DEFINING THE BURDEN OF PULMONARY TUBERCULOSIS AND PROBING THE PREVALENCE OF PNEUMOCOCCAL BACTERIAL CO-INFECTIONS AMONG CHILDREN HOSPITALISED WITH PULMONARY TUBERCULOSIS THAT WERE ENROLLED IN A PNEUMOCOCCAL VACCINE TRIAL

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A research report submitted to the Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg,
in partial fulfilment of the requirements for the degree of
Master of Medicine in the branch of Paediatrics

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DECLARATION

I, David Paul Moore, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……

David Paul Moore

25th day of January, 2009

DEDICATION

To the children of Soweto, in gratitude for what they teach us; in the hope that knowledge imparted to us through them will translate into meaningful and lasting interventions that will improve the well-being of children everywhere.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Oral Presentation

Moore DP, Madhi SA. Defining the burden of tuberculosis in a cohort of children enrolled in a pneumococcal vaccine trial. South African TB Conference, Durban. Presented on Wednesday 2 July 2008.

Poster Presentations

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Moore DP, Klugman KP, Madhi SA. Role of *Streptococcus pneumoniae* co-infections in childhood pulmonary tuberculosis (PTB) determined through a pneumococcal conjugate vaccine (PCV)-probe study design. In: Programme and abstracts of the 27th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID), Brussels, Belgium. Presented *in absentia* on Thursday 11 June 2009.

ABSTRACT

Background

In settings with a high burden of tuberculosis, children with unrecognised culture-confirmed pulmonary tuberculosis (PTB) may be discharged from hospital before mycobacterial culture results are available; in these cases clinical improvement may have been due to successful treatment of an intercurrent viral or bacterial co-infection.

Aim

To estimate the burden of tuberculosis in children who were enrolled in a double-blind, placebo-controlled pneumococcal conjugate vaccine (PCV) trial, and to probe for the presence of pneumococcal co-infection in trial participants who had a hospital-based diagnosis of PTB.

Methods

A retrospective case-finding strategy was adopted in order to define the tuberculosis case load amongst 39 836 children that had been enrolled in a PCV efficacy trial in Soweto, Gauteng Province. The trial follow-up period was 5.3 years. Children with a hospital-based diagnosis of tuberculosis were categorised by strength of evidence for the disease, HIV status and PCV vaccination status. Incidence rates and risk ratio assessments were conducted using standard statistical methods.

Results

Four-hundred and ninety-two episodes of tuberculosis arose amongst 425 of the 39 836 PCV Study participants. Tuberculosis incidence was 1067 per 100 000 children (95% Confidence Interval [CI], 968 – 1173), with the greatest burden observed amongst HIV-infected children (10 633 per 100 000 children [95% CI, 9411 – 11 969]; Risk Ratio [RR] 27.5 [95% CI, 22.6 – 33.5], P<0.001). The burden of PTB in the cohort was 982 cases per 100 000 children (95% CI, 887 – 1084): 9895 per 100 000 (95% CI, 8718 – 11 187) in the HIV-infected children and 352 per 100 000 (95% CI 294 – 417) in the HIV-uninfected children (RR 28.1; 95% CI, 22.9 – 34.6), P<0.001.

PCV recipients exhibited a 44 percent (95% CI, 11 – 65), P=0.010, reduction in incident culture-confirmed PTB compared to placebo recipients; this apparent reduction was demonstrated chiefly in PCV-vaccinated HIV-infected children (RR 0.53; 95% CI, 0.31 – 0.90) compared to HIV-infected placebo recipients, P=0.017.

Conclusions

A high burden of tuberculosis is carried by children under 5.3 years in the study setting, with HIV-infected children bearing the brunt of the morbidity. Pneumococcal co-infections are common in the context of hospitalised PTB in the study setting.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

AFB	acid-alcohol fast bacilli
BCG	bacille Calmette-Guérin
CDC	Centres for Disease Control and Prevention
CHBH	Chris Hani Baragwanath Hospital
CI	Confidence Interval
CMI	cell mediated immunity
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
EPTB	extrapulmonary tuberculosis
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HIV-TB	human immunodeficiency virus and tuberculosis [co-infection]
ICD-9	International Classification of Diseases, 9th Revision
IFN-γ	gamma interferon
IL-12	interleukin-12
IQR	Interquartile Range
IRR	Incidence Rate Ratio
LTBI	latent tuberculosis infection
MMed	Masters of Medicine
MRSA	methicillin-resistant *Staphylococcus aureus*
MTB	*Mycobacterium tuberculosis*
NHLS	National Health Laboratory Service

NTP	National Tuberculosis Programme
OR	Odds Ratio
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
PHRU	Perinatal HIV Research Unit
PMTCT	prevention of mother to child transmission [of HIV]
PTB	pulmonary tuberculosis
RMPRU	Respiratory and Meningeal Pathogens Research Unit
RR	Risk Ratio
SANTP	South African National Tuberculosis Programme
SD	Standard Deviation
TB	tuberculosis
TBCC	TB Care Centre
TBM	tuberculous meningitis
TLR	Toll-like receptor
TST	tuberculin skin test
WHO	World Health Organization

1.0 INTRODUCTION

1.1 The Global and Local Burden of Tuberculosis

Tuberculosis is considered to be the most prevalent infectious disease globally, with a third of the world's population estimated as being infected with the causative organism, *Mycobacterium tuberculosis* (MTB)[1]. In 1989, it was estimated that 1.3 million new paediatric tuberculosis cases and 450 000 tuberculosis-related childhood deaths occur annually, with the overwhelming burden of infection occurring in the developing world[1].

Studies conducted in the Western Cape Province over the past decade have determined that the region has one of the highest global incidences of tuberculosis with incidences in excess of 1000 per 100 000 population in certain communities within the province[2 3]. Based on data from 2006, South Africa is ranked fourth in the world in terms of its tuberculosis disease burden with an incidence of active tuberculosis disease of 940 cases per 100 000 persons per year, and a prevalence of 998 per 100 000[4]. This tuberculosis disease incidence has increased significantly since 1999 when it was 392 per 100 000 population per year[5].

1.2 Tuberculosis in Children

Children under 14 years of age contribute approximately 25 to 40 percent of the burden of notified tuberculosis cases in developing countries, and MTB causes severe disease in the paediatric age group[6]. This is due to numerous factors, primarily immaturity of the developing immune system, but factors such as socio-economic deprivation and malnutrition which impact negatively on childhood well-being are also important[6].

Extrapulmonary tuberculosis (EPTB), including tuberculous meningitis (TBM) and miliary tuberculosis (the most severe manifestations of the disease), is frequently encountered in children in settings with a high burden of tuberculosis.

Risk of progression to active disease after primary infection is increased the younger an individual is at first exposure to MTB: there is a 30 to 40 percent risk for progression to active pulmonary tuberculosis (PTB) and a 10 to 20 percent risk of progression to miliary tuberculosis or TBM after infection with the organism in children under one year of age[7]. This needs to be compared with the estimated 5 to 10 percent lifetime risk for progression to active disease in immunocompetent adults infected with MTB.

Childhood tuberculosis is contracted by means of aerosolisation, primarily from inhalation of droplet nuclei produced by sputum smear positive ('open') adult close contacts with cavitary pulmonary reactivation of a previously quiescent pulmonary focus of latent tuberculosis. Hence, childhood tuberculosis represents the adequacy of local tuberculosis control programmes in detecting and treating existing adult patients with active disease: if these adult cases were identified early and treated effectively there would be fewer childhood tuberculosis cases. Importantly, the current burden of childhood tuberculosis represents concern for future control of this infection as a large population of children with untreated latent tuberculosis infection (LTBI) could ostensibly survive to become a large cohort of tuberculosis-infected adults with propensity to reactivate their tuberculosis in the future[8 9]. The current burden of childhood tuberculosis poses tremendous challenges for future tuberculosis control in South Africa.

1.3 Tuberculosis in the Era of HIV

The human immunodeficiency virus (HIV) pandemic has created a substantial pool of immunocompromised individuals at increased risk for progressing from a state of LTBI to tuberculosis disease through disruption of the cell mediated immune mechanisms responsible for maintaining latency of the infection, and HIV co-infected individuals are at risk for developing severe forms of tuberculosis[10 11]. An intact cell mediated immunity (CMI) has been elucidated as being the most crucial factor in containing tuberculosis infection and preventing progression from latency to disease[12 13 14]. The risk of progression to disease is 10 percent per year in HIV-infected adults co-infected with MTB compared to the 5 to 10 percent lifetime risk of progression to disease in HIV-uninfected adults with LTBI. Untreated tuberculosis is rapidly progressive and fatal in HIV-infected individuals: children with HIV and tuberculosis co-infection have a two- to ten-fold greater risk of early mortality compared to children with tuberculosis alone[15 16 17].

1.4 Tuberculosis Diagnostic Algorithms

It is challenging to make a definitive diagnosis of tuberculosis disease in paediatric patients as representative microbiologic confirmation is difficult to establish, especially in young children. Furthermore, resource limited settings with the greatest burden of childhood tuberculosis are often ill-equipped to investigate possible cases of the disease because of lack of radiological and laboratory facilities[18]. Compared to adult cases, childhood PTB tends to be paucibacillary, as cavitary disease with expectoration of large quantities of acid-

alcohol fast bacilli (AFB) is infrequent in young children: microbiological methods of diagnosing the infection are frequently negative as a result of this phenomenon.

Non-microbiological methods are frequently utilised in order to substantiate a suspected diagnosis of tuberculosis in paediatric patients, with features on patient history, clinical examination and investigations all helping to bring the clinician closer to a likely diagnosis of tuberculosis in situations where microbiological data are lacking.

Diagnostic algorithms, such as the one advocated for use by the South African National Tuberculosis Programme (SANTP) Guidelines (2004) [19] (**Appendix 1**, page 127), are a means of coalescing various features on patient history, examination and clinical response to treatment, in order to arrive at a likely diagnosis of active tuberculosis. These tools are useful in resource-poor settings with low HIV prevalence and limited access to laboratory facilities or chest radiographs[20], but have been found to be lacking in sensitivity and specificity, with both under- and over-diagnosis of tuberculosis being a concern when algorithms are used as the sole means of arriving at a diagnosis of tuberculosis in areas with high HIV prevalence[18 21 22].

