, it is nevertheless important as the only prospective data from Kenya on the proportion of childhood TB cases with MDR-TB

- and, by implication, the proportion of currently circulating strains that are multi-drug resistant.

3.4.3 Case definitions of childhood tuberculosis

Our study does have some limitations. In particular, without a reliable gold standard for diagnosis our case definitions of childhood TB are necessarily imperfect. Like other studies and in keeping with international recommendations,[70, 72] our case definitions reflect a hierarchy of diagnostic certainty, and were based on published case definitions at the time the protocol was written, taking into account all available diagnostic information. New standardized case definitions for childhood TB research have recently been published based on expert consensus, and in many respects are similar to the definitions used in our study.[72] They are however limited to intra-thoracic TB and have not yet been evaluated. While future analyses might explore the performance and implications for our results of these new criteria, the analyses presented in this thesis used the case definitions set out *a priori* in the study protocol.

The definition of confirmed TB as isolation of *M. tuberculosis* in the appropriate clinical context is consistent with other studies and highly specific, but very insensitive. Our detailed definitions of clinically highly probable TB attempt to improve diagnostic sensitivity at minimal cost to specificity. However even these have limitations, including omission of some classic forms of PTB (e.g. expansile pneumonia) and some sites of EPTB (e.g. pericardial and urogenital TB, and non-vertebral osteo-articular TB); a lack of alternative (e.g. imaging) criteria for diagnosis of TBM in children in whom lumbar puncture is contraindicated; and exclusion of HIV positive children from the radiological diagnosis of miliary TB due to overlap of the CXR appearances with conditions such as lymphocytic interstitial pneumonitis (LIP) in this group.

Despite our rigorous protocol there are also aspects of the diagnostic process that might be strengthened, including CXR interpretation. Current data used in these analyses are based on CXR interpretation by the study clinicians rather than specialist paediatric radiologists. Some misclassification is therefore likely despite (informal) training and practice in x-ray interpretation for diagnosis of childhood TB and use of a standardized CXR reporting form.[72-74] Very limited availability of other imaging modalities such as CT (of chest, abdomen or brain), abdominal ultrasound, and echocardiography also limited diagnosis in some cases, particularly in relation to EPTB.

Nevertheless, the non-selective nature of our cohort combined with the clinical, radiological and laboratory diagnostic tools that were available, provides a rare description of the full clinical spectrum of TB among children presenting to two Kenyan hospitals. Future analyses will incorporate improved radiology data following formal review of the CXRs by specialist radiologists; and attempt to improve TB case classification by refining the current definitions and/or applying retrospectively the consensus definitions recently published.

3.4.4 Healthcare burden of childhood TB and the need for better diagnostic tools

Broad screening criteria at KDH, and the easy availability of a paediatric TB diagnostic service at CPGH, allowed us to quantify the burden of childhood TB at each hospital (presented here) and to estimate the incidence of childhood TB in Kilifi (chapter 7). Due to limited available data on the denominator of outpatient presentations we limited our estimates to the proportions of the presenting inpatient caseloads in which TB was suspected or diagnosed. Although only a small proportion of children admitted to hospital are diagnosed with TB (~1% at both hospitals), the true burden of TB on the inpatient services derives from the much larger number of children in whom TB was suspected.

Proper investigation of such large numbers of children to rule TB in or out is difficult and resource intensive with currently available tools (including tuberculin skin tests, chest radiography and appropriate specimen collection and processing), and is not affordable within the already stretched clinical budgets of these and most other hospitals in Africa. This reinforces the very pressing need for better diagnostic tools for childhood TB in low resource settings. It is noteworthy that despite recent advances in specimen collection and processing,[109] diagnosis for the vast majority of children has changed little in the last century. Better and thereby earlier diagnosis should shift the presenting clinical spectrum of childhood TB towards less severe forms of the disease, and thereby improve patient outcomes.

4 RISK FACTORS FOR CHILDHOOD TUBERCULOSIS

4.1 Introduction

A child's risk of infection by *M. tuberculosis* is dependent on the probability, duration, and proximity of exposure to an infectious case, and on the infectiousness of the source.[87, 110-113] Important determinants of exposure include the prevalence of TB in the community and social factors such as population density and crowding, socio-economic status (poverty), and age-related social mobility.[10, 87, 114] Following infection, several host factors including age,[14, 87] and nutritional,[115] vaccination,[107] and immune status[14, 116-119] (and possibly mycobacterial virulence factors[120-122]) then influence the balance of risk between latent tuberculosis infection or progression to active disease.

Although the importance of each of these risk factors is well established, their relative contribution to the burden of childhood TB at a population level is less well described, and is likely to vary between populations with different social, demographic and health indices. We explored socio-demographic and host risk factors for TB among children enrolled in the KIDS TB study, and estimated the impact of each factor on the childhood TB burden in the study population, with the aim of providing data to inform potential public health intervention strategies. Results from this analysis of epidemiological risk factors were also used to develop clinical tools for risk stratifying children with suspected TB as part of an improved diagnostic work up (see chapter 5).

4.2 Methods

4.2.1 Selection of cases and controls for nested case control analyses

We used a nested case control design to explore associations between putative clinical and epidemiological risk factors and a diagnosis of either confirmed or highly probable TB among children recruited to the KIDS TB Study (see Figure 2.4C, page 39).

Choice of cases: We performed three separate case control analyses using each of the following three case definitions:

(a) Confirmed TB (CTB)
(b) Highly probable TB (HPTB)
(c) Confirmed or highly probable TB (CHPTB).

Choice of controls: We selected as controls children in the "Not TB" group, in whom TB had been confidently excluded. Children with "possible TB" whose true disease status is unclear were excluded from the analyses.

Justification for using CHPTB group: To explore the validity of presenting summary effects of each putative risk factor on the combined CHPTB group, we also performed a fourth case control analysis to compare CTB cases with children who had HPTB (the "controls" for this analysis).

4.2.2 Choice and definition of exposure variables

Putative epidemiological and host risk factors for TB were chosen on the basis of current understanding of the pathophysiology of TB and known risk factors in other settings. They included TB contact history, age, nutritional status, HIV co-infection status, BCG vaccination status, and measures of both socio-economic status and domestic crowding. Since vaccination cards were available for a minority of children recruited we used the presence of a BCG scar as a surrogate for BCG vaccination status. We included maternal education history in the analysis as the best available surrogate for socio-economic status; and the number of occupants in the child's household as a crude measure of domestic crowding.

4.2.3 Univariable analysis of risk factors for childhood TB

The distribution of each variable among cases and controls was summarized and crude odds ratios (OR) and 95% confidence intervals (CI) were derived for associations between each exposure variable and TB diagnostic category. Likelihood ratio tests for a general association were performed and p values reported. Comparisons of continuous variables were performed using Student's t test.

Since controls in the active (contact tracing) case detection arm are a different population from controls in the passive case detection arm - the former are mostly well TB contacts followed up from home whereas the latter have presented to hospital because they are sick - we did not assume that disease associations would be the same in each study arm. Instead we performed the analyses separately for children in the active and passive case detection arms of the study and compared our findings.

To explore the association between markers of TB contact and a diagnosis of TB in the passive case detection arm of the study, we used children with no history of TB contact

as our baseline group for comparison with each of the other strata of a particular contact exposure variable. Since, by definition, all children in the active case detection (contact tracing) arm of the study had a history of TB contact, comparisons in this arm of the study were made against the baseline stratum representing 'least' contact for each TB contact variable. Since some children had a history of more than one TB contact, such that contact relationships for each child were not mutually exclusive, we assumed that an individual child had an equal probability of acquiring TB from each of their contacts. We then created a separate record for each child-contact pair, and weighted each of these pairs in the analysis by the reciprocal of the number of TB contacts reported for each child.

We performed χ^2 tests for homogeneity to explore interactions between individual variables, and to assess further whether the effect of each variable differed between the active and passive case detection arms, and between inpatients and outpatients. Where no interaction was demonstrated summary odds ratios were derived using Mantel-Haenszel methods.

4.2.4 Multivariable analysis of risk factors for childhood TB

We then derived multivariable logistic regression models to identify independent risk factors for TB. Categorical variables with at least a weak association with TB in the univariable analysis (likelihood ratio test p value ≤0.1) were included in the model. We performed backwards stepwise logistic regression using standard selection criteria, such that variables that were not significantly associated with TB (Wald p value <0.5) were sequentially dropped from the model. Likelihood ratio tests were used to test for potential interactions in the final model. Based on this model adjusted odds ratios and 95% confidence intervals were derived for the associations with TB of each variable included; p values for each association were derived using the Wald test.

4.2.5 Attributable fractions

The population attributable fraction (PAF) is a measure of the burden of disease associated with a particular exposure (also known as the proportional attributable risk or aetiologic fraction) in a population.[123] We calculated adjusted (population) attributable fractions (PAF_{adj}) for each risk factor in the final model derived for the passive case detection study arm, using the formula

$$\mathrm{PAF}_{adj} = p'(\theta - 1)/\theta,$$

where p' is the proportion of cases with the risk factor and θ is the adjusted odds ratio, under the assumption that the odds ratio is approximately equivalent to the risk ratio for a rare disease.

Since θ represents the odds ratio in the population of children with suspected TB rather than in the community, we also estimated the PAF for contact with a known TB case in the population as a whole. We confined this analysis to children under 5 years old and to the 2 year period of comprehensive enrolment into the passive case detection arm of the study (1 August 2009 to 31 July 2011; see Figure 3.1, page 42). We first determined the number of KHDSS resident children under 5 years old (N), and the number of KHDSS resident TB cases (TB_{khdss}; see section 7.2.4, page 142), during this period; and the mean number of child household contacts under 5 years old per TB case ($\bar{C}_{household}$; see section 7.2.5, page 143). We then estimated the total number of KHDSS resident child contacts of known TB cases ($N_{contacts}$) as

$$N_{contacts} = \frac{TB_{khdss} \times \bar{C}_{household}}{H}$$

where H is the proportion of all documented contacts that came from within a child's household. We estimated the number of person years observation among all KHDSS resident children (pyo_{total}) and among children with a known close TB contact ($pyo_{contacts}$) during the same 2 year recruitment period as:

$$pyo_{total} = N \times 2years$$

$$pyo_{contacts} = N_{contacts} \times 2years.$$

We calculated the rate ($I_{contacts}$) of TB among child contacts of known TB cases as

$$I_{contacts} = \frac{N_{contacts}}{pyo_{contacts}}$$

and the rate ($I_{non\text{-}contacts}$) of TB among children with no known TB contact as

$$I_{non-contacts} = \frac{N - N_{contacts}}{pyo_{total} - pyo_{contacts}}$$

Finally we calculated the PAF for a history of known TB contact as

$$PAF = p_{cases}\left(\frac{I_{contacts}}{I_{non-contacts}} - 1\right)\Big/\left(\frac{I_{contacts}}{I_{non-contacts}}\right)$$

where p_{cases} is the proportion of cases in the passive case detection arm with a known history of TB contact.

4.3 Results

4.3.1 Comparison of confirmed and highly probable TB cases

Children with CTB tended to be older than those with HPTB, but there were no differences between the two groups in any of the putative risk factors of interest (Appendix Table 10.1, page 181). The mean number of household occupants also did not differ between CTB and HPTB cases (5.7 vs. 6.0; p=0.770). These results provide statistical justification for inclusion of the combined CHPTB group in the following analyses of risk factors for TB.

4.3.2 Referral source and socio-demographic and social variables

Associations of referral source and socio-demographic variables with CTB, HPTB and CHPTB are shown in Table 4.1. Cases tended to be older than children in whom TB had been excluded, with median ages of 37 months among CHPTB cases groups compared with 17 months among children without TB (Table 3.4, Table 4.1). However there was no association between the proportion of children in each TB diagnostic category and the child's sex or the mother's educational status. The mean number of occupants in a child's household did not differ between children with CHPTB and those without TB (6.0 vs. 6.3; p=0.764).

4.3.3 Host factors: HIV infection, nutritional status and BCG vaccination status

Associations between each TB category and HIV, malnutrition and BCG scar status are shown for the passive and active case detection arms in Table 4.2. Compared with HIV negative children, those with HIV had double the odds of CHPTB in the passive case detection arm (odds ratio 2.1, 95% confidence interval 1.3 to 3.2, p=0.001).

In the active case detection arm of the study HIV data were not available for 30/87 (34%) controls. None of the 6 CHPTB cases and only 1 of the 57 controls for whom HIV data were available were HIV co-infected, so although there was no demonstrable effect of HIV on the risk of TB among this group the power to detect an effect was small. There was no statistical evidence that the effect of HIV on TB differed between the two study arms (χ^2 test for homogeneity, p=0.641), but power to detect a difference was similarly limited by small numbers.

The odds of TB were higher among children with severe malnutrition in both the passive (OR 1.5, 95% CI 1.0 to 2.1) and active (43, 2.3 to 801) case detection arms. Wide confidence intervals reflected the poor precision of the estimated OR in the active case detection arm due again to small numbers, but the association between malnutrition and TB did nevertheless appear to be stronger among children identified through contact tracing (χ^2 test for homogeneity, p=0.001).

After adjusting for the effect of HIV infection status there was only weak evidence for an independent association between malnutrition and CHPTB (adjusted OR 1.4, 0.9 to 2.0, p=0.104). There was no evidence that malnutrition confounded or modified the effect of HIV infection status on a diagnosis of CHPTB (OR for association of HIV with TB among children with and without severe malnutrition 2.0 (1.1 to 3.7) and 1.9 (1.0 to 3.5), $\chi 2$ test for heterogeneity 0.04, p=0.836).

A BCG scar was present in 1,773/1,979 (89.6%) children enrolled in the study for whom data were available. There was no statistical evidence of an effect of BCG status on any of the TB diagnostic endpoints (Table 4.2), nor in any subgroups defined by study recruitment arm or HIV, malnutrition status, or TB contact status (data not shown).

Table 4.1 Association of socio-demographic variables with confirmed and highly probable TB

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
CONFIRMED TB (CTB)					
Referral source					
Inpatient	55	956	2.5	(0.6 to 10.5)	
Outpatient	13	258	2.0	(0.4 to 9.0)	0.294
Contact tracing	2	87	1.0		
Age category					
< 1 year	11	469	1.0		
1 to 4 years	25	658	1.6	(0.8 to 3.3)	
5 to 9 years	17	139	5.2	(2.4 to 11.4)	<0.001
10 to 14 years	14	50	11.9	(5.1 to 27.7)	
Male sex	38	699	1.1	(0.7 to 1.8)	0.749
Maternal primary education [1]	7/8	197/281 [2]	3.0	(0.4 to 24.8)	0.288
HIGHLY PROBABLE TB (HPTB)					
Referral source					
Inpatient	39	956	0.9	(0.3 to 2.5)	
Outpatient	20	258	1.5	(0.5 to 4.6)	0.178
Contact tracing	2	87	1.0		
Age category					
< 1 year	13	469	1.0		
1 to 4 years	36	658	2.0	(1.0 to 3.8)	
5 to 9 years	9	139	2.3	(1.0 to 5.6)	0.084
10 to 14 years	4	50	2.9	(0.9 to 9.2)	
Male sex	33	699	1.0	(0.6 to 1.6)	0.918
Maternal primary education [1]	10/13 [2]	197/281 [2]	1.4	(0.4 to 5.3)	0.599
CONFIRMED OR HIGHLY PROBABLE TB (CHPTB)					
Referral source					
Inpatient	94	956	1.4	(0.6 to 3.4)	
Outpatient	33	258	1.7	(0.7 to 4.1)	0.473
Contact tracing	2	87	1.0		
Age category					
< 1 year	24	469	1.0		
1 to 4 years	61	658	1.8	(1.1 to 2.9)	
5 to 9 years	26	139	3.7	(2.0 to 6.6)	<0.001
10 to 14 years	18	50	7.0	(3.6 to 13.8)	
Male sex	71	699	1.0	(0.7 to 1.5)	0.875
Maternal primary education [1]	17/21 [2]	197/281 [2]	1.8	(0.6 to 5.6)	0.292

[1] Data on maternal education was only available and analyzed for KHDSS residents.

[2] In cases where data was missing the denominator for which data was available is presented.

Table 4.2 Associations of host factors with confirmed and highly probable TB in the passive and active case detection arms

		TB Cases		Controls				
		factor present	factor absent	factor present	factor absent	Odds ratio for TB (95% CI)		p value
PASSIVE CASE DETECTION ARM	**CONFIRMED TB**							
	HIV infection [1]	17	50	162	1,020	2.2	(1.2 to 3.8)	0.008
	Severe malnutrition	30	38	461	779	1.3	(0.82 to 2.2)	0.250
	BCG vaccination scar	60	7	1,101	116	0.9	(0.4 to 2.0)	0.804
	HIGHLY PROBABLE TB							
	HIV infection [1]	14	45	162	1,020	2.0	(1.1 to 3.7)	0.030
	Severe malnutrition	29	30	461	779	1.6	(0.97 to 2.8)	0.064
	BCG vaccination scar	49	8	1,101	116	0.7	(0.3 to 1.4)	0.272
	CONFIRMED & HIGHLY PROBABLE TB							
	HIV infection [1]	31	95	162	1,020	2.1	(1.3 to 3.2)	0.001
	Severe malnutrition	59	68	461	779	1.5	(1.0 to 2.1)	0.040
	BCG vaccination scar	109	15	1,101	116	0.8	(0.4 to 1.4)	0.360
ACTIVE CASE DETECTION ARM	**CONFIRMED TB**							
	HIV infection [1]	0	2	1	56	0.0		0.851
	Severe malnutrition	0	2	1	86	0.0		0.880
	BCG vaccination scar [2]	2	0	78	9	-		-
	HIGHLY PROBABLE TB							
	HIV infection [1]	0	4	1	56	0.0		0.791
	Severe malnutrition	2	2	1	86	86.0	(2.9 to 2,517)	<0.001
	BCG vaccination scar [2]	4	0	78	9	-		-
	CONFIRMED & HIGHLY PROBABLE TB							
	HIV infection [1]	0	6	1	56	0.0		0.746
	Severe malnutrition	2	4	1	86	43	(2.3 to 801)	<0.001
	BCG vaccination scar [2]	6	0	78	9	-		-

[1] HIV status was missing for 1/68 (1.5%) confirmed TB cases and 58/1240 (4.7%) controls in the passive arm; and for 30/87 (34%) controls in the active arm.

[2] BCG scar status was missing for 1/68 (1.5%) confirmed TB cases and 23/1240 (1.9%) controls in passive arm.

4.3.4 TB contact history

A history of close TB contact was strongly associated with both CTB (OR 6.0, 95% CI 3.5 to 10.0) and HPTB (OR 5.4, 3.1 to 9.5) in the passive case detection arm. Table 4.3 below shows in more detail the associations of each contact history variable with CHPTB in this group. Results of separate analyses for CTB and HPTB were very similar and are presented separately in Table 10.2 and Table 10.3 in the appendix. Contact smear status and proximity both showed a dose response relationship with TB risk. Contact with a smear positive pulmonary TB case increased the odds of TB in a child seven fold, compared with just over 4 fold for contact with a smear negative case. Among children with non-household contact, household contacts, and a contact who slept in the same room, the odds of TB increased two, four and eight fold, respectively. Consistent with the observed high risk among children sharing a room with their contact, TB risk was also highest among children whose parent or sibling had TB. There was no evidence that proximity modified the effect of contact smear status on a child's risk of developing active TB (χ^2 test for heterogeneity, p=0.309). In a multivariable analysis of TB contact proximity, smear status and number, only proximity was independently associated with CHPTB (OR for TB for each level of increasing proximity 1.8, 1.3 to 2.6; p=0.001).

Among children who had a history of TB contact and were admitted to hospital for any reason, one in four (57 of 215, 26.5%) was diagnosed with CHPTB. Crude odds ratios for the association of TB contact with confirmed or highly probable TB were higher among inpatients (OR 7.4, 95% CI 4.5 to 12.0) than among outpatients (OR 95% CI 3.5, 1.6 to 7.7), but with only weak evidence that inpatient status modified the effect (χ^2 test for homogeneity, p=0.110).

For children enrolled in the active case detection arm of the study (all of whom were contacts of smear positive pulmonary TB cases) we explored the effect of TB contact number, domestic proximity, and each contact's relationship to the child on that child's risk of TB. Small numbers and no TB cases in many strata prevented meaningful analysis of associations with confirmed TB and highly probable TB separately (see Appendix **Table 10.4** and **Table 10.5**, page 183). Analysis using the CHPTB endpoint failed to demonstrate any association between contact proximity, relationship or number and TB risk, but was also limited by small numbers (**Table 4.4**).

Table 4.3 Association of TB contact history with CHPTB (passive case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
History of close TB contact [1]					
No history of close TB contact	67	1,058	1.0	(-)	<0.001
Any history of close TB contact	57	158	5.7	(3.8 to 8.5)	
Proximity of TB contact [2]					
Contact outside the household	5	41	2.1	(0.8 to 5.5)	<0.001
Contact sleeps in same household	52	56	4.5	(2.2 to 9.1)	
Contact sleeps in same room	41	148	8.0	(5.1 to 12.5)	
Smear status of TB contact [2]					
Smear positive	33	71	7.3	(4.5 to 11.9)	<0.001
Smear negative	17	59	4.5	(2.5 to 8.2)	
Smear status unknown	7	28	3.9	(1.7 to 9.4)	
Relationship of contact to child [2,3]					
Parent	35	76	7.6	(4.8 to 12.0)	<0.001
Grandparent	5	22	3.6	(1.3 to 9.8)	
Aunt or uncle	11	38	4.9	(2.4 to 9.7)	
Sibling	3	7	6.8	(1.7 to 26.8)	
Other	5	21	3.8	(1.4 to 10.3)	
Number of close TB contacts [2]					
One close TB contact	52	146	5.6	(3.8 to 8.4)	<0.001
More than one close TB contact	5	12	6.6	(2.3 to 19.2)	

[1] TB contact history was not available for 3/59 (5%) HPTB cases and 24/1240 (2%) controls.

[1] compared with children who had no history of TB contact.

[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

Table 4.4 Association of TB contact history with CHPTB (active case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
Proximity of TB contact [1]					
Contact sleeps in same household	1	17	1.0		0.797
Contact sleeps in same room	5	67	1.6	(0.8 to 3.1)	
Relationship of contact to child [2]					
Parent	4	69	1.0		0.260
Grandparent	0	8		-	
Aunt or uncle	1	5	3.5	(0.3 to 37.0)	
Sibling	1	2	8.6	(0.6 to 117)	
Other	0	1		-	
Number of close TB contacts					
One close TB contact	6	85	1.0		-
More than one close TB contact	0	2		-	

[1] Three controls recruited as close non-household contacts excluded from proximity analysis.

[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

4.3.5 Multivariable analysis of risk factors for TB

Due to small numbers of children in the active case detection arm, multivariable analysis of risk factors for TB was confined to children in the passive case detection arm of the study. TB contact was included as a single binary variable. TB contact history and severe malnutrition were independently associated with both CTB and HPTB in separate logistic regression models. In a model using the combined endpoint of CHPTB, HIV infection was also an independent risk factor. Adjusted odds ratios for each diagnostic endpoint derived from each of the 3 models are shown in **Table 4.5**.

Table 4.5 Adjusted odds ratios for risk factors included in the final models

Risk factor	Adjusted Odds Ratio (95% CI)	p value
Confirmed TB		
TB contact	6.5 (3.9 to 10.9)	<0.001
Severe malnutrition	1.7 (1.0 to 2.9)	0.042
Highly Probable TB		
TB contact	6.2 (3.5 to 10.9)	<0.001
Severe malnutrition	2.2 (1.2 to 3.8)	0.007
CHPTB		
TB contact	6.4 (4.2 to 9.6)	<0.001
Severe malnutrition	1.7 (1.1 to 2.7)	0.030
HIV infection	1.7 (1.1 to 2.5)	0.012

4.3.6 Attributable Fractions

Attributable fractions adjusted for each independent risk factor in the final models are presented for CTB, HPTB and CHPTB in Table 4.6. There was only slight variation between each diagnostic endpoint in the PAF derived for each variable, reflecting similar adjusted odds ratios in the three models. A history of a known TB contact was the most important determinant of TB among children in this study, to which ~40% of the caseload was attributed. The attributable fraction of HIV CHPTB cases was 10% in this population.

There were 678 KHDSS resident TB cases during the 2 years enrolment into the passive case detection arm. We identified 362 household contacts under the age of 5 years for 195 index cases, and 343 TB contacts of whom 297 were in the child's household. The estimated total number of KHDSS resident child contacts was therefore calculated as

$$N_{contacts} = 678 \times \frac{362}{195} \times \frac{343}{297} = 1{,}454.$$

Of 17 CHPTB cases among children under 5 years recruited during 2 years passive case enrolment at KDH (**Table 7.3**, page 148) 9 had a known history of TB contact. The total person years observation among all children under 5 years was 89,503 (**Table 7.3**, page 148). Using these figures the estimated population attributable fraction of a known TB contact in this community was 51.4%.

Table 4.6 Adjusted population attributable fraction for risk factors included in the final models

Risk factor	Population attributable fraction, % (95% CI)	
Confirmed TB		
TB contact	39.8	(35.0 to 42.7)
Severe malnutrition	18.2	(0.0 to 28.9)
Highly Probable TB		
TB contact	37.4	(31.9 to 40.5)
Severe malnutrition	26.8	(8.2 to 36.2)
CHPTB		
TB contact	38.8	(35.0 to 41.2)
Severe malnutrition	19.1	(4.2 to 29.3)
HIV infection	10.1	(2.2 to 14.8)

4.4 Discussion

We analyzed associations between putative epidemiological risk factors and active TB among children recruited to the KIDS TB study. We recognized that the nature and magnitude of any effects were unlikely to be the same among children enrolled in the active (contact tracing) and passive case detections arms of the study due to inherent differences in the groups targeted including their TB contact history. We therefore performed analyses separately for each study arm. Although small numbers limited interpretation of some of our findings in the active case detection arm, the observed difference between the two study arms in the effect of malnutrition on TB lends support for this approach. Performing the analyses separately also allowed proper assessment of the effect of TB contact history in the passive case detection arm by removing bias due to inclusion of both cases and controls ascertained purely on the basis of TB contact in the active case detection arm.

For completeness, and to mitigate against any form of incorporation bias inherent in the definitions of highly probable TB, we also performed analyses separately for each TB

diagnostic endpoint: CTB, HPTB, and the combined endpoint of CHPTB. Associations with the epidemiological variables studied were nevertheless very similar for all three endpoints, with no statistical evidence of a difference between CTB and HPTB. Presentation of the combined results for confirmed and highly probable TB (CHPTB) therefore appears justified.

In interpreting our results and their generalizability it is important to keep in mind that each of the children included in the study was enrolled because they fulfilled one or more criteria for suspected TB (see section 2.3, page 28). Particular caution needs to be exercised in interpreting the apparent association of older age with both CTB and HPTB. This result needs to be reconciled with the age-specific incidence estimates derived in this study (chapter 7) and data from several other studies which demonstrate a different pattern of association, with the highest burden of TB among infants and young children, lower rates of disease among school aged children (the so-called "safe school years"), and then a rise in incidence in early adolescence.[12, 87] Two factors are likely to explain this discrepancy. Firstly, age-related differences in microbiological yield and TST responses confound the association, since older children with TB are more likely to be sputum smear or culture positive for *M. tuberculosis*,[8] and to have a positive TST (due to a longer duration of potential exposure and relatively more mature immune responses) - and therefore to meet our stringent criteria for both confirmed and highly probable TB. Secondly, the strong association with young age of conditions such as malnutrition in the differential diagnosis for childhood TB enriched the number of young children in the control group, further confounding the association between age and a diagnosis of TB.

Nevertheless our results do provide important insights into the determinants of childhood TB in the study population. The independent association of TB with both HIV infection and malnutrition in the multivariable analysis is consistent with other studies[104, 115] and current understanding of host determinants of disease.[115, 124, 125] In the case of malnutrition causality cannot be assumed since TB itself causes wasting, so this association is likely to reflect an effect in both directions.[115]

Although wide confidence intervals reflected poor precision of the odds ratio for the association of malnutrition with TB in the active case detection arm, a test for interaction did nevertheless provide evidence that the size of the effect was greater than in the passive case detection arm. This reflects the relatively healthy status of most control children recruited through contact tracing contrasted with the high prevalence

of severe malnutrition among controls in the passive case detection arm. The analyses were not powered to make any inferences about associations with HIV in the active case detection arm.

There was no statistical evidence of an association between TB and the presence or absence of a BCG scar in this study. The protective efficacy of BCG vaccination has been repeatedly demonstrated in other studies,[107] and the point estimate is in the direction anticipated; however high vaccination coverage and a relatively small number of TB cases limit the study's power to detect an effect. Although some children without a discernable scar may have been BCG vaccinated, misclassification is unlikely to account for the lack of an observed effect since the proportion of children without a scar was small.

Poverty and population density/crowding are two of the strongest determinants of tuberculosis risk.[113, 114, 126, 127] In an attempt to measure and control for these in our analyses we used maternal education status and household occupancy as the best easily available surrogate measures of socio-economic status and crowding, respectively. However we were unable to demonstrate any significant association of either variable with TB among children enrolled in the study. This may be because neither measure is a good surrogate marker in the study population, or because neither factor is a strong risk factor for TB in Kilifi, or both. It is certainly plausible that domestic crowding is not an important determinant of risk in the semi-urban and rural populations studied in Kilifi; this combined with a 'dilution' of any effect due to the use of a crude measure of crowding could easily explain the lack of any association with household occupancy. It is interesting to note the suggestion of a trend towards higher risk of TB among children whose mothers had been to school. The most likely explanation is confounding due to a strong association between no maternal schooling and other conditions, including malnutrition, which led to admission of many of the controls.

The most important finding is the very strong association between TB in a child and close contact with a known case of TB. The importance of this association derives not just from the strength of the effect, but also from the high proportion of children who gave a history at presentation of contact with a known TB case. It has two important implications for clinical practice and public health policy.

Firstly, ascertainment of any history of TB contact should become a standard part of the

assessment of every sick child in TB endemic settings. It is remarkable that among inpatients in our study one in four of those who gave a history at presentation of a known close TB contact were found to have either confirmed or highly probable TB. Importantly TB contact was not included in the stringent definitions of highly probable TB, nor did associations with contact history differ between CTB and HPTB, so incorporation bias is unlikely. The strong implication is that if this high-risk group of children could be identified and TB considered and investigated early, clinical benefits would follow from earlier diagnosis and treatment.

Secondly, the need for early identification and provision of isoniazid chemoprophylaxis to child contacts of TB cases through active contact tracing needs to be reinforced and rapidly pushed up the public health agenda. Although this is covered by existing policy recommendations,[21] progress in this area has been slow in many settings including Kenya.[128] One barrier to wider implementation might be a lack until now of published evidence for the likely impact of the intervention on the overall burden of childhood TB at the population level. Our finding that about 40-50% of childhood TB cases could be attributed to a known close TB contact, and are therefore preventable by this strategy, provides strong endorsement for the policy.

Estimation of population attributable fractions assumes that the measured associations are entirely causal, and that removal of a risk factor would remove the risk attributed to it. Having carefully explored and controlled for potential confounders in our multivariate analysis we are confident that both these assumptions hold true for HIV and TB contact. However the association of TB with malnutrition is likely not to be entirely causal as already discussed (and reviewed in detail elsewhere[115]), so the first assumption is violated and the attributable fraction for malnutrition calculated cannot be interpreted.

One further assumption in our analysis, and an important potential limitation, is that the prevalence of risk factors in the study population reflects their community prevalence. This may be a fair assumption for TB contact history, since presentation to hospital is not directly associated with TB contact. However, since both TB prevalence and general health indices (and by implication admission rates) are linked to other factors such as poverty, crowding and HIV, it is possible that the prevalence of TB contact among hospitalized children is higher than the community prevalence; this would probably bias our estimate downwards by reducing the size of the observed effect of TB contact. We

also confined our analysis to children in the passive case detection arm (excluding children with a history of TB contact in the active case detection arm), which is likely to underestimate further the true attributable fraction by reducing the measured prevalence of TB contact history among cases.

We therefore used data from the NTP TB Register and the KHDSS to calculate the attributable fraction using estimates of the number of children in the study population with and without a household TB contacts. We confined this analysis to children under 5 years for two reasons. Firstly, it is well documented from natural history studies in the pre-chemotherapy era that >90% active TB disease in this age group occurs within 2 years of infection.[8] The number of contacts identified during the 2 year recruitment period therefore provided a good estimate of the likely number of contacts putting children at risk, since the overall rate of TB notifications in the study population is constant. Secondly, this is the group targeted for isoniazid chemoprophylaxis since they are the most vulnerable.[21] This method gave a higher estimated population attributable fraction of approximately 50%. Although this second method did not use adjusted rate ratios, the observation that the odds ratio for TB contact as a predictor of TB in the main dataset actually increased (from 5.7 to 6.4) when adjusted for potential confounders in the multivariate analysis, suggests that this is unlikely to have biased our estimate upwards.

The calculated attributable fraction for HIV is also likely to be an underestimate due to bias introduced by the relatively high proportion of HIV among hospital controls who were admitted with other complications of HIV infection. The importance of HIV as a risk factor for TB reinforces the importance of isoniazid chemoprophylaxis in this group, as well as among TB contact.

In conclusion, we sought by analysis of the risk factors for childhood TB to identify the most important determinants of disease in our study population, thereby to inform potential public health intervention strategies and clinical practice. Our results provide further support for the wider implementation of isoniazid chemoprophylaxis among child TB contacts and children infected with HIV. They also emphasize the importance of TB contact history in identifying children at higher risk of TB. The role of this in improving clinical TB diagnosis is explored further in chapter 5.

5 CLINICAL DIAGNOSIS OF CHILDHOOD TUBERCULOSIS

5.1 Introduction

Globally, the greatest burden of tuberculosis among both adults and children occurs in low and middle-income countries where resources for diagnosis are limited.[3] Although diagnosis of TB among adults has been greatly enhanced by increased access to quality assured sputum smear microscopy through the WHO DOTS and Stop TB strategies,[3] this has had little impact on the diagnosis of childhood TB since children rarely expectorate sputum and when samples are obtained they are rarely AFB smear positive.[129] As a result, diagnosis for the large majority of children relies on the presenting clinical features, aided in some settings by plain chest radiography and/or tuberculin skin tests.[21, 130] Diagnosis of TB in children is further complicated by the varied and non-specific nature of many of the presenting clinical features of childhood TB, particularly among children with HIV infection and malnutrition.[12, 53] Deciding whether a child might have TB and whether to start TB treatment is thus a major challenge faced by clinicians throughout the world on a daily basis.

Several structured approaches have therefore been developed and promoted to assist clinicians in the diagnosis of childhood TB.[24] These include numeric clinical scoring systems, whereby points assigned for particular clinical features are summed and TB diagnosis or risk stratification is determined by the total score;[71, 131-135] non-numeric diagnostic classifications;[70, 136-144] algorithms which present a structured approach to investigation and diagnosis;[21] and combined approaches.[132, 145] However the large number of different clinical decision making tools published and in use, and a lack of any consensus regarding their relative merits,[11, 24, 146] are indications of their generally suboptimal performance. This is reflected in their inconsistent use by clinicians, and derives at least in part from methodological inadequacies in their formulation.[24]

Some of these deficiencies have been identified in previous reviews,[24, 146] including the use of suboptimal 'gold standards' (often not incorporating microbiological confirmation);[66, 106, 133, 136, 137, 140, 142, 144, 147] varied and unclear definitions of the clinical characteristics included;[140] reliance on subjective clinical judgment rather than formal statistical methods for inclusion and assignment of weights to clinical parameters;[21, 70, 71, 131, 132, 135-143, 148] and failure to validate most proposed new approaches in an independent dataset or population.[24] Further limitations of many published studies of

proposed clinical tools include narrowly defined inclusion criteria (e.g. exclusion of outpatients[132, 135, 137] or children with HIV[132]) that limit generalizability; poorly defined inclusion criteria, making assessment of generalizability difficult to assess; [106] inclusion/analysis of a limited panel of potential clinical predictors in developing a clinical tool;[133] and a lack in all studies of any follow up data to optimize further the 'gold standard' diagnostic categories against which a new tool is assessed. Finally, even where data that assess the performance of a particular diagnostic approach in an independent population or dataset do exist,[66, 106, 133, 144, 147] the performance of these tools has rarely been formally assessed in the wide range of settings in which they are currently used.

National guidelines for diagnosis and treatment of TB published by the Kenyan Ministry of Public Health and Sanitation include a clinical scoring system for childhood TB diagnosis.[71] This point scoring system is based on the Keith Edwards childhood TB score originally developed among children in Papua New Guinea in the pre-HIV era,[132] modified slightly by the inclusion of abnormal chest x-ray findings. Its performance for diagnosis of TB among children in Kenya has never been properly assessed. New national guidelines for paediatric tuberculosis in Kenya are currently also in draft form, and include completely revised guidelines for childhood TB diagnosis.[149] However there are no published data on the performance of this proposed new approach either from Kenya or elsewhere.

We used detailed clinical data on children enrolled in the KIDS TB Study to explore the predictive value of individual clinical features for childhood TB in our setting. We examined the performance of published clinical diagnostic tools among the same population. Finally we derived a new tool for clinical diagnosis of childhood TB in this and similar settings, and validated it on an independent part of the dataset.

5.2 Methods

Patient selection, clinical and laboratory methods, and definitions of confirmed and highly probable TB are presented in detail in chapter 2.

5.2.1 Choice of cases and controls

Recognizing that populations of children investigated in the active and passive case detection arms are different, we confined the analysis to children identified through the passive case detection arm to ensure the generalizability of our findings to similar

hospital settings. To minimize misclassification bias we excluded from our analysis children with possible TB since their true TB status was uncertain.

We reasoned that confining cases to confirmed TB might introduce important spectrum bias by excluding children from whom microbiological confirmation might be more difficult due to differences in the severity and/or anatomical site of disease. We also recognized that inclusion of highly probable TB might introduce some misclassification bias; however we reasoned that the risk of misclassification bias was much smaller than the risk of spectrum bias given the stringent clinical criteria for HPTB. For our primary analysis we therefore used a combination of confirmed or highly probable TB (CHPTB) as cases, and children in whom TB had confidently been excluded as controls, in a nested case control analysis. For comparison we also performed separate case-control analyses comparing CTB with controls, and CTB with CHPTB, to examine any important differences.

5.2.2 Clinical definitions

Assessment of the utility of individual clinical features and published clinical diagnostic tools for childhood TB diagnosis was a key aim of the KIDS TB Study. We therefore prospectively collected detailed clinical data on all children enrolled in the study, including all the clinical variables required to assess the performance of each published clinical diagnostic tool.