A further shortcoming of diagnostic algorithms in the diagnosis of tuberculosis in children is that the parameters used therein (e.g. weight loss not responding to nutritional rehabilitation, organomegaly with ascites, gibbus) often adopt features encountered in cases of chronic tuberculosis or established EPTB disease, placing the patient presenting with acute PTB at risk of not being diagnosed as such at initial presentation.

1.5 Acute Presentations of PTB

Childhood PTB may present acutely, and the disease can be indistinguishable from acute viral or bacterial pneumonia in its initial presentation as has been demonstrated in three South African studies[23 24 25], where investigators reported that 8 to15 percent of HIV-infected and -uninfected children admitted with acute community-acquired pneumonia had culture-proven tuberculosis, which was predominantly identified by performing gastric aspirates. As the sensitivity of gastric aspirates is only 20 to 40 percent[9 26], these figures may under-represent the true proportion of acute pneumonia which may be due to MTB.

Furthermore, a study conducted by Graham and co-workers in Malawi achieved a diagnosis of PTB in 6 percent of a cohort of infants presenting with *Pneumocystis jiroveci* pneumonia[27]. Although none of these tuberculosis cases were confirmed microbiologically, the diagnosis was inferred because of a history of a positive contact for tuberculosis and suggestive chest radiographic findings.

In a retrospective review of cases admitted to a Durban hospital in 1998 and 1999, Jeena and co-workers demonstrated that 43 percent of a cohort of children with culture-confirmed tuberculosis had in fact presented acutely with symptom duration of less than 10 days. HIV-infected children were equally as likely as HIV-uninfected children to have presented with acute symptoms according to that study[15]. Additional work conducted in Durban in 2002, in which 358 children under 5 years of age were enrolled in a prospective trial investigating the cause of their respiratory illness as well as their response to empiric management according to the World Health Organization (WHO) guideline for severe

community-acquired pneumonia, identified culture-confirmed tuberculosis in 38 (15.7%) of 242 HIV-infected and 15 (12.9%) of 116 HIV-uninfected participants: 45 (84.9%) of these children presented with symptoms of less than 2 weeks' duration[25], providing further evidence to support the importance of acute presentations of childhood tuberculosis.

Complicating the picture, however, is that a study conducted in Soweto at Chris Hani Baragwanath Hospital (CHBH)[28] established that only 49 percent of children with bacteriologically-confirmed tuberculosis had been notified for this disease process and were started on antituberculous treatment. This may suggest that those children with culture-confirmed PTB who were not notified improved because of possible spontaneous resolution of their disease[7], albeit unlikely, or that the precipitating cause for hospitalisation in these children was a superimposed bacterial or viral infection which was successfully treated and resulted in clinical improvement, despite not treating the underlying tuberculosis disease. The long-term outcome of the children with culture-confirmed PTB who were not treated for their disease was not evaluated in that retrospective folder review.

Streptococcus pneumoniae infections in children have been purported to be important immunological disruption trigger events, predisposing to progression from a state of LTBI to active tuberculosis[29]. Additional data that support the importance of acute presentations of PTB and its occurrence in the context of dual infections, particularly *S. pneumoniae*, are well described in the paediatric and adult medical literature: studies from sub-Saharan Africa, North America and Asia have highlighted the fact that PTB can present as acute community-acquired pneumonia in adult and paediatric patients, occasionally with bacterial co-infection [30 31 32 33 34 35 36 37 38].

Adegbola and co-workers documented the occurrence of culture-confirmed tuberculosis in 5 (3.1%) of 159 malnourished children presenting with radiologic evidence of pneumonia in The Gambia, one of these patients having confirmed dual infection with *Haemophilus influenzae*. None of the199 well-nourished children with pneumonia which had been enrolled in the study were found to have tuberculosis[30].

Echave and co-workers enrolled 140 children between the ages of 2 and 59 months in a prospective study in Senegal from March to May 2000 in order to describe the aetiology of paediatric acute community-acquired pneumonia in that setting. Pneumococcus and *H. influenzae* were the most frequently isolated organisms, being identified in 73 percent of the participants. Co-morbidities listed as conferring a poorer outcome in the cohort were tuberculosis, malaria, malnutrition and HIV infection[31].

Chintu *et al.* described autopsy findings confirming the presence of acute pyogenic pneumonia with tuberculosis co-infection in 12 children who died of respiratory illness at the University Teaching Hospital, Lusaka, Zambia between 1997 and 2000, highlighting the fact that this combination of co-infection was the most-frequently encountered one in the study cohort of 264 autopsy cases[32]. Tuberculosis was the fourth most common diagnosis made at autopsy after acute pyogenic pneumonia, *P. jiroveci* pneumonia and cytomegalovirus pneumonitis in this cohort of children, and was noted to occur commonly in all age groups, regardless of HIV-infection status[32].

A prospective study describing the aetiology of acute community-acquired pneumonia in 522 adults admitted to a municipal-county hospital in Seattle from June 1994 to May 1996, identified MTB in 21 (5.1%) of 410 HIV-uninfected patients and 13 (11.6%) of 112 HIV-infected patients[33].

Reporting on their experience in managing 281 adult patients who presented with acute pneumonia to two public hospitals in Kenya from 1994 to 1996, Scott and colleagues were able to identify a defined aetiology in 182 (64.8%) of their patients. Pneumococcus was the most frequently isolated infectious agent (identified in 46 percent of cases) but MTB was identified in 26 (9.3%) of the cases. Twelve patients had dual mycobacterial and pneumococcal infection in this series however the details of the mycobacterial isolates which were identified in co-infections are not fully described in that paper[34].

Nyamande and colleagues, reporting on the aetiology of acute community-acquired pneumonia (with mean symptom duration of 10 days) in a cohort of 430 adult patients admitted to a Durban hospital from June 2000 to October 2001, isolated MTB in 101 study participants (39.6 percent of the 255 positive isolates in their cohort): culture-confirmed tuberculosis was the most frequently identified cause of acute community-acquired pneumonia, regardless of HIV infection status, in this study. Pneumococcus (which accounted for 34.5 percent of the identified pathogens) was the second most frequently isolated organism. Thirty-four of the study participants had polymicrobial disease, 11 (32.4%) of whom had co-infection with MTB and *S. pneumoniae* which was the most frequently encountered combination of pathogens in the patients with identified co-infections[35].

Schleicher and Feldman reported their experience in managing 9 HIV-infected adult patients with dual pneumococcal and MTB infection at the Johannesburg Hospital between January 1999 and March 2003: these patients presented with a 'brief history of respiratory symptoms and fever', all had blood cultures positive for *S. pneumoniae* and all of them had bacteriologically-confirmed tuberculosis. The authors recommended that a diagnosis of tuberculosis should be actively sought in HIV-infected adult patients in sub-Saharan Africa who present with acute community-acquired pneumonia[36].

A study from Korea which draws from a cohort of 1010 adult patients with bacteriologically-confirmed PTB, describes the radiological findings in 17 (1.7%) patients who presented with acute respiratory failure: all of these patients were HIV-uninfected and 13 had underlying chronic disease processes (alcoholism, malnutrition, chronic liver disease or diabetes mellitus) which were likely contributors to the severity of symptoms at presentation and rapid progression of tuberculosis. Symptom duration in these patients ranged from 1 to 7 days (median 3 days) [37]. Likewise, Wei and co-workers, reporting from Taiwan, describe the chest computed tomography findings in 46 HIV-uninfected adult patients who presented with acute pulmonary tuberculosis (symptom duration less than 3 days in each case) and reiterate that underlying illnesses such as diabetes mellitus, malignancies, chronic renal failure and aplastic anaemia are important contributors to rapid disease progression in tuberculosis[38].

1.6 The Use of Vaccines as Probes

A novel manner in which the importance of bacterial co-infections in children with PTB can be evaluated is by using an efficacious vaccine, in the context of a double-blind placebo controlled trial, as a probe in order to elucidate the difference in incidence of PTB between vaccinees and placebo recipients. This strategy has been successfully adopted previously in determining the contribution of *H. influenzae* type b as a cause of radiologically-confirmed pneumonia in The Gambia[39], as well as more recently in defining the minimal role of *S. pneumoniae* in hospitalisation for respiratory virus associated pneumonia[40].

The Gambian study, conducted by Mulholland and co-workers, indicated that at least 20 percent of radiologically-confirmed pneumonia in a cohort of children enrolled in an *H. influenzae* type b vaccine trial was attributable to *H. influenzae* type b, despite the pathogen being infrequently isolated in blood cultures[39].

Madhi and Klugman, by using a 9-valent pneumococcal conjugate vaccine (PCV) as a probe, reported that at least 30 to 40 percent of severe viral-associated pneumonias occur in the context of concurrent pneumococcal infections despite pneumococcus being isolated from blood cultures in fewer than 5 percent of children with community-acquired pneumonia in which respiratory viruses were isolated. As the vaccine included only 9 of the 91 currently-identified pneumococcal serotypes and only prevented 36 percent of all invasive pneumococcal disease, the true burden of pneumococcal co-infection in children with severe viral associated pneumonia may be even higher[40].

In this study, a similar strategy of using the PCV as a probe was used to delineate the role of pneumococcal co-infections in children hospitalised for microbiologically-confirmed and/or clinically diagnosed tuberculosis.

Furthermore, we used the clinical information that was systematically collected during the study to evaluate the usefulness of a widely-used tuberculosis scoring system in diagnosing tuberculosis among hospitalised children with culture-confirmed disease. Hospital-based surveillance of the study cohort, conducted over 5.3 years, also allowed for calculation of the incidence of MTB disease in the study participants.

1.7 Aims and Objectives

1.7.1 Primary objective

To define the burden of tuberculosis (bacteriologically-confirmed, probable PTB, probable EPTB and suspected tuberculosis) in a cohort of HIV-infected and -uninfected children followed up over 5.3 years.

1.7.2 Secondary objectives

1. To define the contribution of pneumococcal co-infections on the burden of hospitalisation amongst children with bacteriologically-confirmed PTB and clinically diagnosed PTB using a double-blind, placebo-controlled trial of PCV as a probe.

2. To evaluate the sensitivity of the diagnostic algorithm utilised by the 2004 SANTP guidelines[19] (**Appendix 1**, page 127) in diagnosing tuberculosis among HIV-infected and -uninfected children hospitalised with culture-confirmed tuberculosis.

3. To describe the clinical manifestations and outcomes of tuberculosis encountered in the study participants.

2.0 MATERIALS AND METHODS

2.1 Study Site and Sample

The Respiratory and Meningeal Pathogens Research Unit (RMPRU), a research unit affiliated with the University of the Witwatersrand, enrolled 39 836 infants from 2nd March 1998 to 30th October 2000, with the primary objective of investigating the efficacy of a 9-valent PCV in preventing invasive pneumococcal disease and pneumonia in a cohort of children resident in Soweto, Gauteng Province. Study participants were between 28 and 84 days of age at enrolment, and were randomised to receive three doses of either a 9-valent PCV or placebo, together with other childhood vaccines including *H. influenzae* type b conjugate vaccine. All children were vaccinated with bacille Calmette-Guérin (BCG) at birth. The detailed demographic information and PCV composition details used in the study have been published [40 41 42].

Information regarding illness in the participants was collected in a systematic manner (see **Appendix 2**, page 128) by RMPRU-affiliated staff. Blood cultures, nasopharyngeal aspirates for respiratory viruses and chest radiographs were performed on children hospitalised with lower respiratory tract infections. The clinical management of the children and the decision as to whether to investigate for tuberculosis was at the discretion of the attending physicians. Tuberculosis was diagnosed based on clinical and radiological suspicion or confirmed bacteriologically.

Most paediatric tuberculosis diagnoses made at CHBH are made in children admitted to the general paediatric wards where an approach utilising the collection of up to three gastric aspirates on three consecutive days (in an attempt to attain a microbiological diagnosis of tuberculosis), is frequently adopted. As there was a possibility that not all PCV Study participants that had been diagnosed with tuberculosis had been identified as such in the PCV Study database, all of the participants admitted to the general paediatric wards at CHBH during the study surveillance period were included for the purposes of this report, and various sources (described in **Section 2.3**) were utilised in order to trace PCV Study participants whose tuberculosis diagnoses had not been identified in the PCV database.

All PCV Study participants with a known discharge diagnosis of tuberculosis as extracted from the electronic PCV Study database by review of discharge diagnoses as recorded using International Classification of Disease, 9th Revision (ICD-9) codes at the RMPRU (n=224, with 302 tuberculosis events recorded between them), formed a reference point from which other cases were sought.

Study participants admitted to CHBH underwent HIV testing at the discretion of the attending physician and/or with parental consent according to the PCV Study protocol. Negative HIV status was confirmed by a negative enzyme-linked immunosorbent assay (ELISA) for HIV antibodies (Axsym system, HIV 1/2, Abbott) or by a negative HIV-1 DNA polymerase chain reaction (PCR) assay (Amplicor version 1.5, Roche) in children who were younger than 18 months of age and whose ELISA was reactive for HIV antibodies.

The prevalence of HIV infection in the study cohort was inferred to be 6.5 percent based upon the 24.9 percent prevailing prevalence of HIV among mothers attending antenatal clinics during the course of the study[43] coupled with a vertical transmission rate of 26.0 percent[44]. This figure was used to estimate the denominators for the number of HIV-infected and HIV-uninfected children in the vaccinated and placebo groups (**Table 1**).

		Sample size		
		PCV group	Placebo group	Total
Study sample population [41]	Intention-to-treat *	19 922	19 914	39 836
HIV-uninfected [40]	Intention-to-treat *	18 633	18 626	37 259
	Per-protocol +	17356	17350	
HIV-infected [40]	Intention-to-treat *	1289	1288	2577
	Per-protocol +	1201	1200	

Table 1: Pneumococcal conjugate vaccine trial randomisation, with projected HIV status in vaccinated and placebo groups

*Intention-to-treat signifies all PCV efficacy trial participants, including those children who did not complete a full course (3 doses) of PCV or placebo.

+ Per-protocol signifies all children who completed 3 doses of PCV or placebo as per study schedule and in whom vaccine efficacy-related events occurred more than 14 days following the third dose of study vaccine.

Surveillance for all-cause hospitalization among children that participated in the study continued until 31st October 2006.

2.2 Ethics and Consent

The PCV Study was approved by the Ethics Committee for research on Human Subjects of the University of the Witwatersrand. Signed informed consent was obtained from legal guardians of the children before their enrolment into the Study.

Human Research Ethics Committee (Medical) of the University of the Witwatersrand granted permission for commencement of this Masters of Medicine (MMed) research report on 3rd April 2006: Protocol M060344 (**Appendix 3**, page 130).

2.3 Data Sources Used

For the purposes of this MMed report, an Excel (Microsoft, Redmond, WA) database was developed by coalescing information from five data sources, the aim being to seek out as far as possible all tuberculosis diagnosis events amongst the PCV-efficacy trial participants that were evaluated at CHBH during the study surveillance period. Sources of data from which the database was compiled are listed below.

1. The RMPRU PCV Study databases

The electronic PCV Study database was used to source all admission events to the general paediatric wards at CHBH amongst Study participants during the period of surveillance, as well as all those with a discharge diagnosis of tuberculosis. Information relevant to the process by which tuberculosis diagnoses were made or excluded in the children as well as details regarding aspects of a family's or participant's past tuberculosis history was obtained through the PCV Study source documents.

2. The TB Care Centre database

The TB Care Centre (TBCC) at CHBH was established in 2003 in response to the finding that only half of all patients who had been diagnosed with tuberculosis at the hospital attended the Soweto Clinics for continuation of their antituberculous therapy within two weeks of discharge, and that 21 percent did not access care at any Soweto clinic[45].

An electronic database has been maintained at the TBCC since November 2003, from which details of all children under 14 years of age (n=2546) registered at the TBCC from November 2003 to July 2006 were extracted. The MMed database and the paediatric data derived from the TBCC were compared in order to incorporate information regarding tuberculosis diagnoses amongst PCV Study participants which may not have been available from review of the RMPRU databases alone.

3. Databases of the paediatric HIV clinics at CHBH

Databases of the two paediatric HIV treatment clinics, namely Harriet Shezi Clinic and the Perinatal HIV Research Unit (PHRU), which supervise the outpatient follow-up of HIV-infected children at CHBH, were utilised in order to ascertain if any of the HIV-infected PCV Study participants attending those clinics had been diagnosed with tuberculosis during the course of their HIV clinic follow-up.

4. Notification booklets

Notification booklets from the general paediatric wards and the paediatric outpatient department at CHBH were used in order to source additional PCV Study participants who were diagnosed with tuberculosis at the hospital. This was a particularly difficult source to

use as there is no central filing system for these booklets at CHBH. Booklets dating from before May 2002 were unobtainable, and many dated subsequent to 2002 were missing. If booklets were available, only patients who were at the anticipated age of the PCV Study participants at tuberculosis notification were evaluated (n=352): details were entered into an Excel database and these were merged against the MMed database.

5. The National Health Laboratory Service databases

The National Health Laboratory Service (NHLS) changed its data recording and retrieval system from the Apex system to the Disa system in 2000 and this posed difficulties when it came to retrieving data for children who had been investigated for tuberculosis prior to 2000. PCV Study participant hospital identification numbers, derived from the participants' source documents, were used to retrieve data from the NHLS database. Positive tuberculosis microscopy and culture results were obtained and entered into the MMed database.

Occasionally, study participants were investigated for tuberculosis at time points other than during hospitalisation. These represented occasions when clinical suspicion had prompted medical personnel to consider a diagnosis of tuberculosis in these children at hospital-based outpatient evaluations. The tuberculosis diagnostic algorithm could not be evaluated in such instances as there was no information regarding the child's clinical status or symptomatology at the time of specimen submission.

The flow of data collection is summarised in **Appendix 4** (page 131).

2.4 Categorisation of Children with Tuberculosis

Children identified as having been diagnosed with tuberculosis during the course of the PCV Study surveillance period were classified using a modification of the WHO definitions for tuberculosis in children[46 47] (**Appendix 5**, page 132) according to the strength of evidence available in arriving at a diagnosis of active tuberculosis in each case.

1. Bacteriologically-confirmed tuberculosis: cases in which MTB was detected on smear or culture from secretions or tissues. This category of patients was further stratified into the following groups based on culture- or smear positivity and site of disease:

    a) Definite PTB: culture confirmation of disease caused by MTB where samples submitted for culture were derived from the respiratory tract. Specimens cultured included gastric aspirates or sputum.

    b) Definite EPTB: culture confirmation of disease caused by MTB where samples were derived from extra-pulmonary sites. Specimens cultured included blood, bone marrow, cerebrospinal fluid, pleural fluid or tissue. According to current convention, cases of definite EPTB in which respiratory specimens were also culture-positive for MTB, were categorised as definite PTB cases.

c) Confirmed PTB: microscopy of respiratory secretions (gastric aspirates and sputum specimens) positive for AFB but without culture confirmation.

2. Probable tuberculosis: these cases were stratified into two groups according to the site of their disease:

a) Probable PTB: children with a respiratory illness suggestive of PTB but without bacteriological confirmation of the disease process. Children with dual (probable) PTB and EPTB were classified as probable PTB cases according to current categorisation policy, because these cases (with respiratory involvement) may pose a public health risk for dissemination of MTB by the production of infectious droplet nuclei.

b) Probable EPTB: those with histological evidence of tuberculous involvement of extrapulmonary sites, but without culture confirmation of tuberculosis.

Both categories of probable tuberculosis patients fitted the WHO definition of suspected tuberculosis (see below), with additional evidence which strengthened the probability of the diagnosis. These included any of the following:

i. A positive tuberculin skin test (TST), defined as being ≥ 5 mm in HIV-infected or malnourished children and ≥ 10 mm in HIV-uninfected children;

ii. A suggestive chest radiograph (for the purposes of this study, this was defined as the presence of hilar adenopathy as this is the only radiographic finding featured for scoring by the tuberculosis score chart);

iii. Suggestive histology;

iv. A favourable response to anti-tuberculous therapy (defined for the purposes of this study as being any improvement in weight gain between the time of tuberculosis diagnosis and a subsequent hospitalisation where weight had been recorded in a child who had been started on anti-tuberculous therapy).