We defined symptom duration as the duration at the point of enrolment. Weight loss duration and severity were assessed from the history provided by the parent or guardian, aided wherever possible by previously documented weights in the child's road to health card, clinical notes or discharge summaries. Regional lymphadenopathy was defined as the presence of one or more palpable lymph nodes ≥1 cm in diameter; and generalized lymphadenopathy as the presence of regional lymphadenopathy involving cervical, axillary and inguinal lymph node groups bilaterally. We defined a reduced level of consciousness as a Blantyre Coma Score <5, and meningism as the presence of a stiff neck, photophobia, or bulging fontanelle. To ensure the relevance of our findings to the vast majority of settings - where sputum induction is not available, but where sputum smear microscopy can be performed if a spontaneous sputum sample is obtained – for this analysis we defined "sputum AFB smear positive" as the existence at least one AFB positive sputum smear result from up to 3 spontaneous sputum samples.

5.2.3 Univariable analysis of individual clinical predictors of childhood TB

We first explored associations between individual clinical variables and childhood TB. Using each case definition we summarized the distribution of each variable with respect to TB and derived crude odds ratios (OR) and 95% confidence intervals (CI) for associations with TB; likelihood ratio tests (LRT) for a general association were performed and p values reported. The sensitivity, specificity, positive and negative likelihood ratios (LR+, LR-), and their 95% CIs were calculated for each categorical variable. To explore further the best cutoffs for continuous and ordered categorical variables as predictors of childhood TB we plotted receiver operator characteristics (ROC) curves.

Important putative confounders and/or effect-modifiers of the associations of specific variables and TB were explored by stratifying by the variable of interest and comparing odds ratios between strata. Tests for interaction were performed using the χ^2 test for heterogeneity. Where significant interaction was demonstrated odds ratios were presented separately for each stratum; otherwise summary odds ratios adjusted for the variable of interest were derived using Mantel-Haenszel methods.

5.2.4 Assessment of the performance of published clinical diagnostic tools

We confined the analysis to diagnostic tools published in the peer reviewed medical literature and to international guidelines from the WHO and Kenyan national guidelines. Published tools that failed to present diagnostic criteria in sufficient detail were excluded. For those tools that included a category of confirmed TB based on microbiological diagnosis we confined our analysis to categories defined by clinical criteria alone.

We created variables for each diagnostic score and/or diagnostic categories with close reference to the published definitions of each variable. In instances where the exact definition of a clinical variable was not clearly specified in the original publication we chose what we judged to be the most likely intended definition for application in the relevant setting and reported the definition we used.

Thus 'unexplained fever' was defined as a fever for >14 days in the absence of malaria parasitaemia or evidence of focal infection; a cutoff of at least one week's duration was

used for a history of night sweats; and 'bulky lymphadenopathy' was defined as the presence of lymph nodes sufficiently large to perform a fine needle aspirate (usually ≥2cm diameter). 'Malnutrition not responding to treatment' was defined as death, or failure to regain 10% body weight (in the case of marasmus) or failure of oedema to resolve (kwashiorkor), in a child admitted with severe malnutrition.

A 'suggestive symptom complex of TB' was included in the Ghidey-Habte diagnostic tool[137] but only vaguely defined as "*non-specific symptoms such as fever, night sweats and loss of weight, and specific symptoms related to the site invaded, e.g. cough, swelling of lymph nodes, abdominal distension, difficulty in walking, etc* ". For the purposes of our analysis we included in this definition fever, cough, night sweats, weight loss, bulky lymphadenopathy, signs of pleural effusion or ascites, gibbus, and a change in temperament or reduced level of consciousness, and required a history of at least 2 weeks duration for fever, cough, night sweats and weight loss. We then compared this tool's performance with a requirement for either ≥2 or ≥3 of these clinical features to define a 'suggestive symptom complex of TB'.

In keeping with published definitions,[72-74] a 'suggestive CXR' for TB was defined as the presence of a Ghon focus or complex, miliary infiltrate, cavities, or a pleural or pericardial effusion - unless an alternative definition was clearly presented for a particular clinical tool in which case the definition presented was used.

We plotted ROC curves to explore the best cutoffs for clinical diagnostic scores, and calculated the sensitivity, specificity, likelihood ratio of a positive test (LR+) and likelihood ratio of a negative test (LR-) and 95% confidence intervals for TB of diagnostic categories defined by each clinical diagnostic tool. We confined each analysis to children for whom data were available for all variables included. In each case we repeated the analysis for the diagnostic endpoints of confirmed TB, and confirmed and highly probable TB.

5.2.5 Derivation and validation of new clinical diagnostic tool for this setting

We used our carefully characterized diagnostic groups and detailed clinical data to develop and validate a new clinical prediction tool to identify children with TB. To allow us to derive and validate our clinical prediction tool in independent datasets we used study data from KDH as a "training dataset" to develop the tool, and then assessed its performance on a "testing dataset" comprising the study data from CPGH. We once again

excluded from the analysis children with 'possible TB' whose true disease status in unknown.

Using the KDH training dataset we developed a multivariable logistic regression model to predict CHPTB using simple clinical explanatory variables. Categorical variables with at least a weak association with TB (LRT p value ≤0.1) in the univariate analyses of clinical predictors and epidemiological risk factors (chapter 4) were included in the model. We excluded variables that were unlikely to be widely generalizable or applicable and justified our choices in each case. For ordered categorical variables we plotted log odds of TB by category and included a variable as a linear effect if this suggested a linear relationship. For those for which the relationship was initially non-linear we adjusted category cutoffs, and weighted categories in proportion to the log odds of TB, to allow their inclusion as a linear effect. In the event that categories of an ordinal categorical variable were concatenated to generate a binary variable for inclusion in the model, choice of cutoff was informed as far as possible by the ROC curves for that variable as a predictor of TB (Figure 5.1).

We then performed backwards stepwise logistic regression using standard selection criteria, such that variables that were not significantly associated with TB (Wald p value <0.5) were sequentially dropped from the model. Likelihood ratio tests were used to test for departure from the linear trend.

The final model therefore took the form: $TB = \alpha + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \ldots + \beta_iX_i$, where α is a constant, β_i are the regression coefficients for each variable (X_i) in the model, and X_i are coded 0 or 1 for binary variables or as integers for ordered categorical variables. We then generated a TB Score for each child using the coefficients of the model, such that TB score $= \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \ldots + \beta_iX_i$. To simplify this TB Score we multiplied the β coefficients by a fixed constant and rounded them for easy interpretation.

The ability of the TB Score correctly to identify children with TB in the KDH training dataset was quantified by plotting a receiver operator characteristics (ROC) curve of score as predictor of TB and calculating the area under the curve (AUC). In each case discriminant analyses were confined to those children for whom data were available for all variables included in the model/score. We used these ROC curves to choose appropriate cutoffs for risk strata identifying children for whom TB was "Unlikely", "Possible", "Likely" and "Very Likely". Variables that are known to be strongly predictive

of TB but which were not included in the model because they perfectly predicted the outcome (i.e. all children with that variable had TB) were then re-incorporated into a final KIDS TB SCORE by giving them a weight equal to the cutoff defined for "TB Very Likely", such that the presence of these clinical features alone would ensure a child is categorized in the "TB Very Likely" group.

We validated the KIDS TB Score by plotting ROC curves for the score as a predictor of TB in the CPGH "testing dataset", both overall and by age group and HIV status. Finally, to explore how the score performed compared to final diagnostic assignments based on all the clinical, laboratory and follow up information, we tabulated risk strata defined by the KIDS TB Score against the study definitions of TB for all children recruited to the passive case detection arm of the KIDS TB Study.

5.3 Results

5.3.1 Univariable analysis of individual clinical predictors of childhood TB

(a) Using composite case definition of CHPTB

Crude associations of individual features of the clinical history are shown in Table 5.1. Several variables in the clinical history were very strongly associated with TB, including many that are well-recognized features of TB such as cough, fever, weight loss, and night sweats. However the effect size (odds ratio) was modest in most cases. Interestingly, a history of lethargy and of change in temperament both also showed a strong association with TB, the former with a clear dose response relationship, despite the inherent subjectivity in the interpretation and reporting of these symptoms. Dyspnoea only appeared to be associated with TB when present for at least a month. Figure 5.1 shows receiver operator characteristics curves of different cutoffs of symptom duration for six of these 'core' variables. Small areas under the curve (AUC) in each case reflect the limited predictive value of these symptoms on their own.

Univariable associations between individual clinical examination findings and TB are shown in Table 5.2. Clubbing, lymphadenopathy, hepatomegaly, splenomegaly and clinical signs of a pleural effusion were all associated with TB. Generalized lymphadenopathy demonstrated the highest odds ratio for TB (OR 5.5, 95% CI 2.6 to 11.8), but this was partially confounded by HIV status (OR adjusted for HIV 4.2, 95% CI 2.0 to 9.0, $p<0.001$). No children in this study had a lymph node sinus. The absolute

association between the presence of a spinal gibbus and TB reflects the incorporation of spinal gibbus in the definition of highly probable TB.

A chest x-ray was performed and available for review for 1,146/1,367 (83%) cases and controls included in the analysis of clinical predictors. Of these, 464 (34%) demonstrated one or more abnormalities. An abnormal chest x-ray *per se* was strongly associated with TB (OR 12.9, 95% CI 7.1 to 23.7, p<0.001). Associations between individual radiological features and TB are shown in Table 5.3. Importantly, several radiological variables associated with TB are incorporated in some way in definitions of highly probable TB (see section 2.8, page 36).

Among 1,308/1,367 (96%) cases and controls included in this analysis for whom TST results were available, 58/119 CHPTB cases and 141/1,189 controls had a positive TST (OR 7.1, 95% CI 4.6 to 10.7, p<0.001; TST sensitivity 0.49, 95% CI 0.40 to 0.58; specificity 0.88, 0.86 to 0.90; LR+ 4.1, 1.3 to 13; LR- 0.58, 0.2 to 1.8). Sensitivity was worst among young children and children with HIV or malnutrition, but even among older children and those with neither HIV nor malnutrition at least one in three cases had a negative TST (**Table 5.4**).

Only one child had an AFB positive smear from sputum,* and 3 children had AFB positive smears from fine needle aspirates of lymph nodes, all of whom were classified as highly probable TB. Microbiological evidence of a focal or invasive bacterial infection other than TB was obtained in 5/127 (3.9%) cases and 94/1,240 (7.6%) controls (OR 0.5, 0.2 to 1.3; p=0.132; LR+ 0.4, 0.37 to 0.46; LR- 1.1, 1.0 to 1.2). A breakdown of the main categories of microbiologically confirmed bacterial infection is given in Table 10.12 of the appendix (page 195).

* Note that this is fewer smear positive cases than there were in KIDS TB study as a whole, since the definition for this analysis of clinical scores is based only on spontaneous sputa (assuming sputum induction is not generally available) and only on the first 3 specimens received (whereas some of the smear positive children in the KIDS TB study had positive smears on specimens taken later in their admission or at follow up).

(b) Comparison of results using confirmed TB case definition

Associations with CTB of each of the clinical, radiological and bacteriological variables examined were similar to associations with CHPTB reported above (Appendix Table 10.6, Table 10.7, Table 10.8, Table 10.13 and Figure 10.1). This is reflected in the similar distribution of most variables among cases of CTB and HPTB (Appendix Table 10.9, **Table 10.10**, **Table 10.11**, and Table 10.14).

A few clinical features were more common among CTB cases than among HPTB cases. These included a history of abdominal pain, change in temperament, and night sweats; and palpable lymphadenopathy. Although this might in part be explained by misclassification of some HPTB cases, it is also consistent with more severe disease in the CTB group. Perihilar lymphadenopathy on CXR was more common among cases of HPTB, probably due to incorporation of this feature in the definition of HPTB (which required the presence of perihilar lymph nodes plus a positive TST), but possibly also reflecting milder disease with lower bacillary loads in some children with early disease. Given the number of comparisons made it is also likely that some of these apparent differences may have occurred by random chance.

Although a miliary pattern on CXR was included in the definition of HPTB, the magnitude of the association with CTB was even greater (OR 79.3, 95% CI 18.9 to 332.1], Table 10.8), again consistent with more severe disease among confirmed cases. The association was stronger among HIV un-infected cases (OR 44.9, 11.7 to 172.7, $p<0.001$) than among HIV co-infected cases (OR 13.2, 2.2 to 78.0, $p<0.001$), providing some support for exclusion of HIV infected children with miliary CXRs from the definition of highly probable TB. Although formal statistical evidence that HIV modifies the association with TB of a miliary CXR pattern is lacking (χ^2 test for heterogeneity, $p=0.270$), this is probably due to the inherently poor power of the test to detect effect modification, and to small numbers.

The sensitivity of a positive TST for diagnosis of active tuberculosis, which was poor for the composite endpoint of CHPTB (see above), was no better when the analysis confined to confirmed TB cases and controls: 30/65 CTB cases had a positive TST (OR 6.4, 3.7 to 10.8; $p<0.001$; sensitivity 0.46, 0.34 to 0.59; specificity 0.88, 0.86 to 0.90; LR+ 3.9, 1.7 to 8.8; LR- 0.6, 0.3 to 1.4; **Table 5.4**).

Table 5.1 Associations of clinical history variables with CHPTB

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Cough & duration					
No cough	221	15	1.0		<0.001
<2 weeks	414	26	0.9	(0.5 to 1.8)	
2-4 weeks	229	27	1.7	(0.9 to 3.4)	
1 month	158	22	2.1	(1.0 to 4.1)	
2 months	52	17	4.8	(2.2 to 10.5)	
≥3 months	105	14	2.0	(0.9 to 4.2)	
Cough productive of sputum					
No	1,168	115	1.0		0.104
Yes	72	12	1.7	(0.9 to 3.2)	
Haemoptysis					
No	1,232	127	1.0		0.364
Yes	8	0	-		
Fever & duration					
No fever	232	22	1.0		<0.001
<2 weeks	469	36	0.8	(0.5 to 1.4)	
2-4 weeks	229	17	0,8	(0.4 to 1.5)	
1 month	135	21	1.6	(0.9 to 3.1)	
2 months	51	11	2.3	(1.0 to 5.0)	
≥3 months	68	14	2.2	(1.0 to 4.5)	
Weight loss & duration					
No weight loss	488	35	1.0		0.008
<1 month	88	11	1.7	(0.9 to 3.6)	
1 month	237	23	1.4	(0.8 to 2.3)	
2 months	107	18	2.3	(1.3 to 4.3)	
≥3 months	224	30	1.9	(1.1 to 3.1)	
Weight loss severity					
No weight loss	488	35	1.0		0.003
Mild	203	22	1.5	(0.9 to 2.6)	
Moderate	247	24	1.4	(0.8 to 2.3)	
Severe	235	37	2.2	(1.3 to 3.6)	
Dyspnoea & duration					
No dyspnoea	881	84	1.0		0.012
<2 weeks	243	24	1.0	(0.6 to 1.7)	
2-4 weeks	32	3	1.0	(0.3 to 3.3)	
≥1 month	29	9	3.3	(1.5 to 7.1)	
Lethargy & duration					
No lethargy	867	67	1.0		<0.001
<2 weeks	121	11	1.2	(0.6 to 2.3)	
2-4 weeks	80	10	1.6	(0.8 to 3.3)	
1 month	57	13	3.0	(1.5 to 5.9)	
2 months	16	5	4.0	(1.4 to 11.4)	
≥3 months	26	10	5.0	(2.3 to 10.8)	

Table 5.1 continued

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Night sweats & duration					
No night sweats	890	73	1.0		
<2 weeks	115	17	1.8	(1.0 to 3.2)	
2-4 weeks	64	4	0.8	(0.3 to 2.2)	
1 month	45	12	3.3	(1.6 to 6.4)	<0.001
2 months	9	5	6.8	(2.2 to 20.9)	
≥3 months	33	6	2.2	(0.9 to 5.5)	
Abdominal pain					
No	959	94	1.0		0.360
Yes	259	31	1.2	(0.8 to 1.9)	
Change in temperament					
No	1,204	120	1.0		0.007
Yes	13	5	3.9	(1.3 to 11.0)	
Seizures					
No	732	75	1.0		0.892
Yes	42	4	0.9	(0.3 to 2.7)	
History of previous TB treatment					
No	1,146	112	1.0		0.001
Yes	26	9	3.5	(1.6 to 7.8)	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Figure 5.1 Receiver operator characteristic (ROC) curves for six core symptoms showing the sensitivity and specificity of different cutoffs of symptom duration (in weeks) as predictors of CHPTB. Arrows indicate the chosen cutoffs for inclusion in the mulitivariable model.

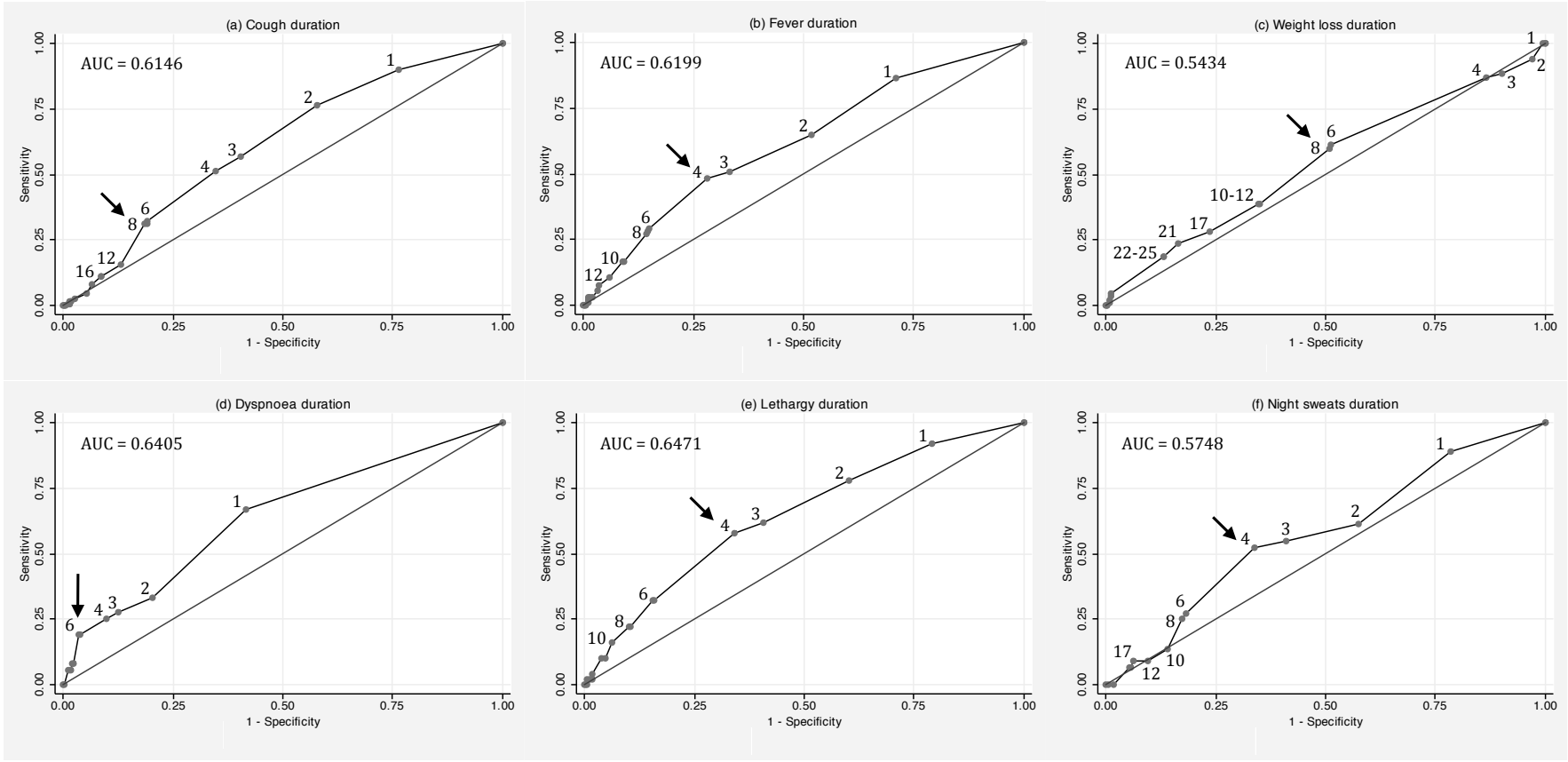


Table 5.2 Associations of clinical examination findings with CHPTB

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Clubbing					
No	581	60	1.0		0.004
Yes	11	5	4.4	(1.5 to 13.2)	
Any lymphadenopathy					
No	910	66	1.0		<0.001
Yes	330	61	2.5	(1.8 to 3.7)	
Cervical lymphadenopathy					
None	1,028	76	1.0		<0.001
Unilateral	60	9	2.0	(1.0 to 4.3)	
Bilateral	152	42	3.7	(2.5 to 5.7)	
Axillary lymphadenopathy					
None	1,151	103	1.0		<0.001
Unilateral	28	8	3.2	(1.4 to 7.2)	
Bilateral	61	16	2.9	(1.6 to 5.3)	
Inguinal lymphadenopathy					
None	1,086	99	1.0		0.003
Unilateral	0	0	-		
Bilateral	150	27	1.9	(1.2 to 3.1)	
Generalized lymphadenopathy					
No	1,215	115	1.0		<0.001
Yes	21	11	5.5	(2.6 to 11.8)	
Stridor					
No	1,221	125	1.0		0.749
Yes	1	0	-		
Wheeze					
No	1,173	122	1.0		0.435
Yes	46	3	0.6	(0.2 to 2.0)	
Clinical signs of pleural effusion					
No	1,193	116	1.0		0.002
Yes	29	9	3.2	(1.5 to 6.9)	
Abdominal mass					
No	1,214	122	1.0		0.013
Yes	6	3	5.0	(1.2 to 20.2)	
Hepatomegaly					
No	1,009	88	1.0		0.001
Yes	212	37	2.0	(1.3 to 3.0)	
Splenomegaly					
No	1,173	109	1.0		<0.001
Yes	47	16	3.7	(2.0 to 6.7)	
Ascites					
No	1,209	122	1.0		0.116
Yes	11	3	2.7	(0.7 to 9.8)	

Table 5.2 continued

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Reduced consciousness					
No	1,201	121	1.0		0.148
Yes	18	4	2.2	(0.7 to 6.6)	
Meningism					
No	1,209	124	1.0		0.842
Yes	12	1	0.8	(0.1 to 6.3)	
Focal neurological signs					
No	1,205	122	1.0		0.326
Yes	16	3	1.9	(0.5 to 6.5)	
Gibbus					
No	1,221	123	1.0		<0.001
Yes	0	2	-		
Firm, non-traumatic joint swelling					
No	1,237	126	1.0		0.279
Yes	3	1	3.3	(0.3 to 31.8)	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 5.3 Associations of chest x-ray features with CHPTB

Radiological feature	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Abnormal chest x-ray					
No	668	14	1.0		<0.001
Yes	365	99	12.9	(7.1 to 23.7)	
Airspace shadowing					
No	845	50	1.0		<0.001
Yes	225	54	4.1	(2.7 to 6.2)	
Expansile lobar pneumonia					
No	976	88	1.0		
Yes	1	0	-		0.764
Bronchopneumonia					
No	943	80	1.0		0.012
Yes	48	10	2.5	(1.2 to 5.1)	
Cavities					
No	976	76	1.0		<0.001
Yes	2	14	89.9	(18.1 to 446)	
Nodular infiltrate (nodules >2mm)					
No	975	86	1.0		0.025
Yes	4	2	5.7	(1.0 to 31.5)	
Miliary infiltrate (nodules <2mm)					
No	975	77	1.0		0.001
Yes	3	15	63.3	(16.5 to 243)	
Volume loss					
No	974	88	1.0		0.603
Yes	3	0	-		
Calcification					
No	977	88	1.0		-
Yes	0	0	-		
Perihilar lymphadenopathy					
No	950	66	1.0		<0.001
Yes	69	39	8.1	(5.0 to 13.3)	
Paratracheal lymphadenopathy					
No	970	72	1.0		<0.001
Yes	10	19	25.6	(10.9 to 60.1)	
Mediastinal lymphadenopathy					
No	943	56	1.0		<0.001
Yes	78	50	10.8	(6.7 to 17.4)	
Perihilar streakiness					
No	976	85	1.0		<0.001
Yes	1	3	34.4	(3.4 to 344)	
Airway compression					
No	976	83	1.0		<0.001
Yes	2	5	29.4	(5.4 to 158)	

Table 5.3 continued

Radiological feature	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Pleural effusion					
No	949	77	1.0		<0.001
Yes	35	17	6.0	(3.2 to 11.3)	
Pneumothorax					
No	976	88	1.0		0.764
Yes	1	0	-		

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 5.4 TST responses among cases and controls, overall and among subgroups defined by age and HIV/malnutrition status

	CASES		CONTROLS		Sensitivity (%) (95% CI)		Specificity (%) (95% CI)		OR (%) (95% CI)		p value
	TST+	TST-	TST+	TST-							
CONFIRMED TB (CTB)											
Age category											
0-4 years	14	20	135	866	41.2	(24.6-59.3)	86.5	(84.2-88.6)	4.5	(2.2-9.2)	0.011*
5-9 years	8	8	4	125	50.0	(24.7-75.3)	96.9	(92.3-99.1)	31.3	(6.0-163.9)	
10-14 years	8	4	1	47	66.7	(34.9-90.1)	97.9	(88.9-99.9)	94.0	(3.2-2770)	
HIV status											
HIV infected	4	12	13	138	25.0	(7.3-52.4)	91.4	(85.7-95.3)	3.5	(1.0-12.8)	0.224*
HIV un-infected	26	22	121	865	54.2	(39.2-68.6)	87.7	(85.5-89.7)	8.4	(4.5-15.7)	
Nutrition status											
Severely malnourished	10	19	46	400	34.5	(17.9-54.3)	89.7	(86.5-92.3)	4.6	(2.0-10.6)	0.266*
Not severely malnourished	20	16	95	648	55.6	(38.1-72.1)	87.2	(84.6-89.5)	8.5	(4.2-17.4)	
HIV & Nutrition status combined											
HIV infected AND severely malnourished	2	6	7	67	25.0	(3.2-65.1)	90.5	(81.5-96.1)	3.2	(0.5-19.5)	0.184
NEITHER HIV infected NOR malnourished	18	10	82	539	64.3	(44.1-81.4)	86.8	(83.9-89.4)	11.8	(5.1-27.5)	<0.001
All children	**30**	**35**	**141**	**1048**	**46.2**	**(33.7-59.0)**	**88.1**	**(86.2-89.9)**	**6.4**	**(3.7-10.8)**	**<0.001**
CONFIRMED & HIGHLY PROBABLE TB (CHPTB)											
Age category											
0-4 years	37	38	135	866	49.3	(37.6-61.1)	86.5	(34.2-88.6)	6.2	(3.8-10.3)	0.030*
5-9 years	12	13	4	125	48.0	(27.8-68.7)	96.9	(92.3-99.1)	28.8	(6.2-131.1)	
10-14 years	9	6	1	47	60.0	(32.3-83.7)	97.9	(88.9-99.9)	70.5	(3.3-1490)	
HIV status											
HIV infected	8	22	13	138	26.7	(12.3-45.9)	91.4	(85.7-95.3)	3.9	(1.4-10.6)	0.115*
HIV un-infected	50	38	121	865	56.8	(45.8-67.3)	87.7	(85.5-89.7)	9.4	(5.8-15.4)	
Nutrition status											
Severely malnourished	22	35	46	400	38.6	(26.0-52.4)	89.7	(86.5-92.3)	5.5	(2.9-10.3)	0.212*
Not severely malnourished	36	26	95	648	58.1	(44.8-70.5)	87.2	(84.6-89.5)	9.4	(5.3-16.9)	
HIV & Nutrition status combined											
HIV infected AND severely malnourished	3	15	7	67	16.7	(3.6-41.4)	90.5	(81.5-96.1)	1.9	(0.4-8.4)	0.381
NEITHER HIV infected NOR malnourished	31	19	82	539	62.0	(47.2-75.3)	86.8	(83.9-89.4)	10.7	(5.6-20.7)	<0.001
All children	**58**	**61**	**141**	**1048**	**48.7**	**(39.5-58.1)**	**88.1**	**(86.2-89.9)**	**7.1**	**(4.6-10.7)**	**<0.001**

* $\chi 2$ test for heterogeneity

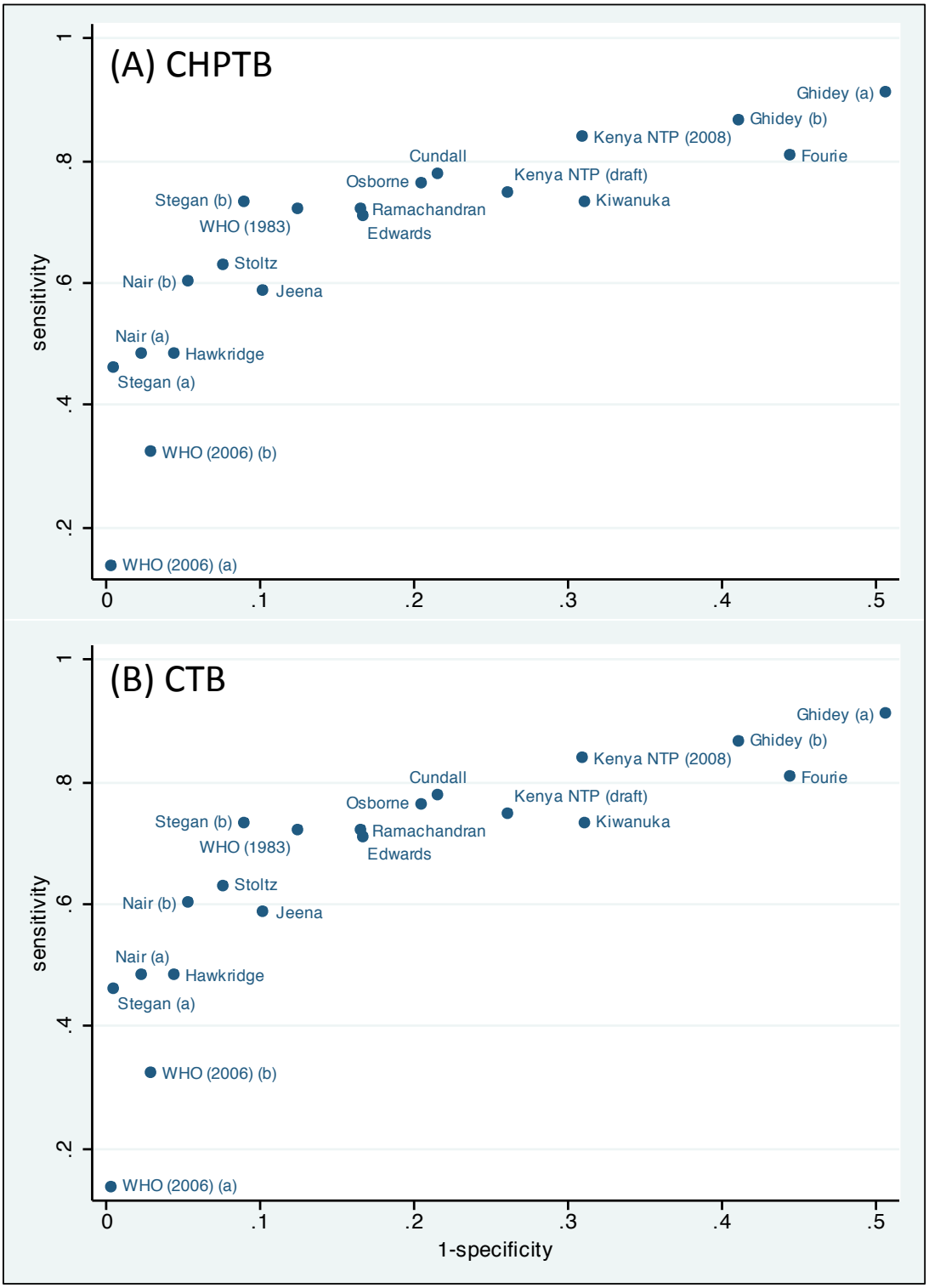
5.3.2 Assessment of the performance of published clinical diagnostic tools

Published clinical diagnostic tools assessed in this analysis are shown in Table 5.5, which summarizes the origin, diagnostic approach, and clinical information required for each tool. Three peer reviewed scores were omitted from the analysis: one[140] because it provided insufficient detail to re-apply the diagnostic criteria proposed; a second[131] because it relied on routine availability of TB culture for diagnosis; and a third[144] because it differed from the Ghidey-Habte score[137] (already included) only by the addition of sputum microscopy on gastric washings, which were not done routinely in our study. The performances of all other published clinical tools in identifying CTB and CHPTB in our cohort are summarized graphically in Figure 5.2. The sensitivity, specificity, LR+ and LR- of each tool are tabulated in more detail in Table 10.15 and Table 10.16 of the appendix (pages 198 and 199).

The WHO (2006) guidelines for childhood TB diagnosis[21] are considered separately (see below). Among the remaining clinical tools assessed there was a wide range of performance in identification of both CTB and CHPTB. As expected, higher specificity came at the cost of lower sensitivity: thus Ghidey & Habte's[137] 'Probable TB' (definition "(a)") had the highest sensitivity (91.2% and 87.4% for CTB and CHPTB, respectively), but the lowest specificity (49.4%); and 'Highly Probable TB' as defined by Stegen *et al*[150] had the highest specificity (99.6%) but the lowest sensitivity (46.2% and 33.9% for CTB and CHPTB, respectively).

ROC curves and published cutoffs are shown for the six numerical diagnostic scores as predictors of CHPTB and CTB in Figure 5.3 and Figure 5.4, respectively. Areas under the ROC curves (AUC) were generally slightly greater for CTB than for CHPTB (reflecting slightly better discriminatory power for CTB), and highest for the Stegen-Jones score[150] as a predictor of CTB (AUC = 0.8828). The exception was the Keith Edwards score, [132] for which the AUC for CHPTB (0.8774) was greater than that for CTB and greater than all the other ROC curves for CHPTB. Although choosing a single best cutoff from most of the ROC curves is difficult, based on their performance in our dataset the published cutoffs do appear reasonable for five of the six scores. However examination of the ROC curves for the Nair & Phillips score suggests a cutoff of 4 for probable TB might be more appropriate in this population (Figure 5.3 and Figure 5.4).

Figure 5.2 Receiver operator characteristics curves showing the performance of each clinical tool for diagnosis of (A) CHPTB and (B) CTB



All the clinical diagnostic tools examined incorporated the results of a clinical assessment and TST; the majority also incorporated the results of chest radiography; in addition seven included results of AFB microscopy (Table 5.5). Definitions that incorporated microscopy for AFBs tended to be more specific but slightly less sensitive than those that did not include AFB microscopy (median specificity 91.1% c.f. 79.6%; median sensitivity for CTB/CHPTB 72.1/68.5% c.f. 75.0/74.0%; Appendix Table 10.15 and Table 10.16). Of two clinical tools that used neither CXR nor AFB microscopy, the better performance of the Keith Edwards score over that of Fourie *et al* is illustrated in Figure 5.2, and by more clinically useful likelihood ratios of a positive and negative test for both CTB and CHPTB (Appendix Table 10.15 and Table 10.16).

The performance of the current[71] and draft[149] Kenyan guidelines with respect to identification of CHPTB was similar (Figure 5.2). Compared with the prototype Keith Edwards score on which it is based, the discriminant power of the current Kenyan guideline was slightly better for both CHPTB (AUC 0.8224 c.f. 0.7747; Figure 5.3) and for CTB (AUC 0.8413 c.f. 0.8020; Figure 5.4). The choice of a cutoff of ≥7 points for a diagnosis of TB using both the Keith Edwards score and the current Kenyan guidelines appeared appropriate (Figure 5.3 and Figure 5.4).

The WHO (2006) guidelines for childhood TB diagnosis do not purport to be diagnostic definitions *per se*, but rather a detailed approach to diagnosis.[21] Nevertheless the statements they make regarding the likelihood of TB given combinations of clinical features lend themselves to analysis and they are therefore included as the only current published international guidelines. According to these guidelines, "*The presence of three or more of the following should strongly suggest a diagnosis of TB: chronic symptoms suggestive of TB; physical signs highly of suggestive of TB; a positive tuberculin skin test; chest X-ray suggestive of TB."* Each of these components is then defined in detail. Although these clinical features defined as "strongly suggestive of TB" proved highly specific (specificity 99.7%, 95% CI 99.1 to 99.9), they identified only 13.8% CTB cases and 12.6% CHPTB cases. Futhermore, diagnostic sensitivity only improved slightly when we broadened the criteria to include under "physical signs highly suggestive of TB" all the other "suggestive clinical signs" listed as requiring investigation for TB (sensitivity 26.9% and 32.3% for CHPTB and CTB, respectively; Appendix Table 10.15, Table 10.16).

Table 5.5 Existing clinical diagnostic algorithms whose performance was assessed among children recruited to the KIDS TB study

	Author(s) and reference	Country	Year	Diagnostic approach	Includes:			Lineage
					CXR	TST	AFB	
[1]	Ramachandran[142]	India	1968	Binary classification	✓	✓	✗	Original prototype
[2]	Stegan *et al.*[150]	Chile	1969	Numerical score + hierarchical classification	✓	✓	✓	Original prototype
[3]	Nair & Philip[148]	India	1981	Numerical score + hierarchical classification	✓	✓	✓	Modification of [2]
[4]	Ghidey & Habte[137]	Ethiopia	1983	Binary classification	✓	✓	✓	Original prototype
[5]	WHO [70]	(WHO)	1983	Hierarchical classification	✓	✓	*(✓)*[3]	Original prototype
[6]	Cundall[136]	Kenya	1986	Hierarchical classification§	✓	✓	*(✓)*[3]	Modification of [5]
[7]	Edwards[132]	PNG[1]	1987	Numerical score + binary classification	✗	✓	✗	Original prototype
[8]	Stoltz *et al.*[143]	South Africa	1990	Hierarchical classification	✓	✓	*(✓)*[3]	Modification of [5]
[10]	Osborne[141]	Zambia	1995	Hierarchical classification	✓	✓	✗	Modification of [5]
[11]	Jeena *et al.*[138]	South Africa	1996	Binary classification	✓	✓	✗	Original prototype
[12]	Fourie *et al.*[133]	International	1998	Numerical score + binary classification	✗	✓	✗	Original prototype
[13]	Kiwanuka *et al.*[139]	Malawi	2001	Hierarchical classification§	✓	✓	*(✓)*[3]	Modification of [5]
[14]	Hawkridge *et al.*[145]	South Africa	2006	Hierarchical classification§	✓	✓	✓	Original prototype
[15]	WHO guidelines to NTPs[21]	(WHO)	2006	Diagnostic approach	✓	✓	✗	Current WHO guidelines
[16]	Kenya NTP Guidelines[71]	Kenya	2008	Numerical score + binary classification	✓	✓	✗	Modification of [7]
[17]	Kenya draft paediatric guidelines[149]	Kenya	2012	Binary classification§	✓	✓	✗	Original prototype

[1] PNG, Papua New Guinea

[2] AFB, Microscopy for acid fast bacilli on clinical specimens

[3] Microscopy (± culture) of clinical specimens for TB only required as part of the definition of confirmed tuberculosis.

Figure 5.3 Receiver operator characteristic (ROC) curves for numerical diagnostic scores showing the sensitivity and specificity of different score cutoffs for a diagnosis of CHPTB. Arrows indicate the recommended cutoffs used in the published scores.