3. Suspected tuberculosis: according to the WHO definition, this category applies to:

a) Any ill child with a history of contact with a confirmed case of PTB, or

b) Any child:

i. Not regaining health after an episode of measles or pertussis;

ii. With weight loss, cough or wheeze not responding to antibiotic therapy for a respiratory illness;

iii. With painless swelling in a group of superficial lymph nodes.

4. Same-episode tuberculosis: children with a previous diagnosis of tuberculosis who required hospitalisation within six months of that diagnosis (six months being the usual time period over which anti-tuberculous therapy is administered for drug-susceptible disease), and who had been classified on the subsequent discharge as

having tuberculosis were included in this category. This classification was adopted in order not to over-estimate the number of tuberculosis episodes identified in the cohort.

5. Recurrent tuberculosis: children who had a previous diagnosis of tuberculosis (more than six months previously) who were subsequently diagnosed with tuberculosis, regardless of HIV infection status. The exception to this rule were 2 cases of probable PTB, i.e. not bacteriologically confirmed, who were completing a course of anti-tuberculous therapy but who had culture-confirmed disease at a point beyond 5 months into the course of therapy for the previous episode of tuberculosis; whilst these may reflect failing therapy in children with drug-resistant disease, they were placed in this category in order to limit the number of disease categories.

6. Untreated tuberculosis: children with bacteriological evidence of tuberculosis who were not diagnosed with the disease at hospital discharge as evidenced by review of the PCV source documents and who were not started on anti-tuberculous treatment based on review of the other data sources mentioned in **Section 2.3**.

2.5 Assessment of the SANTP Tuberculosis Scoring System

The SANTP Guidelines (2004) recommend the use of a scoring chart to guide clinical staff in achieving a diagnosis of tuberculosis in children[19]. The system is tabulated in **Appendix 1**, page 127.

Parameters on patient history and examination as recorded in PCV study source documents were used to score participants using the tuberculosis scoring chart. The chart emphasises six general features and six local features which are scored in order to arrive at a threshold total score of at least 7 (the cut-off which suggests active tuberculosis according to the scoring system).

General features, obtained from patient history, anthropometric evaluation and vital signs include:

1. Duration of illness (for the purposes of this study, where clinical information was derived from study source documents only, duration of illness was taken to represent the duration of cough in days as reported to study investigators);
2. Percentage of expected weight for age. Children who were born prematurely were scored according to their corrected weight for age, in order to minimise the effect of prematurity on age-related weight trends;
3. Family history of tuberculosis;
4. TST reaction;
5. Response of poor weight gain to nutritional supplementation: response in weight in terms of mass (in kilograms) and percentile (as percentage of expected weight for age) were calculated in children who had multiple admission episodes with weights recorded in PCV Study source documents at each admission;
6. Unexplained fever.

Local features (which screen for the presence of different forms of EPTB) include assessment for the presence of:

1. Lymphadenitis;
2. Arthritis;
3. Features suggestive of abdominal tuberculosis;
4. Features suggestive of TBM;
5. Chest radiographic changes indicative of intrathoracic hilar adenitis;
6. Spinal tuberculosis as evidenced by gibbus deformity on lateral chest radiograph.

For the purposes of this study, where assessment of the sensitivity of the score chart was one of the research objectives, a potential confounding factor which would impact on the score sensitivity analysis would be that of scoring children where all parameters had not been fully assessed: if score sensitivity were routinely evaluated using few of the parameters, there would be a tendency for the score chart to under-perform. In order to achieve as accurate a score sensitivity evaluation as possible, only children with known HIV status whose score could be evaluated using at least 9 of the 12 parameters and whose score included the result of a TST, were assessed.

Children were scored at the index tuberculosis admission episode if clinical data were available: children classified as having ‘same-episode tuberculosis’ were not scored.

2.6 Statistical Analysis

Anthropometric data were analysed using Epi Info version 6.04d (Centres for Disease Control and Prevention [CDC], Atlanta, Georgia, USA) to calculate weight-for-age z-scores (using 1978 CDC/WHO Growth Reference Curves) for study participants whose weight data was recorded in PCV Study source documents.

Application of the 'vaccine efficacy' calculation[48] was made in order to derive the Incidence Rate Ratio (IRR)[49] for tuberculosis between BCG-vaccinated HIV-infected and -uninfected children (**Appendix 6**, page 133); this was used to gauge the degree of risk which an HIV-affected immune system confers to a BCG-vaccinated child for developing active tuberculosis, relative to HIV-uninfected children.

STATA version 9.2 (StataCorp LP, College Station, Texas, USA) was used for the following analyses:

1. Measuring the relative burden of tuberculosis (all categories) in HIV-infected compared to HIV-uninfected children using risk ratio methods;

2. Probing the importance of pneumococcal co-infections in children hospitalised with tuberculosis by stratifying data based on PCV status and comparing the incidence rate of tuberculosis in vaccinees with that observed in placebo recipients; means and 95 percent confidence intervals for disease incidences were calculated using the Poisson distribution. The importance of pneumococcal co-infections was attributed

based on the difference observed in the incidence of hospitalisation for tuberculosis between the PCV vaccinated and unvaccinated children. Data was also stratified by HIV infection status and the degree of certainty of the tuberculosis diagnosis;

3. Sensitivity calculations relating to the SANTP diagnostic algorithm, calculated using standard methods[50];

4. Use of the unpaired Student's t test for continuous variables with normal distributions. Numerical variables with skewed distributions were analysed using the two-sample Wilcoxon rank-sum test;

5. Analysis of categorical variables using the Pearson χ^2 test. Fisher's exact test was used if the anticipated observation in any cell was less than or equal to 5.

An $\alpha \leq 0.05$ was considered as being significant (all quoted P-values are two-tailed).

3.0 RESULTS

3.1 The Spectrum of Tuberculosis Disease Encountered in the Study Cohort

3.1.1 General demographics of tuberculosis cases

Four hundred and twenty-five PCV Study participants were identified as ever having tuberculosis following admission to CHBH. Data sources from which patients with tuberculosis were identified are tabulated below (**Table 2**).

Data source utilised to identify tuberculosis cases arising in the PCV Study cohort	PCV Study patient tuberculosis episodes identified at each data source *	
	First tuberculosis diagnosis episodes: 'incident episodes'	All tuberculosis episodes
1. PCV Study databases	332	375
2. TBCC database	14	20
3. (a). Harriet Shezi database	11	13
3. (b). PHRU database	4	4
4. Notification booklets	5	11
5. NHLS database	59	69
Total number of tuberculosis episodes identified	425	492

Table 2: Data sources used, and yield of tuberculosis-related episodes from each source

* Cases listed as having been identified through each data source are exclusive to each source, and have been tabulated once only.

Two hundred and thirty-one (54.4%) first-episode tuberculosis cases arose in boys and 194 (45.6%) occurred in girls.

Two hundred and seventy-four (64.5%) of the children who had ever been diagnosed with tuberculosis were HIV-infected, 144 (33.9%) were HIV-uninfected and 7 (1.6%) had no definitive HIV result (**Table 3**).

Median age at first tuberculosis episode was older in HIV-infected children (19.8 months: Interquartile Range [IQR] 7.7 – 44.3) compared to HIV-uninfected children (12.3 months: IQR 6.0 – 26.3), P<0.001, indicating that HIV-infected children had an ongoing risk beyond early childhood of developing active tuberculosis over HIV-uninfected children.

3.1.2 Categorisation of tuberculosis cases by HIV status

One hundred and eighteen (27.7%) of all first episode tuberculosis cases were confirmed bacteriologically, with no significant difference in rates of culture- (P=0.801) or smear-positivity (P=0.351) between HIV-infected and -uninfected children. The proportions of the different categorisations of tuberculosis between HIV-infected and -uninfected children for definite tuberculosis, confirmed PTB and probable EPTB were similar (**Table 3**). HIV-infected children were less likely to have been diagnosed with probable PTB (Odds Ratio [OR] 0.49; 95% Confidence Interval [CI], 0.31 – 0.78), P=0.001, and more likely to have been diagnosed with suspected tuberculosis (OR 2.00; 95% CI, 1.27 – 3.17), P=0.002, compared to HIV-uninfected children. When taking all episodes of tuberculosis into account, 141 (28.7%) of 492 episodes were bacteriologically confirmed. No significant difference in the yield of bacteriologically proven tuberculosis could be demonstrated between HIV-infected and -uninfected children when analysing for all tuberculosis episodes (**Table 3**).

Tuberculosis categories, n (%)*	First episode of tuberculosis					All tuberculosis episodes				
	HIV-infected	HIV-uninfected	No HIV result	Total	P **	HIV-infected	HIV-uninfected	No HIV result	Total	P **
Definite PTB	58 (21.2)	28 (19.4)	0 (0.0)	86 (20.2)	0.679	70 (21.0)	29 (19.2)	0 (0.0)	99 (20.1)	0.657
Definite EPTB	5 (1.8)	3 (2.1)	0 (0.0)	8 (1.9)	1.000 §	6 (1.7)	3 (2.0)	0 (0.0)	9 (1.9)	1.000 §
Confirmed PTB	15 (5.5)	8 (5.6)	1 (14.3)	24 (5.6)	0.972	24 (7.2)	8 (5.3)	1 (14.3)	33 (6.7)	0.438
Bacteriologically-confirmed tuberculosis +	78 (28.5)	39 (27.1)	1 (14.3)	118 (27.7)	0.765	100 (29.9)	40 (26.5)	1 (14.3)	141 (28.7)	0.438
Probable PTB	64 (23.4)	55 (38.2)	3 (42.8)	122 (28.7)	0.001	75 (22.5)	58 (38.4)	3 (42.8)	136 (27.6)	< 0.001
Probable EPTB	13 (4.7)	10 (6.9)	2 (28.6)	25 (5.9)	0.349	21 (6.3)	10 (6.6)	2 (28.6)	33 (6.7)	0.889
Probable tuberculosis ++	77 (28.1)	65 (45.1)	5 (71.4)	147 (34.6)	< 0.001	96 (28.8)	68 (45.0)	5 (71.4)	169 (34.3)	< 0.001
Suspected tuberculosis	119 (43.4)	40 (27.8)	1 (14.3)	160 (37.7)	0.002	138 (41.3)	43 (28.5)	1 (14.3)	182 (37.0)	0.007
All tuberculosis	274 §§ (100.0)	144 (100.0)	7 (100.0)	425 (100.0)	-	334 (100.0)	151 (100.0)	7 (100.0)	492 (100.0)	-

Table 3: Proportions of tuberculosis episodes and categories according to HIV status

* Percentage (%) of cases in each category of tuberculosis, according to HIV status.

** P-value derived by comparison of proportions of different tuberculosis categories in groups with known HIV status.

+ Bacteriologically-confirmed tuberculosis comprises 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

++ Probable tuberculosis comprises 'Probable PTB' and 'Probable EPTB' cases. § Fisher's exact test.

§§ HIV-infected children contributed to 65.6% of all tuberculosis cases where a definitive HIV result was available in the analysis of 'first-episode' tuberculosis.

Analysis of all tuberculosis episodes in the cohort by HIV status (**Table 3**) reiterates the findings that HIV-infected children were more likely to be diagnosed with suspected PTB than were HIV-uninfected children (OR 1.77; 95% CI, 1.15 – 2.75), P=0.007, and that HIV-uninfected children more frequently had a tuberculosis diagnosis which fulfilled criteria for classification as probable PTB (OR 2.15; 95% CI, 1.39 – 3.33), P<0.001.

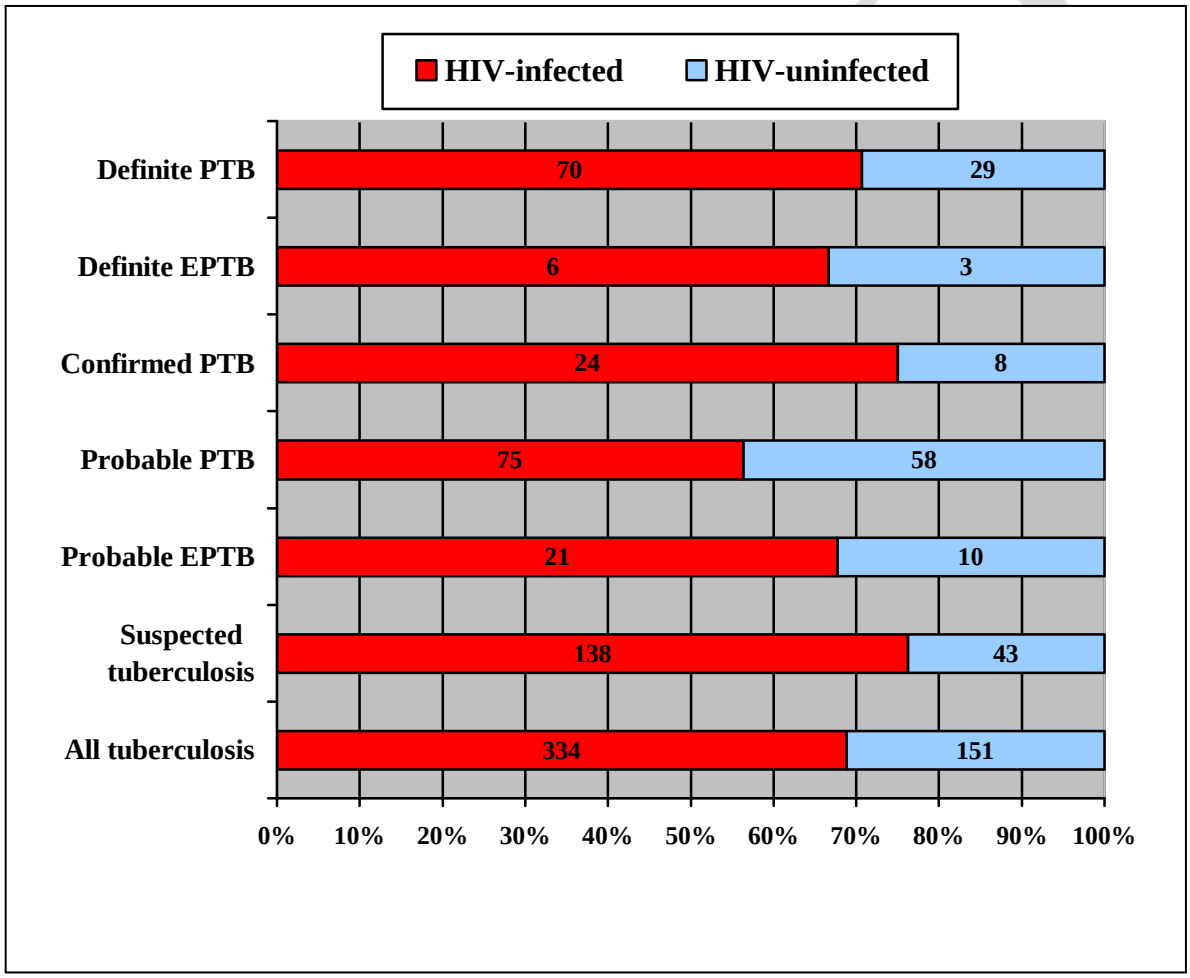


Figure 1: Classification of tuberculosis in children with defined HIV status: all tuberculosis episodes

The proportions of children classified according to each tuberculosis diagnosis category are illustrated in **Figure 1**, above (7 tuberculosis episodes in children with undefined HIV status are not represented). In children with defined HIV status, PTB accounted for 427

(88.0%) of all cases (n=485), PTB with concomitant EPTB was encountered in 18 (3.8%) and EPTB occurred in 40 (8.2%) of all cases, with no significant difference in proportions of each type of disease when considering HIV status. Of these 485 episodes, 140 (28.9%) were bacteriologically confirmed (highlighted in yellow in **Table 3**). Of the confirmed cases 32 (22.9%) were smear positive alone and 108 (77.1%) were culture-confirmed. Culture-confirmation of tuberculosis was achieved in 108 (22.3%) of all tuberculosis cases identified (n=485) in the cohort of hospitalised children with known HIV status (**Table 3**).

3.1.3 Categorisation of tuberculosis cases by PCV vaccination status

Table 4 illustrates the proportions of tuberculosis encountered in children according to vaccination status (PCV versus placebo), and reveals that the PCV-vaccinated children had a significantly lower burden of culture-confirmed PTB than did the placebo recipients for first-episode tuberculosis episodes (OR 0.53; 95% CI 0.32 – 0.89), P=0.011.

This analysis was not extended to include all tuberculosis episodes by vaccination status, as it would be expected that the vaccinated children would have had a lasting vaccination effect which would lead to a systematic bias in favour of PCV when conducting this comparison.

This finding was not due to over-investigation of the placebo group or inability to retrieve laboratory results for PCV-vaccinated children, both of which sampling errors would lead to relatively poor detection rates of culture-confirmed tuberculosis in the PCV group compared to placebo recipients: median number of specimens sent for tuberculosis

microscopy and culture in the PCV recipients was 2 (IQR 0 – 3) versus 2 specimens (IQR 0 – 3) in the placebo group, P=0.388. Similarly, laboratory results were retrievable for 171 (73.4%) of all 233 tuberculosis episodes identified in PCV recipients and 193 (74.5%) of all 259 tuberculosis episodes identified in placebo recipients with tuberculosis, P=0.776.

Tuberculosis categories, n (%)*	First episode of tuberculosis			
	PCV	Placebo	Total	P **
Definite PTB	31 (15.1)	55 (25.0)	86 (20.2)	0.011
Definite EPTB	2 (1.0)	6 (2.7)	8 (1.9)	0.287 §
Confirmed PTB	14 (6.8)	10 (4.5)	24 (5.6)	0.308
Bacteriologically-confirmed tuberculosis +	47 (22.9)	71 (32.2)	118 (27.7)	0.032
Probable PTB	60 (29.3)	62 (28.2)	122 (28.7)	0.805
Probable EPTB	17 (8.3)	8 (3.7)	25 (5.9)	0.042
Probable tuberculosis ++	77 (37.6)	70 (31.9)	147 (34.6)	0.214
Suspected tuberculosis	81 (39.5)	79 (35.9)	160 (37.7)	0.444
All tuberculosis	205 (100.0)	220 (100.0)	425 (100.0)	-

Table 4: Proportions of tuberculosis episodes and categories according to PCV vaccination status: incident tuberculosis

* Percentage (%) of cases in each category of tuberculosis, according to PCV vaccination status.

** P-value derived by comparison of proportions of different tuberculosis categories in groups according to vaccination status.

+ Bacteriologically-confirmed tuberculosis comprises 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

++ Probable tuberculosis comprises 'Probable PTB' and 'Probable EPTB' cases.

§ Fisher's exact test.

3.1.4 Recurrent tuberculosis episodes

Seventy recurrent tuberculosis episodes* occurred in 58 children: these occurred in 50 (18.2%) of the 274 HIV-infected children who ever had tuberculosis compared to 8 (5.6%) of 144 HIV-uninfected children who ever had tuberculosis, (Risk Ratio [RR] 3.3; 95% CI, 1.6 – 6.7), P<0.001.Whereas all of the HIV-uninfected children with tuberculosis recurrences had only one recurrent episode, 11 (22.0%) of the 50 HIV-infected children with recurrences had at least 2 recurrent episodes of tuberculosis (**Table 5**). Twenty-two (35.5%) of the 62 recurrent episodes amongst HIV-infected children were confirmed bacteriologically compared to 1 (12.5%) of 8 episodes in HIV-uninfected children (OR 3.9; 95% CI, 0.4 – 181.2), P=0.257 (Fisher's exact test).

Comparing between children according to HIV status, those who were HIV-infected more frequently developed recurrent tuberculosis after a previous episode of culture-confirmed tuberculosis (P=0.016, Fisher's exact test), whilst HIV-uninfected children developed a recurrent episode after a previous episode of probable PTB (P=0.030, Fisher's exact test) (**Table 6**). Thirteen (41.9%) of the 31 recurrent episodes which occurred subsequent to a previous bacteriologically-confirmed disease episode (in the initial tuberculosis episode or the recurrences) amongst HIV-infected children, were themselves bacteriologically-confirmed. Extending this analysis to highlight culture-confirmed recurrences in cases with previous culture-confirmed tuberculosis, 5 (23.8%) culture-confirmed episodes arose subsequent to 21 episodes of culture-confirmed disease in the HIV-infected children.