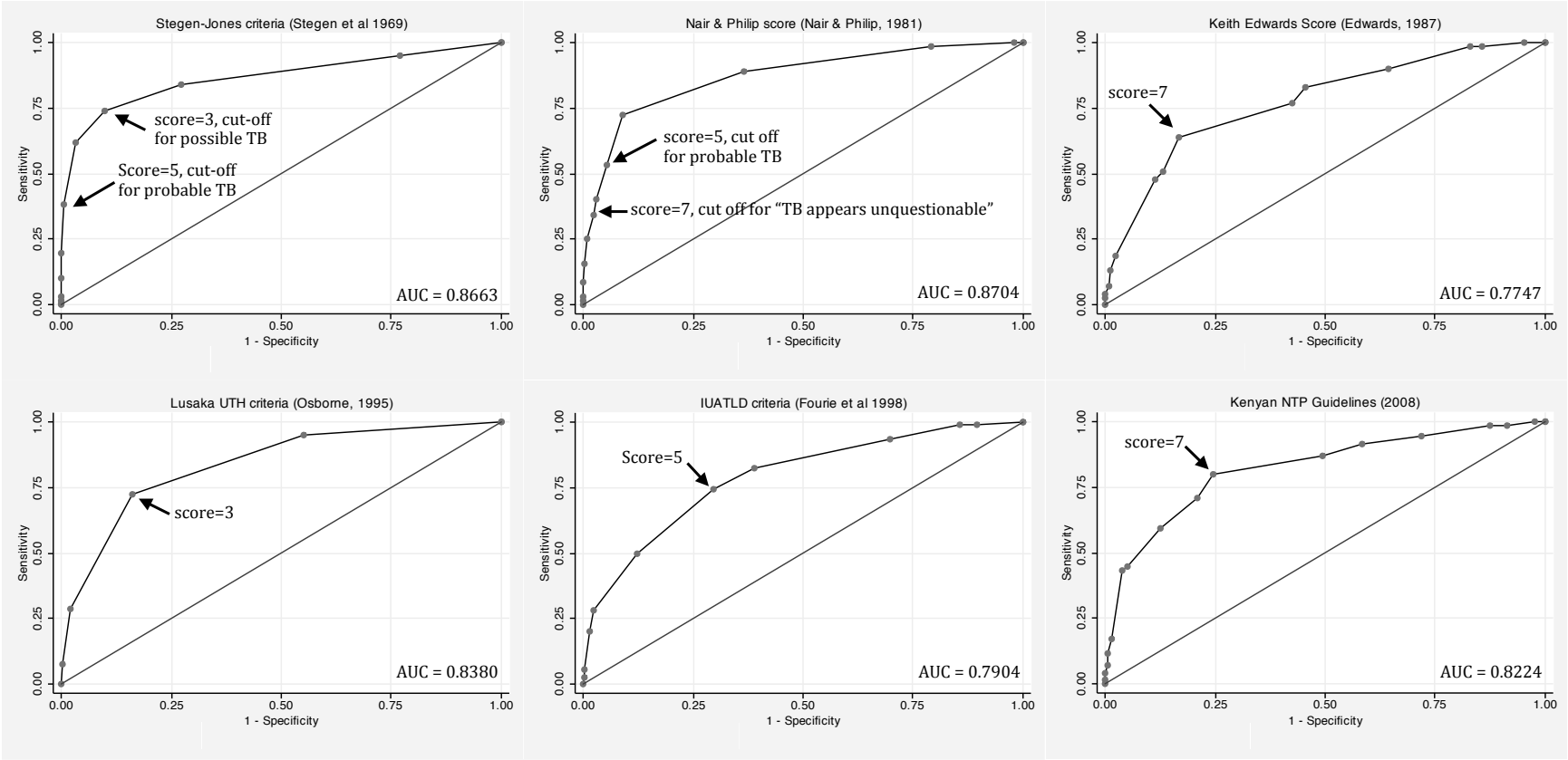
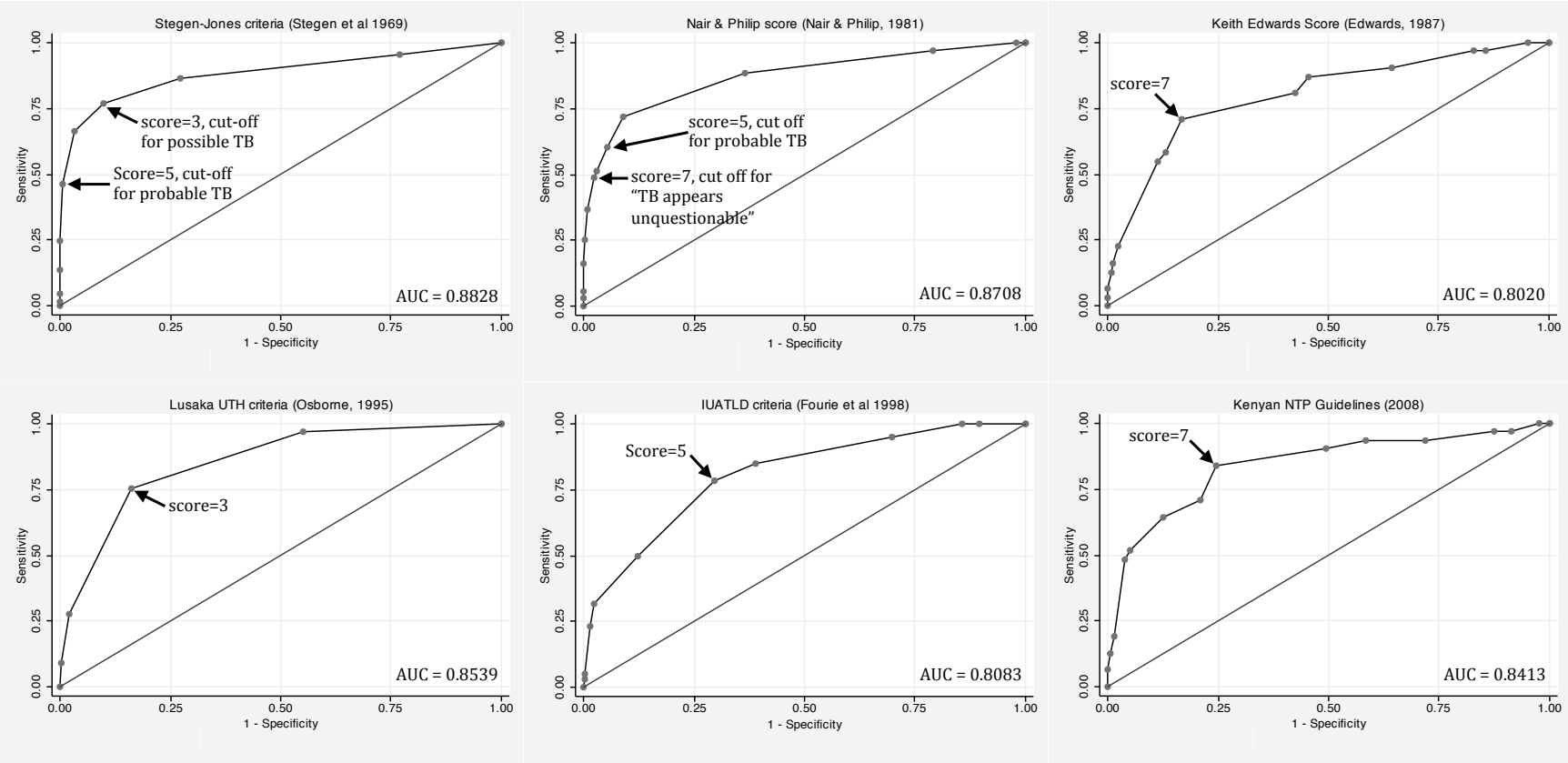


Figure 5.4 Receiver operator characteristic (ROC) curves for numerical diagnostic scores showing the sensitivity and specificity of different score cutoffs for a diagnosis of Confirmed TB. Arrows indicate the recommended cutoffs used in the published scores.



5.3.3 Derivation and validation of a new clinical diagnostic tool for this setting

Categories and weightings of each variable included in generating the model are shown in Table 10.17 in the appendix (page 201). Graphs of the log odds of TB for ordered categorical variables supported a linear association in each case (appendix Figure 10.2, page 203). We chose to exclude a history of previous TB treatment from the model since we reasoned that the effect of this variable on TB risk depends on local prescribing practices, drug regimens, the prevalence of drug resistance among circulating strains, and social determinants of patient compliance, and is therefore likely to vary widely. We also excluded those chest x-ray features that are unlikely to be interpreted reliably by the non-expert clinicians responsible for patient care in most settings, confining radiological features in our model to an abnormal CXR, and the presence of alveolar consolidation, cavities, pleural effusion, or a miliary pattern.

Regression coefficients and adjusted odds ratios and 95% CIs for each variable included in the final model are given in **Table 5.6**. To generate a simplified TB Score we multiplied the β coefficients by 5 and rounded them to the nearest integer or round number. ROC curves for this score as a predictor of CTB and CHPTB in the training (KDH) dataset are shown in Figure 5.5. The areas under each of these curves are summarized in Table 5.7.

Table 5.6 Regression coefficients and adjusted odds ratios for variables in the final model

Variables	β	Adjusted Odds ratio (95% CI)	p value
TB contact proximity	0.661267	1.9 (1.4 to 2.6)	<0.001
Lethargy history & duration	0.459377	1.6 (1.2 to 2.0)	<0.001
Axillary lymphadenopathy	1.280832	3.6 (1.3 to 9.8)	0.012
Abnormal CXR	1.372135	3.9 (1.7 to 9.2)	0.002
Cavities on CXR	4.279877	72.2 (6.8 to 766)	<0.001
Miliary infiltrate on the CXR	3.439185	31.2 (2.5 to 386)	0.007
Tuberculin skin test result	3.343981	28.3 (10.8 to 74.5)	<0.001

Table 5.7 Area under ROC curves for the simplified TB score as a predictor of confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets

	Area under ROC curve (95% CI)	
	Confirmed TB	Confirmed & highly probable TB
Training dataset	0.924 (0.874 to 0.974)	0.922 (0.881 to 0.963)
Testing dataset	0.883 (0.825 to 0.941)	0.872 (0.799 to 0.946)

Using these ROC plots we defined score cutoffs for “TB Possible” (score ≥10), “TB Likely” (score ≥25) and “TB Very Likely”(score ≥40); a score <10 was designated “TB Unlikely”. Two important variables that are strongly predictive of TB - the presence of a spinal gibbus and a positive smear from sputum or FNA - were not incorporated in the model because they perfectly predicted the outcome (i.e. all children with these features had a diagnosis of highly probable or confirmed TB). We therefore incorporated these features into the final KIDS TB SCORE (Table 5.8) by assigning each a value of 40, such that the presence of these features alone would ensure a child is categorized in the “TB Likely” group.

The areas under the ROC curves for this KIDS TB SCORE as a predictor of Confirmed TB and CHPTB in the testing (CPGH) dataset were 0.891 (95% CI 0.834 to 0.947) and 0.9130 (0.876 to 0.951), respectively (Figure 5.6B). ROC curves for the KIDS TB SCORE as a predictor of TB among subgroups defined by age and HIV status shown in Figure 5.7 and Figure 5.8; areas under each of these ROC curves are summarized in Table 5.9. Together these results validate the KIDS TB Score in an independent dataset, both overall and each of the subgroups tested.

Table 5.10 shows the distribution of risk defined by the KIDS TB SCORE tabulated against final TB diagnostic categories in the passive case detection arm of the study. The sensitivity of the “TB Very Likely”, “TB Likely” and “TB Possible” categories combined for identifying children with CTB and CHPTB was 81.7% (69.6 to 90.5) and 87.9% (80.6 to 93.2), respectively. Among 1,151 children assigned to the “TB unlikely” risk stratum (64.6% of children investigated) only 1.2% had CHPTB (negative predictive value 98.8%, 95% CI 98.0 to 99.3%). The positive predictive values of “TB Possible”, “TB Likely” and “TB Very Likely” for CHPTB were 8.0% (5.8 to 10.8%), 37.0% (27.1 to 47.7%) and 68.3% (51.9 to 81.9%), respectively.

Table 5.8 The KIDS TB SCORE

Clinical finding	Description	Score
TB contact & proximity	No known TB contact	**0**
	Non-household contact	**4**
	Household contact, sleeps in different room	**8**
	Household contact, sleeps in same room	**12**
Lethargy history & duration	No lethargy or lethargy for <2 weeks	**0**
	Lethargy for 2-4 weeks	**6**
	Lethargy for 1-<2 months	**9**
	Lethargy for ≥2 months	**15**
Axillary lymphadenopathy	No axillary lymphadenopathy	**0**
	Axillary lymphadenopathy present	**8**
Gibbus	Absent	**0**
	Present	**40**
Abnormal chest x-ray (CXR)	Normal CXR (no abnormality detected)	**0**
	Abnormal CXR (≥1 abnormality)	**8**
Cavities on CXR	Absent	**0**
	Present	**22**
Miliary infiltrate on CXR	Absent	**0**
	Present	**18**
Positive tuberculin skin test	TST negative	**0**
	TST positive	**18**
AFB positive sputum/FNA smear	No positive smear result	**0**
	Positive smear result	**40**

KIDS TB SCORE = Total Score for all clinical findings

Score interpretation:		
	<10	**TB Unlikely**
	10-29	**TB Possible**
	30-39	**TB Likely**
	≥40	**TB Very Likely**

Table 5.9 Area under ROC curves for the KIDS TB SCORE as a predictor of TB among subgroups defined by age category and HIV status in the testing (CPGH) dataset

	No. children			Area under ROC curve (95% CI)			
	CTB	HPTB	Not TB	CTB		CHPTB	
HIV status							
HIV negative	30	16	529	0.899	(0.836-0.962)	0.921	(0.880- 0.962)
HIV positive	10	5	82	0.848	(0.695-1.000)	0.876	(0.774-0.977)
Age category							
<5 years	20	17	558	0.869	(0.784-0.954)	0.901	(0.854-0.949)
5-9 years	9	2	51	0.932	(0.837-1.000)	0.941	(0.861-1.000)
10-14 years	8	1	23	0.932	(0.809-1.000)	0.941	(0.833-1.000)

Table 5.10 Distribution of final TB diagnostic categories across risk strata defined by the KIDS TB SCORE among all children enrolled in the passive case detection arm of the study

	No. children in risk strata each risk stratum (% by category in each column/stratum)				
	Total	Unlikely	Possible	Likely	Very Likely
Confirmed TB	**60**	11 (0.96)	9 (2.3)	25 (13.0)	15 (36.6)
Highly Probable TB	**56**	3 (0.26)	12 (3.0)	28 (14.6)	13 (31.7)
Possible TB cases treated for TB	**132**	63 (5.5)	45 (11.3)	20 (10.4)	4 (9.8)
Possible TB cases not treated for TB	**369**	222 (19.3)	87 (21.9)	54 (28.1)	6 (14.6)
Not TB (TB excluded)	**1,165**	852 (74.0)	245 (61.6)	65 (33.9)	3 (7.3)
Total	**1,782**	**1,151**	**398**	**192**	**41**

Figure 5.5 Receiver operator characteristics curves for the simplified TB score as a predictor of Confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets.

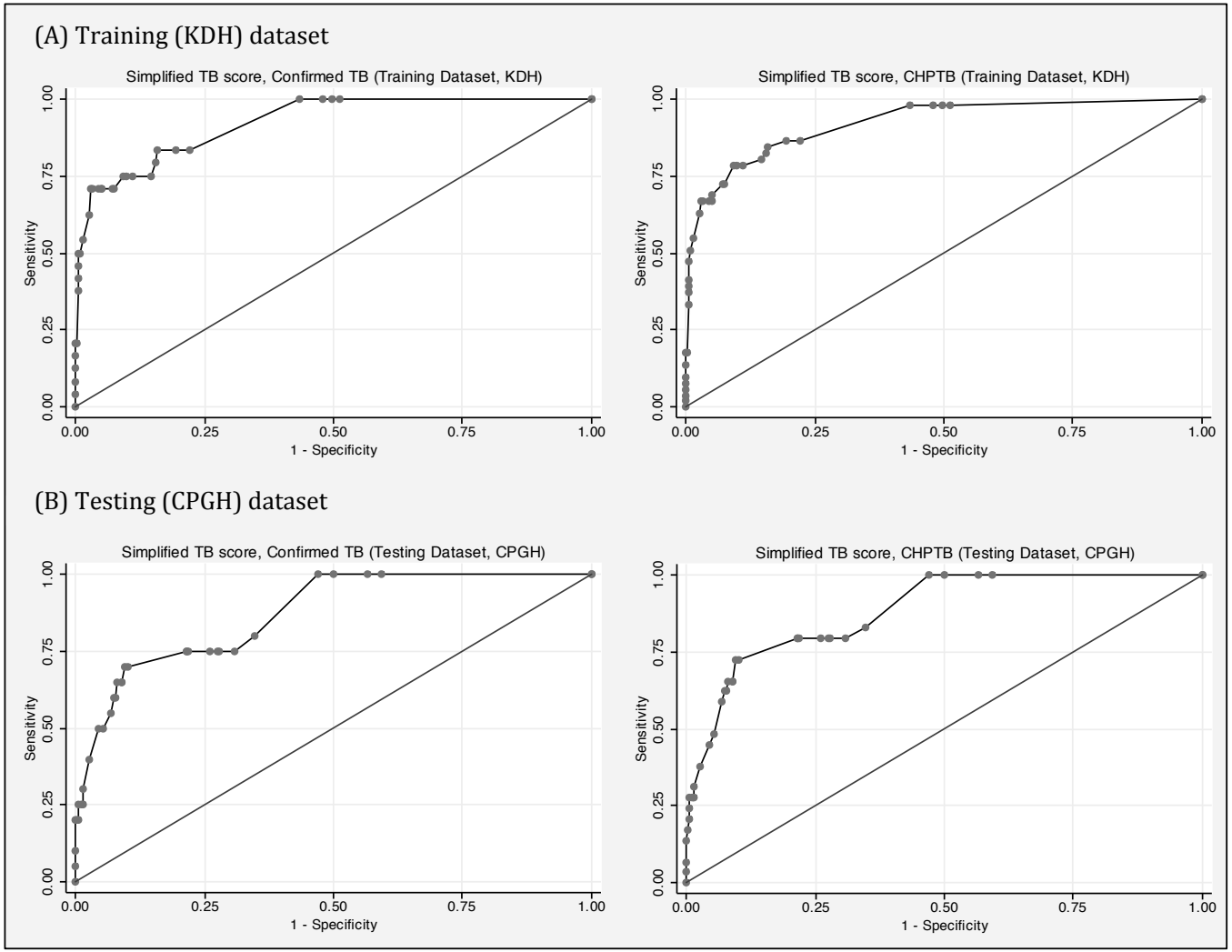


Figure 5.6 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB and CHPTB in the training (KDH) and testing (CPGH) datasets.

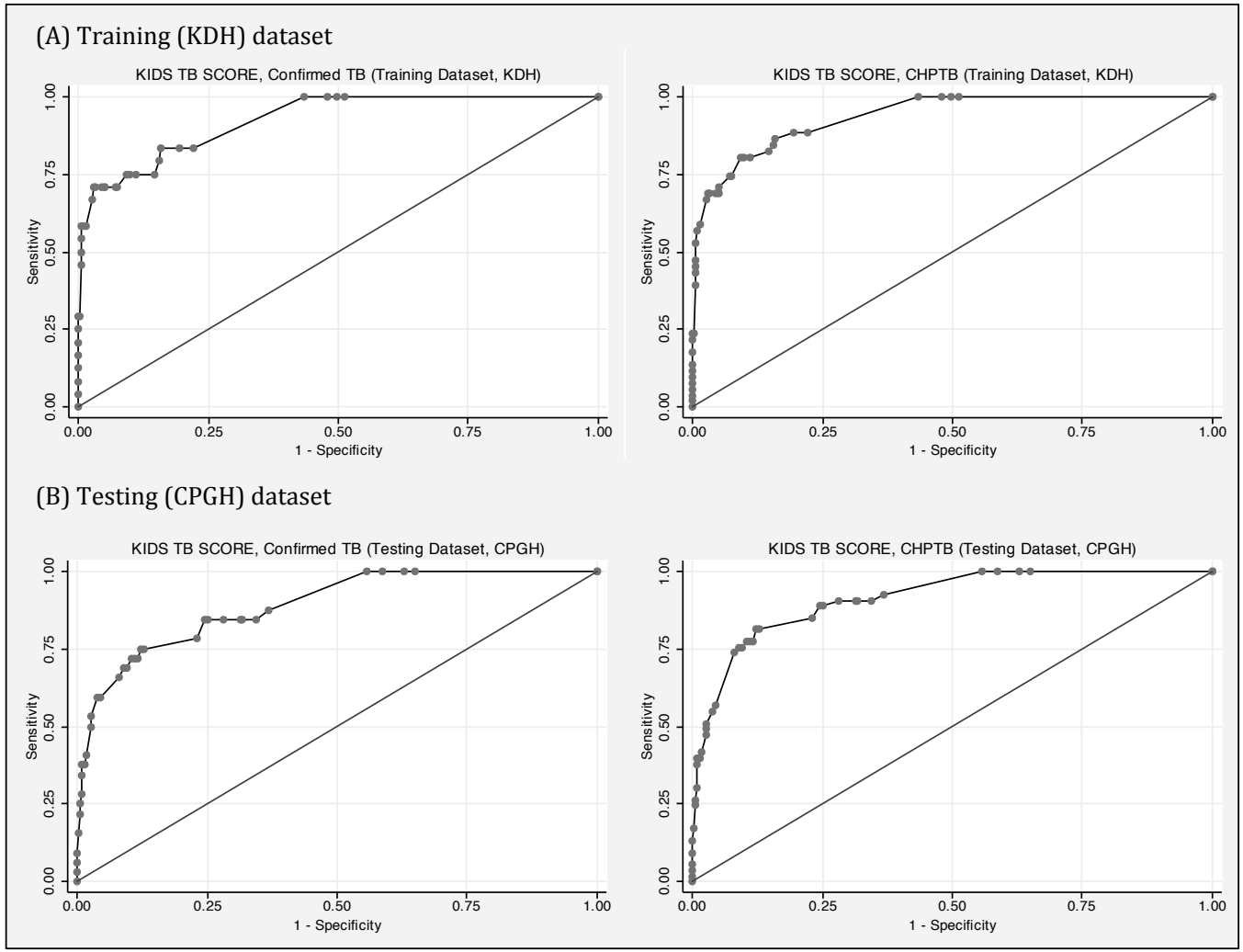


Figure 5.7 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB and CHPTB in the testing (CPGH) dataset, by age category.

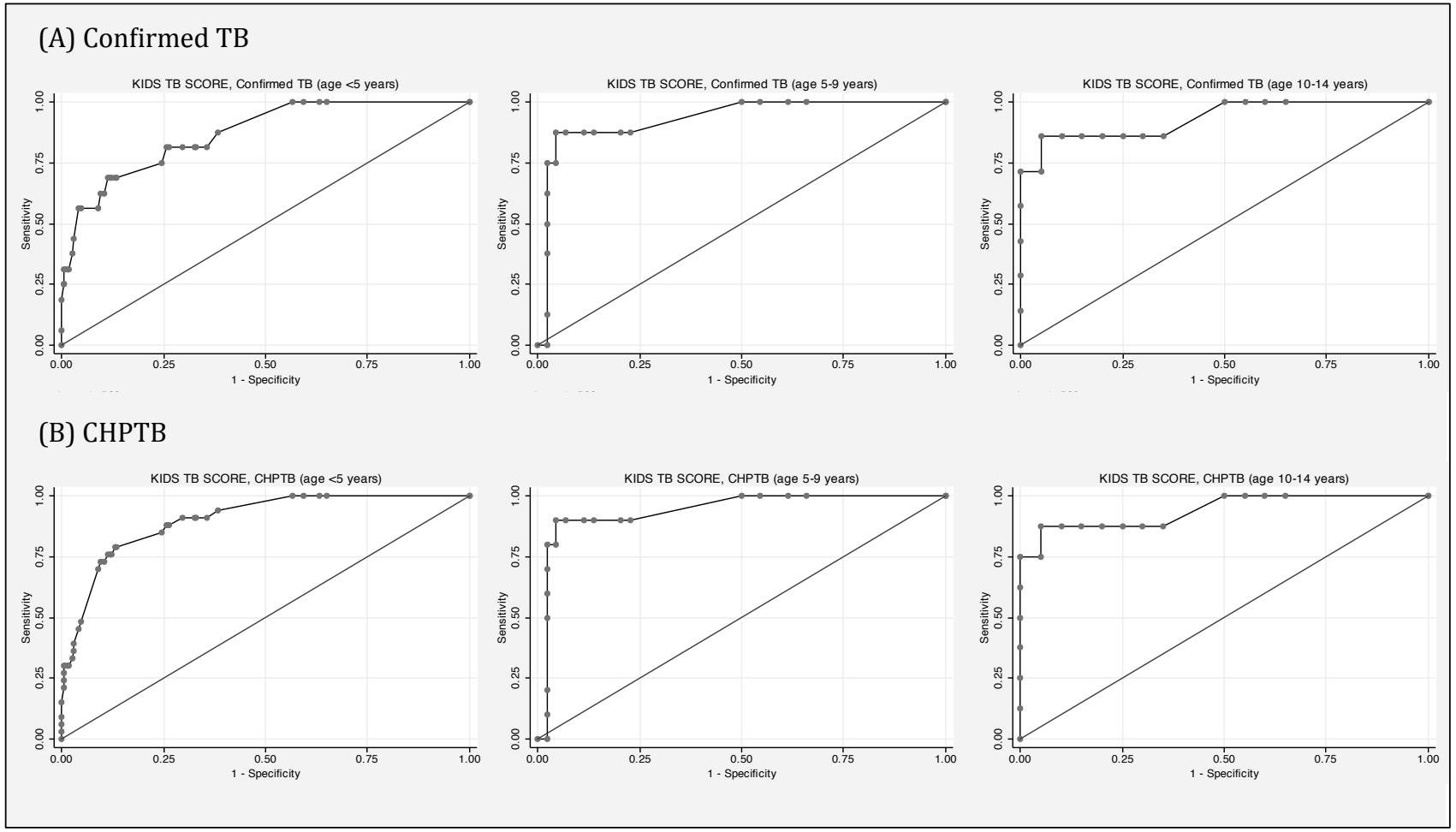
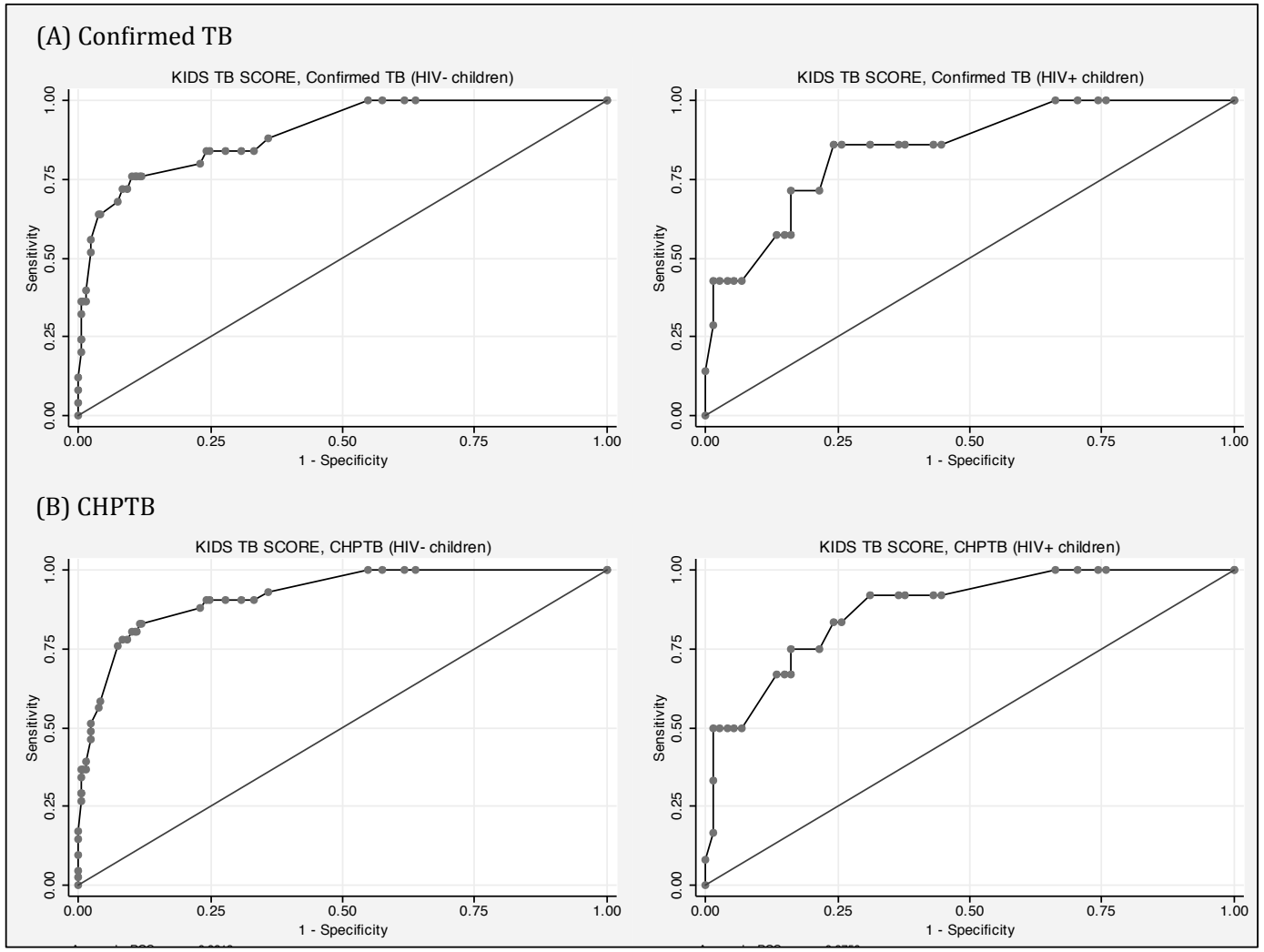


Figure 5.8 Receiver operator characteristics curves for the KIDS TB SCORE as a predictor of Confirmed TB & CHPTB in the testing (CPGH) dataset, by HIV status.



5.4 Discussion

Clinical diagnosis of childhood TB remains one of the most difficult challenges in clinical paediatrics in many parts of the world. In the absence of more sophisticated tests, even relatively modest improvements in clinical diagnosis could have substantial benefits for both case management and surveillance in many high TB burden settings. We evaluated the diagnostic performance of individual clinical signs and clinical decision-making tools among a large group of children representative of the presenting caseload with suspected TB at two Kenyan hospitals.

5.4.1 Individual clinical and radiological features

Our results reinforce the nature and the size of the diagnostic problem. Although some individual clinical features were strongly associated with a diagnosis of confirmed and/or highly probable TB, in most cases the effect size is small. As expected some radiological features were very strongly predictive of TB, including the presence of cavities, miliary shadowing, paratracheal lymphadenopathy, and airway compression. Although associations of the first two with highly probable TB may be put down to incorporation bias (both cavities and miliary shadowing are incorporated in the definition of highly probable TB), similar independent associations of all these features with confirmed TB validates their clinical predictive value.

Of particular note is the poor sensitivity of the TST for diagnosis of both CTB (46.2%) and CHPTB (48.7%). This is despite incorporation of a positive TST in some of the case definitions of highly probable TB, which one would expect to bias sensitivity estimates upwards. Although sensitivity was slightly better among older children and those with neither HIV nor malnutrition, these groups represent a small minority of the caseload. Overall the results are in stark contrast to current WHO guidance on the diagnosis of active TB in children, which states that "*In the greatest majority, infection with M. tuberculosis can be demonstrated by a TST*".[21]

Measured sensitivity is critically dependent on the definition and clinical spectrum of cases that define the denominator. There are few published studies with inclusion criteria that are well enough defined to compare our results. However one contemporary study from South Africa does provide a useful comparison.[151] Among 1024 children aged <13 years who presented to a primary care clinic with a persistent

cough for > 2 weeks, 197 were diagnosed with pulmonary TB (PTB), of whom 96 (49%) were bacteriologically confirmed and 75 (38%) were termed "radiologically certain". The sensitivity of TST among HIV un-infected children aged <3 and ≥3 years old was 89.3% and 81.9%, respectively. A study of 'suspected TB' cases in Malawi reported 79% and 54% sensitivity of the TST among HIV uninfected and HIV infected children with confirmed or clinically highly probable TB, respectively.[139] Both results are considerably higher than in our Kenyan study, and underpin the current WHO guidance.[21]

Possible reasons for the lower sensitivity we observed might include a higher proportion of severe and extra-pulmonary cases in our hospital based study, and/or higher rates of malnutrition. It is noteworthy that among HIV infected children in the South African study the sensitivity (17.6%) was very similar to that among children with HIV and severe malnutrition in our study (16.7% for CHPTB), suggesting that HIV-infected children in the South African study may also have had advanced disease with wasting. Other studies have found a similarly poor sensitivity among HIV infected children.[103, 152] Whatever the underlying cause of the differences, our results suggest that TST performance may vary considerably between settings, and support a re-evaluation of the WHO guidance as it is currently stated.

5.4.2 Clinical diagnostic tools

While underlining the clinical problem, the poor predictive value of individual clinical signs comes as no surprise, and it is for this reason that decision-making tools incorporating clinical and radiological features have been developed to aid diagnosis. This is the first prospective comparison of the large number of published clinical tools, and for many of these tools represents the first attempt to validate them in an independent dataset. The results reveal a wide range in diagnostic performance, and reinforce the inevitable trade off between diagnostic sensitivity and specificity. The very poor sensitivity of current WHO (2006) guidance[21] on childhood TB is particularly noteworthy, and suggests that in the desire to make the guidelines as specific as possible, diagnostic sensitivity has been over-compromised.

Two previous retrospective comparisons of clinical diagnostic tools have been published. One used data collected from a TB vaccine study to compare burden of

disease estimates based on each of a combination of South African and international tools, and found a similarly wide range in diagnostic performance.[146] A second examined the hospital notes of paediatric TB cases in the Democratic Republic of Congo and by comparison reported good sensitivity for most of the tools evaluated.[106] However the analysis was limited by the poorly defined diagnostic criteria for inclusion, a lack of microbiological confirmation in the large majority of cases, as well as the retrospective nature of the data collection.

Strengths of our analysis of clinical diagnostic tools include the broad and clearly defined inclusion criteria which facilitate generalization of the results; plus stringent case definitions that incorporate bacteriological confirmation and follow up, and prospective collection of the data required for each of the scores assessed - both of which minimize misclassification bias.

Another strength is the control group, all of whom presented with clinical features of suspected TB but were later classified as Not TB after investigation and follow up. By comparing our TB cases with a group of children who also required investigation for TB, we ensure our measures of diagnostic performance are generalizable to the population for whom the clinical tools are intended. This avoids the 'spectrum bias' that would ensue were one to select controls from other groups (e.g. well children or children with other conditions).[153]

To minimize misclassification bias we excluded from these analyses children with possible TB, since their true disease status is unknown. We recognize, however, that this may inadvertently introduce a form of spectrum bias if there are important differences in the clinical features between CHPTB cases and any 'true' TB cases among those classified as possible TB. Nevertheless we believe that any bias thus introduced is likely to be small in comparison to the misclassification bias that would result if we included the possible TB cases among our controls. Related to this, it is also important to note that the sensitivity and specificity of these diagnostic tools are dependent in a particular setting on the clinical spectrum of both TB and other diseases from which it must be distinguished, so our results may not be generalizable to all clinical contexts. This is in addition to variations in their positive and negative predictive values that depend on the local disease prevalence.

Countering a criticism of many similar diagnostic studies,[154] we first assessed the performance of these existing diagnostic tools before attempting to develop a new model. In order not to ignore information from the studies in which these tools were developed, we might then have attempted to update (improve) one or more of the better performing models by recalibrating it in the light of our data.[154] However the absence in the vast majority of cases of any formal statistical method for deriving these tools, and therefore of a published model which we might update, precluded these analyses. We therefore proceeded formally to develop our own KIDS TB Score.

5.4.3 The KIDS TB Score

This is the first study formally to derive and then to validate a clinical decision making tool for childhood TB. The ability of the KIDS TB Score to distinguish TB cases from controls is given by the *c* index - a measure of concordance that for logistic regression models is equivalent to the area under the ROC curves (AUC) - and is best assessed in the testing dataset.[155, 156] The AUCs for diagnosis of CHPTB (AUC = 0.913) and CTB (AUC = 0.891) are higher than all six of the other numeric scores assessed, demonstrating better discriminative ability. Comprising nine components - all of which are simple and seven of which are binary - the KIDS TB Score is also less complex than some of the better published numeric scores.[71, 133-135] Not surprisingly there was overlap in the constituent clinical features of each of these numeric scores, although only TB contact history and TST results were universally included.

To optimize the clinical utility of diagnostic categories derived using scoring tools, choice of appropriate numeric cutoffs is essential. This determines not just their diagnostic performance but also their usability in the field settings for which they are intended. Binary ('decisive') decision-making tools that use a single definition or cutoff are simplest to apply in practice (e.g. the Keith Edwards Score[132]),[154] but dichotomization forfeits potentially useful information on what is actually a continuous spectrum of risk.[157] Multiple risk strata in an "assistive" decision making tool allow a more accurate assessment of an individual child's risk; however they rely on higher level interpretation by the clinician[154] and/or distinct, evidence-based management guidelines for each stratum, if these benefits are to be translated into improved patient care.

We chose to define four risk strata based on the KIDS TB Score, which we term "TB Very Likely", "TB Likely", "TB Possible", and "TB Unlikely", loosely describing the hierarchy of risk that they encapsulate. Each of these is inevitably imperfect. The ability of the "TB Unlikely" category to exclude CHPTB with reasonable certainty (NPV 98.8%) in two thirds of children with suspected tuberculosis is potentially most useful. However 12% of CHPTB cases would still have been missed using this cutoff. The positive predictive value for TB of the "TB Very Likely" group (PPV 68.3%) is also useful to guide treatment decisions but only captures a quarter of CHPTB cases. Intermediate positive and negative predictive values for the "TB Possible" group and the "TB Likely" group will require additional guidelines for investigation and/or follow up of these children to further narrow down a group for treatment.

Further calibration to optimize the tool's performance depends on the perceived importance of sensitivity versus specificity, which in turn may be context specific. Some published tools are highly specific, and as a result less sensitive.[21, 135] These might be most appropriate in clinical settings where close follow up is possible and/or the prior probability (prevalence) of disease is low, so that the threshold for treatment may be set slightly higher. Other tools with a lower specificity but better sensitivity might be more suitable in settings where access to care and follow up are more challenging and/or the disease prevalence is higher.[137]

A final issue relates to our case definition for TB in the absence of a reliable gold standard. Inclusion of highly probable TB cases risks both misclassification bias (if some of these children did not actually have active TB) and incorporation bias (because some of the candidate predictors form part of the definition of highly probable TB). On the other hand, limiting cases to those with microbiologically confirmed TB will introduce important spectrum bias, since they are not representative of all childhood TB cases in either disease site (PTB or EPTB) or severity. Furthermore, even in the absence of bacteriological proof, the clinical case for TB treatment of those who meet the stringent definitions of HPTB is very strong. Similar associations of CTB and CHPTB with TB risk factors (see chapter 4) and with most clinical variables (allowing for confounding by disease severity; see section 5.3.1) also provide evidence for the specificity of the definition of HPTB. We therefore elected to use the composite case definition of confirmed or highly probable TB (CHPTB) in deriving our model.

5.4.4 Conclusion

Clinical diagnosis of TB in children remains an important but difficult area. Furthermore, progress in developing and validating clinical diagnostic tools has been limited in the past by a lack of methodological rigor.[24] Our results reinforce the inherent challenges, but also demonstrate how application of a rigorous statistical approach to carefully characterized groups of children with and without TB can lead to an improved diagnostic tool. We were able to derive and independently validate a KIDS TB Score that outperforms other published clinical diagnostic tools in our setting, and has promise as a useful risk stratification tool. Further research is now required to validate the performance and impact of the score in different populations and settings.

Clinical diagnostic tools such as the KIDS TB Score have an important place in clinical decision making, especially in low resource settings where specialist facilities for further investigation are lacking. Nevertheless, despite the improved performance of the KIDS TB Score, the ability of all these clinical tools correctly to identify children with TB remains modest. Indeed their performance may reflect a 'ceiling' of what can be achieved by this approach due to the inherently poor specificity of available clinical predictors. Further significant gains in diagnostic performance are likely to require more specialist tests including bacteriology. The performance of microbiological methods for childhood TB diagnosis among Kenyan children is investigated in the next chapter.

6 MICROBIOLOGICAL DIAGNOSIS OF CHILDHOOD TUBERCULOSIS

6.1 Introduction

Definitive diagnosis of tuberculosis in both children and adults rests on detection of *M. tuberculosis* from clinical specimens in the appropriate clinical context. Although microbiological confirmation is more difficult in children due to the paucibacillary nature of paediatric disease and the inherent challenges to appropriate specimen collection, it is arguably even more important than in adults due to more frequent disease misclassification when diagnosis relies on clinical features alone (see chapter 5).

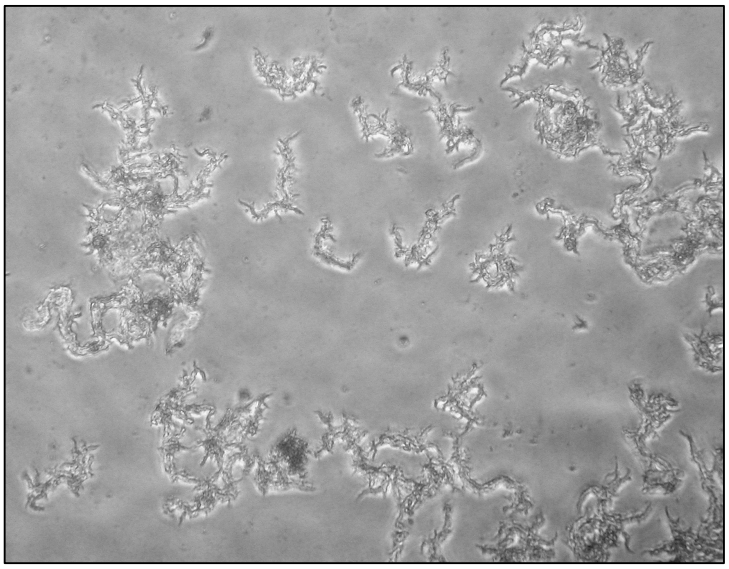
In adults, microbiological confirmation rests on sputum smear microscopy in most settings, and quality assured smear microscopy is a key component underpinning the WHO DOTS and Stop TB strategies.[158] In children, however, the yield of smear microscopy alone is very poor due to small numbers of bacilli in clinical specimens. Microbiological diagnosis has therefore traditionally depended on mycobacterial culture.

Mycobacterial culture affords several other advantages in addition to higher diagnostic sensitivity, including the potential for diagnosis earlier in the clinical course of disease (thereby potentially reducing transmission), and the ability definitively to identify isolates and perform drug susceptibility testing (DST).[55] Strengthening laboratory capacity including expansion of mycobacterial culture facilities in low resource, high burden countries, is therefore a key component of the Stop TB Strategy and Global Plan to Stop TB.[54, 158] Commercial liquid culture methods offer higher sensitivity and more rapid results than traditional solid media, are the standard of care for TB diagnosis in industrialized countries, and have been endorsed by the WHO for wider implementation.[54, 55, 159] However, barriers to their uptake include cost, a lack of technically trained personnel, and the need for biosafety level 3 facilities.[55]

Microcolony culture techniques provide culture and drug susceptibility results at a fraction of the cost of commercial liquid culture systems and do not require expensive biosafety level 3 laboratory facilities. These methods have therefore generated interest as a potential alternative to traditional culture methods in low resource settings. The

Microscopic Observation Drug Susceptibility (MODS) assay is one such novel microcolony culture technique that identifies *M. tuberculosis* by direct observation of its characteristic cording pattern of growth in liquid culture (Figure 6.1). Potential advantages over conventional methods in low resource settings include higher sensitivity, more rapid results that include DST, lower cost, and the lack of a requirement for biosafety level 3 facilities.[80]

Figure 6.1 Characteristic cord-like growth of *M. tuberculosis* in liquid culture (MODS culture from the KIDS TB Study)



The WHO has endorsed the use of MODS where genotypic and automated liquid culture and DST are not available,[160] and there is an accumulating body of literature validating it against other culture methods.[80, 161-170] However there are only limited data from children,[166, 169, 170] and no published studies evaluating the performance of MODS on induced sputum samples for paediatric TB diagnosis, despite the fact that sputum induction is now the preferred method for specimen collection from children.[21]

Given the paucibacillary nature of childhood TB there has long been hope that diagnosis would be revolutionized by nucleic acid amplification tests (NAATs) and the increased sensitivity they should afford. However the performance of NAATs for TB diagnosis among both adults and children has until recently been disappointing.[171] Interest in NAATs has been reignited by the publication in 2010 of data from a large multi-centre trial demonstrating the much better diagnostic performance of the Xpert MTB/RIF real time PCR assay for *M. tuberculosis*.[172] In this study of adult TB suspects the sensitivity of

the Xpert MTB/RIF assay compared to mycobacterial liquid culture was 98.2% and 72.5% among sputum smear positive and smear negative patients, respectively. A subsequent smaller study in South Africa derived sensitivity estimates of 100% and 58.1% respectively, among smear positive and negative children with culture confirmed TB.[57] Importantly, the feasibility of implementing the Xpert MTB/RIF assay in low resource settings has been demonstrated in a large multi-national operational study,[172] and the assay has been endorsed by the WHO for wider scale implementation.[173] However published data on its performance for childhood TB diagnosis remain limited, and there is a recognized need for further studies from other settings.[57, 174]

We compared the performance of microscopy, conventional liquid culture, MODS culture and the Xpert MTB/RIF assay for the diagnosis of TB among children recruited to the KIDS TB Study with suspected TB.

6.2 Methods

6.2.1 Processing of specimens for TB microscopy and culture

Specimen collection, transport and processing were closely coordinated to mitigate against bacterial overgrowth arising from delays and/or high ambient temperatures between collection and processing, thereby to minimize culture contamination. Specimen collection at both study sites took place on weekday mornings so that samples could be processed the same afternoon. Following collection all samples were immediately kept refrigerated at 4ºC, and then transported to the laboratory in dedicated cool boxes, where they were processed as soon as possible the same day.

We routinely decontaminated all specimens for TB culture, with the exception of specimens from normally sterile sites (e.g. CSF, pleural/ascitic fluid, tissue samples) that did not demonstrate growth on routine bacterial culture. Larger volume fluid specimens were pre-concentrated by centrifugation to optimize culture yield. Specimens were decontaminated by the modified Petroff's method, using a standard 4% sodium hydroxide (NaOH) and 15 minute decontamination protocol.[77] Decontaminated specimens were then centrifuged and the sediment re-suspended for preparation of smears and inoculation of culture media.

Initially we re-suspended the sediment in 1.0 ml phosphate buffer, and used 0.5 ml for mycobacterial culture using the Mycobacterial Growth Inhibitor Tube (MGIT) 960

system (see below); the remaining 0.5ml re-suspended sediment was archived at -80ºC for later real-time mycobacterial PCR using the Xpert MTB/RIF assay. Following a protocol amendment in April 2010 the sediment was re-suspended in 2.0 ml 7H9-OADC-PANTA culture broth containing Middlebrook 7H9 medium, oxalic acid, albumin, dextrose, and catalase (OADC, Becton Dickinson), and polymyxin, amphotercin B, nalidixic acid, trimethoprim, and azlocillin (PANTA, Becton Dickinson). This was then vortexed vigorously to ensure homogenization of the sample, two drops were used to make a smear, and the remainder was divided between MGIT culture (0.5 ml), MODS culture (0.5 ml) and the Xpert MTB/RIF assay (0.5 ml). A 0.5 ml aliquot was kept as a 'backup specimen' in case of MODS culture contamination, in keeping with the published MODS protocol.[79]

6.2.2 Microscopy for Acid Fast Bacilli (AFB)

Smears were prepared from each specimen, stained using the Ziehl Neelson (ZN) method, and a minimum of 300 high power fields examined for AFB using the x100 objective. Results were reported based on WHO guidelines (Table 6.1).[78]

Table 6.1 Reporting of AFB microscopy results

No. acid fast bacilli (AFB)	Report
No AFB's seen in entire smear	No AFB observed
1-9 AFB's seen in entire smear	Actual no. AFB seen reported
10-99 AFB's per 100 high power fields	1+
1-10 AFB's per filed in at ≥ 50 high power fields	2+
> 10 AFB's per field in ≥20 high power fields	3+

6.2.3 Automated liquid mycobacterial culture: BD MGIT 960 system

Automated liquid mycobacterial culture was performed using the Becton Dickinson Mycobacterial Growth Inhibitor Tube (MGIT) 960 system (BD Diagnostics, Sparks, MD, USA). Protocols were based closely on published guidelines.[77] From all cultures that flagged positive a smear was made for ZN microscopy and a sample inoculated onto 5% blood agar and incubated at 37ºC for 48-72 hours to check for bacterial contamination. Positive mycobacterial cultures were confirmed by the presence of typical AFB on ZN microscopy. Contamination was defined as the presence of colonial growth on blood agar inoculated from the MGIT tube, in the absence of AFB on ZN microscopy. Cultures that were flagged positive by the MGIT incubator but were AFB negative and demonstrated no growth on blood agar were re-incubated and re-examined following a

further 7 days culture incubation. If they remained AFB and bacterial growth negative after a maximum of 2 re-incubation periods they were reported as culture negative.

6.2.4 Microscopic Observation Drug Susceptibility (MODS) assay

In order to compare the performance of MGIT culture with the MODS assay, following the protocol amendment in April 2010, a proportion of the re-suspended, decontaminated sediment was also cultured by MODS. Protocols were based closely on published guidelines.[79] Briefly, the re-suspended sediment was further diluted in 7H9-OADC-PANTA media and then used to inoculate pre-prepared 24-well tissue-culture plates. 900 µl of each sample was inoculated into each of 4 wells: 2 containing 100 µl 7H9-OADC culture broth, 1 containing 100 µl rifampicin 10 µg/ml, and 1 containing 100 µl isoniazid 4 µg/ml. Each plate also included negative control wells containing only 7H9-OADC-PANTA, and each batch a positive control plate containing a known concentration of *M. tuberculosis* in culture broth. Plates were sealed in a ziplock back after inoculation and incubated at 37^0C.

The cultures were examined under an inverted light microscope at x100 magnification on day 5, and at x40 magnification every Monday, Wednesday and Friday thereafter, until either positive or contaminated, or the 21 day incubation period was complete. Positive cultures were defined by the growth of 2 or more cords characteristic of *M. tuberculosis* in both drug-free wells. An indeterminate result was defined as growth of <2 cords in one or both drug-free wells; and contamination as florid overgrowth of the wells with clouding. Drug susceptibility was defined as growth in the drug-free wells but not in the drug-containing wells. Contaminated cultures were repeated using the re-decontaminated MODS backup specimen.

In order to compare the performance of MGIT and MODS culture methods and to mitigate against possible observer bias in the interpretation of either result, laboratory technologists were blinded to the identify of the MODS portion of each specimen. Specimens were instead identified by an electronically generated random numeric code, the key to which was held by the Principal Investigator.

6.2.5 Mycobacterial speciation & identification of *M. tuberculosis* complex (MTBC)

We identified positive MGIT and MODS mycobacterial cultures as *M. tuberculosis* complex (MTBC) or non-MTBC in the first instance using the BD MGIT TBc Identification

test (TBc ID, Becton Dickinson, Sparks, MD, USA) according to the manufacturer's instructions. Further confirmation and speciation of all positive mycobacterial cultures was then performed using the Hain GenoType® lineprobe assay platform (Hain Lifescience Gmbh, Nehren, Germany). Cultures that tested positive for MTBC with the TBc ID test were tested using the Genotype® MTBC assay, which permits genetic differentiation of the following species/strains belonging to the *Mycobacterium tuberculosis* complex: *M. africanum*, *M. bovis* BCG, *M. bovis* ssp. bovis, *M. bovis* ssp. caprae, M. microti, and *M. tuberculosis*/"*M. canettii*". Cultures that were TBc ID test negative were tested using the Genotype® Mycobacterium CM assay, followed by the Genotype® Mycobacterium AS assay if the CM assay did not provide a definitive species identification. Using this methodology we were able to validate the performance of the TBc ID test for identification of MTBC against a gold standard defined by the GenoType® lineprobe assays, utilizing the Xpert MTB/RIF assay and the available clinical data to adjudicate in the case of discrepancies. This data has been published elsewhere.[175]

6.2.6 Xpert MTB/RIF Assay

Frozen aliquots of the decontaminated and re-suspended sediments were assayed for the presence of MTBC DNA using the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions. Since resources did not allow the Xpert MTB/RIF assay to be performed on all clinical specimens we selected samples for analysis as follows. Firstly, to mitigate against reduced diagnostic yield due to mycobacterial culture contamination, we performed the Xpert MTB/RIF assay on a stored aliquot of all specimens whose MGIT culture was contaminated, and in a smaller subset compared yield from the original specimen with that from the contaminated culture broth. Secondly, we performed the assay on all specimens from children with confirmed or highly probable TB, or who were treated empirically for TB or died during follow up, in order to maximize detection of *M. tuberculosis* among the highest risk children, and to allow estimation of the test sensitivity. Thirdly, to investigate test specificity we tested a random selection of samples from children in whom TB had been excluded (Not TB). Finally, we performed the assay on a random selection of samples that had grown non-tuberculous mycobacteria (NTM), to investigate whether *M. tuberculosis* culture was inhibited by growth of NTM.

6.2.7 Quality assurance

All laboratory procedures incorporated detailed quality control procedures that met international standards for Good Clinical Laboratory Practice (GCLP), and included external quality assurance monitoring by the United Kingdom National External Quality Assessment Scheme (UK NEQAS). Formal external GCLP accreditation was granted to the laboratory in April 2010 and renewed annually.

6.2.8 Statistical Methods

Analyses were performed per specimen to reflect routine practice in a diagnostic TB laboratory.[80] We used a composite of MGIT and MODS culture and the Xpert MTB/RIF assay to define the gold standard, such that a specimen in which MTBC was detected by any one of these tests was considered MTBC positive. We calculated the sensitivity and specificity of AFB microscopy, MGIT and MODS culture, and the Xpert MTB/RIF assay for detection of MTBC in paediatric clinical specimens against this gold standard. To facilitate comparability with other studies we also calculated the sensitivity of the Xpert MTB/RIF assay against mycobacterial culture alone (isolation of MTBC by either MGIT or MODS). Finally we calculated the sensitivity of each method among all specimens from (a) confirmed TB cases; and (b) confirmed and highly probable TB cases. We used McNemar's χ^2 test to compare proportions between tests on paired aliquots of the same sample, and the Wilcoxon signed rank test to compare time to detection of MTBC by each culture method. Independent associations between smear and HIV status and a positive Xpert MTB/RIF assay were explored using multivariable logistic regression.

6.3 Results

6.3.1 Microscopy and culture yield

A breakdown of AFB smear microscopy and mycobacterial culture results is given by specimen type in Table 6.2. Induced sputa accounted for the majority of specimens (83%), followed by spontaneous sputum samples (5.7%) and CSF (3.0%). Overall, only 2% specimens were AFB smear positive; the proportion was highest among spontaneous sputum samples (10%) and fine needle aspirates of lymph nodes (5%).

123 (4%) specimens were culture positive for MTBC, including 41/50 (82%) AFB smear positive specimens. Of the 9 AFB smear positive specimens that were not culture positive for MTBC, 2 were FNA slides for microscopy unsuitable for culture, 3 were from patients on TB treatment who had had MTBC isolated from other specimens, 2 were culture positive for non-tuberculous mycobacteria (NTM), and 2 specimens were mycobacterial culture negative, both of which were scanty smear positive. Overall, in the absence of TB treatment, 41/45 AFB smear positive specimens were culture positive for MTBC (PPV 91.1%, [78.8 to 97.5]).

6.3.2 Diagnostic performance of MGIT and MODS culture methods

A total of 1,899 specimens were cultured using both MGIT and MODS methods, of which 1,788 (94%) were respiratory specimens (spontaneous or induced sputa or gastric aspirates). The vast majority of specimens were induced sputa (1,647, 87%).

MGIT culture was positive for 180 of these specimens, of which 90 (50%) were MTBC (PPV for MTBC of a positive AFB smear from MGIT culture *without additional confirmatory biochemical or molecular tests*, 50% [95% CI 42.5 to 57.5%]).

Mycobacterial growth with cording (Figure 6.1) was observed in 90/1,899 (4.7%) specimens cultured using the MODS method. Growth of MTBC was confirmed (using the TBc ID test and PCR) in 77/90 of these specimens, giving a PPV of cording growth alone for MODS isolation of MTBC of 85.6% (76.6 to 92.1%). NTM were isolated from the remaining 13 MODS positive culture broths. Thus the specificity of microscopic observation of cording growth for isolation of MTBC by the MODS method was 1809/1822, 99.3% (98.8 to 99.6%).

Mycobacterial species for non-MTBC isolates are summarized in Table 6.3.

Table 6.2 AFB microscopy and culture yield by specimen type

Specimen type	No. Specimens (% of total)	culture positive		culture negative		total smear positive (%)	Total culture positive (% specimens cultured)[1,3]
		smear positive (%)	smear negative (%)	smear positive (%)	smear negative (%)		
abscess fluid/pus	38 (1.3%)	1 (3%)	2 (6%)	1 (3%)	34 (89%)	2 (5%)	3 (8%)
ascitic fluid	23 (0.8%)	0 (0%)	1 (4%)	0 (0%)	22 (96%)	0 (0%)	1 (4%)
bone marrow[2]	6 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CSF	87 (3.0%)	0 (0%)	1 (1%)	1 (1%)	85 (98%)	1 (1%)	1 (1%)
FNA lymph node[3]	57 (2.0%)	1 (2%)	0 (0%)	0 (0%)	4 (7%)	3 (5%)	1 (20%)
gastric aspirate	29 (1.0%)	0 (0%)	1 (3%)	0 (0%)	28 (97%)	0 (0%)	1 (3%)
induced sputum	2,388 (83%)	21 (1%)	58 (2%)	5 (0%)	2,304 (96%)	26 (1%)	79 (3%)
joint aspirate	1 (<0.1%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
lymph node biopsy	5 (0.2%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)	0 (0%)	0 (0%)
pericardial fluid	3 (0.1%)	0 (0%)	1 (33%)	0 (0%)	2 (67%)	0 (0%)	1 (33%)
pleural fluid	34 (1.2%)	1 (3%)	5 (15%)	0 (0%)	28 (82%)	1 (3%)	6 (18%)
sputum	163 (5.7%)	17 (10%)	12 (7%)	0 (0%)	134 (82%)	17 (10%)	29 (18%)
tissue biopsy	4 (0.1%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)
urine	22 (0.8%)	0 (0%)	1 (5%)	0 (0%)	20 (91%)	0 (0%)	1 (5%)
Total[3]	2,860 (100%)	41 (1%)	82 (3%)	7 (0%)	2,672 (93%)	50 (2%)	123 (4%)

[1] Percentage of the total number of specimens cultured

[2] Bone marrow specimens not cultured

[3] Only 5/57 (9%) FNA specimens were suitable for culture; the remainder were smears for AFB microscopy alone.

Table 6.3 Identity of non-MTBC isolates from MGIT and MODS culture

Identity of non-MTBC isolates	MGIT N (%)	MODS N (%)
Mycobacterium fortuitum	22 (24%)	3 (23%)
Mycobacterium simiae	10 (11%)	3 (23%)
Mycobacterium asiaticum	4 (4%)	0 (0%)
Mycobacterium gordonae	2 (2%)	0 (0%)
Mycobacterium malmoense	3 (3%)	0 (0%)
Mycobacterium intracellulare	3 (3%)	0 (0%)
Mycobacterium kansasii	1 (1%)	0 (0%)
Mycobacterium szulgai / M. intermedium	1 (1%)	0 (0%)
Non-tuberculous mycobacteria – species not identified	29 (32%)	4 (31%)
Mycobacteria not identified	6 (7%)	1 (8%)
PCR results pending	9 (10%)	2 (15%)
Total	**90**	**13**

The distribution of MTBC culture positive results and sensitivity estimates for MTBC culture for each method are summarized in **Table 6.4**. Culture yield was higher from MGIT than from MODS (sensitivity 87% [79 to 93%] compared with 72% [62 to 80%], p=0.047), with evidence for a similar difference in culture yield when the analysis was confined to all respiratory specimens (p=0.05). Although a similar trend was observed for induced sputum specimens alone, analytical power was limited by the smaller number of positive cultures (p=0.248, **Table 6.4**).

Table 6.4 Summary of MGIT & MODS MTBC culture positive results

	MGIT positive		MGIT negative		MGIT Sensitivity, % (95% CI)	MODS Sensitivity, % (95% CI)	p value
	MODS positive	MODS negative	MODS positive	MODS negative			
All specimens	65	25	12	1,797	87 (79 to 93)	72 (62 to 80)	0.047
Respiratory specimens	58	22	10	1,698	87 (78 to 93)	72 (61 to 81)	0.050
Induced sputum	37	17	10	1,583	84 (73 to 92)	70 (58 to 81)	0.248

Twelve specimens that were MODS culture positive were not isolated by MGIT. The Xpert MTBC assay confirmed the presence of MTBC in the original specimen for 6 of these, 3 of which had grown NTM and 3 of which were contaminated on MGIT culture. Among the remaining 6 specimens for which the Xpert MTBC assay was negative, 3 were from patients who had MTBC isolated by MODS from another specimen. The remaining 3 specimens were treated as MTBC culture positive for the purposes of this analysis to generate the most positive estimates for MODS sensitivity pending definitive investigation of possible cross-contamination by molecular typing; however they were not considered definitive evidence of MTBC for patient diagnostic classification. Direct evidence for cross contamination was detected by growth in the negative control wells in only one of 605 MODS plates over the entire study period.

We explored whether the lower culture yield for MODS may have been due to a learning effect as laboratory staff became more experienced with performing MODS. Table 6.5 below shows the number of MTBC isolates obtained by each culture method in each quarter over the 2 years during which both methods were used. There was no evidence of any trend in the proportion of isolates identified by MODS over this period (χ^2 test for trend, p=0.476).

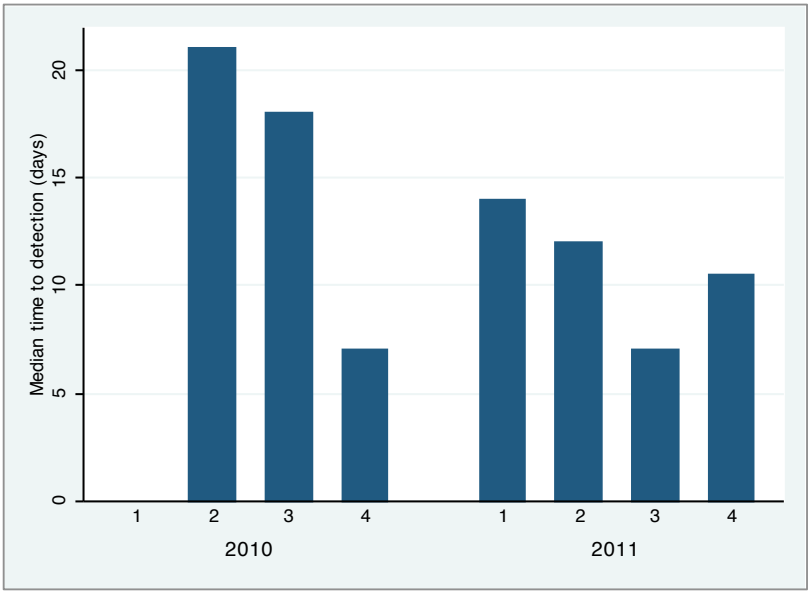
Table 6.5 Number of MTBC isolates from MGIT and MODS by quarter (2010 to 2011)

	2010				2011			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
MGIT	3	12	9	12	7	13	18	16
MODS	2	12	6	5	6	9	23	14

There was evidence, however, that the time to detection of MTBC positive MODS cultures did decrease over time (Figure 6.2), with a median time of 16 days (IQR 9 to 21) in 2010 compared with 10 days (7 to 14) in 2011 (p=0.002).The median time to detection of MTBC was shorter for MODS culture than for MGIT culture (11 days [IQR 7 to 16 days] overall for MODS versus 13 days [7 to 18 days] for MGIT, p=0.007).

The proportion of cultures that were contaminated was dramatically lower for MODS than for MGIT (8.5% versus 21%, p<0.01).

Figure 6.2 Median time to detection of MTBC positive MODS cultures, by yearly quarter (2010 to 2011)



6.3.3 Diagnostic performance of the Xpert MTB/RIF assay

Xpert MTB/RIF assay results were available for 122 of 125 MTBC culture positive specimens (98%), among which 87 were Xpert MTB/RIF positive, giving an overall sensitivity *compared to culture* of 71% (95% CI 62 to 79%). The majority were respiratory specimens, among which the sensitivity was 73% (63 to 81%). Sensitivity was 95% (83 to 99%) among smear positive specimens, but only 60% (48 to 70%) among smear negative specimens (p<0.001).

We investigated the specificity of the Xpert MTB/RIF assay by performing the test on 472 specimens from children in whom TB had been excluded (Not TB cases), including 101 culture negative specimens, 321 culture-contaminated specimens, and 50 specimens from which NTM had been isolated. The assay was negative in all 472 cases, giving a specificity of 100% (one sided 97.5% CI 99.2 to 100%).

Table 6.6 Breakdown of Xpert MTB/RIF results by mycobacterial culture result

Culture result	No. tested	Xpert MTB/RIF positive (%)
MTBC *	122	87 (71%)
NTM	124	3 (2%)
Contaminated	559	3 (0.5%)
Negative	411	1 (0.2%)
Total	1,215	94 (8%)

** Includes 3 samples that were contaminated by MGIT but MTBC culture positive by MODS*

Having confirmed the very high specificity of the Xpert MTB/RIF assay, we calculated the test sensitivity against the composite gold standard defined by a positive culture or Xpert MTB/RIF result. A breakdown of Xpert MTB/RIF results by mycobacterial culture result is shown in Table 6.6. Seven specimens from five children – all of whom had clinically highly probable TB - were MTBC culture negative but Xpert MTB/RIF positive. **Table 6.7** shows a breakdown of the test sensitivity overall and by smear status, HIV status and specimen type when these specimens are incorporated in the denominator. Sensitivity was slightly improved overall and among respiratory specimens as expected (73% and 75%, respectively). It remained higher among smear positive specimens than among smear negative specimens, and there was also a trend towards higher sensitivity among specimens from HIV positive children. In a multivariable logistic regression model including smear and HIV status as predictors of Xpert MTB/RIF test positivity, smear status was strongly associated with a positive test (OR 10.0 [95% CI 2.2 to 44.6; p=0.003]), but there was no good evidence of an independent association between HIV status and test positivity (OR 1.9 [0.6 to 5.7; p=0.226]).

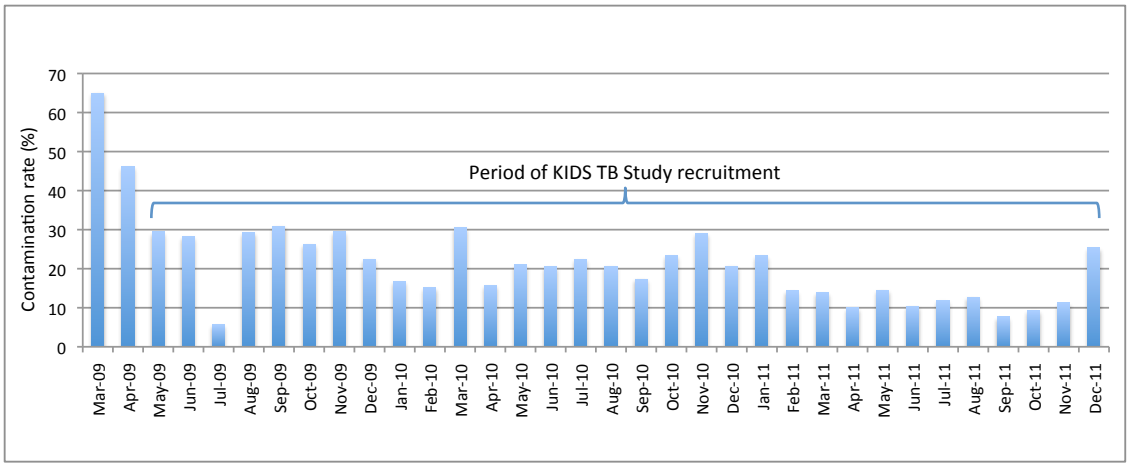
Table 6.7 Sensitivity of the Xpert MTB/RIF assay against the composite gold standard

	No. specimens	Xpert MTB/RIF positive	Sensitivity, % (95% CI)	p value
Smear status				
smear positive	40	38	95 (83 to 99)	<0.001
smear negative	89	56	63 (52 to 73)	
HIV status				
HIV positive	34	29	85 (69 to 95)	0.058
HIV negative	95	65	68 (58 to 78)	
Specimen type				
respiratory specimens	114	85	75 (66 to 82)	0.233
non-respiratory specimens	15	9	60 (32 to 84)	
All MTBC positive specimens	129	94	73 (64 to 80)	

6.3.4 Culture contamination

A relatively high proportion of MGIT cultures were contaminated throughout most of the KIDS TB study period (Figure 6.3). Following establishment of the laboratory, very high initial contamination rates were quickly reduced prior to commencement of the study, due to a combination of training and improved processes for specimen collection and transport driven by the study protocols. However the proportion of cultures that were contaminated remained relatively high throughout the study despite regular audit, feedback, reagent review, and rigorous implementation of laboratory protocols including both internal and external quality assurance systems.

Figure 6.3 Monthly MGIT contamination rate as a percentage of samples processed (2009-2011)



The majority of specimens were processed on the day of collection, and there was no association between the number days between collection and processing and risk of contamination. However samples transported from Coast Provincial Hospital in Mombasa were more likely to be contaminated than those collected at Kilifi District Hospital (24% versus 15%, $p<0.001$). The median time to detection of contaminated cultures was 7 days (IQR 3 to 20 days). All of the 416 negative control specimens processed with every culture batch over the study period were culture negative at 42 days, arguing against laboratory cross-contamination as an important contributing factor.

To investigate whether the observed high rates of contamination were related to specimen collection practices, in late 2010 we independently audited sputum induction by direct observation of the process. This revealed generally good adherence to the standard operating procedure by the clinical team; however it was noted that in practice many children were not fasted for the stipulated 3 hours prior to specimen collection since mothers frequently gave them breakfast despite instructions to postpone feeding until after the procedure. Feedback from this audit was incorporated into comprehensive, formal retraining of the clinical team in specimen collection practices in early 2011, following which (with the exception of December 2011) the contamination rate has remained between 8 and 15%; this reduction has been sustained in 2012 (data not shown).

Since bacterial contamination may outcompete and thereby inhibit growth of mycobacteria in liquid culture, we performed the Xpert MTB/RIF assay on the stored aliquot of specimens whose MGIT cultures were contaminated, in order partially to mitigate this effect. The Xpert MTB/RIF assay was positive in 6/561 (1%) specimens tested. Three of these specimens were MODS culture positive for MTBC; the remaining three came from patients with clinically highly probable TB who had been treated empirically for tuberculosis.

6.3.5 Test sensitivity among samples from confirmed and highly probable TB cases

The sensitivity of each method for detection of MTBC among specimens from confirmed TB cases and CHPTB cases is shown in Table 6.8. Overall the sensitivity of MGIT was higher than that of both MODS (p=0.001) and the Xpert MTB/RIF assay (p=0.007). There was no evidence that the sensitivity of MODS and the Xpert MTB/RIF assay differed among the 161 samples for which paired results from both tests were available (60 positive by both tests, 16 positive by MODS only, 24 positive by Xpert MTB/RIF only, and 61 negative by both tests; McNemar's χ^2 test, p=0.268).

Table 6.8 Test sensitivity among specimens from confirmed and highly probable TB cases

	Specimens from Confirmed TB cases				Specimens from CHPTB cases			
	N	test +ve	Sensitivity, % (95% CI)		N	test +ve	Sensitivity, % (95% CI)	
Smear microscopy								
All specimens	200	42	21	(16 - 27)	348	47	14	(10 - 18)
Respiratory specimens	164	37	23	(16 - 30)	288	40	14	(10 - 18)
Induced sputum	128	20	16	(10 - 23)	238	23	10	(6 - 14)
MGIT culture								
All specimens	194	114	59	(51 - 56)	338	114	34	(29 - 39)
Respiratory specimens	164	101	62	(54 - 69)	288	101	35	(30 - 41)
Induced sputum	128	71	55	(46 - 64)	238	71	30	(24 - 36)
MODS culture								
All specimens	167	77	46	(38 - 54)	261	77	30	(24 - 35)
Respiratory specimens	142	68	48	(39 - 56)	230	68	30	(24 - 36)
Induced sputum	110	47	43	(33 - 53)	189	47	25	(19 - 32)
Any positive culture*								
All specimens	194	123	63	(56 - 70)	338	123	36	(31 - 42)
Respiratory specimens	164	109	66	(59 - 74)	288	109	38	(32 - 44)
Induced sputum	128	79	62	(53 - 70)	238	79	33	(27 - 40)
Xpert MTB/RIF assay								
All specimens	185	92	50	(42 - 57)	319	92	29	(24 - 34)
Respiratory specimens	157	84	54	(45 - 61)	275	84	31	(25 - 36)
Induced sputum	121	58	48	(39 - 57)	225	58	26	(20 - 39)

*MGIT and/or MODS culture positive

6.4 Discussion

6.4.1 Current challenges, opportunities and evidence gaps

The single greatest obstacle to improving both childhood TB surveillance and the clinical management of children with TB is the lack of reliable diagnostic tools, particularly in low resource settings where the disease burden is greatest.[130] Crude estimates suggest that in the 22 highest burden TB countries the great majority of childhood cases are currently not diagnosed and/or not reported.[176] This observation, combined with the suboptimal performance of clinical and radiological features alone for diagnosis of TB among children (see chapter 5), highlights the urgent need for better diagnostic tools in most settings. While the ultimate goal must be the development of a reliable and affordable point of care diagnostic test, to date progress in this area has been slow. Meanwhile the expansion of access to existing laboratory methods for TB diagnosis championed by the WHO[54, 55] provides an opportunity to improve both diagnosis and surveillance of childhood TB in many settings. Good quality data evaluating and comparing the performance of these laboratory methods among paediatric specimens are however needed to optimize their future use and interpretation in the range of settings in which they will increasingly become available.

Culture and NAATs for *M. tuberculosis* are currently the mainstay of TB laboratory diagnosis,[109, 177] but in contrast to adult TB there is a paucity of studies evaluating the available methods for diagnosis of TB in children. This lack of data is further compounded in many of the available studies by small numbers of confirmed TB cases and *M. tuberculosis* positive specimens,[166, 169, 170 178] even smaller numbers of cases in the under 5 year age group among whom diagnosis is most challenging,[166, 169, 170, 178] and the omission in some studies of currently recommended 'gold standard' methods for specimen collection and mycobacterial culture. [166, 169, 170]

6.4.2 Strengths of the KIDS TB Study for evaluation of laboratory diagnostic methods

Strengths of the KIDS TB Study for evaluation and comparison of laboratory methods for childhood TB diagnosis derive from the comprehensive inclusion criteria and careful diagnostic work up of a large number of children. Prospective recruitment of all children who met broad, pre-defined inclusion criteria ensures generalizability to a wide range of settings and clinical syndromes, including among children under 5 years who accounted

for 81% of those investigated and more than half of confirmed cases (see chapter 3). Importantly, attempts to achieve a microbiological diagnosis in as many children as possible followed current international recommendations for specimen collection and culture, including the use of sputum induction[21] and liquid mycobacterial culture.[159]

Our study compared automated liquid mycobacterial culture using the MGIT 960 system with two promising newer methods: the MODS microcolony culture technique and the Xpert MTB/RIF assay. Both MODS and the Xpert MTB/RIF assay have already demonstrated much higher sensitivity than sputum smear microscopy, and more rapid results than conventional culture methods, without the requirement for a biosafety level 3 laboratory.[80, 169, 170, 172, 179] We therefore evaluated their performance among the relatively paucibacillary specimens obtained from children to explore their potential role in expanding laboratory diagnosis of childhood TB in resource limited settings.

6.4.3 Smear microscopy and culture yield

The low proportion of smear positive specimens from children with confirmed and highly probable TB in our study underlines the severe limitations of smear microscopy alone for childhood TB diagnosis, even when appropriate specimens can be obtained, and is consistent with a recent study from the region which used similar definitions for highly probable TB.[178] However this and another recent study[166] both found a higher overall yield of liquid TB culture among confirmed and probable paediatric TB cases (55% and 49%, respectively, versus 38% in our study). This might reflect the greater proportion of young children in our study, who are more likely to have paucibacillary disease, and the application of broad screening criteria that may have identified children earlier in the course of their disease. In general, comparison of diagnostic yield between studies is hampered by widely varying clinical definitions, specimen types and culture methods. Our findings are nevertheless consistent with other studies, lying in the middle of the published range of liquid culture yield from paediatric specimens.[16, 166, 178, 180, 181]

We report overall culture yield based on the combination of MGIT and MODS culture, since the yield from each method is likely to have been reduced by the reduction in sample volume inherent in splitting each specimen. Stochastic variation in culture positivity by each method is also likely, despite careful homogenization of the samples prior to aliquoting, due to the small numbers of bacilli present in paediatric specimens,

such that the threshold for culture positivity might only be met in one of the two aliquots by random chance. Splitting the specimen into equal aliquots nevertheless allows fair comparison of the sensitivity of each culture method when performed on a large number of samples.