* Sixty-seven children had first and recurrent tuberculosis episodes diagnosed at CHBH; 3 (all HIV-infected) had the first episode of tuberculosis diagnosed in Soweto Clinics, and recurrences diagnosed at CHBH.

	HIV-infected *				HIV-uninfected *	
Tuberculosis episodes	Initial episode of tuberculosis in children who had recurrent tuberculosis [n=50]	1st recurrent episode [n=50]	2nd recurrent episode [n=11]	3rd recurrent episode [n=1]	Initial episode of tuberculosis in children who had recurrent tuberculosis [n=8]	1st recurrent episode [n=8]
Definite tuberculosis, n (%)	20 (39.0)	10 (20.0)	2 (18.2)	1 (100.0)	0 (0.0)	1 (12.5)
Confirmed PTB, n (%)	4 (8.0)	8 (18.0)	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)
Probable PTB, n (%)	11 (22.0)	10 (20.0)	1 (9.1)	0 (0.0)	4 (50.0)	3 (37.5)
Probable EPTB, n (%)	0 (0.0)	6 (12.0)	2 (18.2)	0 (0.0)	1 (12.5)	0 (0.0)
Suspected tuberculosis, n (%)	15 (29.0)	16 (30.0)	5 (45.4)	0 (0.0)	3 (37.5)	4 (50.0)
Total, n (%)	50 (100.0)	50 (100.0)	11 (100.0)	1 (100.0)	8 (100.0)	8 (100.0)

Table 5: Recurrent tuberculosis episodes according to HIV status

* Columns represent the proportions of tuberculosis disease encountered at initial episode and each recurrence by category of tuberculosis.

		Initial tuberculosis episode in children who had recurrent tuberculosis				Recurrent tuberculosis episodes									
						1st				2nd				3rd	
		n	Confirmed n (%)	Probable n (%)	Suspected n (%)	n	Confirmed n (%)	Probable n (%)	Suspected n (%)	n	Confirmed n (%)	Probable n (%)	Suspected n (%)	n	Confirmed n (%)
HIV-uninfected	Confirmed * n (%)	8	0 (0.0)	-	-	0	-	-	-	0	-	-	-	0	-
	Probable n (%)		-	5 (62.5)	-	5	1 (12.5)	3 (37.5)	1 (12.5)		-	-	-		-
	Suspected n (%)		-	-	3 (37.5)	3	-	-	3 (37.5)		-	-	-		-
HIV-infected	Confirmed * n (%)	50	24 (48.0)	-	-	24	**11** (22.0)	**8** (16.0)	**5** (10.0)	5	**1** (9.1)	**1** (9.1)	**3** (27.2)	1	**1** (100.0)
	Probable n (%)		-	11 (22.0)	-	11	4 (8.0)	4 (8.0)	3 (6.0)	3	1 (9.1)	**1** (9.1)	1 (9.1)	0	-
	Suspected n (%)		-	-	15 (30.0)	15	3 (6.0)	4 (8.0)	8 (16.0)	3	1 (9.1)	1 (9.1)	1 (9.1)		-
P		<0.001 +	0.016 §	0.030 §	0.694 §										

Table 6: Categorisation of initial and recurrent tuberculosis episodes in children who were diagnosed with recurrent disease

* 'Confirmed' represents tuberculosis cases with bacteriologically-confirmed (culture- and/or smear-positive) disease.

+ P-value derived from proportions of children ever identified with tuberculosis: HIV-infected (n=274) and HIV-uninfected (n=144).

§ Fisher's exact test: P-value is derived by comparison of proportions of recurrent tuberculosis according to HIV status.

Numbers highlighted in **RED** represent recurrent tuberculosis episodes which arose subsequent to a previous bacteriologically-confirmed tuberculosis episode.

Median time between initial tuberculosis episode and first recurrence was 14.7 months (IQR 9.4 – 24.3) in HIV-infected children and 15.9 months (IQR 6.7 – 48.3) in HIV-uninfected children, P=0.793.

Median time between first and second tuberculosis recurrence episodes in the HIV-infected children was 18.9 months (IQR 8.0 – 25.0). The time interval between the second and third tuberculosis recurrence episodes in the only child (HIV-infected) in the cohort that had 3 tuberculosis recurrences after an initial episode of tuberculosis was 7.3 months.

Time to tuberculosis recurrence in the HIV-infected children with previous bacteriologically-confirmed tuberculosis was 16.1 months (IQR 8.4 – 23.4). Amongst HIV-infected children, median time to recurrence was significantly shorter in children who had a previous episode of smear-positive PTB (10.0 months; IQR 7.3 – 11.4) compared to those who had previous culture-confirmed tuberculosis (19.0 months; IQR 8.5 – 26.8), P=0.039.

3.1.5 Evaluation of the tuberculosis diagnostic algorithm

Assessment of the tuberculosis score chart advocated for use by the 2004 SANTP Guidelines[19] (**Appendix 1**, page 127) was useful in gaining insight into the clinical characteristics of the children who had been identified as having active tuberculosis.

Four hundred and fifty-four (93.6%) of the 485 identified tuberculosis episodes in children with known HIV status were scored using the diagnostic algorithm (**Table 7**): 401 (95.9%) of the 418 first-episode cases and 53 (75.7%) of the 70 recurrent cases. Four hundred and forty-five

(98.0%) of the 454 scored episodes were evaluated by assessment of at least 9 of the 12 parameters used by the scoring system: 165 (36.3%) of these were scored with the TST result as part of the assessment, and were therefore suitable for use in order to evaluate the sensitivity of the scoring system as per study criteria.

	HIV-infected	HIV-uninfected	Odds Ratio (95% CI)	P
1. Tuberculosis episodes	334	151	-	-
2. Score assessed, n (%) *	304 (91.0)	150 (99.3)	0.07 (0.001 – 0.42)	< 0.001
3. Score with ≥ 9 parameters, n (%) [+]	296 (97.4)	149 (99.3)	0.25 (0.01 – 1.89)	0.283 [§]
4. Score with ≥ 9 parameters (including TST), n (%) [++]	85 (28.7)	80 (53.7)	0.35 (0.23 – 0.53)	< 0.001

Table 7: Proportions of children who were evaluated using the tuberculosis score chart according to HIV status: General Features

* Expressed as a percentage of the number of admission episodes as tabulated in row 1.

[+] Expressed as a percentage of row 2. [++] Expressed as a percentage of row 3.

[§] Fisher's exact test.

A smaller proportion of HIV-infected children (304 [91.0%] of those who were diagnosed with tuberculosis) could be scored using the diagnostic algorithm compared to HIV-uninfected children (150 [99.3%] of those with tuberculosis), P<0.001, because clinical information regarding the tuberculosis diagnosis was not always available from the data sources used: this reflects the fact that HIV-infected children were either investigated for tuberculosis or had tuberculosis diagnoses made in outpatient settings e.g. paediatric HIV clinics at the hospital.

3.1.5.1 Scoring 'General Features' – all tuberculosis episodes

Three hundred and eighty-five (85.9%) of 448 children with defined HIV status who were diagnosed with tuberculosis and had been scored presented with an acute duration (< 2 weeks) of cough: median cough duration was 4 days (IQR 2 – 7) in HIV-infected and -uninfected children, P=0.725. In children with PTB, 199 (80.6%) of 247 cases in the HIV-infected and 98 (88.3%) of 111 cases in the HIV-uninfected presented with a cough duration of less than 14 days' duration (P=0.072).

HIV-infected children with tuberculosis were 2.40 times more likely to be underweight (95% CI, 1.56 – 3.71), P<0.001 and 9.84 times more frequently assessed as being marasmic (95% CI, 3.88 – 31.80), P<0.001 than were HIV-uninfected children (**Table 8**).

There was no significant difference in the proportions of tuberculosis cases with a family history of tuberculosis according to HIV status: absence of a positive family history of tuberculosis was noted in 221 (72.9%) of 303 HIV-infected children diagnosed with tuberculosis in whom a history of family contact was enquired about, compared to 105 (70.5%) of 149 HIV-uninfected children, P=0.582.

HIV-infected children diagnosed with tuberculosis were more likely to exhibit TST anergy compared to HIV-uninfected children (OR 8.56; 95% CI, 3.83 – 19.44), P<0.001. TST responses in all children diagnosed with tuberculosis that were tested using the Mantoux method are reflected in **Figures 2** and **3**. Median TST induration was 0 mm (Range 0 – 25) in HIV-infected and 15 mm (Range 0 – 25) in HIV-uninfected children, P<0.001.

Parameter	Score	HIV-infected		HIV-uninfected		Odds Ratio (95% CI)	P
Weeks of illness score, n (%)	0	251 (84.0)	n=299	134 (89.9)	n=149	0.59 (0.29 – 1.11)	0.086
	1	27 (9.0)		11 (7.4)		1.25 (0.58 – 2.87)	0.555
	3	21 (7.0)		4 (2.7)		2.74 (0.90 – 11.16)	0.059
Nutrition score, n (%)	0	62 (20.9)	n=297	95 (64.2)	n=148	0.15 (0.09 – 0.23)	< 0.001
	1	159 (53.5)		48 (32.4)		2.40 (1.56 – 3.71)	< 0.001
	3	76 (25.6)		5 (3.4)		9.84 (3.88 – 31.80)	< 0.001
Family history of tuberculosis score, n (%)	0	221 (72.9)	n=303	105 (70.5)	n=149	1.13 (0.71 – 1.78)	0.582
	1	81 (26.8)		43 (28.9)		0.90 (0.57 – 1.43)	0.634
	3	1 (0.3)		1 (0.6)		0.49 (0.01 – 38.72)	0.551 §
TST Score, n (%)	0	69 (81.2)	n=85	25 (31.2)	n=80	9.49 (4.37 – 20.90)	< 0.001
	3	16 (18.8)		55 (68.8)		0.11 (0.05 – 0.23)	< 0.001
Malnutrition (weight) not improving score, n (%)	3	55 (39.9)	n=138	4 (11.8)	n=34	4.97 (1.61 – 20.34)	0.002
Malnutrition (centile) not improving score, n (%)	3	89 (65.4)	n=136	16 (47.1)	n=34	2.13 (0.93 – 4.90)	0.049
Fever Score, n (%)	2	10 (71.4)	n=14	3 (50.0)	n=6	2.5 (0.22 – 27.07)	0.613 §

Table 8: General Features as scored in PCV Study participants with tuberculosis

§ Fisher's exact test.