6.4.4 Comparison of MGIT and MODS culture for detection of MTBC

We found that MGIT was more sensitive than MODS for detection of MTBC both overall and among respiratory samples. This finding is in keeping with the only other published study to compare MGIT with MODS for detection of MTBC in paediatric specimens.[166] No previous study has evaluated the performance of MODS for induced sputum samples, despite the fact that sputum induction is now the recommended standard of care for obtaining respiratory specimens from young children.[21] Although our study was not powered to answer the question definitively, the trend towards lower MODS yield among induced sputum specimens is nevertheless highly suggestive of a real difference between the two methods in the context of similar results for respiratory specimens overall.

Despite the lower sensitivity, the time to detection of MTBC was shorter for MODS than MGIT, and shortened over time as the experience of the laboratory personnel increased. Since detection of MTBC by MODS depends on direct visualization of the growing mycobacteria, time to detection is inherently dependent on the frequency with which the MODS plates are examined, which in turn depends on available staffing, since examination of large numbers of plates is time consuming.[109] The median time to detection of MTBC by MODS in our study (11 days overall; 10 days in 2011) is similar to the 10 days reported in another study of MODS for childhood TB diagnosis in which plates were also read on alternate days.[170]

Both sensitivity and time to detection of positive cultures are also a function of the number of viable mycobacteria present in the culture inoculum. This may also partly explain the shorter times to detection reported for several studies of adult specimens,[80, 161, 165, 167, 182] although in a recent systematic review of 9 adult studies the mean time to detection was 9.9 days, similar to our findings among paediatric specimens. Some of the heterogeneity observed between studies is likely to be explained by variations between laboratories in experience performing the assay, since MODS relies of the subjective

assessment of typical mycobacterial growth in the culture wells. It is noteworthy that MODS performed best against a gold standard that included MGIT in the studies from Lima, Peru, where the assay was developed,[80, 183] with lower assay sensitivity reported from other centres.[165, 166, 168, 182] Nevertheless we cannot exclude the possibility that MODS might be inherently less sensitive compared to MGIT among paucibacillary specimens such as those from children.

Two notable advantages of MODS over MGIT are the lower proportion of NTM isolated, most of which are of likely no clinical relevance (author, unpublished data), and the much lower proportion of contaminated cultures. The former derives from the characteristic cording growth of *M. tuberculosis* in liquid culture, which differs from other commonly isolated mycobacteria, notably *M. avium* and *M. kansasii*.[183] However, although the specificity of a MODS positive culture for MTBC was >99%, consistent with the high specificity reported in other studies,[80, 165, 166, 168, 182] the predictive value for the presence of MTBC of a positive MODS assay alone (i.e. the presence of cording growth) was only 86% in our study. This reflects the high proportion of NTM isolated (50% of positive MGIT cultures). While the study's broad inclusion criteria and resulting low prevalence of TB among the subjects investigated may partly explain the relatively high proportion of NTM isolates, high rates of NTM have also been reported from other centres in Africa.[184-186] Our findings would therefore support recommendations from the WHO that the presence of MTBC in MODS cultures be further confirmed, for example by the inclusion for each sample of a well containing *p*-nitrobenzoic acid in which MTBC growth is inhibited.[160] The advantage of this method over other biochemical or molecular tests is that microtitre plates containing concentrated mycobacterial cultures need not be opened, thereby reducing the biosafety risk and obviating the need for biosafety level 3 facilities.

6.4.5 Performance of the Xpert MTB/RIF assay

The lack of a requirement for complex laboratory facilities is one of many potential advantages of the Xpert MTB/RIF assay. In our study the performance of the Xpert MTB/RIF assay was as good as that of MODS, with a sensitivity against the composite gold standard of 73%. This represents a small milestone in the development of NAATs for diagnosis of TB, since historically NAATs have performed inferiorly to liquid mycobacterial culture methods. Importantly we were also able to confirm the assay's

very high specificity in a large sample of specimens from children in whom TB had been excluded. The sensitivity among both smear positive and negative samples was similar to two previous paediatric studies.[57, 178] Although a higher sensitivity among HIV infected children has been reported,[57] we were unable to demonstrate an independent association between HIV status and test sensitivity in our dataset.

6.4.6 Study limitations

Our analysis of laboratory methods for identification of *M. tuberculosis* in paediatric clinical specimens does have some important potential limitations. The first of these is the high proportion of contaminated MGIT cultures, since this may limit the sensitivity of our gold standard for detection of MTBC. Arguably, however, this might be considered a strength of the study, since it reflects the situation (and the performance of the 'gold standard') in many parts of Africa, where the proportion of contaminated MGIT cultures in published studies varies from 12% to 55%[184, 187-191] - much higher than the average of 8.4% reported in a meta-analysis of European and North American data,[192] and the target of 7-8% recommended by the Centers for Disease Control and Prevention (CDC). We chose not to increase the harshness of the decontamination protocol since paediatric specimens are likely to be particularly sensitive to over-decontamination due to the paucibacillary nature of childhood TB. A recent study in South Africa in which a reduction in NaOH concentration from 6% to 4% led to a 60% increase in culture yield from paediatric specimens appears to provide support for this approach.[193] In theory application of the Xpert MTB/RIF assay - which had a sensitivity of 63% among AFB smear negative specimens - to all culture contaminated specimens should have mitigated most of the expected reduction in culture sensitivity due to contamination.

Due to finite resources we were unable to perform the Xpert MTB/RIF assay on all specimens in our study. Nevertheless by applying the assay to all specimens from confirmed and highly probable cases, and to nearly 500 specimens from children without TB, were able to derive reasonably precise estimates for the sensitivity of the assay and a very precise estimate of assay specificity. The high precision with which we were able to demonstrate 100% specificity of the assay supports its incorporation in a composite gold standard of culture and/or Xpert MTB/RIF positivity for assessing the performance of each test. By only performing Xpert MTB/RIF on a proportion of

specimens we may have excluded other culture negative / Xpert MTB/RIF positive specimens, thereby underestimating the true sensitivity of the Xpert MTB/RIF assay.

A weakness of our MGIT-MODS comparison is the omission for the current analysis of molecular typing of isolates derived from specimens with discordant results between the two methods. Although Xpert MTB/RIF and isolation of MTBC from other specimens from the same patients provided corroborative evidence for the presence of MTBC in the majority of cases, molecular typing would help rule out cross-contamination as the culprit. No evidence for cross-contamination was detected by negative control specimens included in every MGIT batch and MODS plate throughout the study, and although cross contamination is more likely to occur for MODS due to the proximity of the wells on the plate, this has been shown to be rare.[194] Nevertheless any bias arising from a failure to identify such isolates is likely to favour MODS, so the finding that MGIT was superior to MODS for detection of MTBC is unlikely to be altered.

6.4.7 Conclusion

Our study reinforces previous findings that automated liquid mycobacterial culture remains the best available method for detection of MTBC in paediatric clinical specimens. Evaluations of both the MODS and Xpert MTB/RIF assays for diagnosis of childhood TB are broadly similar to the limited existing published data, and provide the first direct comparison of the two methods. Interestingly the sensitivity of MODS and the Xpert MTB/RIF assay were similar in our study.

In practice choice of MTBC detection method(s) in a particular setting will depend not just on sensitivity but on a combination of other factors including cost, time to detection, and the availability and requirements for drug susceptibility testing (DST), trained staff, and laboratory infrastructure. Each method has advantages that might make it most suitable in a particular setting. MGIT culture offers the best sensitivity, but cost and the need for high level laboratory equipment and training currently limit expansion beyond a few urban centres in most high burden countries. Although less sensitive than MGIT, the much lower cost and lower biosafety risks of MODS might make it more suitable at the district level; however MODS roll out would still entail significant investment in laboratory staff and training, and would require good external quality assurance. The appeal of the Xpert MTB/RIF assay is its speed and simplicity, obviating the need for

complex laboratory equipment or training, but this comes at a higher cost - which remains prohibitive in many settings, despite reduced prices for developing countries.

Ultimately the cost effectiveness of all three methods will depend on the pre-test probability of TB in the populations on which they are used, which in turn determines the number needed to test to identify one MTBC positive sample. To maximize benefit in resource limited settings they should ideally be used in conjunction with clinical risk stratification to identify for microbiological investigation those children most at risk of TB. Further research is needed to define appropriate management protocols incorporating these tests.

7 COMMUNITY INCIDENCE OF CHILDHOOD TUBERCULOSIS

Chapter overview

Despite Kenya having one of the highest national TB incidence rates per capita, there are no good quality data from either Kenya or other east African countries on the burden of childhood tuberculosis. We utilized the TB diagnostic infrastructure established for the KIDS TB Study, combined with unique linkage of KDH clinical episodes and KHDSS population data, to estimate the community incidence of childhood TB in Kilifi, and to infer the likely national burden of childhood TB. An overview of the methods is shown schematically in Figure 7.1.

We calculated the crude incidence of childhood TB in the KHDSS as the number of childhood TB cases detected divided by the number of person-years observation in each age group. Because recruitment started and ended later in the active case detection arm we calculated incidence separately for each study arm and combined the results.[1]

Since not all incident cases of childhood TB are captured at KDH we estimated the case detection rate (CDR) as the proportion of all KHDSS resident children with TB that were captured by the study, in order to adjust our crude incidence estimates accordingly. As the true number of children with TB is unknown, we used data from National Tuberculosis Programme (NTP) notifications, KHDSS mortality surveillance, and a contemporaneous verbal autopsy (VA) study to estimate, respectively, the proportions of notified TB cases, all-cause childhood deaths, and deaths among TB cases and suspects that were captured by the study (Figure 7.1). Although no single method is without limitations, confidence in the case detection rates derived is strengthened by similar estimates using each method.

Final adjusted estimates of the incidence of childhood TB in Kilifi were then calculated by dividing the crude incidence estimates by the case detection rate. In order to provide the most conservative incidence estimates we used the highest estimate of the case detection rate, derived using KHDSS mortality surveillance data (CDR = 0.35); this method is also most easily transferrable to other settings, facilitating comparability between sites.

Finally we estimated the national burden of childhood TB in Kenya by cautiously extrapolating our results from Kilifi, assuming that the paediatric proportion of the total TB caseload is similar.

[1] For comparison, we also calculated the incidence of childhood TB in Kilifi using the published clinical diagnostic tools assessed in chapter 5 (this is not shown in Figure 7.1).

Figure 7.1 Chapter overview: Calculating the community incidence of childhood TB

Crude estimate of childhood TB incidence in KHDSS (*I*)

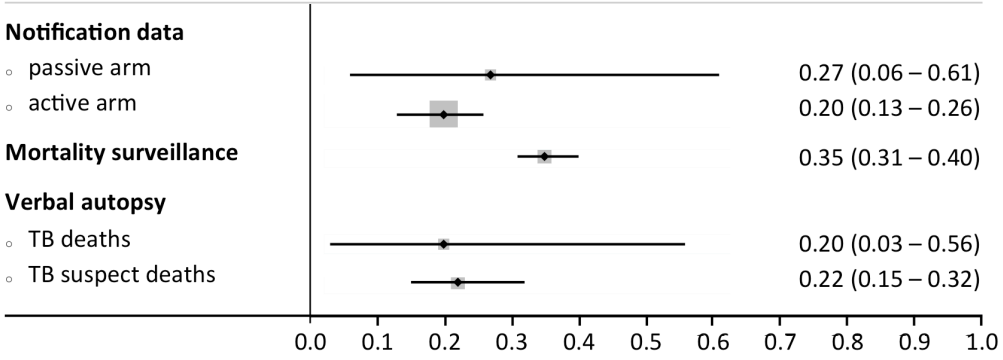
$$I = \left(\frac{TB\ cases\ detected}{Person-years\ observation}\right)_{\substack{passive\ case\\ detection\ arm}} + \left(\frac{TB\ cases\ detected}{Person-years\ observation}\right)_{\substack{active\ case\\ detection\ arm}}$$

Estimation of the case detection rate (CDR)

All methods for estimation of the CDR are subject to different potential biases (see table 7.1). Estimates therefore derived using 3 independent methods and compared:

1. NTP Notification Data[1]	2. Mortality Surveillance	3. Verbal Autopsy (VA) Study[2]
$CDR = \frac{\text{TB cases detected}}{\text{All notified TB cases}}$	$CDR = \frac{\text{All deaths detected}}{\text{All recorded deaths}}$	$CDR = \frac{\text{TB deaths detected}}{\text{All TB deaths}}$

Broadly similar estimates derived using each of the 3 methods (point estimates 0.20 to 0.35):



Mortality surveillance CDR estimate (0.35) chosen for adjustment of crude incidence estimates due to applicability in other settings, greater precision, and to provide the most conservative adjusted estimate of childhood TB incidence.

Adjusted estimate of childhood TB incidence in KHDSS (I_{adj})

Final adjusted incidence estimates calculated by dividing crude incidence estimates by the case detection rate:

$$I_{adj} = \frac{Crude\ incidence\ estimate\ (I)}{Case\ detection\ rate\ (CDR)}$$

Estimated national burden of childhood TB in Kenya

National burden of childhood TB estimated assuming the paediatric proportion of the total TB caseload is the same in the KHDSS and nationally.

[1] In the active case detection arm CDR estimated as the proportion of TB *contacts* captured/investigated.
[2] Verbal autopsy data used to estimate CDR using both TB deaths and deaths among TB suspects.

7.1 Introduction

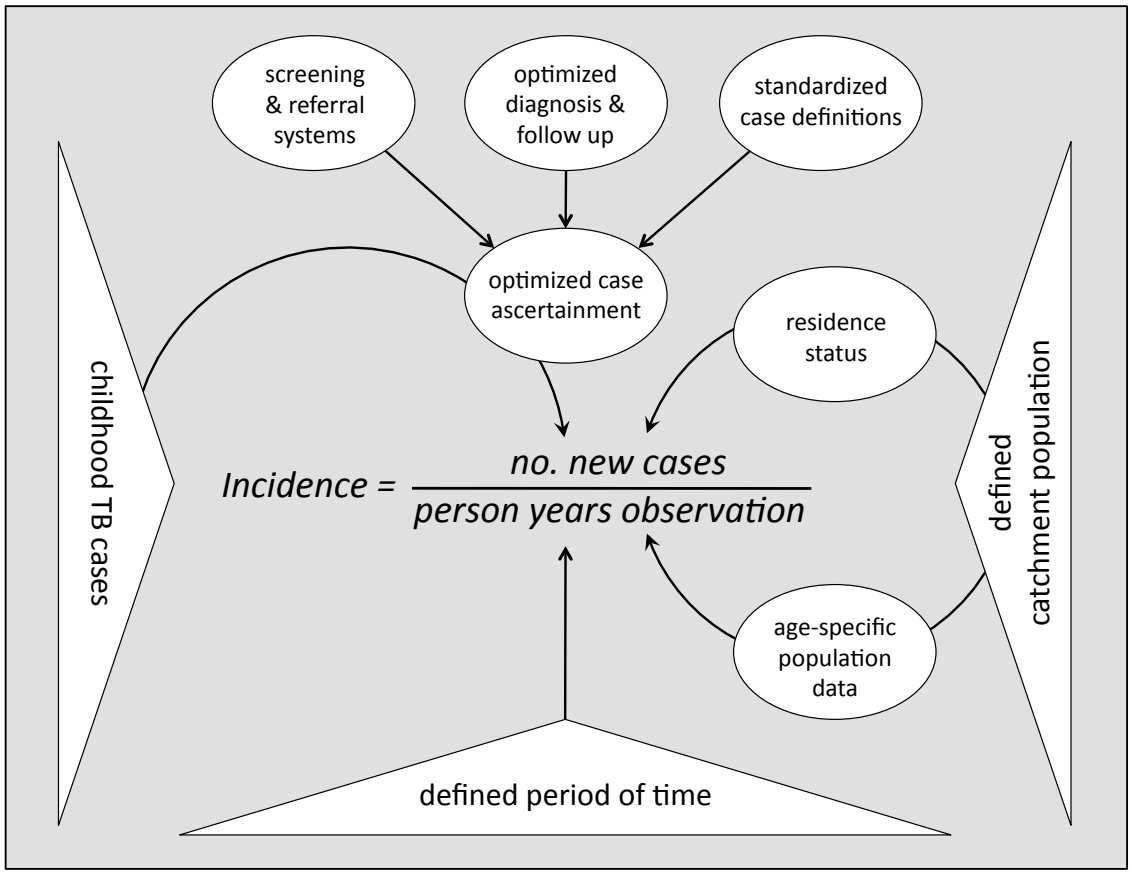
Despite Kenya having one of the highest national TB incidence rates per capita, both overall[3] and among children,[10] there are no good quality data from either Kenya or other east African countries on the burden of childhood tuberculosis. Published estimates based on routine notification data suggested a very high national incidence of 167 per 100,000 among children <15 years old in 2000, which was exceeded only in South Africa, Zimbabwe and Afghanistan (Table 1.1).[10] However there are no published studies from the region that provide good quality prospective surveillance data to assess the accuracy of these estimates.

One of the chief obstacles to such studies is a lack of the resources and infrastructure required for good quality childhood TB surveillance in most settings. High quality community TB prevalence surveys require huge resources even among adults,[195] and are an even greater undertaking among children, given the additional complexity of childhood TB diagnosis. Hospital based studies provide a convenience sample, but in most cases the community burden of disease is difficult to extrapolate from hospital data due to poorly defined hospital catchment populations, incomplete community case ascertainment at single health facilities, and a lack of systematic procedures for patient screening, referral and diagnostic work up. Further challenges include sub-optimal resources for childhood TB diagnosis in most hospitals in the region, frequent loss to follow up that prevents longitudinal assessment of children whose TB status is not clear at initial presentation, and varying and often ill-defined case definitions. Accurate estimates of the community incidence of childhood TB require systematic and transparent procedures for identifying and investigating TB suspects underpinned by high quality diagnostics and standardized case definitions, combined with knowledge of the age structure of the catchment population and the residence location of each case (**Figure 7.2**).

The KEMRI-WT Research Programme at Kilifi combines several of the components necessary for improving childhood TB surveillance. In addition to unique linkage of clinical episodes at Kilifi District Hospital (KDH) with population data from the Kilifi Health and Demographic Surveillance System (KHDSS), existing clinical and laboratory facilities and state of the art TB diagnostic facilities established for this study allowed

careful clinical classification of children into those with and without TB. We utilized this infrastructure to investigate the burden of childhood TB in Kilifi.

Figure 7.2 Schematic of infrastructure and resources required for accurate estimation of community childhood TB incidence



7.2 Methods

7.2.1 Crude estimation of the community incidence of childhood TB

Children with active TB were identified as described in chapters 2 and 3. The incidence of childhood TB in the KHDSS was estimated using numerator and population denominator data from 2 complete years of recruitment in both the passive and active case detection arms of the study. We used clinical data from KDH and event data from the KHDSS to compile for every KHDSS resident child a life history record consisting of birth, death, migration, enumeration or hospital presentation events recorded during this period of study enrolment. We then used these event data to generate for each individual a series of chronological time-span records representing the period from one event to the next, so that together these time-span records encompassed the total period of observation within the KHDSS for that individual. We used Lexis expansion to further split these periods of observation by each child's age category at the time, in order to calculate incidence estimates stratified by age (see below). We calculated TB incidence as the number of TB cases among KHDSS residents divided by the total person years of observation summed across all KHDSS resident children, and presented 95% confidence intervals assuming a Poisson distribution.

Because recruitment started later and ended later in the active case detection arm than in the passive case detection arm (Section 3.3.1, Figure 3.1) we calculated TB incidence separately for each study arm and combined the results to generate overall incidence estimates, using the following formula for incidence, I:

$$I = I_{\text{active case detection arm}} + I_{\text{passive case detection arm}} = I_{\text{a}} + I_{\text{p}}$$
$$= \left(\frac{\text{No. TB cases from KHDSS area}}{\text{No. person years observation}} \right)_{\substack{\text{passive case} \\ \text{detection arm}}} + \left(\frac{\text{No. TB cases from KHDSS area}}{\text{No. person years observation}} \right)_{\substack{\text{active case} \\ \text{detection arm}}}$$

To compute 95% confidence intervals for these combined incidence estimates we assumed TB incidence in each study arm was independent to derive the following formula for the standard error (s.e) of a combined rate:

$$\text{s.e.}\left\{\ln(I)\right\} = \text{s.e.}\left\{\ln\left(I_a + I_p\right)\right\} = \frac{a/T_a^2 + p/T_p^2}{\left(I_a + I_p\right)^2},$$

where a and p are the number of cases, and T_a and T_p the person years observation, in the active and passive case detection arms respectively. The derivation of this formula is given in Appendix 10.1.

Minimum incidence estimates and 95% confidence intervals (per 100,000 children per year) were calculated for confirmed TB, confirmed plus highly probable TB (CHPTB), and for All TB. In addition, within each of these diagnostic categories we derived estimates for all children <15 years, and for children in each of the following age strata, reflecting groups with different epidemiological risks for TB in previous studies:[12]

- 0 to 4 years (young children)
- 5 to 9 years (school age children)
- 10 to 14 years (young adolescents)

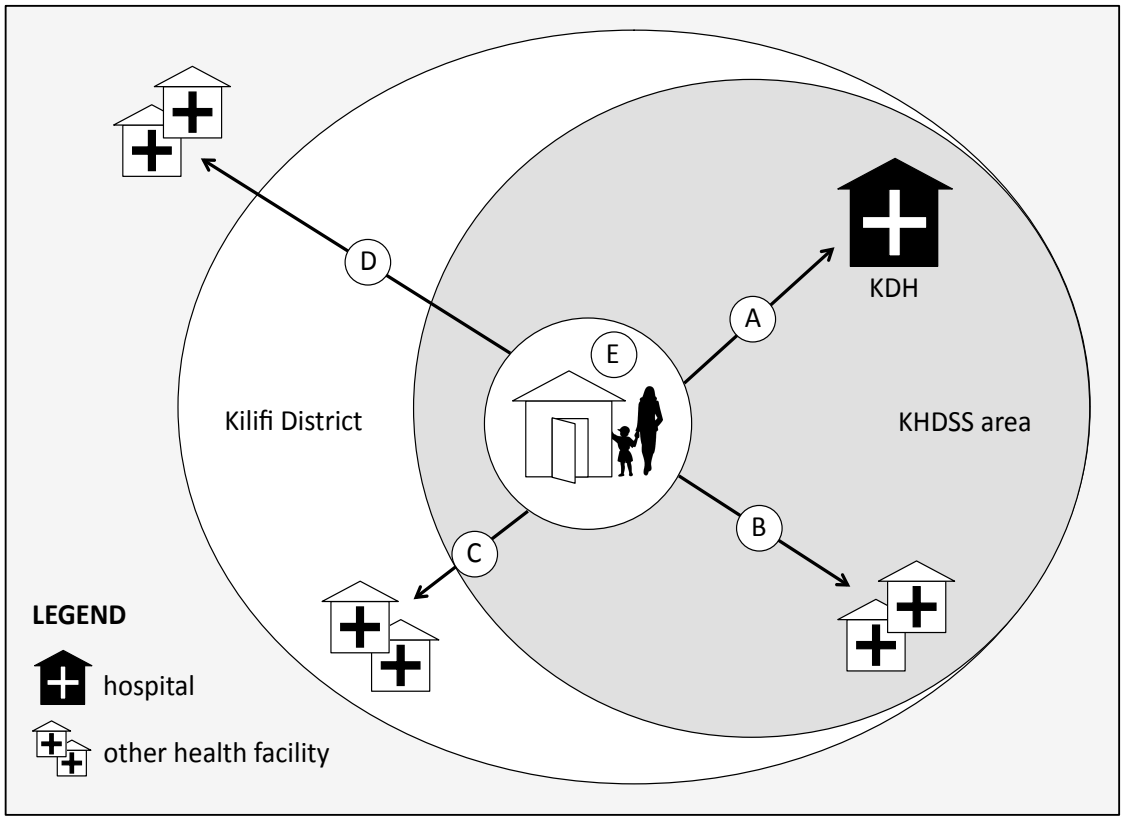
7.2.2 Application of clinical diagnostic tools to estimate incidence of childhood TB

For comparison, we also calculated the incidence of childhood TB in Kilifi using the range of published clinical diagnostic tools reviewed and assessed in chapter 5. We confined each analysis to children identified through the passive case detection arm of the study, since most of these clinical tools were designed for use among children presenting unwell with suspected tuberculosis. In each case, to derive the incidence of presentation to hospital with TB we applied the published clinical definition to our dataset of KHDSS resident children enrolled in KIDS TB Study in order to calculate the numerator, and used as the denominator the corresponding person years of observation among children <15 years old during the 2 years of case ascertainment for the incidence study. We derived 95% confidence intervals assuming a Poisson distribution.

7.2.3 Estimating the case detection rate

Incidence estimates derived as described above assume that all incident cases of childhood TB within the KHDSS are captured at KDH through either the passive or the active case detection arm of the KIDS TB study. KDH is the only hospital in the area and it provides both primary care and secondary level care to the local population. However, children with suspected TB may present to a lower level health facility in the KHDSS area, or to another hospital or health facility located outside the KHDSS area. Some children may not seek medical attention at all. These patterns are summarized schematically in Figure 7.3.

Figure 7.3 Potential healthcare seeking behaviour of children resident in the KHDSS. A, presents to KDH; B, presents to another healthcare facility within the KHDSS area; C, presents to another healthcare facility outside the KHDSS area but within Kilifi district; D, presents to another healthcare facility outside Kilifi district; E, does not present to a healthcare facility.



We estimated the case detection rate (CDR) as the proportion of all KHDSS resident children with TB captured by the KIDS TB study. As the true number of KHDSS resident children with TB is unknown (this is what we are seeking to determine), we considered a variety of methods to estimate this proportion. Table 7.1 summarizes the numerator and denominator, advantages and disadvantages of each method. All have limitations. Since no single method appeared obviously superior to all the others *a priori*, we performed the analysis using each method, so that by triangulating their results we might derive as accurate an estimate as possible of the case detection rate. More detailed descriptions of each method are presented in the sections below.

Table 7.1 Comparison of methods for estimating the sensitivity of hospital-based surveillance for childhood TB

Method	Denominator	Numerator	Advantages & disadvantages
NTP notification (passive arm)	TB cases notified to NTP whose address lay within the KHDSS	Members of the denominator who were investigated for TB within the KIDS TB study	***Advantages***: Uses an objective denominator outside the project system ***Disadvantages***: Frequent diagnostic misclassification outside KDH, and ascertainment bias due to enhanced surveillance at KDH, preclude meaningful interpretation of notification data among younger children. Analysis therefore limited to adolescents. This limits precision, and ascertainment of adolescents cannot necessarily be extrapolated to younger children. This method also estimates sensitivity loss associated with presentation to another health facility, but not that associated with 'no presentation', or presentation to a facility outside the district. Therefore likely to overestimate sensitivity.
NTP notification (active arm)	KHDSS household contacts, aged <5 years, of smear positive pulmonary TB index cases notified to NTP	Members of the denominator who were investigated for TB within the active surveillance arm of the KIDS TB study	***Advantages***: Uses an objective denominator outside the project system ***Disadvantages***: Exact number of household contacts <5 years unknown for index cases not captured by the KIDS TB Study. Denominator must therefore be imputed by assuming that index cases that were and were not captured had the same average number of child household contacts. The method also assumes TB risk among contacts was independent of whether the child was brought to the paediatric TB clinic for investigation; might under-estimate sensitivity if TB cases were more likely to be brought for review.
Mortality surveillance	All deaths occurring among children <15 years old in the KHDSS	Members of the denominator whose death was recorded at KDH.	***Advantages***: Simple, potentially applicable in other settings. A good measure of the sensitivity of hospital surveillance for acute life threatening syndromes. ***Disadvantages***: Validity as a measure of the sensitivity of hospital surveillance for more chronic syndromes like TB has not yet been established.
Verbal autopsy (VA) study - **TB deaths**	TB deaths among children aged <15 years identified in KHDSS VA study.	Members of the denominator who were investigated for TB within the KIDS TB study.	***Advantages***: One of the few methods to estimate insensitivity of hospital based TB recruitment attributable to sub-groups who do not present to hospital for medical care. ***Disadvantages***: The number of deaths confidently diagnosed as attributable to TB by a retrospective history method (VA) is likely to be small, so precision of the sensitivity estimate will be poor.
Verbal autopsy study - TB suspects	Deaths identified by the KHDSS VA study that were associated with features of suspected TB.	Members of the denominator who were investigated for TB within the KIDS TB study.	***Advantages***: Circumvents the difficulty determining TB by verbal autopsy. ***Disadvantages***: Suspected TB is poorly specific for actual TB; assumes that among TB suspects who died the proportion who actually had TB was the same in the group captured by the KIDS TB Study as in the group who were not captured by the study.

* Numerator and denominator were limited in all cases to the 2 year period of recruitment into the KIDS TB incidence study (Section 3.3.1, Figure 3.1).

7.2.4 Using NTP notification data to estimate the case detection rate in the passive arm

The District TB Register contains details of all TB patients notified to the NTP by each designated TB treatment facility in Kilifi district. In keeping with the WHO DOTS strategy for TB case notification it includes data on the name, age, sex, address, referral source, treatment facility, TB clinical syndrome (pulmonary or extra-pulmonary TB), sputum smear status, treatment and outcome of each patient. We used notification data from the Kilifi District TB Register to estimate the proportions of KHDSS resident child TB cases captured at KDH in the passive case detection arm of the study.

Data from the Kilifi District TB register were double entered into a bespoke electronic database using Filemaker Pro version 10 (Filemaker Inc, CA, USA). The KHDSS residence status (resident or non-resident) of each patient in the register was then coded manually by a senior demographer with several years of local experience and detailed knowledge of the KHDSS area, using the address documented in the register. All KHDSS resident childhood TB cases notified between August 2009 and July 2011 were identified from this database. We then manually cross-referenced the name, age and treatment date of each of these cases against the KIDS TB Study database to identify children that had also been captured by the passive case detection arm of the study. We calculated the case detection rate as:

$$CDR = \frac{\text{No. KHDSS resident child TB cases captured by the KIDS TB Study}}{\text{Total no. KHDSS resident child TB cases}}$$

We reasoned that, while NTP notification data was likely to be reasonably reliable for adults and adolescents (among whom TB presentation is similar and diagnosis relatively more straightforward), frequent diagnostic misclassification and ascertainment bias introduced by enhanced surveillance at KDH would prevent meaningful interpretation of notification data among younger children. We therefore limited calculations to children aged 10-14 years. For consistency with study definitions we considered notification of smear positive pulmonary TB in a young adolescent equivalent to a diagnosis of highly probable TB, and estimated the case detection rate for CHPTB and All TB.

7.2.5 Using NTP notification data to estimate the case detection rate in the active arm

Case ascertainment of children aged 0 to 4 years in the active (contact tracing) case detection arm depended firstly on identification of all KHDSS resident cases of smear positive pulmonary TB; and secondly on each of these smear positive index cases bringing their child household contacts to the paediatric TB clinic for investigation. By linking smear and residence data from the Kilifi District TB Register with data from our register of all smear positive pulmonary TB patients seen in the KDH TB clinic we determined the proportion of all notified smear positive pulmonary TB cases from the KHDSS area that were captured at KDH during the period of enrolment into the active case detection arm of the study (analogous to A/(A+B+C) in Figure 7.3). We identified from the KHDSS census the number of child household contacts under 5 years old for each index case, and thereby the total number of eligible child household contacts under 5 years old and resident in the KHDSS area. We assumed for the purpose of this analysis that the average number of child household contacts was similar among index cases who presented to KDH and elsewhere, and that the risk of TB among contacts was independent of where the index case presented or whether the child was brought to the paediatric TB clinic for investigation. The case detection rate in the active case detection arm was then calculated as:

$$CDR = \frac{\text{No. index cases captured at KDH}}{\text{Total no. index cases}} \times \frac{\text{No. eligible child contacts investigated}}{\text{Total no. eligible child contacts identified at KDH}}$$

We derived 95% confidence intervals based on the variance of the product of these two proportions using standard methods (see appendix 10.1.2, page 179).

7.2.6 Using hospital-based mortality surveillance to estimate the case detection rate

We used vital status data from KHDSS census rounds combined with outcome data for paediatric admissions at KDH to calculate the proportion of all childhood deaths in the KHDSS area that were captured at KDH during the study period. We calculated the case detection rate as:

$$CDR = \frac{\text{No. KHDSS resident paediatric inpatient deaths at KDH}}{\text{Total no. childhood deaths among KHDSS residents}}$$

7.2.7 Using verbal autopsy (VA) to estimate the case detection rate

A better approach to estimating the sensitivity of hospital-based surveillance is to use *disease specific* mortality data to calculate the proportion of childhood TB deaths that were captured by the study. Poor quality vital registration data in Kilifi District make these data unsuitable for this analysis, which requires more robust data on the number and causes of death in the population of interest. We therefore made use of data from an ongoing verbal autopsy (VA) study within the KHDSS.

Details of the Kilifi verbal autopsy study, including validation of the methodology using hospital records of the cause of death, have been published elsewhere.[196] Deaths among KHDSS residents are identified by the thrice yearly enumeration rounds, and relatives of the deceased are then visited at home as soon as possible after the locally accepted one month bereavement period. Following consent, verbal autopsy is performed using the WHO Sample Vital Registration with Verbal Autopsy (SAVVY) tool. Structured questionnaires include an initial narrative section with open questions about the circumstances of death, followed by a series of closed questions that provide detailed information about the medical history and associated clinical features. Two independent clinicians then code the causes of death in each case according to a standard rubric and the WHO International Statistical Classification of Diseases Version 10 (ICD10). In the case of a discrepancy between the two clinicians, a third clinician reviews the case blind in order to adjudicate, and if there is no agreement between the three reviewers they meet to discuss the case in order to form a consensus.

Using each child's unique KHDSS personal identification (PID) number we merged VA and KIDS TB records to calculate the proportion of TB deaths among KHDSS resident children with that were captured by the KIDS TB study. We defined TB deaths as those whose cause was coded as TB or which occurred in a patient with documented tuberculosis according to the respondent and/or any available supporting documentation, including death certificates, burial permits and post mortem reports.

We then estimated the case detection rate as

$$CDR = \frac{\text{No. TB deaths in VA study that occurred in children captured by KIDS TB Study}}{\text{No. TB deaths in VA study}}$$

Although the true mortality burden of TB among children in Kilifi District was not known, we predicted that the number of child TB cases diagnosed by VA was likely to be small (since TB is responsible for a minority of childhood deaths and is even more difficult to diagnose retrospectively by VA than in clinical practice); and that the precision of our case detection rate estimate was therefore likely to be poor.

To mitigate this, we also used the VA study to identify the much larger group of children whose reported clinical features before death met the KIDS TB Study criteria for suspected TB ("TB suspect deaths"; see section 2.3, page 28 for definition of suspected TB*). Healthcare-seeking behavior in Kilifi is usually determined by the clinical features of an illness, rather than the diagnosis *per se*.[197, 198] We reasoned, therefore, that of all children with clinical features of suspected TB who died, the proportion captured by the KIDS TB Study would provide a surrogate measure of the case detection rate:

$$CDR = \frac{\text{No. TB suspect deaths in VA study that were captured by KIDS TB Study}}{\text{No. TB suspect deaths in VA study}}$$

In each case we confined analysis to children recruited during the period of passive enrolment for the incidence analysis (1 August 2009 to 31 July 2011). Results were reported overall and by age category.

7.2.8 Final adjusted estimates of the incidence of childhood TB in Kilifi

Case detection rate point estimates and 95% confidence intervals derived using each of the methods above were compared. We selected the best CDR estimate for adjustment of measured incidence rates on the basis of their likely biases (Table 7.1), relative magnitude, precision, and potential applicability in other settings. We then divided our observed caseload and incidence rates by this CDR estimate to derive estimated annual caseload and adjusted incidence rates by age category and TB diagnostic group.

7.2.9 Estimating the national burden of childhood TB in Kenya

Estimating the national childhood TB burden by extrapolating data from a single district is speculative since the community TB burden varies geographically, in part due to geographical differences in the prevalence of social and biological risk factors for TB (such as poverty, crowding, HIV and malnutrition). Nevertheless, placed in appropriate

* Criteria for suspected TB include '*pneumonia that fails to respond to first line antibiotics*', which we defined in the VA dataset as death due to pneumonia despite reported treatment.

context, the burden of childhood TB in Kilifi may provide valuable insights into the likely burden at a national level.

We estimated the national burden of childhood TB in Kenya by assuming that the proportion of the national TB caseload that occurs in children is equal to the proportion of TB cases among KHDSS residents that occurred among children. We also assumed that published estimates for the TB burden and case detection rate among Kenyan adults are reasonably reliable. For each age category of children we then estimated the number of new TB cases annually in Kenya (TB_c), and the corresponding national incidence of childhood TB (I_{Kenya}), using the following formulae (derived in appendix 10.1.3, page 180):

$$TB_c = \frac{TB'_a}{\left[{(tb'_a + tb_c)}\Big/{tb_c} - 1\right]}$$

and

$$I_{Kenya} = \frac{TB_a}{N_c R \left\{\frac{[(tb_a/R)+tb_c]}{tb_c} - 1\right\}}$$

where N_c is the population of Kenyan children in each age category (data from 2009);[59] R is the estimated national TB case detection rate among adults in Kenya in 2010;[58] TB_a is the number of new notified TB cases among Kenyan adults in 2010;[58] tb_a is the number of new notified TB cases among KHDSS residents in the KIDS TB incidence study; $TB'_a = \frac{TB_a}{R}$ and $tb'_a = \frac{tb_a}{R}$; and tb_c is the estimated number of new TB cases among KHDSS resident children in the same period, adjusted for the study case detection rate.

7.3 Results

7.3.1 Crude incidence of childhood TB

During the two years of comprehensive inpatient and outpatient screening for TB among children presenting to KDH (see section 3.3.1 and Figure 3.1, page 42), 443 KHDSS resident children were enrolled in the passive case detection arm and 104 in the active (contact tracing) arm of the study. Final diagnostic categories of these children are shown in Table 7.2.

Table 7.2 TB diagnostic category of children enrolled during two years' comprehensive inpatient and outpatient, active and passive screening for childhood TB at KDH

	Passive case detection arm	Active case detection arm	Total
Confirmed TB (CTB)	12	2	14
Highly Probable TB (HPTB)	16	4	20
Possible TB cases treated for TB	31	0	31
Possible TB cases not treated for TB	88	15	103
Not TB (TB confidently excluded)	296	83	379
Total	**443**	**104**	**547**

Incidence estimates for CTB, HPTB and All TB during this period are shown in Table 7.3. The incidence of both CTB and HPTB was highest among children aged 0-4 years, and lowest among the 11-14 year age group. TB cases identified through the active (contact tracing) arm of the study added only marginally to the total incidence estimates. The crude incidence of All TB among children <15 years was 27.2 (21.3 to 34.7) per 100,000 per year during the study period.

Table 7.3 Crude incidence of TB by diagnostic certainty and age category in the active and passive case detection arms and overall

Age group	KDH passive case detection arm				KDH active case detection arm				KHDSS incidence per 100,000 children / year (95% CI)	
	pyo[1]	No. TB cases	Incidence per 100,000 children / year (95% CI)		pyo[1]	No. TB cases	Incidence per 100,000 children / year (95% CI)			
Confirmed TB										
0-4 years	89,503	5	5.6	(2.3 to 13.4)	91,746	2	2.2	(0.3 to 7.9)	7.8	(3.7 to 16.3)
5-9 years	79,170	5	6.3	(2.6 to 15.2)	*	*		*	6.3	(2.6 to 15.2)
10-14 years	70,073	2	2.9	(0.7 to 11.4)	*	*		*	2.9	(0.7 to 11.4)
<15 years	238,746	12	5.0	(2.9 to 8.9)	241,538	2	0.8	(0.1 to 3.0)	5.9	(3.5 to 9.9)
Confirmed & Highly Probable TB										
0-4 years	89,503	17	19.0	(11.8 to 30.6)	91,746	6	6.5	(2.4 to 14.2)	25.5	(35.7 to 64.4)
5-9 years	79,170	8	10.1	(5.1 to 20.2)	*	*		*	10.1	(6.9 to 24.7)
10-14 years	70,073	3	4.3	(1.4 to 13.3)	*	*		*	4.3	(3.1 to 18.6)
<15 years	238,746	28	11.7	(8.1 to 17.0)	241,538	6	2.5	(0.9 to 5.4)	14.2	(19.6 to 32.4)
All TB										
0-4 years	89,503	34	38.0	(27.1 to 53.2)	91,746	6	6.5	(2.4 to 14.2)	44.5	(32.7 to 60.7)
5-9 years	79,170	19	24.0	(15.3 to 37.6)	*	*		*	24.0	(15.3 to 37.6)
10-14 years	70,073	6	8.6	(3.8 to 19.1)	*	*		*	8.6	(3.8 to 19.1)
<15 years	238,746	59	24.7	(19.1 to 31.9)	241,538	6	2.5	(0.9 to 5.4)	27.2	(21.3 to 34.7)

[1] pyo, person years observation = age-specific KHDSS population at midpoint of enrolment of each study arm x duration of enrolment (2 yrs)

* Recruitment in the active case detection arm was confined to children <5 years old.

Table 7.4 Incidence of childhood TB derived by applying published clinical definitions to children enrolled in the passive case detection arm.
Results presented in order of ascending incidence. Incidence based on KIDS TB Study definitions and KIDS TB Score also presented for comparison.

Published clinical definition		No. TB cases by age category & overall				Incidence per
Author	**Outcome(s) defined**	**0-4 y**	**5-9 y**	**10-14 y**	**Total**	**100,000/y (95% CI)[1]**
WHO (2006) [21]	(a) Strongly suggestive of TB [4]	6	0	0	6	3 (1 to 6)
KIDS TB Study definition	***Confirmed TB***	**5**	**5**	**2**	**12**	**5** (3 to 9)
KIDS TB Study definition	***Confirmed or highly probable TB***	**17**	**8**	**3**	**28**	**12** (8 to 17)
Stegan [150]	(a) Probable TB	19	9	1	29	12 (8 to 17)
Nair [148]	(a) "TB appears unquestionable"	20	10	5	35	15 (10 to 20)
WHO (2006) [21]	(b) Requires investigation for TB [4]	21	12	7	40	17 (12 to 23)
KIDS TB Score	***Very Likely TB***	**27**	**8**	**7**	**42**	**18** (13 to 24)
KIDS TB Study definition	***All TB***	**34**	**19**	**6**	**59**	**25** (19 to 32)
Kenya NTP (draft) [149]	Criteria for TB treatment	38	16	5	59	25 (19 to 32)
Hawkridge [145]	Probable TB	39	10	11	60	25 (19 to 32)
Nair [148]	(b) TB probable or "unquestionable"	45	16	8	69	29 (23 to 37)
Graham [72]	Probable TB	57	22	6	85	36 (28 to 44)
Stoltz [143]	Probable TB	62	24	13	99	42 (34 to 51)
Stegan [150]	(b) Probable or Possible TB	70	27	6	103	43 (35 to 52)
WHO (1983) [70]	Probable TB	73	27	16	116	49 (40 to 58)
Ramachandran [142]	Criteria for TB treatment	76	27	16	119	50 (41 to 60)
Jeena [138]	Probable TB	90	29	11	130	55 (46 to 65)
Edwards [132]	Criteria for TB treatment	139	21	6	166	70 (59 to 81)
Cundall [136]	Probable TB	133	39	15	187	78 (68 to 90)
Kenya NTP (2008) [71]	Criteria for TB treatment	180	28	7	215	90 (78 to 103)
KIDS TB Score	***Likely TB***	**183**	**29**	**14**	**226**	**95** (83 to 108)
Osborne [141]	Probable TB	165	44	19	228	96 (84 to 109)
Kiwanuka [139]	Probable TB	170	42	20	232	97 (85 to 111)
Ghidey [137]	(b) Criteria for TB treatment [2]	303	72	23	398	167 (151 to 184)
Fourie [133]	High probability of TB [3]	339	65	20	424	178 (161 to 195)
Ghidey [137]	(a) Criteria for TB treatment [2]	351	79	29	459	192 (175 to 210)

1 Denominator for incidence calculations is the total person years observation among children under 15 years = 238,746 (see Table 7.2).

2 Results for Ghidey & Habte tool presented separately using requirement for (a) ≥2 and (b) ≥3 symptoms & signs to define a 'suggestive symptom complex of TB' (see section 5.2.4)

3 For the purposes of our analyses we used 'score 2' proposed by Fourie *et al*, which was derived in high TB burden settings in South Africa, Madagascar and Nicaragua.

4 Results presented separately for (a) children whose clinical features "strongly suggest a diagnosis of TB" according to the guidelines, and (b) using broader criteria that included under "physical signs highly suggestive of TB" all the other "suggestive clinical signs" listed as requiring investigation for TB.

7.3.2 Application of clinical diagnostic tools to estimate incidence of childhood TB

Table 7.4 shows childhood TB incidence estimates for Kilifi derived using published clinical definitions. Incidence estimates derived using the KIDS TB study definitions and the KIDS TB Score are also included in the table for comparison. There was a very wide range in the estimated incidence of childhood TB, spanning almost two orders of magnitude (3 to 192 per 100,000 per year), with a median estimated incidence of 49 per 100,000 per year. When compared with estimates based on the published clinical definitions, incidence estimates derived using the KIDS TB Study definitions were among the most conservative.

7.3.3 Using NTP notification data to estimate the case detection rate in the passive arm

The Kilifi District TB register contained 21 young adolescents aged 10-14 years who had been diagnosed and started on TB treatment during the two year period of interest. Of these, 6/21 had been captured by the KIDS TB study (2 with confirmed TB, 1 with highly probable TB and 3 who were treated for TB but did not meet criteria for highly probable TB), giving an estimated case detection rate for All TB of 29% (11 to 52%). Of the remaining 15 children not captured by the KIDS TB Study, 11 (73%) were diagnosed at another health facility in the district, 8 (53%) had smear positive pulmonary TB, 4 (27%) had smear negative pulmonary TB, and 3 (20%) had extra-pulmonary TB. Thus the estimated case detection rate for CHPTB was 3/11, 27% (6 to 61%).

7.3.4 Using NTP notification data to estimate the case detection rate in the active arm

There were 678 notified cases of TB among KHDSS resident adults between January 2010 and December 2011, of which 297 (44%) were sputum smear positive. 195 (66%) of these sputum smear positive patients presented to the KDH TB clinic where they were screened for child household contacts in the active contact tracing arm of the study. A total of 362 child household contacts of these 195 index cases less than 5 years old were identified on the KHDSS census database, of whom 108 (30%) were brought to the KDH paediatric TB clinic for investigation. The case detection rate was therefore calculated as:

$$CDR = \left(\frac{195}{297} \times \frac{108}{362}\right)$$

giving an estimated case detection rate of 20% (13 to 26%).

7.3.5 Using hospital-based mortality surveillance to estimate the case detection rate

Between 1 August 2009 and 31 July 2011 there were 514 deaths recorded among children <15 years old in the KHDSS area. Of these, 182 were recorded at KDH, giving an overall case detection rate of 35% (31 to 40%). Case detection rate estimates are presented by age category in Table 7.5.

Table 7.5 Estimated case detection rates by age category (mortality surveillance)

	Deaths among KHDSS resident children	Deaths captured at KDH	Sensitivity, % (95% CI)
0-4 years	414	152	37 (32 to 42)
5-9 years	49	15	31 (18 to 45)
10-14 years	51	15	29 (17 to 44)
<15 years	514	182	35 (31 to 40)

7.3.6 Using verbal autopsy (VA) to estimate the case detection rate

Of the 514 deaths recorded among KHDSS resident children under the age of 15 years between 1 August 2009 and 31 July 2011 (section 7.3.5 above), verbal autopsy was performed for 382 (74%). There were 10 TB deaths (2.6%), only 2 of which occurred in children who had been captured by the KIDS TB Study, giving an estimated case detection rate for fatal TB cases of 20% (3 to 56%).

Based on the clinical description of the illness at VA, 107 (28%) cases met the definition of suspected TB, of whom 24 had been captured by the KIDS TB Study. Thus overall the overall case detection rate for children with features of suspected TB who subsequently died was 22% (15 to 32%); age category specific estimates are shown in Table 7.6.

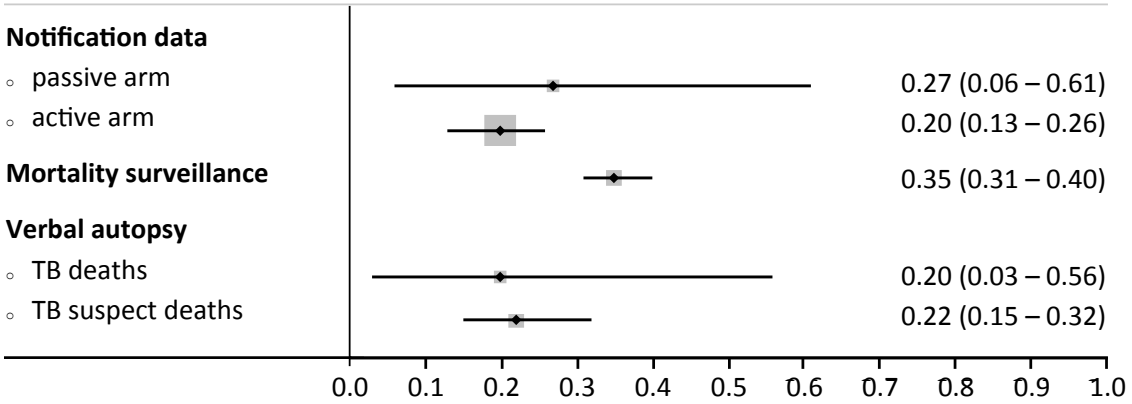
Table 7.6 Estimated CDR for fatal illness associated with clinical features of suspected TB (by age category)

	Deaths among KHDSS resident children	Deaths captured at KDH	Sensitivity, % (95% CI)
0-4 years	77	15	19 (11 to 30)
5-9 years	15	6	40 (16 to 68)
10-14 years	15	3	20 (4 to 48)
<15 years	107	24	22 (15 to 32)

7.3.7 Adjusted incidence estimates for childhood TB in Kilifi

Case detection rate estimates derived by each method are plotted for comparison in Figure 7.4.

Figure 7.4 Case detection rate estimates and 95% confidence intervals derived by each method



The proportion of TB deaths in the KHDSS VA study that were captured by the KIDS TB study arguably provides the least biased method for estimating the case detection rate. However the small number of TB cases identified by verbal autopsy severely limited the precision of this analysis. Very poor precision also limited the utility of the CDR estimate derived using NTP notification data for young adolescents. Although the proportion of TB suspect deaths in the VA study that was captured at KDH provides a surrogate for the case detection rate, we elected for our incidence adjustments below to use the CDR estimate derived using KHDSS mortality surveillance data, for the following reasons.

Firstly, the VA method is very resource intensive, so is not easily transferrable to other settings. On the other hand, the proportion of childhood deaths that are captured at hospital is relatively straightforward to estimate even in sites without a demographic surveillance system, so the method facilitates comparison between sites. Secondly, the larger number of deaths leads to better precision of the estimate based on deaths from all causes. Finally, of all the methods, this provides the most conservative estimate of case under-ascertainment in this study, so is unlikely to over-estimate the factor by which we need to adjust the crude measured incidence.

Only one method estimated the case detection rate in the active case detection arm of the study, and we used this estimate to adjust the incidence component derived in that arm of the study. Adjusted incidence estimates and projected annual paediatric TB caseload are shown for the KHDSS by age category and overall in Table 7.7.

Table 7.7 Adjusted incidence and projected annual caseload of childhood TB in the KHDSS

	Estimated annual caseload (N) and adjusted incidence (I_{adj}) per 100,000 children per year					
	KDH passive case detection arm		KDH active case detection arm		KHDSS (total)	
Age group	N	I_{adj}	N	I_{adj}	N	I_{adj}
Confirmed TB						
0-4 years	14	15.1	10	11.0	24	26.1
5-9 years	16	20.3	*	*	16	20.3
10-14 years	7	10.0	*	*	7	10.0
<15 years	34	14.3	10	4.0	44	18.3
CHPTB						
0-4 years	46	51.4	30	32.5	76	83.9
5-9 years	26	32.6	*	*	26	32.6
10-14 years	10	14.8	*	*	10	14.8
<15 years	80	33.4	30	12.5	110	45.9
All TB						
0-4 years	92	102.7	30	32.5	122	135.2
5-9 years	61	77.4	*	*	61	77.4
10-14 years	21	29.7	*	*	21	29.7
<15 years	169	70.6	30	12.5	199	83.1

* Recruitment in the active case detection arm was confined to children <5 years old.

7.3.8 Kenyan national burden of childhood TB

Between 1 August 2009 and 31 July 2011 there were 678 new TB cases notified among KHDSS resident adults. In the same period there were an estimated 110 new CHPTB cases among KHDSS resident children (Table 7.11). Children therefore represented 11.7% of the estimated total case burden in the KHDSS. According to official TB notification data for Kenya there were 89,883 new TB cases among adults, and 5,721 cases among children in 2010; the estimated overall case detection rate was 82%.[58] There are an estimated 17.6 million children under the age of 15 years in Kenya in 2009.[59]

Based on these figures, and assuming children accounted for a similar proportion of the TB caseload in the KHDSS and nationally, the estimated number of new TB cases among children in 2010 was 14,583 - more than twice the official figure of 5,721 notified cases. This equates to a national incidence of childhood TB in Kenya of 83 per 100,000 per year. Estimates of the national burden of childhood TB are shown by age category in Table 7.8.

Table 7.8 Estimated national case burden and incidence of childhood TB by age group

Age group	Estimated no. childhood CHPTB cases in the KHDSS[1]	Proportion of the total TB caseload in the KHDSS[1]	National population in each age group (millions)	Estimated no. childhood CHPTB cases each year	Incidence per 100,000/y
0-4 years	76	8.1%	6.2	9,672	156
5-9 years	26	2.8%	6.0	3,120	52
10-14 years	10	1.2%	5.4	1,188	22
<15 years	110	11.7%	17.6	14,608	83

[1] during the 2 years of the KHDSS incidence study

7.4 Discussion

7.4.1 Crude incidence estimates & definitions of childhood TB

This is the first prospective study from East Africa to estimate the incidence of childhood TB, and one of very few prospective incidence studies from high burden countries. As a community-based study nested in a demographic surveillance survey, and underpinned by linked hospital, laboratory and demographic data, it is unique. The validity of the results derives from a combination of all these strengths.

In keeping with recommendations for childhood TB surveillance[199] and research[72] we used a hierarchical diagnostic classification. Incidence estimates are therefore derived for groups of increasing diagnostic specificity. Although the specificity of confirmed TB is highest, the poor sensitivity of mycobacterial culture for childhood TB diagnosis means that incidence estimates based only on confirmed cases under-estimate the true disease burden. In the absence of a definitive gold standard it is impossible to know the true diagnostic sensitivity of culture for childhood TB, but estimates vary between 10-30%, depending on the denominator used.[12, 16, 109, 180, 200]

The best published estimate is probably that derived from a study of 250 children admitted to hospital in South Africa with clinical evidence of pulmonary TB (defined as cough >28 days plus at least one of the following: household TB contact; loss of weight or failure to thrive during the previous 3 months; a positive TST; or radiographic signs suggestive of pulmonary TB).[16] A quarter of children in that study had a positive AFB smear or TB culture from one or more gastric lavage and/or induced sputum specimens. However sensitivity was also related to the number of specimens cultured: a single induced sputum sample identified only 66% of culture positive cases; this increased to 79% for 2 specimens. Culture sensitivity in our study is therefore likely to be a product of both optimal mycobacterial culture sensitivity for childhood TB (estimated as 25% in the above study) and the sensitivity of our two (pooled) induced sputum samples for culture positive pulmonary TB (79% in that study).

At the other end of the spectrum of diagnostic specificity is our group of All TB, which encompasses all children who were diagnosed and/or treated presumptively for TB. This group includes children in whom TB was thought to be the most likely diagnosis clinically, but who did not meet the deliberately stringent criteria for highly probable

TB; as well as cases for whom TB was only one of the differential diagnoses, but in whom empiric treatment was indicated due to the severity of a child's illness. It is relevant to note in this context that the threshold for treatment at KDH was higher during the study than in routine clinical practice because study resources combined with detailed information from the KHDSS about each child's residence location allowed very careful follow up in children whose disease status was unclear at initial presentation. Nevertheless it is likely that many of these cases did not actually have active TB, so incidence estimates based on this group probably provide an over-estimate of the true burden of childhood TB in Kilifi.

By contrast, the proportion of children in the HPTB group that did not actually have TB is likely to be very small, due to the stringent clinical definition used. Although not all children with TB will have been captured by this group (see Chapter 3, section 3.4.3 for a more detailed discussion of the potential limitations of this definition), it is likely to have captured many of the true cases of active TB for whom culture confirmation was not obtained. Therefore, although imperfectly sensitive, the combination of confirmed and highly probable TB (CHPTB) has greatest validity as a measure of the childhood TB burden in this study.

Compared with estimates based on published clinical definitions, our estimate of the incidence of CHPTB was among the most conservative. Even when we included All TB cases in the numerator, effectively doubling the estimated incidence, it remained smaller than that estimated by the majority of published clinical tools. This suggests that most published definitions would tend to over-diagnose TB in this and similar settings if they were to be applied routinely in clinical practice. The vast range in incidence estimates is itself staggering (3 to 192 per 100,000/year), and underlines the need for reliable data based on accurate definitions.

7.4.2 Case detection rate

Robust community incidence estimates depend on high quality diagnosis to minimize misclassification, and a high case detection rate. Achievement of both is very challenging in high burden settings. A comprehensive clinical, radiological, and laboratory workup combined with careful follow up of children enrolled in the KIDS TB Study ensured diagnostic classifications were optimized within the limitations of currently available diagnostic tools. Broad screening criteria for all children admitted to hospital with any

features of suspected TB, plus active case detection through contact tracing, ensured case ascertainment at KDH was also optimal. Nevertheless the social, financial and geographic barriers to presentation to hospital in this setting mean that many sick KHDSS resident children do not present to KDH.[198, 201, 202] Furthermore, the non-specific presentation of TB in children combined with a lack of diagnostic resources mean that surveillance data from other health facilities is unreliable. In recognition of this we attempted to quantify the sensitivity of hospital-based surveillance at KDH.

Each of the methods used to estimate the case detection rate has advantages and disadvantages as summarized in Table 7.1. No one method appeared clearly superior to all the others *a priori*. We therefore estimated the case detection rate using each method, in order to infer the 'true' case detection rate by triangulating our results.

Estimates of the sensitivity of passive surveillance at KDH ranged from 20% to 35% (Figure 7.4). Due to heterogeneity in the estimates derived, and because each method measures something slightly different (and therefore incorporates different potential biases) we did not attempt to calculate a single summary CDR estimate. Instead, on the basis of the likely biases, relative magnitude, precision and external applicability of each of the estimates, we selected the CDR estimate derived using KHDSS all-cause mortality surveillance data to adjust hospital-based incidence rates. We justify this choice in section 7.3.7 above.

The sensitivity of hospital-based all-cause mortality surveillance has also been used to estimate the sensitivity of hospital based surveillance for invasive bacterial disease among children.[203, 204] A potential criticism of the approach is that the more chronic nature of TB compared with many of the commonest causes of death (such as pneumonia, diarrhoea and meningitis), might allow more opportunity for TB patients to reach hospital before they die. In theory the relative 'acuteness' of hospital presentation due to TB and these other causes might be explored by comparing the timing of inpatient deaths in each group; however bias due to the exclusion of children with suspected TB who died early in their admission - and could not therefore be investigated adequately - precludes meaningful analysis. Some insight into the healthcare seeking behavior of children with chronic illness is nonetheless afforded by the large number of children who present to KDH with severe malnutrition, among whom the early inpatient mortality is very high.[205] This suggests that the likelihood of presentation to hospital with severe disease is not simply a function of disease tempo. Indeed careful analysis of

treatment seeking behavior in Kilifi reveals a complex interplay of social, cultural and economic factors, among which financial constraints feature prominently.[197, 198, 201, 206] Given the strong association of TB with poverty, and its greater associated household economic burden compared with many other illnesses,[207, 208] it is plausible that hospital based mortality surveillance may be even poorer for TB than for many other more acute childhood illnesses. The consistently lower CDR estimates derived using each of the other methods applied in this study would support this, and suggest that the CDR estimate based on all-cause mortality surveillance is likely to represent a conservative estimate of the true case detection rate.

Only one method was available to estimate the case detection rate in the active case detection (contact tracing) arm of the study. The result suggests only 20% of TB cases among child household contacts were captured for investigation by the KIDS TB Study. This is partly explained by loss to contact tracing of the third of KHDSS resident smear positive index cases that did not present to KDH. However an even greater operational challenge was failure of those index cases that were seen at KDH to bring their household contacts to the clinic for investigation. Two thirds of child household contacts identified were not brought for investigation, despite provision of the fare to return to hospital and a subsequent visit by a field worker at home to remind those who did not present to bring the children to the clinic - and to provide the fare again if required. This suggests barriers to presentation other than cost. Operational research in this area will be important to understand and overcome these barriers in order to realize the potential of contact tracing and isoniazid chemoprophylaxis to prevent TB in children.[32] The importance of this is emphasized by our finding that 40-50% of childhood TB cases in Kilifi could be attributed to a known close TB contact (chapter 4).

We assumed in our analysis of the case detection rate in the active case detection arm of the study that presentation of a child for investigation was independent of his or her risk of having TB. While this may have led to an under-estimate of surveillance sensitivity if a parent or guardian were more likely to bring a sick child for review, two factors argue against major bias in this direction. Firstly, the proportion of TB cases identified among household contacts <5 years old investigated in our study (6/104, 5.8%) is less than the pooled yield of 8.5% (95% CI 7.4 to 9.7%) found by a meta-analysis of contact tracing studies.[209] Secondly, due to finite resources we limited contact tracing to the most vulnerable group of children, namely those under 5 years old living in the household of a

smear positive index case. Exclusion from contact tracing of smear negative index cases, child contacts over 5 years old, and non-household-resident child contacts almost certainly under-estimates the true number of child contact TB cases, thereby over-estimating the case detection rate.

7.4.3 Adjusted incidence estimates for childhood TB in Kilifi & nationally

We applied our estimate of the case detection rate to the crude hospital-based incidence rates to estimate the true community incidence of childhood TB in the KHDSS; and then used these adjusted incidence rates for Kilifi to infer the likely national burden of childhood TB.

Caution must be exercised in extrapolating results from a single district to the national TB burden, since the incidence of childhood TB and the contribution of children to the total TB burden are likely to be affected by many factors which vary geographically within Kenya. These include the community TB prevalence;[32, 60] social and demographic factors such as the degree of urbanization that affect the risk of tuberculosis infection;[59] the prevalence of host factors such as BCG vaccination, HIV infection and nutritional status;[12, 59, 60] and local population structures.[59] For this reason we did not attempt simply to age-standardize the Kilifi incidence rates to the national population of children in Kenya. We reasoned instead that the proportion of the total TB caseload accounted for by children was likely to be less prone to geographical variation, and estimated the national incidence of childhood TB by assuming this proportion is equal to that observed in the KHDSS.

Final adjusted estimates of the incidence of CHPTB in the KHDSS and nationally were 46 and 83 per 100,000 per year, respectively. The proportion of the TB case load accounted for by children in our study (11.7%) is similar to the global average reported for 2000,[10] but lower than the 16% estimate for Kenya in the same year and the global estimate of 15-20% quoted more recently,[11, 210] and substantially lower than the paediatric proportion of cases in some high burden settings.[12, 32-34] This suggests our results are unlikely to be a gross over-estimate of the childhood TB burden in Kenya. Other factors that suggest that these estimates may actually be conservative include restriction of TB cases to those that met the stringent criteria of confirmed and highly probable TB; and adjustment of hospital-based incidence rates using the highest estimate of passive surveillance sensitivity.

The much lower estimate of childhood TB incidence in Kilifi compared with our national estimate is in keeping with data from other sources. National tuberculin surveys consistently demonstrate a lower annual risk of tuberculosis infection (ARTI) in Kilifi district than in most other districts surveyed.[211, 212] Although direct extrapolation of infection risk to TB incidence (the 'Styblo rule') is no longer supported by empiric epidemiological data,[213] variations in ARTI are nevertheless informative of geographical and temporal trends in transmission.[214] The HIV prevalence is also lower in Coast Province than in most other parts of the country,[59] and a clear geographical association between HIV and community TB prevalence has been reported at district level in Kenya.[60] The combination in Kilifi of a low ARTI and HIV prevalence, combined with relatively high BCG vaccine coverage and a more rural population than the national average,[59, 62] are all in keeping with the lower estimated childhood TB incidence.

Our national incidence estimates are roughly half the previous annual estimate of 167 per 100,000 extrapolated from NTP notification data in 2000.[10] This difference is likely to reflect a real reduction in TB burden as well as differences in the methodology used. Over the same period the overall reported incidence of TB in Kenya fell from 450 to 298 per 100,000 per year.[10, 58] Global estimates of the burden of childhood TB have also been revised downwards,[10, 11] although the lack of formal published figures from the WHO reflects uncertainty due to the limited surveillance data available.[3] Almost 350,000 cases of childhood TB are estimated to have gone undiagnosed in the 22 highest burden countries in 2010.[53, 176] Comparison of our projections for the number of childhood TB cases with the number of notified cases among Kenyan children[58] suggests almost 9000 cases may have gone undiagnosed and/or unreported in the same year.

7.4.4 Public health implications & lessons for childhood TB surveillance in Kenya

Nevertheless, despite some limitations, our estimates of the incidence of TB among Kenyan children are still better (and, a decade later, more up to date) than the only previous estimates. Based on enhanced surveillance that utilized both state of the art diagnostics and an almost unique system of linkage of clinical data to demographic surveillance, they provide a valuable source of information for policy makers and clinicians in Kenya and the region. Furthermore they emphasize the continued high burden of childhood TB, reinforcing the case for development and introduction of new TB vaccines.[215]

We effectively established for the KIDS TB Study a sentinel site for childhood TB surveillance in Kilifi. To estimate and monitor the disease burden more widely requires high quality paediatric TB surveillance data from other sites. Given the many competing priorities and already over-stretched public health budgets it is unlikely that a similar surveillance platform could be established outside a research setting. Ultimately, high quality incidence estimates beyond a research environment will require better diagnostic tools that are simple, affordable, and appropriate for use at the point of care in resource poor settings.

8 CONCLUDING REMARKS

Children are among the most vulnerable to TB, and one of the most challenging groups for both diagnosis and disease surveillance. The need for improved diagnostic tools and better surveillance data are therefore among the top research priorities for childhood TB.[32] Building on the existing clinical and demographic research infrastructure at the KEMRI-Wellcome Trust Research Programme, we established a state of the art diagnostic TB laboratory, and inpatient and outpatient paediatric TB clinical services at KDH and CPGH. Utilizing this platform, and combining broad and clearly defined inclusion criteria with a comprehensive clinical, radiological, and laboratory workup, and careful follow up, we aimed in the KIDS TB Study to improve our understanding of the epidemiology of childhood TB, and to investigate methods for improving childhood TB diagnosis, in a low resource, high burden setting in Kenya.

8.1.1 Clinical spectrum and risk factors of childhood TB

We have provided the first comprehensive description from the region of the burden and clinical spectrum of childhood tuberculosis, including the proportions of pulmonary and extra-pulmonary TB cases, HIV co-infection, and MDR-TB (chapter 3). Our data confirm the known associations of HIV and malnutrition with TB (chapter 4). Of much more immediate practical importance, however, is the very strong association we have demonstrated in Kilifi between a history of close TB contact and presentation with active TB, which has implications for both clinical practice and public health policy. In particular, our finding that 40-50% of childhood TB cases can be attributed to a known close TB contact - and were therefore potentially preventable - provides strong endorsement for expansion of programmes to deliver isoniazid chemoprophylaxis to child TB contacts in similar settings.

8.1.2 Clinical diagnosis of childhood TB

Our analysis of clinical parameters also has practical implications for diagnosis in routine clinical practice (chapter 5). In particular, the poor sensitivity (<50%) of the TST for both confirmed and highly probable TB undermines current WHO guidance that "the greatest majority" of cases should have a positive TST.[21] Perhaps even more importantly we have demonstrated the very poor sensitivity of existing WHO case definitions for childhood TB in our setting. Both observations support a re-evaluation of the WHO guidelines.

In addition to evaluating WHO and Kenyan national TB guidelines, we have presented the first comprehensive analysis of the performance of the wide range of published clinical tools for childhood TB diagnosis. Wide variation in their performance underlines the diagnostic challenge, but also reflects methodological limitations in their development and, in most cases, the lack until now of any formal validation in an independent population. Using prospective clinical data from the KIDS TB Study we have developed and independently validated our own prognostic model to risk stratify children with suspected TB. The KIDS TB Score derived outperforms other more complex diagnostic scores in our setting, allowing TB to be effectively ruled out in two thirds of suspects (NPV 98.8%), and stratifying the remaining children into groups of increasing risk to guide further investigation and/or treatment.

8.1.3 Microbiological diagnosis of childhood TB

While clinical tools are important to guide management, particularly in low resource settings, definitive diagnosis of TB relies on bacteriological confirmation. Our study is the first to compare the MGIT, MODS and Xpert MTB/RIF assays, three methods that have attracted most interest for childhood TB diagnosis (chapter 6). Interestingly - and contrary to early experience of MODS for adult TB diagnosis[80] - MODS was inferior to MGIT, supporting the findings of the only other study to compare the two methods for paediatric TB diagnosis.[166] The suboptimal positive predictive value for MTBC of MODS alone (86% in our study, due to the large number of NTM culture isolates) also supports a WHO recommendation for inclusion in the assay of a well containing *p*-nitrobenzoic acid to improve specificity.[160]

The Xpert MTB/RIF assay has generated great excitement as a potential method for enhancing diagnosis of TB in resource poor settings. Our study adds to the limited existing data from children in two ways. Firstly, it confirms the assay sensitivities previously reported among both smear positive and smear negative induced sputum specimens. Indeed overall sensitivity in our study was as good as that of MODS culture. Secondly, we were able to confirm the assay's very high specificity (100% in this study) with good precision, by analyzing a large number of samples from children in whom TB had been definitively excluded.

Although MGIT culture provided the highest bacteriological yield, our experience adds to that of other centres in Africa where high contamination rates have been reported. It

highlights the challenges of wide scale implementation of liquid mycobacterial culture facilities, and emphasizes the importance of careful protocol adherence in specimen collection including sputum induction.

8.1.4 Community incidence of childhood TB

Finally, we present the only prospective estimates outside South Africa of the incidence of TB in African children (chapter 7). Ours is one of only very few such studies globally and the only study from anywhere in the world to meet the basic criteria set out for prospective community incidence studies in the *WHO Research Agenda for Childhood TB*, since the document was published in 2006.[32] National projections based on data from Kilifi suggest that less than half of all TB cases in Kenyan children are diagnosed and treated within the National TB Programme, with ~9,000 cases per year unaccounted for by official notification data.

8.1.5 Conclusion and directions for future research

We hope that publication and dissemination of our study findings will inform future public health policy and clinical guidelines on childhood TB in Kenya and in similar settings elsewhere. Future research to build on these findings should include operational studies to understand better the barriers and potential solutions to improving contact tracing and provision of isoniazid chemoprophylaxis; evaluation of the performance and impact of the KIDS TB Score in other settings; and analyses of the performance, impact and cost effectiveness of combining initial clinical risk stratification using the KIDS TB Score with culture and/or PCR for TB in broader management algorithms.

9 REFERENCES

1. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proceedings of the National Academy of Sciences of the United States of America. 2009; **106**(33): 13980-5.
2. Dye C, Williams BG. Eliminating human tuberculosis in the twenty-first century. Journal of the Royal Society, Interface / the Royal Society. 2008; **5**(23): 653-62.
3. World Health Organization. Global Tuberculosis Control 2011. Geneva: World Health Organization; 2011.
4. World Health Organization. World Health Statistics 2010. Geneva: World Health Organization; 2010.
5. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet. 2005; **365**(9465): 1147-52.
6. World Health Organization. World Health Statistics 2011. Geneva: World Health Organization; 2011.
7. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012; **379**(9832): 2151-61.
8. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, 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**(4): 392-402.
9. 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**(9517): 1173-80.
10. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis. 2004; **8**: 636-47.
11. Getahun H, Sculier D, Sismanidis C, Grzemska M, Raviglione M. Prevention, diagnosis, and treatment of tuberculosis in children and mothers: evidence for action for maternal, neonatal, and child health services. J Infect Dis. 2012; **205 Suppl 2**: S216-27.
12. Newton SM, Brent AJ, Anderson S, Whittaker E, Kampmann B. Paediatric tuberculosis. The Lancet infectious diseases. 2008; **8**(8): 498-510.
13. Schaaf HS, Michaelis IA, Richardson M, Booysen CN, Gie RP, Warren R, et al. Adult-to-child transmission of tuberculosis: household or community contact? Int J Tuberc Lung Dis. 2003; **7**(5): 426-31.
14. Guwatudde D, Nakakeeto M, Jones-Lopez EC, Maganda A, Chiunda A, Mugerwa RD, et al. Tuberculosis in household contacts of infectious cases in Kampala, Uganda. American journal of epidemiology. 2003; **158**(9): 887-98.
15. Jones C, Whittaker E, Bamford A, Kampmann B. Immunology and pathogenesis of childhood TB. Paediatric respiratory reviews. 2011; **12**(1): 3-8.
16. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for micorbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet. 2005; **2005**(365): 130-4.
17. Ling DI, Zwerling AA, Steingart KR, Pai M. Immune-based diagnostics for TB in children: what is the evidence? Paediatric respiratory reviews. 2011; **12**(1): 9-15.
18. English M, Esamai F, Wasunna A, Were F, Ogutu B, Wamae A, et al. Delivery of paediatric care at the first-referral level in Kenya. Lancet. 2004; **364**: 1622-9.

19. English M, Esamai F, Wasunna A, Were F, Ogutu B, Wamae A, et al. Assessment of inpatient paediatric care in first referral level hospitals in 13 districts in Kenya. Lancet. 2004; **363**: 1948-53.
20. International Union Against Tuberculosis and Lung Disease. Desk-guide for diagnosis and management of TB in children. Paris: International Union Against Tuberculosis and Lung Disease; 2010.
21. World Health Organisation. Guidance for national tuberculosis programmes on the management of tuberculosis in children. Geneva: World Health Organisation; 2006.
22. World Health Organization. Use of interferon gamma release assays (IGRAs) in tuberculosis control in low and middle income countries. Geneva, Switzerland: World Health Organization; 2011.
23. Marais BJ, Obihara CC, Gie RP, Schaaf HS, Hesseling AC, Lombard C, et al. The prevalence of symptoms associated with pulmonary tuberculosis in randomly selected children from a high burden community. Arch Dis Child. 2005; **90**(11): 1166-70.
24. 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**(12): 1038-45.
25. World Health Organization. Global Tuberculosis Control 2010. Geneva: World Health Organization; 2010.
26. Enarson DA, Rieder HL, Arnadottir T, Trebucq A. Management of tuberculosis. A guide for low income countries. Fifth ed. Paris, France: International Union Against Tuberculosis and Lung Disease; 2000.
27. Dunbar R, Lawrence K, Verver S, Enarson DA, Lombard C, Hargrove J, et al. Accuracy and completeness of recording of confirmed tuberculosis in two South African communities. Int J Tuberc Lung Dis. 2011; **15**(3): 337-43.
28. Teo SS, Alfaham M, Evans MR, Watson JM, Riordan A, Sonnenberg P, et al. An evaluation of the completeness of reporting of childhood tuberculosis. Eur Respir J. 2009; **34**(1): 176-9.
29. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med. 2003; **163**(9): 1009-21.
30. Sandgren A, Hollo V, Quinten C, Manissero D. Childhood tuberculosis in the European Union/European Economic Area, 2000 to 2009. Euro Surveill. 2011; **16**(12).
31. Menzies HJ, Winston CA, Holtz TH, Cain KP, Mac Kenzie WR. Epidemiology of tuberculosis among US- and foreign-born children and adolescents in the United States, 1994-2007. American journal of public health. 2010; **100**(9): 1724-9.
32. Donald P, Maher D, Qazi S. A research agenda for childhood tuberculosis. Improving the management of childhood tuberculosis within national tuberculosis programmes: research priorities based on a literature review. Geneva: World Health Organisation; 2007.
33. 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. Archives of disease in childhood. 1999; **80**(5): 433-7.
34. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of community-based surveillance data. Int J Tuberc Lung Dis. 2006; **10**(3): 259-63.
35. Oren E, Winston CA, Pratt R, Robison VA, Narita M. Epidemiology of urban tuberculosis in the United States, 2000-2007. American journal of public health. 2011; **101**(7): 1256-63.