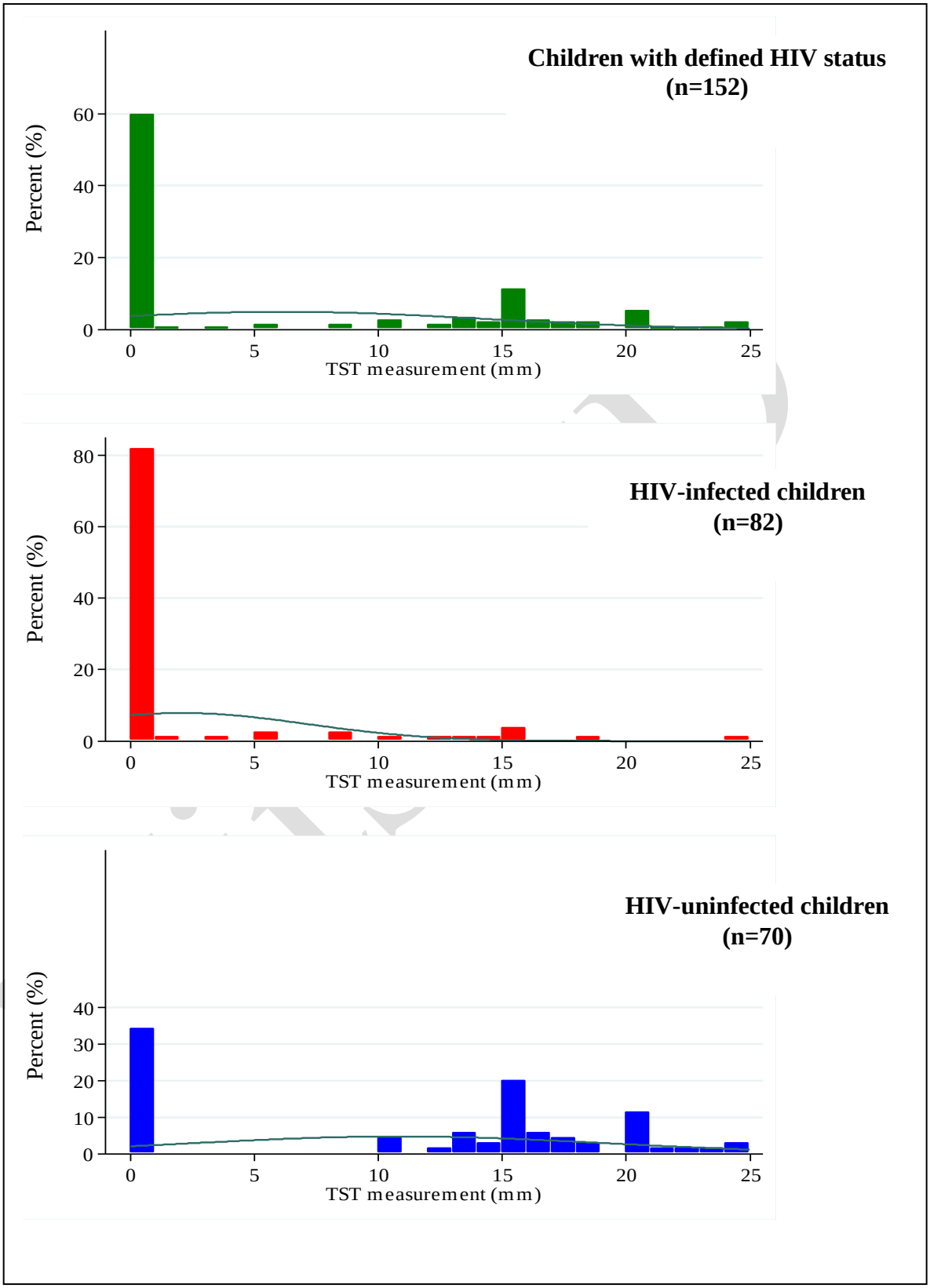


Figure 2: TST responses in children with defined HIV status who were diagnosed with tuberculosis, inclusive of anergic TST responses

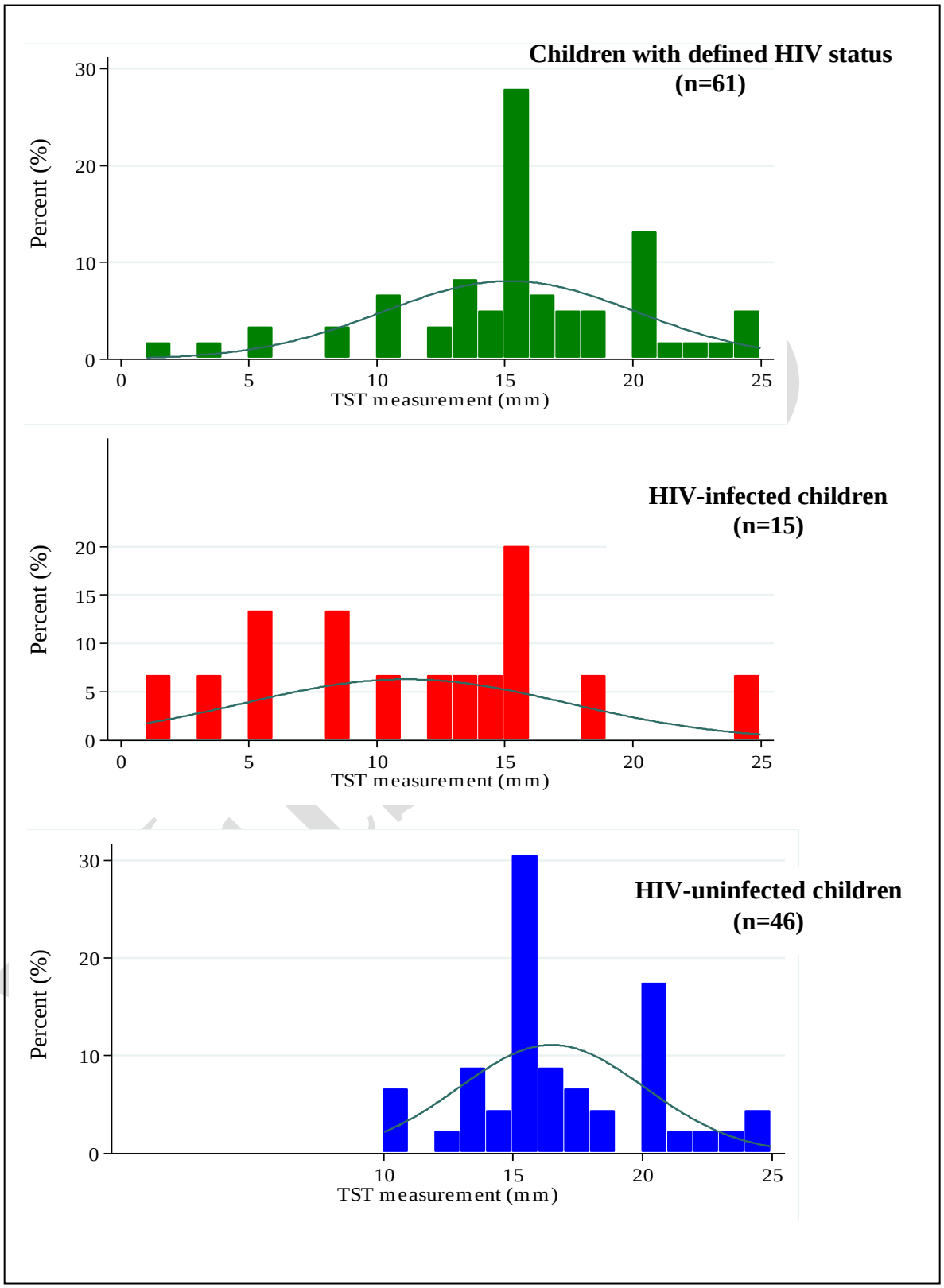


Figure 3: TST responses in children with defined HIV status who were diagnosed with tuberculosis, omitting anergic TST responses

Analysis of TST induration with exclusion of all negative responses was performed in order to determine the degree of 'reactivity' of the TST in the two groups, according to HIV status (**Figure 3**). Although the HIV-infected children had less TST-induced induration compared to HIV-uninfected children, with a mean positive induration diameter of 11.1 mm (Standard Deviation [SD] 6.3; Range 7.6 – 14.6) compared to 16.5 mm (SD 3.6; Range 15.4 – 17.6) in the HIV-uninfected children, P<0.001, it is important to emphasise that the mean induration size in HIV-infected children was nevertheless ≥ 10 mm.

When analysing for 'weight not improving', HIV-infected children were 5 times (95% CI, 1.61 – 20.34) more likely not to have exhibited weight gain compared to HIV-uninfected children, P=0.002. When analysing for 'centile not improving' HIV-infected children were twice (95% CI, 0.93 – 4.90) as likely not to have exhibited an improvement in their weight-for-age centiles compared to HIV-uninfected children, P=0.049. Mean weight-for-age z-scores were significantly reduced in HIV-infected children diagnosed with tuberculosis (-2.80, SD 1.36) compared to HIV-uninfected children (-1.23, SD 1.39), P<0.001.

There was no significant difference in fever duration between HIV-infected and -uninfected children diagnosed with tuberculosis, P=0.613 (Fisher's exact test).

3.1.5.2 Scoring 'Local Features' – all tuberculosis episodes

The only local feature which exhibited a significant difference between HIV-infected and -uninfected children diagnosed with tuberculosis was that of lymphadenopathy: this feature was encountered 5-fold more frequently (95% CI, 3.34 – 8.84) in the HIV-infected children compared to HIV-uninfected children, P<0.001 (**Table 9**).