36. Cruz-Ferro E, Fernandez-Nogueira E. Epidemiology of tuberculosis in Galicia, Spain, 1996-2005. Int J Tuberc Lung Dis. 2007; **11**(10): 1073-9.
37. Krogh K, Suren P, Mengshoel AT, Brandtzaeg P. Tuberculosis among children in Oslo, Norway, from 1998 to 2009. Scandinavian journal of infectious diseases. 2010; **42**(11-12): 866-72.
38. Viney K, O'Connor J, Wiegandt A, Lambert M, Cox H, Downing S. Tuberculosis trends in the Pacific: 2000-2006. Pacific health dialog. 2010; **16**(1): 157-71.
39. Abreu G, Gonzalez JA, Gonzalez E, Bouza I, Velazquez A, Perez T, et al. Cuba's strategy for childhood tuberculosis control, 1995-2005. MEDICC review. 2011; **13**(3): 29-34.
40. Jurcev-Savicevic A, Katalinic-Jankovic V, Gjenero-Margan I, Simunovic A, Vidic D, Gunjaca J, et al. Epidemiological patterns of tuberculosis in Croatia in the period 1996-2005. Collegium antropologicum. 2011; **35**(2): 523-8.
41. Chang YM, Shen CK, Chiu CH, Chiang HJ, Lu LC, Liou SH. Burden of tuberculosis among aboriginal and non-aboriginal Taiwanese, 1996-2006. Int J Tuberc Lung Dis. 2011; **15**(4): 471-7.
42. Middelkoop K, Bekker LG, Morrow C, Zwane E, Wood R. Childhood tuberculosis infection and disease: a spatial and temporal transmission analysis in a South African township. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 2009; **99**(10): 738-43.
43. Romero-Sandoval NC, Flores-Carrera OF, Sanchez-Perez HJ, Sanchez-Perez I, Mateo MM. Pulmonary tuberculosis in an indigenous community in the mountains of Ecuador. Int J Tuberc Lung Dis. 2007; **11**(5): 550-5.
44. Hesseling AC, Cotton MF, Jennings T, Whitelaw A, Johnson LF, Eley B, et al. High incidence of tuberculosis among HIV-infected infants: evidence from a South African population-based study highlights the need for improved tuberculosis control strategies. Clin Infect Dis. 2009; **48**(1): 108-14.
45. Wang PD. Epidemiological trends of childhood tuberculosis in Taiwan, 1998-2005. Int J Tuberc Lung Dis. 2008; **12**(3): 250-4.
46. Gninafon M, Trebucq A, Rieder HL. Epidemiology of tuberculosis in Benin. Int J Tuberc Lung Dis. 2011; **15**(1): 61-6.
47. Braitstein P, Nyandiko W, Vreeman R, Wools-Kaloustian K, Sang E, Musick B, et al. The clinical burden of tuberculosis among human immunodeficiency virus-infected children in Western Kenya and the impact of combination antiretroviral treatment. The Pediatric infectious disease journal. 2009; **28**(7): 626-32.
48. Styblo K, Sutherland I. [Epidemiology of tuberculosis in the child]. Bulletin of the International Union against Tuberculosis. 1982; **57**(2): 134-41.
49. McNally LM, Jeena PM, Gajee K, Thula SA, Sturm AW, Cassol S, et al. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. Lancet. 2007; **369**(9571): 1440-51.
50. Zar HJ, Hanslo D, Tannenbaum E, Klein M, Argent A, Eley B, et al. Aetiology and outcome of pneumonia in human immunodeficiency virus-infected children hospitalized in South Africa. Acta Paediatr. 2001; **90**(2): 119-25.
51. Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet. 2002; **360**(9338): 985-90.
52. Cain KP, McCarthy KD, Heilig CM, Monkongdee P, Tasaneeyapan T, Kanara N, et al. An algorithm for tuberculosis screening and diagnosis in people with HIV. The New England journal of medicine. **362**(8): 707-16.

53. Perez-Velez CM, Marais BJ. Tuberculosis in children. The New England journal of medicine. 2012; **367**(4): 348-61.
54. World Health Organization. A Roadmap for Ensuring Quality Tuberculosis Diagnostics Services within National Laboratory Strategic Plans Geneva: World Health Organization; 2010 20 January 2011.
55. World Health Organization. Policy framework for implementing new tuberculosis diagnostics. Geneva: World Health Organization; 2010 March 2010.
56. Leung E, Minion J, Benedetti A, Pai M, Menzies D. Microcolony culture techniques for tuberculosis diagnosis: a systematic review. Int J Tuberc Lung Dis. 2011.
57. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, 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. The Lancet infectious diseases. 2011.
58. World Health Organization. Global Tuberculosis Control 2011: Kenya country profile. Geneva: World Health Organization; 2011.
59. Kenya National Bureau of Statistics. Kenya Demographic and Health Survey 2008-09. Nairobi: Kenya National Bureau of Statistics; 2010.
60. World Health Organization. A brief history of tuberculosis control in Kenya. Geneva: WHO; 2009.
61. World Health Organization. Global Tuberculosis Control 2009. Geneva: World Health Organization; 2009.
62. Scott JAG, Bauni E, Moisi J, Ojal J, Nyaga V, Gatakaa H, et al. Cohort Profile: The Kilifi Health and Demographic Surveillance System. Manuscript submitted for publication. 2012.
63. Sanitation KMoPHa. DLTLD Guidelines. In: Division of Leprosy TaLDD, editor. Nairobi; 2008.
64. Kenya Ministry of Health. Guidelines for tuberculosis and leprosy control for health workers in Kenya. Nairobi: National Leprosy and Tuberculosis Control Programme; 2003.
65. Marais BJ, Gie RP, Obihara CC, Hesseling AC, Schaaf HS, Beyers N. Well defined symptoms are of value in the diagnosis of childhood pulmonary tuberculosis. Archives of disease in childhood. 2005; **90**(11): 1162-5.
66. Schaaf HS, Beyers N, Gie RP, Nel ED, Smuts NA, Scott FE, et al. Respiratory tuberculosis in childhood: the diagnostic value of clinical features and special investigations. The Pediatric infectious disease journal. 1995; **14**(3): 189-94.
67. WHO. Treatment of tuberculosis: guidelines for national programmes. 3rd edition ed. Geneva: World Health Organization; 2003.
68. WHO. Pocket book of hospital care for children: guidelines for the managment of common illnesses with limited resources. Geneva: World Health Organization; 2005.
69. WHO. Guidance for national tuberculosis programmes on the management of tuberculosis in children. Geneva; 2006.
70. World Health Organisation. Provisional guidelines for the diagnosis and classification of the EPI target diseases for primary health care, surveillance and special studies. Geneva: World Health Organization; 1983. Report No.: EPI/GEN/83/4.
71. Division of Leprosy Tuberculsosis and Lung Diseases. DLTLD Guidelines. Nairobi: Kenya Ministry of Public Health and Sanitiation; 2008.
72. Graham SM, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis. 2012; **205 Suppl 2**: S199-208.

73. Marais BJ, Gie RP, Schaaf HS, Starke JR, Hesseling AC, Donald PR, et al. A proposed radiological classification of childhood intra-thoracic tuberculosis. Pediatr Radiol. 2004; **34**(11): 886-94.
74. Gie RP. Diagnostic atlas of intrathoracic tuberculosis in children. A guide for low income countries. Paris: International Union Against Tuberculosis and Lung Disease (IUATLD); 2003.
75. World Health Organisation. Pocket book of hospital care for children: guidelines for the management of common illnesses with limted resources. Geneva: World Health Organisation; 2005.
76. Kenya Ministry of Public Health and Sanitation. National Guidelines for HIV Testing and Counselling in Kenya. In: (NASCOP) NAaSCP, editor. 2nd Edition ed. Nairobi; 2010.
77. Siddiqi SH, Ruesch-Gerdes S. MGIT Procedure Manual: Foundation for Innovative New Diagnostics (FIND); 2006.
78. World Health Organization. Laboratory Services in Tuberculosis Control. Part II. Microscopy. Geneva: WHO; 1998.
79. Coronel J, Roper M, Caviedes L, Moore DA. MODS: A user guide. Microscopic observation drug susceptibility assay. Lima, Peru; 2008.
80. Moore DA, Evans CA, Gilman RH, Caviedes L, Coronel J, Vivar A, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. The New England journal of medicine. 2006; **355**(15): 1539-50.
81. Hill PC, Jackson-Sillah D, Fox A, Franken KL, Lugos MD, Jeffries DJ, et al. ESAT-6/CFP-10 fusion protein and peptides for optimal diagnosis of mycobacterium tuberculosis infection by ex vivo enzyme-linked immunospot assay in the Gambia. Journal of clinical microbiology. 2005; **43**(5): 2070-4.
82. Scholvinck E, Wilkinson KA, Whelan AO, Martineau AR, Levin M, Wilkinson RJ. Gamma interferon-based immunodiagnosis of tuberculosis: comparison between whole-blood and enzyme-linked immunospot methods. Journal of clinical microbiology. 2004; **42**(2): 829-31.
83. Liebeschuetz 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**(9452): 2196-203.
84. Kenya Ministry of Health. National Tuberculosis and Leprosy Guidelines. Nairobi: National Leprosy and Tuberculosis Programme; 2006.
85. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007; **370**(9596): 1453-7.
86. Bossuyt PM, Reitsma JB. The STARD initiative. Lancet. 2003; **361**(9351): 71.
87. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Nelson LJ, et al. The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis. 2004; **8**(3): 278-85.
88. Beyers N, Gie RP, Schaaf HS, Van Zyl S, Talent JM, Nel ED, 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**(1): 38-43.
89. Holt PG. Postnatal maturation of immune competence during infancy and childhood. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 1995; **6**(2): 59-70.

90. Upham JW, Rate A, Rowe J, Kusel M, Sly PD, Holt PG. Dendritic cell immaturity during infancy restricts the capacity to express vaccine-specific T-cell memory. Infection and immunity. 2006; **74**(2): 1106-12.
91. Kampmann B, Tena-Coki G, Anderson S. Blood tests for diagnosis of tuberculosis. Lancet. 2006; **368**(9532): 282; author reply -3.
92. Marias BJ. Intrathoracic tuberculosis in children. Arch Dis Child Educ Pract Ed. 2006; **91**: ep1-7.
93. Marais BJ, Donald PR, Gie RP, Schaaf HS, Beyers N. Diversity of disease in childhood pulmonary tuberculosis. Annals of tropical paediatrics. 2005; **25**(2): 79-86.
94. Marais BJ, Gie RP, Hesseling AH, Beyers N. Adult-type pulmonary tuberculosis in children 10-14 years of age. The Pediatric infectious disease journal. 2005; **24**(8): 743-4.
95. Madhi SA, Huebner RE, Doedens L, Aduc T, Wesley D, Cooper PA. HIV-1 co-infection in children hospitalised with tuberculosis in South Africa. Int J Tuberc Lung Dis. 2000; **4**(5): 448-54.
96. Hussey G, Chisholm T, Kibel M. Miliary tuberculosis in children: a review of 94 cases. The Pediatric infectious disease journal. 1991; **10**(11): 832-6.
97. van den Bos F, Terken M, Ypma L, Kimpen JL, Nel ED, Schaaf HS, et al. Tuberculous meningitis and miliary tuberculosis in young children. Trop Med Int Health. 2004; **9**(2): 309-13.
98. Thwaites GE, Tran TH. Tuberculous meningitis: many questions, too few answers. Lancet neurology. 2005; **4**(3): 160-70.
99. 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**(7): 732-8.
100. Gutman LT, Moye J, Zimmer B, Tian C. Tuberculosis in human immunodeficiency virus-exposed or -infected United States children. The Pediatric infectious disease journal. 1994; **13**(11): 963-8.
101. Jeena PM, Pillay P, Pillay T, Coovadia HM. Impact of HIV-1 co-infection on presentation and hospital-related mortality in children with culture proven pulmonary tuberculosis in Durban, South Africa. Int J Tuberc Lung Dis. 2002; **6**(8): 672-8.
102. Mukadi YD, Wiktor SZ, Coulibaly IM, Coulibaly D, Mbengue A, Folquet AM, et al. Impact of HIV infection on the development, clinical presentation, and outcome of tuberculosis among children in Abidjan, Cote d'Ivoire. AIDS. 1997; **11**(9): 1151-8.
103. Palme IB, Gudetta B, Bruchfeld J, Muhe L, Giesecke J. Impact of human immunodeficiency virus 1 infection on clinical presentation, treatment outcome and survival in a cohort of Ethiopian children with tuberculosis. The Pediatric infectious disease journal. 2002; **21**(11): 1053-61.
104. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. Clin Infect Dis. 2000; **31**(1): 170-6.
105. Marais BJ, Graham SM, Cotton MF, Beyers N. Diagnostic and management challenges for childhood tuberculosis in the era of HIV. J Infect Dis. 2007; **196 Suppl 1**: S76-85.
106. Edwards DJ, Kitetele F, Van Rie A. Agreement between clinical scoring systems used for the diagnosis of pediatric tuberculosis in the HIV era. Int J Tuberc Lung Dis. 2007; **11**(3): 263-9.
107. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. Jama. 1994; **271**(9): 698-702.

108. Faurholt-Jepsen D, Range N, Praygod G, Jeremiah K, Faurholt-Jepsen M, Aabye MG, et al. BCG protects against tuberculosis irrespective of HIV status: a matched case-control study in Mwanza, Tanzania. Thorax. 2012.
109. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. Paediatric respiratory reviews. 2011; **12**(1): 16-21.
110. Almeida LM, Barbieri MA, Da Paixao AC, Cuevas LE. Use of purified protein derivative to assess the risk of infection in children in close contact with adults with tuberculosis in a population with high Calmette-Guerin bacillus coverage. The Pediatric infectious disease journal. 2001; **20**(11): 1061-5.
111. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet. 2003; **361**(9364): 1168-73.
112. Grzybowski S, Barnett GD, Styblo K. Contacts of cases of active pulmonary tuberculosis. Bulletin of the International Union against Tuberculosis. 1975; **50**(1): 90-106.
113. Lienhardt C, Sillah J, Fielding K, Donkor S, Manneh K, Warndorff D, et al. Risk factors for tuberculosis infection in children in contact with infectious tuberculosis cases in the Gambia, West Africa. Pediatrics. 2003; **111**(5 Pt 1): e608-14.
114. Marais BJ, Obihara CC, Warren RM, Schaaf HS, Gie RP, Donald PR. The burden of childhood tuberculosis: a public health perspective. Int J Tuberc Lung Dis. 2005; **9**(12): 1305-13.
115. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. Int J Tuberc Lung Dis. 2004; **8**(3): 286-98.
116. Barnes PF, Bloch AB, Davidson PT, Snider DE, Jr. Tuberculosis in patients with human immunodeficiency virus infection. The New England journal of medicine. 1991; **324**(23): 1644-50.
117. Chen ZW. Immunology of AIDS virus and mycobacterial co-infection. Current HIV research. 2004; **2**(4): 351-5.
118. Cooper AM. Cell-mediated immune responses in tuberculosis. Annual review of immunology. 2009; **27**: 393-422.
119. Schluger NW, Rom WN. The host immune response to tuberculosis. American journal of respiratory and critical care medicine. 1998; **157**(3 Pt 1): 679-91.
120. van der Spuy GD, Kremer K, Ndabambi SL, Beyers N, Dunbar R, Marais BJ, et al. Changing Mycobacterium tuberculosis population highlights clade-specific pathogenic characteristics. Tuberculosis (Edinb). 2009; **89**(2): 120-5.
121. Dormans J, Burger M, Aguilar D, Hernandez-Pando R, Kremer K, Roholl P, et al. Correlation of virulence, lung pathology, bacterial load and delayed type hypersensitivity responses after infection with different Mycobacterium tuberculosis genotypes in a BALB/c mouse model. Clin Exp Immunol. 2004; **137**(3): 460-8.
122. Hesseling AC, Marais BJ, Kirchner HL, Mandalakas AM, Brittle W, Victor TC, et al. Mycobacterial genotype is associated with disease phenotype in children. Int J Tuberc Lung Dis. 2010; **14**(10): 1252-8.
123. Kirkwood B, Sterne J. Essential Medical Statistics. 2nd ed. Oxford, UK: Blackwell Science Ltd; 2006.
124. Tena GN, Young DB, Eley B, Henderson H, Nicol MP, Levin M, et al. Failure to control growth of mycobacteria in blood from children infected with human immunodeficiency virus and its relationship to T cell function. J Infect Dis. 2003; **187**(10): 1544-51.

125. Elenga N, Kouakoussui KA, Bonard D, Fassinou P, Anaky MF, Wemin ML, et al. Diagnosed tuberculosis during the follow-up of a cohort of human immunodeficiency virus-infected children in Abidjan, Cote d'Ivoire: ANRS 1278 study. The Pediatric infectious disease journal. 2005; **24**(12): 1077-82.
126. Bates I, Fenton C, Gruber J, Lalloo D, Medina Lara A, Squire SB, et al. Vulnerability to malaria, tuberculosis, and HIV/AIDS infection and disease. Part 1: determinants operating at individual and household level. The Lancet infectious diseases. 2004; **4**(5): 267-77.
127. Cantwell MF, McKenna MT, McCray E, Onorato IM. Tuberculosis and race/ethnicity in the United States: impact of socioeconomic status. American journal of respiratory and critical care medicine. 1998; **157**(4 Pt 1): 1016-20.
128. Rutherford ME, Hill PC, Triasih R, Sinfield R, van Crevel R, Graham SM. Preventive therapy in children exposed to Mycobacterium tuberculosis: problems and solutions. Trop Med Int Health. 2012.
129. Brent AJ, Anderson ST, Kampmann B. Childhood tuberculosis: out of sight, out of mind? Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008; **102**(3): 217-8.
130. Brent AJ. Childhood TB Surveillance: Bridging the Knowledge Gap to Inform Policy. Journal of tropical medicine. 2012; **2012**: 865436.
131. Bergman NJ. A 'treatment score' for primary and pulmonary tuberculosis. Cent Afr J Med. 1995; **41**: 1-6.
132. Edwards K. The diagnosis of childhood tuberculosis. Papua New Guinea Med J. 1987; **30**: 169-78.
133. Fourie PB, Becker PJ, Festenstein F, Migliori GB, Alcaide J, Antunes M, et al. Procedures for developing a simple scoring method based on unsophisticated criteria for screening children for tuberculosis. Int J Tuberc Lung Dis. 1998; **2**(2): 116-23.
134. Nair PM, Philip E. A scoring system for the diagnosis of tuberculosis in children. Indian pediatrics. 1981; **18**: 299-303.
135. Stegen G, Jones K, Kaplan P. Criteria for guidance in the diagnosis of tuberculosis. Pediatrics. 1969; **43**(2 260-3).
136. Cundall DB. The diagnosis of pulmonary tuberculosis in malnourished Kenyan children. Annals of tropical paediatrics. 1986; **6**: 249-55.
137. Ghidey Y, Habte D. Tuberculosis in childhood: an analysis of 412 cases. Ethiopian medical journal. 1983; **21**: 161-7.
138. Jeena PM, Mitha T, Bamber S, Wesley A, Coutsoudis A, Coovadia HM. Effects of the human immunodeficiency virus on tuberculosis in children. Tubercle and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 1996; **77**(5): 437-43.
139. Kiwanuka J, Graham SM, Coulter JB, Gondwe JS, Chilewani N, Carty H, et al. Diagnosis of pulmonary tuberculosis in children in an HIV-endemic area, Malawi. Annals of tropical paediatrics. 2001; **21**(1): 5-14.
140. Okeahialam TC. Diagnostic criteria of tuberculosis in malnourished children. East Afr Med J. 1974; **51**: 79-89.
141. Osborne CM. The challenge of diagnosing childhood tuberculosis in a developing country. Archives of disease in childhood. 1995; **72**: 369-74.
142. Ramachandran RS. Tuberculosis in children: experience with 1284 cases. Indian pediatrics. 1968; **5**: 564-71.
143. Stoltz AP, Donald PR, Strebel PM, Talent JMT. Criteria for the notification of childhood tuberculosis in a high-incidence area of the Western Cape Province. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 1990; **77**: 385-6.

144. Migliori GB, Borghesi A, Rossanigo P, Adriko C, Neri M, Santini S, et al. Proposal of an improved score method for the diagnosis of pulmonary tuberculosis in childhood in developing countries. Tubercle and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 1992; **73**(3): 145-9.
145. Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahomed H, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. BMJ. 2008; **337**: a2052.
146. Hatherill M, Hanslo M, Hawkridge T, Little F, Workman L, Mahomed H, et al. Structured approaches for the screening and diagnosis of childhood tuberculosis in a high prevalence region of South Africa. Bulletin of the World Health Organization. 2010; **88**(4): 312-20.
147. Houwert KA, Borggreven PA, Schaaf HS, Nel E, Donald PR, Stolk J. Prospective evaluation of World Health Organization criteria to assist diagnosis of tuberculosis in children. Eur Respir J. 1998; **11**(5): 1116-20.
148. Nair PH, Philip E. A scoring system for the diagnosis of tuberculosis in children. Indian pediatrics. 1981; **18**(5): 299-303.
149. Division of Leprosy Tuberculsosis and Lung Diseases. Draft guidelines for the management of childhood TB. Nairobi: Kenya Ministry of Public Health and Sanitiation; 2012.
150. Stegen G, Jones K, Kaplan P. Criteria for guidance in the diagnosis of tuberculosis. Pediatrics. 1969; **43**(2): 260-3.
151. 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**(5): e1350-9.
152. Espinal MA, Reingold AL, Perez G, Camilo E, Soto S, Cruz E, et al. Human immunodeficiency virus infection in children with tuberculosis in Santo Domingo, Dominican Republic: prevalence, clinical findings, and response to antituberculosis treatment. Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association. 1996; **13**(2): 155-9.
153. Lachs MS, Nachamkin I, Edelstein PH, Goldman J, Feinstein AR, Schwartz JS. Spectrum bias in the evaluation of diagnostic tests: lessons from the rapid dipstick test for urinary tract infection. Annals of internal medicine. 1992; **117**(2): 135-40.
154. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. BMJ. 2009; **338**: b606.
155. Royston P, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. BMJ. 2009; **338**: b604.
156. Steyerberg EW, Eijkemans MJ, Harrell FE, Jr., Habbema JD. Prognostic modeling with logistic regression analysis: in search of a sensible strategy in small data sets. Medical decision making : an international journal of the Society for Medical Decision Making. 2001; **21**(1): 45-56.
157. Altman DG, Royston P. The cost of dichotomising continuous variables. BMJ. 2006; **332**(7549): 1080.
158. World Health Organization, Stop TB Partnership. The Global Plan to Stop TB 2011-2015: Transforming the fight towards elimination of tuberculosis. Geneva: WHO Press; 2010.
159. World Health Organization. Use of liquid TB culture and drug susceptibility testing (DST) in low and medium income countries: Summary report of the expert group meeting on the use of liquid culture media. Geneva: World Health Organization; 2007 26 March 2007.

160. World Health Organization. Non-commercial culture and drug-susceptibility testing methods for screening patients at risk for multi-drug resistant tuberculosis: Policy statement. Geneva: WHO Press; 2011.
161. Bwanga F, Haile M, Joloba ML, Ochom E, Hoffner S. Direct nitrate reductase assay versus microscopic observation drug susceptibility test for rapid detection of MDR-TB in Uganda. PloS one. 2011; **6**(5): e19565.
162. Ha DT, Lan NT, Kiet VS, Wolbers M, Hang HT, Day J, et al. Diagnosis of pulmonary tuberculosis in HIV-positive patients by microscopic observation drug susceptibility assay. Journal of clinical microbiology. **48**(12): 4573-9.
163. Minion J, Pai M. Expanding the role of the microscopic observation drug susceptibility assay in tuberculosis and HIV management. Clin Infect Dis. 2010; **50**(7): 997-9.
164. Shah NS, Moodley P, Babaria P, Moodley S, Ramtahal M, Richardson J, et al. Rapid diagnosis of tuberculosis and multidrug resistance by the microscopic-observation drug-susceptibility assay. American journal of respiratory and critical care medicine. 2011; **183**(10): 1427-33.
165. Caws M, Dang TM, Torok E, Campbell J, Do DA, Tran TH, et al. Evaluation of the MODS culture technique for the diagnosis of tuberculous meningitis. PloS one. 2007; **2**(11): e1173.
166. Ha DT, Lan NT, Wolbers M, Duong TN, Quang ND, Thi Van Thinh T, et al. Microscopic observation drug susceptibility assay (MODS) for early diagnosis of tuberculosis in children. PloS one. 2009; **4**(12): e8341.
167. Arias M, Mello FC, Pavon A, Marsico AG, Alvarado-Galvez C, Rosales S, et al. Clinical evaluation of the microscopic-observation drug-susceptibility assay for detection of tuberculosis. Clin Infect Dis. 2007; **44**(5): 674-80.
168. Michael JS, Daley P, Kalaiselvan S, Latha A, Vijayakumar J, Mathai D, et al. Diagnostic accuracy of the microscopic observation drug susceptibility assay: a pilot study from India. Int J Tuberc Lung Dis. 2010; **14**(4): 482-8.
169. Oberhelman RA, Soto-Castellares G, Caviedes L, Castillo ME, Kissinger P, Moore DA, et al. Improved recovery of Mycobacterium tuberculosis from children using the microscopic observation drug susceptibility method. Pediatrics. 2006; **118**(1): e100-6.
170. Oberhelman RA, Soto-Castellares G, Gilman RH, Caviedes L, Castillo ME, Kolevic L, et al. Diagnostic approaches for paediatric tuberculosis by use of different specimen types, culture methods, and PCR: a prospective case-control study. The Lancet infectious diseases. 2010; **10**(9): 612-20.
171. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess. 2007; **11**(3): 1-196.
172. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet. 2011; **377**(9776): 1495-505.
173. World Health Organization. Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System. Policy Statement. . Geneva: World Health Organization; 2011.
174. Gotuzzo E. Xpert MTB/RIF for diagnosis of pulmonary tuberculosis. The Lancet infectious diseases. 2011; **11**(11): 802-3.
175. Brent AJ, Mugo D, Musyimi R, Mutiso A, Morpeth S, Levin M, et al. Performance of the MGIT TBc identification test and meta-analysis of MPT64 assays for identification of

the Mycobacterium tuberculosis complex in liquid culture. Journal of clinical microbiology. 2011; **49**(12): 4343-6.
176. du Cros P, Nyang'wa BT, Gale M, Venis S, Ford N. Counting children: comparing reporting for paediatric HIV and tuberculosis. Bulletin of the World Health Organization. 2011; **89**(12): 855.
177. 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.
178. Rachow A, Clowes P, Saathoff E, Mtafya B, Michael E, Ntinginya EN, et al. Increased and Expedited Case Detection by Xpert MTB/RIF Assay in Childhood Tuberculosis: A Prospective Cohort Study. Clin Infect Dis. 2012.
179. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. The New England journal of medicine. 2010; **363**(11): 1005-15.
180. 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? Archives of disease in childhood. 2009; **94**(3): 195-201.
181. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis. 2006; **42**(8): e69-71.
182. Ha DT, Lan NT, Kiet VS, Wolbers M, Hang HT, Day J, et al. Diagnosis of pulmonary tuberculosis in HIV-positive patients by microscopic observation drug susceptibility assay. Journal of clinical microbiology. 2010; **48**(12): 4573-9.
183. Caviedes L, Lee TS, Gilman RH, Sheen P, Spellman E, Lee EH, et al. Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. Journal of clinical microbiology. 2000; **38**(3): 1203-8.
184. Verweij KE, Kamerik AR, van Ingen J, van Dijk JH, Sikwangala P, Thuma P, et al. Application of modern microbiological diagnostic methods for tuberculosis in Macha, Zambia. Int J Tuberc Lung Dis. 2010; **14**(9): 1127-31.
185. Buijtels PC, van der Sande MA, Parkinson S, Verbrugh HA, Petit PL, van Soolingen D. Isolation of non-tuberculous mycobacteria at three rural settings in Zambia; a pilot study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2010; **16**(8): 1142-8.
186. Buijtels PC, van-der-Sande MA, de-Graaff CS, Parkinson S, Verbrugh HA, Petit PL, et al. Nontuberculous mycobacteria, zambia. Emerging infectious diseases. 2009; **15**(2): 242-9.
187. Apers L, Mutsvangwa J, Magwenzi J, Chigara N, Butterworth A, Mason P, et al. A comparison of direct microscopy, the concentration method and the Mycobacteria Growth Indicator Tube for the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis. 2003; **7**(4): 376-81.
188. Chihota VN, Grant AD, Fielding K, Ndibongo B, van Zyl A, Muirhead D, et al. Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. Int J Tuberc Lung Dis. 2010; **14**(8): 1024-31.
189. Githui W, van't Hoog AH, Orina F, Karimi F, Meme H, Bii C. High MGIT contamination rates of sputum specimens from a tuberculosis prevalence survey in Western Kenya, 2007. 39th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease; 2008; Paris: International Journal of Tuberculosis & Lung Disease; 2008. p. S248.

190. Muyoyeta M, Schaap JA, De Haas P, Mwanza W, Muvwimi MW, Godfrey-Faussett P, et al. Comparison of four culture systems for Mycobacterium tuberculosis in the Zambian National Reference Laboratory. Int J Tuberc Lung Dis. 2009; **13**(4): 460-5.
191. Otu J, Antonio M, Cheung YB, Donkor S, De Jong BC, Corrah T, et al. Comparative evaluation of BACTEC MGIT 960 with BACTEC 9000 MB and LJ for isolation of mycobacteria in The Gambia. Journal of infection in developing countries. 2008; **2**(3): 200-5.
192. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. Journal of clinical microbiology. 2004; **42**(5): 2321-5.
193. Whitelaw AC, Mentoor K, Zar HJ, Nicol M. Standard Mycobacterial Decontamination Protocols May Be Inappropriate For Samples From Paediatric Patients 41st Union World Conference on Lung Health; 2010 11-15 November 2010; Berlin: International Union Against Tuberculosis And Lung Disease; 2010.
194. Moore DA, Caviedes L, Gilman RH, Coronel J, Arenas F, LaChira D, et al. Infrequent MODS TB culture cross-contamination in a high-burden resource-poor setting. Diagnostic microbiology and infectious disease. 2006; **56**(1): 35-43.
195. World Health Organization. Tuberculosis prevalence surveys: a handbook. Geneva: World Health Organization; 2011.
196. Bauni E, Ndila C, Mochamah G, Nyutu G, Matata L, Ondieki C, et al. Validating physician-certified verbal autopsy and probabilistic modeling (InterVA) approaches to verbal autopsy interpretation using hospital causes of adult deaths. Popul Health Metr. 2011; **9**: 49.
197. Molyneux CS, Mung'Ala-Odera V, Harpham T, Snow RW. Maternal responses to childhood fevers: a comparison of rural and urban residents in coastal Kenya. Trop Med Int Health. 1999; **4**(12): 836-45.
198. Molyneux CS, Murira G, Masha J, Snow RW. Intra-household relations and treatment decision-making for childhood illness: a Kenyan case study. Journal of biosocial science. 2002; **34**(1): 109-31.
199. Keja J. Provisional guidelines for the diagnosis and classification of the EPI target diseases for primary health care, surveillance and special studies. Geneva, Switzerland: World Health Organization; 1983.
200. Kumar A, Upadhyay S, Kumari G. Clinical Presentation, treatment outcome and survival among the HIV infected children with culture confirmed tuberculosis. Current HIV research. 2007; **5**(5): 499-504.
201. Chuma J, Gilson L, Molyneux C. Treatment-seeking behaviour, cost burdens and coping strategies among rural and urban households in Coastal Kenya: an equity analysis. Trop Med Int Health. 2007; **12**(5): 673-86.
202. Moisi JC, Nokes DJ, Gatakaa H, Williams TN, Bauni E, Levine OS, et al. Sensitivity of hospital-based surveillance for severe disease: a geographic information system analysis of access to care in Kilifi district, Kenya. Bulletin of the World Health Organization. 2011; **89**(2): 102-11.
203. Berkley JA, Lowe BS, Scott JAG. Bacteremia among Kenyan Children. The New England journal of medicine. 2005; **352**: 1380-1.
204. Brent AJ, Ahmed I, Ndiritu M, Lewa P, Ngetsa C, Lowe B, et al. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. Lancet. 2006; **367**(9509): 482-8.
205. Maitland K, Berkley JA, Shebbe M, Peshu N, English M, Newton CR. Children with severe malnutrition: can those at highest risk of death be identified with the WHO protocol? PLoS medicine. 2006; **3**(12): e500.

206. Molyneux C, Hutchison B, Chuma J, Gilson L. The role of community-based organizations in household ability to pay for health care in Kilifi District, Kenya. Health policy and planning. 2007; **22**(6): 381-92.
207. McIntyre D, Thiede M, Dahlgren G, Whitehead M. What are the economic consequences for households of illness and of paying for health care in low- and middle-income country contexts? Soc Sci Med. 2006; **62**(4): 858-65.
208. Russell S. The economic burden of illness for households in developing countries: a review of studies focusing on malaria, tuberculosis, and human immunodeficiency virus/acquired immunodeficiency syndrome. The American journal of tropical medicine and hygiene. 2004; **71**(2 Suppl): 147-55.
209. Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. The Lancet infectious diseases. 2008; **8**(6): 359-68.
210. Marais BJ, Gupta A, Starke JR, El Sony A. Tuberculosis in women and children. Lancet. 2010; **375**(9731): 2057-9.
211. Kwamanga D, Chakaya J, Sitienei J, Kalisvaart N, L'Herminez R, van der Werf MJ. Tuberculosis transmission in Kenya: results of the third National Tuberculin Survey. Int J Tuberc Lung Dis. 2010; **14**(6): 695-700.
212. Odhiambo JA, Borgdorff MW, Kiambih FM, Kibuga DK, Kwamanga DO, Ng'ang'a L, et al. Tuberculosis and the HIV epidemic: increasing annual risk of tuberculous infection in Kenya, 1986-1996. American journal of public health. 1999; **89**(7): 1078-82.
213. van Leth F, van der Werf MJ, Borgdorff MW. Prevalence of tuberculous infection and incidence of tuberculosis: a re-assessment of the Styblo rule. Bulletin of the World Health Organization. 2008; **86**(1): 20-6.
214. Dye C. Breaking a law: tuberculosis disobeys Styblo's rule. Bulletin of the World Health Organization. 2008; **86**(1): 4.
215. Hatherill M. Prospects for elimination of childhood tuberculosis: the role of new vaccines. Archives of disease in childhood. 2011; **96**(9): 851-6.

10 APPENDICES

10.1 Statistical derivations

10.1.1 Derivation of the confidence interval for the sum of two rates

Total incidence estimates for childhood TB in the KHDSS area were calculated as the sum of the incidence in the active (contact tracing) and passive case detection arms of the study. The confidence interval about the total combined rate was derived as follows:

Notation

a number of TB cases in the active case detection study arm

p number of TB cases in the passive case detection study arm

T_a denominator in the active case detection study arm (person years observation)

T_p denominator in the passive case detection study arm (person years observation)

I_a incidence rate in the active case detection study arm $= a / T_a$

I_p incidence rate in the passive case detection study arm $= p / T_p$

I total combined incidence rate $= I_a + I_p$

Variance of the combined rate

We first computed the variance of the natural logarithm of the combined rate:

$$Var\{\ln(I)\} = Var\{\ln(I_a + I_p)\}$$

$$= \frac{Var(I_a + I_p)}{(I_a + I_p)^2}, \text{ since } Var(\ln x) \cong \frac{1}{x^2} Var(x) \text{ via the delta method}$$

$$= \frac{Var(I_a) + Var(I_p)}{(I_a + I_p)^2}, \text{ assuming independence of the two rates}$$

$$= \frac{Var\left(\frac{a}{T_a}\right) + Var\left(\frac{p}{T_p}\right)}{(I_a + I_p)^2}$$

$$= \frac{\left(\frac{Var(a)}{T_a^2}\right) + \left(\frac{Var(p)}{T_p^2}\right)}{(I_a + I_p)^2}$$

$$= \frac{\left(\frac{a}{T_a^2}\right) + \left(\frac{p}{T_b^2}\right)}{(I_a + I_p)^2}$$

Confidence interval for the combined rate

We then calculated the confidence interval for the combined rate using the standard formula for the confidence interval about a rate:

$$\begin{aligned} 95\%\ \text{CI} &= \exp\left\{\ln(I) \pm 1.96 \times \text{s.e.}\left\{\ln(I)\right\}\right\} \\ &= \exp\left\{\ln(I) \pm 1.96 \times \sqrt{Var\left\{\ln(I)\right\}}\right\} \\ &= \exp\left\{\ln(I) \pm 1.96 \times \sqrt{\frac{\left(\frac{a}{T_a^2}\right) + \left(\frac{p}{T_b^2}\right)}{\left(I_a + I_p\right)^2}}\right\} \end{aligned}$$

10.1.2 Estimating 95% confidence intervals for the sensitivity of active case ascertainment

The sensitivity of case ascertainment in the active case detection arm of the study is given by:

$$S = \frac{\text{No. index cases captured at KDH}}{\text{Total no. index cases}} \times \frac{\text{No. eligible child contacts investigated}}{\text{Total no. eligible child contacts identified at KDH}}$$

It is thus the product of two independent proportions, each of which has its own variance, given by the formula:

$$Variance\ of\ a\ proportion = \frac{p(1-p)}{n}$$

where p is the value of the proportion and n is the denominator in each case.

The variance of the product of the two proportions (X and Y) is then given by the formula:

$$Var(XY) = [E(X)]^2 Var(Y) + [E(Y)]^2 Var(X) + Var(X)Var(Y)$$

The standard error is then calculated as the square root of the variance and used to derive upper and lower 95% confidence intervals in the usual way.

10.1.3 Estimating the national burden of childhood TB in Kenya

The formula for estimating the national burden of childhood TB was derived as follows:

Notation:

tb_t = estimated no. new TB cases in KHDSS (adults and children)*

tb_c = estimated no. new TB cases among KHDSS resident children*

tb_a = no. notified TB cases among KHDSS resident adults*

TB_t = no. new TB cases notified annually in Kenya (adults and children)

TB_a = no. new TB cases notified annually among Kenyan adults

TB_c = estimated no. new TB cases annually among Kenyan children

R = estimated national TB case detection/notification rate

N_c = population of children <15 years old in Kenya

**Figures for KHDSS derived for period 1 August 2009 to 31 July 2011.*

The incidence of childhood TB in Kenya was calculated as

$$(1)\ I_{Kenya} = \frac{TB_c}{N_c}$$

We estimated the true number of adult TB cases in the KHDSS (tb'_a) and nationally (TB'_a) by dividing the number of notified adult cases by the published national TB case detection rate:

$$(2)\ TB'_a = \frac{TB_a}{R}$$

$$(3)\ tb'_a = \frac{tb_a}{R}$$

We assumed that the paediatric proportions of TB cases among KHDSS residents and nationally were the same:

$$(4)\ \frac{tb_c}{tb_t} = \frac{TB_c}{TB_t} => \frac{tb_c}{tb'_a + tb_c} = \frac{TB_c}{TB'_a + TB_c}$$

Rearranging this we get:

$$(5)\ TB_c = \frac{TB'_a}{\left[{(tb'_a + tb_c)}\Big/{tb_c} - 1\right]}$$

Substituting formulae (2), (3) and (5) into formula (1) gives:

$$(5)\ I_{Kenya} = \frac{TB'_a}{N_c\left\{\frac{(tb'_a + tb_c)}{tb_c} - 1\right\}} = \frac{TB_a}{N_c R\left\{\frac{[(tb_a/R) + tb_c]}{tb_c} - 1\right\}}$$

10.2 Supplementary tables

10.2.1 Chapter 4 supplementary tables

Table 10.1 Effect of TB contact history on confirmed versus highly probable TB in the passive case detection arm of the study

	Confirmed TB (n=70)	Highly Probable TB (n=63)	Odds ratio for confirmed TB (95% CI)		p value
SOCIO-DEMOGRAPHIC FACTORS					
Referral source					
Inpatient	55	39	2.8	(0.5 to 16.5)	
Outpatient	13	20	1.3	(0.2 to 8.4)	0.103
Contact tracing	2	4	1.0		
Age category					
< 1 year	11	13	1.0		
1 to 4 years	25	36	0.8	(0.3 to 2.1)	
5 to 9 years	19	10	2.2	(0.7 to 7.0)	0.010
10 to 14 years	15	4	4.4	(1.0 to 19.2)	
Male sex	38	33	1.1	(0.6 to 2.1)	0.826
Maternal primary education [1]	7/8 [2]	10/13 [2]	2.1	(0.2 to 24.6)	0.555
HOST FACTORS					
HIV infection	17/69 [2]	14	1.1	(0.5 to 2.6)	0.745
Severe malnutrition	30	31	0.8	(0.4 to 1.5)	0.465
BCG scar	62/69 [2]	53/61 [2]			
TB CONTACT HISTORY					
History of close TB contact					
No history of close TB contact	36	31	1.0		0.789
Any history of close TB contact	32	25	1.1	(0.5 to 2.2)	
Proximity of TB contact [3]					
Contact outside the household	2	3	0.6	(0.1 to 3.7)	
Contact sleeps in same household	9	2	3.9	(0.8 to 19.3)	0.235
Contact sleeps in same room	21	20	0.9	(0.4 to 2.0)	
Smear status of TB contact [3]					
Smear positive	21	12	1.5	(0.6 to 3.5)	
Smear negative	8	9	0.8	(0.3 to 2.2)	0.594
Smear status unknown	3	4	0.6	(0.1 to 3.1)	
Relationship of contact to child [3,4]					
Parent	19	16	1.1	(0.5 to 2.4)	
Grandparent	1	4	0.2	(0.0 to 2.0)	
Aunt or uncle	7	4	1.7	(0.5 to 6.3)	0.504
Sibling	3	0		-	
Other	3	2	1.3	(0.2 to 8.2)	
Number of close TB contacts [3]					
One close TB contact	28	24	1.0	(0.5 to 2.1)	0.485
More than one close TB contact	4	1	3.4	(0.4 to 32.5)	

[1] Data on maternal education was only available and analyzed for KHDSS residents.
[2] In cases where data was missing the denominator for which data was available is presented.
[3] compared with children who had no history of TB contact.
[4] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

Table 10.2 Effect of TB contact history on confirmed TB (passive case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
History of close TB contact					
No history of close TB contact	36	1,058	1.0	(-)	<0.001
Any history of close TB contact	32	158	6.0	(3.5 to 10.0)	
Proximity of TB contact [1]					
Contact outside the household	2	41	1.5	(0.4 to 6.7)	<0.001
Contact sleeps in same household	9	56	6.8	(3.1 to 15.1)	
Contact sleeps in same room	23	148	7.6	(4.3 to 13.7)	
Smear status of TB contact [1]					
Smear positive	21	71	8.7	(4.8 to 15.7)	<0.001
Smear negative	8	59	4.0	(1.8 to 9.0)	
Smear status unknown	3	28	3.1	(0.9 to 10.8)	
Relationship of contact to child [1,2]					
Parent	19	76	7.3	(4.0 to 13.5)	<0.001
Grandparent	1	22	1.5	(0.2 to 11.4)	
Aunt or uncle	7	38	6.0	(2.5 to 14.4)	
Sibling	3	7	11.3	(2.5 to 50.5)	
Other	3	21	4.3	(1.2 to 15.2)	
Number of close TB contacts [1]					
One close TB contact	28	146	5.6	(3.3 to 9.6)	<0.001
More than one close TB contact	4	12	9.8	(3.0 to 31.9)	

[1] compared with children who had no history of TB contact.
[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

Table 10.3 Effect of TB contact history on highly probable TB (passive case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
History of close TB contact					
No history of close TB contact	31	1,058	1.0	(-)	<0.001
Any history of close TB contact	25	158	5.4	(3.1 to 9.5)	
Proximity of TB contact [1]					
Contact outside the household	3	41	2.7	(0.8 to 9.2)	<0.001
Contact sleeps in same household	22	56	1.8	(0.4 to 7.6)	
Contact sleeps in same room	20	148	8.4	(4.6 to 15.4)	
Smear status of TB contact [1]					
Smear positive	12	71	5.8	(2.8 to 11.7)	<0.001
Smear negative	9	59	5.2	(2.4 to 11.4)	
Smear status unknown	4	28	4.9	(1.6 to 14.7)	
Relationship of contact to child [1,2]					
Parent	16	76	7.3	(3.9 to 13.8)	<0.001
Grandparent	4	22	6.2	(2.0 to 19.1)	
Aunt or uncle	4	38	3.5	(1.2 to 10.4)	
Sibling	0	7		-	
Other	2	21	3.3	(0.7 to 14.5)	
Number of close TB contacts [1]					
One close TB contact	24	146	5.6	(3.2 to 9.8)	<0.001
More than one close TB contact	1	12	2.8	(0.4 to 22.6)	

[1] compared with children who had no history of TB contact.
[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

Table 10.4 Effect of TB contact history on confirmed TB (active case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
Proximity of TB contact [1]					
Contact sleeps in same household	0	17	-		
Contact sleeps in same room	2	67	-		
Relationship of contact to child [2]					
Parent	2	69	1.0		
Grandparent	0	8	-		
Aunt or uncle	0	5	-		-
Sibling	0	2	-		
Other	0	1	-		
Number of close TB contacts					
One close TB contact	2	85	1.0		
More than one close TB contact	0	2	-		-

[1] Three controls recruited as close non-household contacts excluded from proximity analysis.
[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

Table 10.5 Effect of TB contact history on highly probable TB (active case detection arm)

	TB cases	Controls	Odds ratio for TB (95% CI)		P value
Proximity of TB contact [1]					
Contact sleeps in same household	1	17	-		
Contact sleeps in same room	3	67	-		
Relationship of contact to child [2]					
Parent	2	69	1.0		
Grandparent	0	8	-		
Aunt or uncle	1	5	6.9	(0.5 to 89.8)	-
Sibling	1	2	17.3	(1.1 to 279)	
Other	0	1	-		
Number of close TB contacts					
One close TB contact	4	85	1.0		
More than one close TB contact	0	2	-		-

[1] Three controls recruited as close non-household contacts excluded from proximity analysis.

[2] Actual numbers of children presented, but weighted analysis used to derive OR (see section 4.2.1).

10.2.2 Chapter 5 supplementary tables and figures

Table 10.6 Associations of clinical history variables with confirmed TB

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Cough & duration					
No cough	221	8	1.0		0.005
<2 weeks	414	16	1.1	(0.4 to 2.5)	
2-4 weeks	229	15	1.8	(0.8 to 4.4)	
1 month	158	10	1.7	(0.7 to 4.5)	
2 months	52	10	5.3	(1.9 to 14.5)	
≥3 months	105	8	2.1	(0.8 to 5.8)	
Cough productive of sputum					
No	1168	63	1.0		0.598
Yes	72	5	1.3	(0.5 to 3.3)	
Haemoptysis					
No	1232	68	1.0		0.507
Yes	8	0	-		
Fever & duration					
No fever	233	11	1.0		<0.001
<2 weeks	469	15	0.7	(0.3 to 1.5)	
2-4 weeks	229	13	1.2	(0.5 to 2.7)	
1 month	135	13	2.0	(0.9 to 4.7)	
2 months	51	7	2.9	(1.1 to 7.9)	
≥3 months	68	8	2.5	(1.0 to 6.5)	
Weight loss & duration					
No weight loss	488	16	1.0		0.028
<1 month	88	7	2.4	(1.0 to 6.1)	
1 month	237	13	1.7	(0.8 to 3.5)	
2 months	107	9	2.6	(1.1 to 6.0)	
≥3 months	224	16	2.2	(1.1 to 4.4)	
Weight loss severity					
No weight loss	488	16	1.0		0.002
Mild	203	11	1.7	(0.8 to 3.6)	
Moderate	247	14	1.7	(0.8 to 3.6)	
Severe	235	22	2.9	(1.5 to 5.6)	
Dyspnoea & duration					
No dyspnoea	885	42	1.0		0.007
<2 weeks	243	16	1.4	(0.8 to 2.5)	
2-4 weeks	32	3	2.0	(0.6 to 6.7)	
≥1 month	29	5	3.6	(1.3 to 9.9)	
Lethargy & duration					
No lethargy	869	33	1.0		<0.001
<2 weeks	121	5	1.1	(0.4 to 2.8)	
2-4 weeks	80	9	3.0	(1.4 to 6.4)	
1 month	57	5	2.3	(0.9 to 6.1)	
2 months	16	2	3.3	(0.7 to 14.9)	
≥3 months	26	6	6.1	(2.3 to 15.9)	

Table 10.6 continued

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Night sweats & duration					
No night sweats	892	32	1.0		<0.001
<2 weeks	115	13	3.1	(1.6 to 6.2)	
2-4 weeks	64	3	1.3	(0.4 to 4.4)	
1 month	45	9	5.6	(2.5 to 12.5)	
2 months	9	4	12.4	(3.6 to 43.0)	
≥3 months	33	4	3.4	(1.1 to 10.1)	
Abdominal pain					
No	959	45	1.0		0.015
Yes	259	23	1.9	(1.1 to 3.2)	
Change in temperament					
No	1204	63	1.0		<0.001
Yes	13	5	7.4	(2.5 to 21.4)	
Seizures					
No	732	31	1.0		0.398
Yes	42	3	1.7	(0.5 to 5.8)	
History of previous TB treatment					
No	1146	63	1.0		0.052
Yes	26	4	2.8	(0.9 to 8.3)	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.7 Associations of clinical examination findings with confirmed TB

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Clubbing					
No	581	26	1.0		0.057
Yes	11	2	4.1	(0.9 to 19.4)	
Any lymphadenopathy					
No	910	29	1.0		<0.001
Yes	330	39	3.7	(2.2 to 6.1)	
Cervical lymphadenopathy					
None	1028	35	1.0		<0.001
Unilateral	60	5	2.5	(3.4 to 9.9)	
Bilateral	152	29	5.8	(2.5 to 5.1)	
Axillary lymphadenopathy					
None	1151	53	1.0		<0.001
Unilateral	28	2	1.6	(0.4 to 6.7)	
Bilateral	61	13	4.6	(2.4 to 9.0)	
Inguinal lymphadenopathy					
None	1086	50	1.0		0.001
Unilateral	0	0	-		
Bilateral	150	18	2.6	(1.5 to 4.6)	
Generalized lymphadenopathy					
No	1215	58	1.0		<0.001
Yes	21	10	10.0	(4.4 to 22.5)	
Stridor					
No	1221	68	1.0		0.814
Yes	1	0	0.0		
Wheeze					
No	1173	68	1.0		0.103
Yes	46	0	0.0		
Clinical signs of pleural effusion					
No	1193	62	1.0		0.001
Yes	29	6	4.0	(1.6 to 10.0)	
Abdominal mass					
No	1214	65	1.0		<0.001
Yes	6	3	9.3	(2.3 to 38.5)	
Hepatomegaly					
No	1009	43	1.0		<0.001
Yes	212	25	2.8	(1.6 to 4.6)	
Splenomegaly					
No	1173	57	1.0		<0.001
Yes	47	11	4.8	(2.4 to 98)	
Ascites					
No	1209	66	1.0		0.102
Yes	11	2	3.3	(0.7 to 15.4)	

Table 10.7 continued

Clinical variable	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Reduced consciousness					
No	1201	65	1.0		0.063
Yes	18	3	3.1	(0.9 to 10.7)	
Meningism					
No	1209	67	1.0		0.695
Yes	12	1	1.5	(0.2 to 11.7)	
Focal neurological signs					
No	1205	67	1.0		0.910
Yes	16	1	1.1	(0.1 to 8.6)	
Gibbus					
No	1221	68	1.0		-
Yes	0	0	-		
Firm, non-traumatic joint swelling					
No	1237	67	1.0		0.074
Yes	3	1	6.2	(0.6 to 60.2)	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.8 Associations of chest x-ray features with confirmed TB

Radiological feature	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Abnormal chest x-ray					
No	668	9	1.0		<0.001
Yes	365	53	10.8	(5.1 to 22.6)	
Airspace shadowing					
No	845	24	1.0		<0.001
Yes	225	32	5.0	(2.9 to 8.8)	
Expansile lobar pneumonia					
No	976	48	1.0		0.825
Yes	1	0	0.0		
Bronchopneumonia					
No	943	45	1.0		0.298
Yes	48	4	1.7	(0.6 to 5.1)	
Cavities					
No	976	44	1.0		<0.001
Yes	2	6	66.5	(12.1 to 364.9)	
Nodular infiltrate (nodules >2mm)					
No	975	47	1.0		0.104
Yes	4	1	5.2	(0.6 to 47.5)	
Miliary infiltrate (nodules <2mm)					
No	975	41	1.0		<0.001
Yes	3	10	79.3	(18.9 to 332.1)	
Volume loss					
No	974	48	1.0		0.701
Yes	3	0	0.0		
Calcification					
No	977	48	1.0		-
Yes	0	0	-		
Perihilar lymphadenopathy					
No	950	39	1.0		<0.001
Yes	69	15	5.3	(2.8 to 10.2)	
Paratracheal lymphadenopathy					
No	970	38	1.0		<0.001
Yes	10	13	33.2	(12.8 to 85.8)	
Mediastinal lymphadenopathy					
No	943	34	1.0		<0.001
Yes	78	21	7.5	(4.1 to 13.7)	
Perihilar streakiness					
No	976	48	1.0		0.825
Yes	1	0	0.0		
Airway compression					
No	976	45	1.0		<0.001
Yes	2	3	32.5	(5.1 to 206.1)	

Table 10.8 continued

Radiological feature	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Pleural effusion					
No	949	41	1.0		<0.001
Yes	35	12	7.9	(3.8 to 16.7)	
Pneumothorax					
No	976	48	1.0		0.825
Yes	1	0	0.0		

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.9 Comparison of proportions of each clinical history variable among confirmed and highly probable TB cases.

Clinical variable	Confirmed TB cases	Highly probable TB cases	p value
Cough & duration			
No cough	8	7	
<2 weeks	16	10	
2-4 weeks	15	12	0.924
1 month	10	12	
2 months	10	7	
≥3 months	8	6	
Cough productive of sputum			
No	63	52	0.386
Yes	5	7	
Haemoptysis			
No	68	59	-
Yes	0	0	
Fever & duration			
No fever	11	11	
<2 weeks	15	21	
2-4 weeks	13	4	0.241
1 month	13	8	
2 months	7	4	
≥3 months	8	6	
Weight loss & duration			
No weight loss	16	19	
<1 month	7	4	
1 month	13	10	0.846
2 months	9	9	
≥3 months	16	14	
Weight loss severity			
No weight loss	16	19	
Mild	11	11	0.634
Moderate	14	10	
Severe	22	15	
Dyspnoea & duration			
No dyspnoea	42	42	
<2 weeks	16	8	0.201
2-4 weeks	3	0	
≥1 month	5	4	
Lethargy & duration			
No lethargy	33	34	
<2 weeks	5	6	
2-4 weeks	9	1	
1 month	5	8	0.175
2 months	2	3	
≥3 months	6	4	

Table 10.9 **continued**

Clinical variable	Confirmed TB cases	Highly probable TB cases	p value
Night sweats & duration			
No night sweats	32	41	0.051
<2 weeks	13	4	
2-4 weeks	3	1	
1 month	9	3	
2 months	4	1	
≥3 months	4	2	
Abdominal pain			
No	45	49	0.011
Yes	23	8	
Change in temperament			
No	63	57	0.037
Yes	5	0	
Seizures			
No	31	44	0.185
Yes	3	1	
History of previous TB treatment			
No	63	49	0.493
Yes	4	5	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.10 Comparison of proportions of each clinical examination variable and TST among confirmed and highly probable TB cases.

Clinical variable	Confirmed TB cases	Highly probable TB cases	p value
Clubbing			
No	26	34	0.885
Yes	2	3	
Any lymphadenopathy			
No	29	37	0.024
Yes	39	22	
Cervical lymphadenopathy			
None	35	42	
Unilateral	5	4	0.040
Bilateral	29	13	
Axillary lymphadenopathy			
None	53	50	
Unilateral	2	6	0.021
Bilateral	13	3	
Inguinal lymphadenopathy			
None	50	49	
Unilateral	0	0	0.135
Bilateral	18	9	
Generalized lymphadenopathy			
No	58	57	0.010
Yes	10	1	
Stridor			
No	68	57	-
Yes	0	0	
Wheeze			
No	68	54	0.056
Yes	0	3	
Clinical signs of pleural effusion			
No	62	54	0.443
Yes	6	3	
Abdominal mass			
No	65	57	0.108
Yes	3	0	
Hepatomegaly			
No	43	45	0.055
Yes	25	12	
Splenomegaly			
No	57	52	0.217
Yes	11	5	

Table 10.10 continued

Clinical variable	Confirmed TB cases	Highly probable TB cases	p value
Ascites			
No	66	56	0.666
Yes	2	1	
Reduced consciousness			
No	65	56	0.400
Yes	3	1	
Meningism			
No	67	57	0.358
Yes	1	0	
Focal neurological signs			
No	67	55	0.458
Yes	1	2	
Gibbus			
No	68	55	0.119
Yes	0	2	
Firm, non-traumatic joint swelling			
No	67	59	0.350
Yes	1	0	
Tuberculin skin test			
No	35	26	0.536
Yes	30	28	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.11 Comparison of proportions of each radiological variable among confirmed and highly probable TB cases.

Radiological feature	Confirmed TB cases	Highly probable TB cases	p value
Abnormal chest x-ray			
No	9	5	0.449
Yes	53	46	
Airspace shadowing			
No	24	26	0.250
Yes	32	22	
Expansile lobar pneumonia			
No	48	40	-
Yes	0	0	
Bronchopneumonia			
No	45	35	0.331
Yes	4	6	
Cavities			
No	44	32	0.298
Yes	6	8	
Nodular infiltrate (nodules >2mm)			
No	47	39	0.896
Yes	1	1	
Miliary infiltrate (nodules <2mm)			
No	41	36	0.339
Yes	10	5	
Volume loss			
No	48	40	-
Yes	0	0	
Calcification			
No	48	40	-
Yes	0	0	
Perihilar lymphadenopathy			
No	39	27	0.041
Yes	15	24	
Paratracheal lymphadenopathy			
No	38	34	0.222
Yes	13	6	
Mediastinal lymphadenopathy			
No	34	22	0.054
Yes	21	29	
Perihilar streakiness			
No	48	37	0.054
Yes	0	3	

Table 10.11 continued

Radiological feature	Confirmed TB cases	Highly probable TB cases	p value
Airway compression			
No	45	38	0.801
Yes	3	2	
Pleural effusion			
No	41	36	0.192
Yes	12	5	
Pneumothorax			
No	48	40	-
Yes	0	0	

Note that the total number of children in all categories of a particular variable is equal to the number for which data were available for that variable.

Table 10.12 Associations of available microbiology results with CHPTB*

Microbiology result	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Bacteraemia					
No	1,184	124	1.0		0.256
Yes	56	3	0.5	(0.2 to 1.7)	
Positive bacterial sputum culture					
No	1,204	126	1.0		0.162
Yes	36	1	0.3	(0.0 to 2.0)	
Confirmed pyogenic abscess					
No	1,228	127	1.0		0.238
Yes	12	0	0.5	(0.2 to 1.6)	
Confirmed empyema					
No	1,237	127	1.0		0.579
Yes	3	0	-		
Bacterial gastroenteritis					
No	1,235	126	1.0		0.533
Yes	5	1	2.0	(0.2 to 16.9)	
Any focal or invasive bacterial infection					
No	1,146	122	1.0		0.132
Yes	94	5	0.5	(0.2 to 1.3)	

*Note TB microscopy and culture results are not included in this table.

Table 10.13 Associations of available microbiology results with confirmed TB*

Microbiology result	No. controls	No. cases	Crude odds ratio (95% CI)		p value
Bacteraemia					
No	1184	68	1.0		0.073
Yes	56	0	0.0		
Positive bacterial sputum culture					
No	1204	67	1.0		0.488
Yes	36	1	0.5	(0.1 to 3.7)	
Confirmed pyogenic abscess					
No	1228	68	1.0		0420
Yes	12	0	0.5	(0.1 to 2.6)	
Confirmed empyema					
No	1237	68	1.0		0.685
Yes	3	0	0.0		
Bacterial gastroenteritis					
No	1235	67	1.0		0.205
Yes	5	1	3.7	(0.4 to 32.1)	
Any focal or invasive bacterial infection					
No	1146	66	1.0		0.153
Yes	94	2	0.4	(0.1 to 1.5)	

*Note TB microscopy and culture results are not included in this table.

Table 10.14 Comparison of proportions of each microbiology variable among confirmed and highly probable TB cases.

Microbiology result	Confirmed TB cases	Highly probable TB cases	p value
Bacteraemia			
No	68	56	0.060
Yes	0	3	
Positive bacterial sputum culture			
No	67	59	0.350
Yes	1	0	
Confirmed pyogenic abscess			
No	68	59	-
Yes	0	0	
Confirmed empyema			
No	68	59	-
Yes	0	0	
Bacterial gastroenteritis			
No	67	59	0.350
Yes	1	0	
Any focal or invasive bacterial infection			
No	66	56	0.536
Yes	2	3	

*Note TB microscopy and culture results are not included in this table.

Table 10.15 Performance of existing clinical diagnostic tools in identifying children with CHPTB in this study

Clinical diagnostic tool		True pos[1]	False pos[1]	True neg[1]	False neg[1]	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR+[2] (95% CI)	LR-[2] (95% CI)
Author	**Outcomes(s) defined**								
Ramachandran [142]	Criteria for TB treatment	93	205	1035	34	73.2 (64.6-80.7)	83.5 (81.3-85.5)	4.43 (1.24-15.8)	0.32 (0.09-1.15)
Stegan [150]	(a) Probable TB	45	5	1120	73	38.1 (29.4-47.5)	99.6 (99.0-99.9)	85.8 (30.3-243)	0.62 (0.22-1.76)
Stegan [150]	(b) Probable or Possible TB	87	110	1130	40	68.5 (59.7-76.5)	91.1 (89.4-92.7)	7.72 (2.20-27.2)	0.35 (0.10-1.22)
Nair [148]	(a) "TB appears unquestionable"	43	28	1212	84	33.9 (25.7-42.8)	97.7 (96.8-98.5)	15.0 (5.32-42.3)	0.68 (0.24-1.91)
Nair [148]	(b) TB probable or "unquestionable"	67	60	1174	66	52.8 (43.7-61.7)	94.7 (93.3-95.9)	10.4 (3.15-34.0)	0.52 (0.16-1.71)
Ghidey [137]	(a) Criteria for TB treatment [3]	111	627	613	16	87.4 (80.3-92.6)	49.4 (46.6-52.3)	1.73 (0.47-6.34)	0.25 (0.07-0.94)
Ghidey [137]	(b) Criteria for TB treatment [3]	101	510	730	26	79.5 (71.5-86.2)	58.9 (56.1-61.6)	1.93 (0.54-6.98)	0.35 (0.10-1.26)
WHO (1983) [70]	Probable TB	91	154	1086	36	71.7 (63.0-79.3)	87.7 (85.6-89.4)	5.77 (1.62-20.5)	0.32 (0.09-1.15)
Cundall [136]	Probable TB	96	267	973	31	75.6 (67.2-82.8)	78.5 (76.1-80.7)	3.51 (0.98-12.6)	0.31 (0.09-1.12)
Edwards [132]	Criteria for TB treatment	44	128	642	25	63.8 (51.3-75.0)	83.4 (80.6-85.9)	3.84 (1.51-9.78)	0.43 (0.47-1.11)
Stoltz [143]	Probable TB	78	94	1146	49	61.4 (52.4-69.9)	92.4 (90.8-93.8)	8.10 (2.38-27.6)	0.42 (0.12-1.42)
Osborne [141]	Probable TB	94	253	987	33	74.0 (65.5-81.4)	79.6 (77.2-81.8)	3.63 (1.01-13.0)	0.33 (0.09-1.17)
Jeena [138]	Probable TB	73	127	1113	54	57.5 (48.4-66.2)	89.8 (87.9-91.4)	5.61 (1.68-18.8)	0.47 (0.14-1.58)
Fourie [133]	High probability of TB [5]	99	552	668	28	78.0 (69.7-84.8)	55.5 (52.7-58.3)	1.72 (0.48-6.17)	0.40 (0.11-1.44)
Kiwanuka [139]	Probable TB	99	385	855	23	78.0 (69.7-84.8)	69.0 (66.3-71.5)	2.61 (0.73-9.37)	0.27 (0.08-0.98)
Hawkridge [145]	Probable TB	54	53	1187	73	42.5 (33.8-51.6)	95.7 (94.4-96.8)	9.95 (3.27-30.3)	0.60 (0.20-1.83)
WHO (2006) [21]	(a) Strongly suggestive of TB [5]	15	4	1185	104	12.6 (7.23-19.9)	99.7 (99.1-99.9)	37.5 (20.4-69.0)	0.88 (0.48-1.61)
WHO (2006) [21]	(b) Requires investigation for TB [5]	32	35	1154	87	26.9 (19.2-35.8)	97.1 (95.9-97.9)	9.14 (3.64-22.9)	0.75 (0.30-1.89)
Kenya NTP (2008) [71]	Criteria for TB treatment	55	188	582	14	79.7 (68.3-88.4)	69.1 (65.7-72.3)	3.26 (1.21-8.82)	0.27 (0.10-0.72)
Kenya NTP (draft) [149]	Criteria for TB treatment	101	323	917	26	76.0 (64.7-85.1)	73.9 (69.9-77.6)	3.05 (0.84-11.1)	0.28 (0.08-1.01)

1. Available data for each tool are presented; for most tools this is less than the total number of children (1,367) included in the analysis due to missing data.
2. LR+ = Likelihood ratio of a positive test; LR- = likelihood ratio of a negative test.
3. Results for Ghidey & Habte tool presented separately using requirement for (a) ≥2 and (b) ≥3 symptoms and signs to define a 'suggestive symptom complex of TB' (see section 5.2.4, page 77).
4. For the purposes of our analyses we used 'score 2' proposed by Fourie *et al*, which was derived in high TB burden settings in South Africa, Madagascar and Nicaragua.
5. Results based on the WHO *Guidance for national tuberculosis programmes on the management of tuberculosis in children* (2006) are presented separately for (a) children whose clinical features "strongly suggest a diagnosis of TB" according to the guidelines, and (b) using broader criteria that included under "physical signs highly suggestive of TB" all the other "suggestive clinical signs" listed as requiring investigation for TB.

Table 10.16 Performance of existing clinical diagnostic tools in identifying children with confirmed TB in this study

Clinical diagnostic tool		True pos[1]	False pos[1]	True neg[1]	False neg[1]	Sensitivity (%) (95% CI)	Specificity (%)[2] (95% CI)	LR+[3] (95% CI)	LR-[3] (95% CI)
Author	**Outcomes(s) defined**								
Ramachandran [142]	Criteria for TB treatment	49	205	1035	19	72.1 (59.9-82.3)	83.5 (81.3-85.5)	4.36 (1.64-11.6)	0.33 (0.13-0.89)
Stegan [150]	(a) Probable TB	30	5	1120	35	46.2 (33.7-59.0)	99.6 (99.0-99.9)	104 (45.7-236)	0.54 (0.24-1.23)
Stegan [150]	(b) Probable or Possible TB	50	110	1130	18	73.5 (61.4-83.5)	91.1 (89.4-92.7)	8.29 (3.10-22.2)	0.29 (0.11-0.78)
Nair [148]	(a) "TB appears unquestionable"	33	28	1212	35	48.5 (36.2-61.0)	97.7 (96.8-98.5)	21.5 (9.10-50.8)	0.53 (0.22-1.24)
Nair [148]	(b) TB probable or "unquestionable"	41	66	1174	27	60.3 (47.7-72.0)	94.7 (93.9-95.9)	11.3 (4.48-28.6)	0.42 (0.17-1.06)
Ghidey [137]	(a) Criteria for TB treatment [3]	62	627	613	6	91.2 (81.8-96.7)	49.4 (46.6-52.3)	1.80 (0.64-5.04)	0.18 (0.06-0.50)
Ghidey [137]	(b) Criteria for TB treatment [3]	59	510	730	9	86.8 (76.4-93.8)	58.9 (56.1-61.6)	2.11 (0.76-5.85)	0.22 (0.08-0.62)
WHO (1983) [70]	Probable TB	49	154	1086	19	72.1 (59.9-82.3)	87.6 (85.6-89.4)	5.80 (2.18-15.4)	0.32 (0.12-0.85)
Cundall [136]	Probable TB	53	267	973	15	77.9 (66.2-87.1)	78.5 (76.1-80.7)	3.62 (1.34-9.81)	0.28 (0.10-0.76)
Edwards [132]	Criteria for TB treatment	22	128	642	9	71.0 (52.0-85.8)	83.4 (80.6-85.9)	4.27 (2.38-7.67)	0.35 (0.19-0.63)
Stoltz [143]	Probable TB	43	94	1146	25	63.2 (50.7-74.6)	92.4 (90.8-93.8)	8.34 (3.26-21.4)	0.40 (0.16-1.02)
Osborne [141]	Probable TB	52	253	987	16	76.5 (64.6-85.9)	79.6 (77.2-81.8)	3.75 (1.39-10.1)	0.30 (0.11-0.80)
Jeena [138]	Probable TB	40	127	1113	28	58.8 (46.2-70.6)	89.8 (87.9-91.4)	5.74 (2.29-14.4)	0.46 (0.18-1.15)
Fourie [133]	High probability of TB [5]	55	552	688	13	80.9 (69.5-89.4)	55.5 (52.7-58.3)	1.82 (0.67-4.49)	0.34 (0.13-0.94)
Kiwanuka [139]	Probable TB	50	385	855	18	73.5 (61.4-83.5)	69.0 (66.3-71.5)	2.37 (0.89-6.31)	0.38 (0.14-1.02)
Hawkridge [145]	Probable TB	33	53	1187	35	48.5 (36.2-61.0)	95.7 (94.4-96.8)	11.4 (4.81-26.8)	0.54 (0.23-1.27)
WHO (2006) [21]	(a) Strongly suggestive of TB [5]	9	4	1185	56	13.8 (6.5-24.7)	99.7 (99.1-99.9)	41.2 (28.9-58.7)	0.86 (0.61-1.23)
WHO (2006) [21]	(b) Requires investigation for TB [5]	21	35	1154	44	32.3 (21.2-45.1)	97.1 (95.9-97.9)	11.0 (5.47-22.0)	0.70 (0.35-1.40)
Kenya NTP (2008) [71]	Criteria for TB treatment	26	188	582	5	83.9 (66.3-94.5)	69.1 (65.7-72.3)	3.44 (1.83-6.45)	0.21 (0.11-0.40)
Kenya NTP (draft) [149]	Criteria for TB treatment	36	135	382	12	75.0 (60.4-86.4)	73.9 (69.9-77.6)	2.87 (1.30-6.36)	0.34 (0.15-0.75)

1. Available data for each tool are presented; for most tools this is less than the total number of children (1,367) included in the analysis due to missing data.
2. Note that estimates of the specificity of each clinical tool for identifying children with confirmed TB are identical to estimates derived using as cases all children with confirmed + highly probable TB since the control group ('Not TB') was identical in each case.
3. LR+ = Likelihood ratio of a positive test; LR- = likelihood ratio of a negative test.
4. Results for Ghidey & Habte tool presented separately using requirement for (a) ≥2 and (b) ≥3 symptoms and signs to define a 'suggestive symptom complex of TB' (see section 5.2.4, page 77).
5. For the purposes of our analyses we used 'score 2' proposed by Fourie *et al*, which was derived in high TB burden settings in South Africa, Madagascar and Nicaragua.
6. Results based on the WHO *Guidance for national tuberculosis programmes on the management of tuberculosis in children* (2006) are presented separately for (a) children whose clinical features "strongly suggest a diagnosis of TB" according to the guidelines, and (b) using broader criteria that included under "physical signs highly suggestive of TB" all the other "suggestive clinical signs" listed as requiring investigation for TB.

Figure 10.1 Receiver operator characteristic (ROC) curves for six core symptoms showing the sensitivity and specificity of different cutoffs of symptom duration (in weeks) as predictors of confirmed TB.

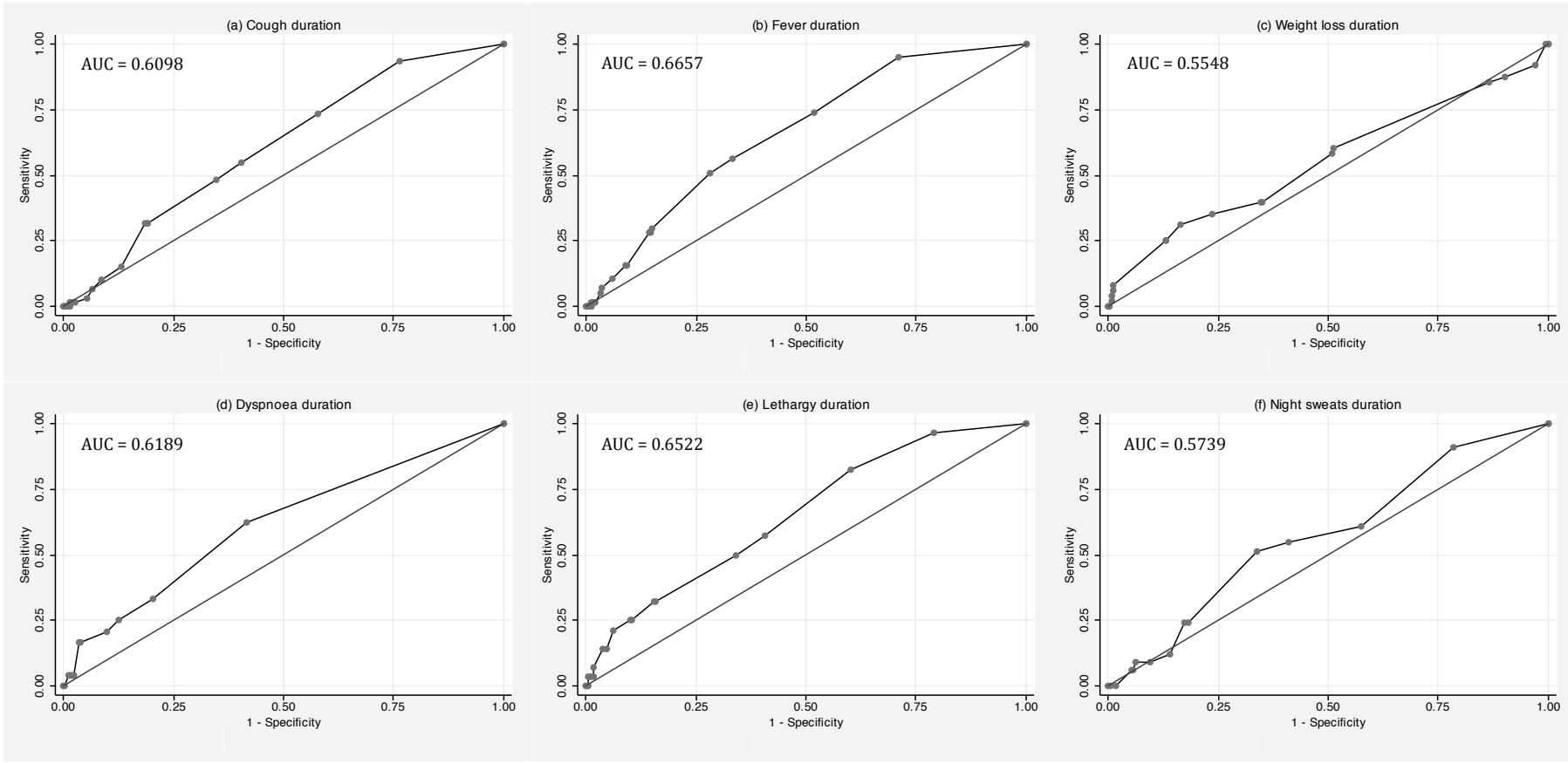


Table 10.17 Variables included in generating a logistic regression model for TB and their categories and weightings.

Variable	Category & weighting	
Severe malnutrition	Not severely malnourished	0
	Severely malnourished	1
HIV infection	HIV negative	0
	HIV positive	1
TB contact	No known TB contact	0
	History of TB contact	1
TB contact proximity	No known TB contact	0
	Non-household contact	1
	Household contact, sleeps in different room	2
	Household contact, sleeps in same room	3
TB contact smear status	No known TB contact	0
	Contact smear status negative or unknown	2
	Contact known to be smear positive	3
Number of TB contacts	No known TB contact	0
	One TB contact	1
	More than one TB contact	2
Cough history & duration	No cough	0
	Cough <1 month	1
	Cough 1-2 months	3
	Cough >2 months	4
Cough productive of sputum	No productive cough	0
	Cough productive of sputum	1
Fever history & duration	No fever or fever <1 month	0
	Fever for 1-2 months	3
	Fever for >2 months	4
Weight loss history & duration	No weight loss	0
	Weight loss for <2 months	1
	Weight loss for >2 months	2
Weight loss severity	No weight loss	0
	Non severe weight loss	2
	Severe weight loss	3
Night sweats & duration	No night sweats or night sweats for <4 weeks	0
	Night sweats for >4 weeks	1
Dyspnoea history & duration	No dyspnoea or dyspnoea for <6 weeks	0
	Dyspnoea for ≥6 weeks	1
Lethargy history & duration	No lethargy or lethargy for <2 weeks	0
	Lethargy for 2-4 weeks	2
	Lethargy for 1-<2 months	3
	Lethargy for ≥2 months	5
Finger clubbing	Absent	0
	Present	1
Any lymphadenopathy	No lymphadenopathy	0
	Some lymphadenopathy	1
Cervical lymphadenopathy	No cervical lymphadenopathy	0
	Unilateral cervical lymphadenopathy	1
	Bilateral cervical lymphadenopathy	2
Axillary lymphadenopathy	No axillary lymphadenopathy	0
	Some axillary lymphadenopathy	1

Table 10.17 **continued**

Variable	Category & weighting	
Inguinal lymphadenopathy	No inguinal lymphadenopathy	0
	Some inguinal lymphadenopathy	1
Clinical signs of pleural effusion	Absent	0
	Present	1
Abdominal mass	Absent	0
	Present	1
Hepatomegaly	Absent	0
	Present	1
Splenomegaly	Absent	0
	Present	1
Abnormal chest x-ray (CXR)	Normal CXR (no abnormality detected)	0
	Abnormal CXR (≥1 abnormality)	1
Alveolar consolidation on CXR	Absent	0
	Present	1
Cavities on CXR	Absent	0
	Present	1
Miliary infiltrate on CXR	Absent	0
	Present	1
Radiological pleural effusion	Absent	0
	Present	1
Positive tuberculin skin test	TST negative	0
	TST positive	1
Microbiological evidence of focal / invasive bacterial infection	None	0
	Evidence of focal/invasive bacterial infection	1

Figure 10.2 Log odds of TB plotted across categories of ordinal categorical variables included in generation of the model