Parameter	Score	HIV-infected	HIV-uninfected	Odds Ratio (95% CI)	P
1. Tuberculosis episodes	-	334	151	-	-
2. Local features assessed, n (%) *	-	299 (89.5)	149 (98.7)	0.11 (0.01 – 0.46)	< 0.001
Lymphadenopathy, n (%) +	3	258 (86.3)	80 (53.7)	5.43 (3.34 – 8.84)	< 0.001
Joint or bone swelling, n (%) +	3	1 (0.3)	1 (0.7)	0.50 (0.01 – 39.24)	1.000 §
Abdominal mass or ascites, n (%) +	3	3 (1.0)	5 (3.4)	0.29 (0.04 – 1.53)	0.123 §
CNS signs or abnormal CSF, n (%) +	3	33 (11.0)	13 (8.7)	1.30 (0.64 – 2.78)	0.448
Hilar adenopathy on chest radiograph, n (%) +	3	44 (14.7)	17 (11.4)	1.34 (0.72 – 2.60)	0.336
Angle deformity of spine, n (%) +	4	1 (0.3)	0 (0.0)	-	1.000 §

Table 9: Local Features as scored in PCV Study participants with tuberculosis

* Expressed as a percentage of Row 1. + Expressed as a percentage of Row 2. § Fisher's exact test.

3.1.5.3 Scoring 'General' and 'Local' Features in cases with culture-confirmed tuberculosis

When assessing the clinical parameters of children who had definite tuberculosis (**Table 10**), 56 (77.8%) of 72 children with culture-confirmed PTB presented with an acute duration of cough.

Family history of tuberculosis was equally encountered in children with culture-confirmed disease, regardless of HIV status (P=0.987).

Median TST induration was 0 mm in children with culture-confirmed tuberculosis, regardless of HIV status (P=0.792). Seven (17.5%) of 40 children with definite tuberculosis who had a TST (five of them HIV-uninfected) had reactive TST responses.

HIV-infected children were 2.8 times (95% CI, 1.1 – 7.5), more likely to be underweight than were HIV-uninfected children, P=0.021, although there was no significant difference in proportions of marasmic children with culture-confirmed disease by HIV status (OR 2.5; 95% CI, 0.6 – 14.5), P=0.248 (Fisher's exact test). Poor weight gain (P=0.212, Fisher's exact test) and failure to thrive (P=1.000, Fisher's exact test) were observed with equal frequency in HIV-infected and -uninfected children with definite tuberculosis.

As was demonstrated in the scoring of 'Local Features' in all tuberculosis cases, the only finding on clinical examination of HIV-infected children which was consistently encountered over HIV-uninfected children, was that of lymphadenopathy (OR 15.8; 95% CI, 4.7 – 57.1), P<0.001.

Parameter	HIV-infected n (%)		HIV-uninfected n (%)		OR (95% CI)	P
PTB cough duration less than 14 days	41 (78.8)	n=52	15 (75.0)	n=20	1.2 (0.3 – 4.7)	0.757 §
Median duration of cough (IQR)	4 days (2 – 7)		4 days (3 – 7)		-	0.652
Underweight	38 (59.4)	n=64	11 (34.4)	n=32	2.8 (1.1 – 7.5)	0.021
Marasmic	13 (20.3)		3 (9.4)		2.5 (0.6 – 14.5)	0.248 §
Mean weight-for-age z-score (SD)	- 2.62 ± 1.23		- 1.37 ± 1.57		-	<0.001
Family history of tuberculosis	15 (22.7)	n=66	7 (22.6)	n=31	1.0 (0.3 – 3.3)	0.987
Median TST response (Range)	0 (0 – 15)		0 (0 – 22)		-	0.792
No improvement in nutritional status	Weight * 11 (44.0)	n=25	Weight * 1 (14.3)	n=7	4.7 (0.4 – 236.2)	0.212 §
	Centile ** 16 (64.0)		Centile ** 5 (71.4)		0.7 (0.1 – 5.6)	1.000 §
Fever	-	n=0	-	n=0	-	-
Lymphadenopathy	60 (90.9)	n=66	12 (38.7)	n=31	15.8 (4.7 – 57.1)	<0.001
Bone involvement	0 (0.0)		0 (0.0)		-	-
Abdominal signs	2 (3.0)		3 (9.7)		0.3 (0.0 – 2.7)	0.323 §
CNS signs	11 (16.7)		3 (9.7)		1.9 (0.4 – 11.2)	0.538 §
X-ray changes	9 (13.6)		3 (9.7)		1.5 (0.3 – 9.1)	0.747 §
Spine changes	0 (0.0)		0 (0.0)		-	-

Table 10: Scoring clinical parameters for children with culture-confirmed tuberculosis

* 'Weight' reflects no improvement or a decline in weight (in kilograms) between a previous admission episode and the admission episode in which tuberculosis was diagnosed.

** 'Centile' reflects a downward trend on weight-for-age growth reference curves between a previous admission episode and the admission episode in which tuberculosis was diagnosed.

§ Fisher's exact test.

3.1.5.4 Sensitivity of the diagnostic algorithm

Sensitivity of the algorithm in terms of substantiating a diagnosis of tuberculosis in the cohort is explored in **Table 11**. The sensitivity analyses were conducted by identifying those tuberculosis episodes which were scored and attained a score of at least 7 (the cut-off for positivity according to the algorithm) and calculating what proportion those cases contributed to the total number of tuberculosis episodes which were scored in each disease category. This analysis was conducted using only those patients who had been scored using at least 9 of the 12 parameters stipulated in the algorithm and in whom the TST was included as part of the tuberculosis diagnostic work-up. From **Table 7**, 85 HIV-infected and 80 HIV-uninfected children fulfilled the criteria for this analysis.

Forty instances of definite tuberculosis (culture-proven cases) were scored using at least 9 parameters, including the TST. The score equalled or exceeded the cut-off value of 7 in 15 (37.5%; 95% CI, 22.7 – 54.2) of the 40 culture-positive tuberculosis episodes, i.e. sensitivity of the score was 37.5 percent for culture-confirmed tuberculosis in this cohort (**Table 11**).

The scoring system was not superior in sensitivity in HIV-infected children compared to HIV-uninfected children when analysing for all forms of tuberculosis: overall, a score compatible with a clinical diagnosis of tuberculosis (according to the score chart criteria) was achieved in HIV-infected children as frequently as in HIV-uninfected children (OR 1.05; 95% CI, 0.54 – 2.05), P=0.878.

	All children with defined HIV status			HIV-infected			HIV-uninfected			Odds Ratio (95% CI)	P
	Score ≥ 7	All cases*	Sensitivity (%) (95% CI)	Score ≥ 7	All cases*	Sensitivity (%) (95% CI)	Score ≥ 7	All cases*	Sensitivity (%) (95% CI)		
Definite tuberculosis	15	40	37.5 (22.7 – 54.2)	8	23	34.8 (16.4 – 57.3)	7	17	41.2 (18.4 – 67.1)	0.76 (0.17 – 3.38)	0.680
Confirmed PTB	2	8	25.0 (3.2 – 65.1)	1	4	25.0 (0.6 – 80.6)	1	4	25.0 (6.3 – 80.6)	1.00 (0.01 – 104.37)	1.000 §
Bacteriologically-confirmed tuberculosis	17	48	35.4 (22.2 – 50.5)	9	27	33.3 (16.5 – 54.0)	8	21	38.1 (18.1 – 61.6)	0.81 (0.21 – 3.17)	0.732
Probable PTB	38	76	50.0 (38.3 – 61.7)	17	26	65.4 (44.3 – 82.8)	21	50	42.0 (28.2 – 56.8)	2.61 (0.88 – 7.95)	0.053
Probable EPTB	6	8	75.0 (34.9 – 96.8)	3	5	60.0 (14.7 – 94.7)	3	3	100.0 (29.2 – 100.0)	-	0.464 §
Suspected tuberculosis	6	33	18.2 (7.0 – 35.5)	6	27	22.2 (8.6 – 42.3)	0	6	0.0 (0.0 – 45.9)	-	0.563 §
All tuberculosis	67	165	40.6 (33.0 – 48.5)	35	85	41.2 (30.6 – 52.4)	32	80	40.0 (29.2 – 51.6)	1.05 (0.54 – 2.05)	0.878

Table 11: Sensitivity of the diagnostic score chart in instances where a diagnosis of tuberculosis was achieved

* Denominators are tuberculosis cases which were scored according to the diagnostic algorithm in children with defined HIV status, where at least 9 of the parameters (including the TST) were scored.

§ Fisher's exact test.

3.1.5.5 Comparison of the sensitivities of the algorithm and the Mantoux test

It is interesting to compare the sensitivity of the diagnostic algorithm with the sensitivity of the TST response in the children who were subjected to tuberculin skin testing: 71 positive TST responses occurred in 165 children with defined HIV status who were diagnosed with tuberculosis and were tested using the Mantoux method (**Table 12**). The sensitivity of the TST in HIV-infected children (2 positive TSTs out of 27 bacteriologically-confirmed tuberculosis episodes that were screened with TST) was 7.4 percent (95% CI, 0.9 – 24.3) compared to 38.1 percent (8 positive TSTs out of 21 bacteriologically-confirmed tuberculosis episodes where TST results were available: 95% CI, 18.1 – 61.6) in HIV-uninfected children.

TST anergy in children with bacteriologically-confirmed tuberculosis was 3-fold (95% CI, 0.49 – 22.46) more frequent in HIV-infected children, P=0.235 (Fisher's exact test), although small sample size failed to secure statistical significance in this analysis.

Overall, sensitivity of the TST was significantly greater in HIV-uninfected compared to HIV-infected children (68.8 versus 18.8 percent: OR 9.49; 95% CI 4.37 – 20.90), P<0.001.

A comparison of the sensitivities of the tuberculosis score chart and the TST in children according to tuberculosis disease category and HIV status is tabulated below (**Table 13**): this indicates that the tuberculosis score chart sensitivity was significantly better than the TST sensitivity in HIV-infected children with bacteriologically-confirmed tuberculosis (P=0.039, Fisher's exact test) and suggests that the score chart may be of use in augmenting the sensitivity of TST in providing evidence for tuberculosis in HIV-infected children.

	All children			HIV-infected			HIV-uninfected			Odds Ratio (95% CI)	P
	TST Pos	All cases	Sensitivity (%) (95% CI)	TST Pos	All cases	Sensitivity (%) (95% CI)	TST Pos	All cases	Sensitivity (%) (95% CI)		
Definite tuberculosis	7	40	17.5 (7.3 – 32.8)	2	23	8.7 (1.1 – 28.0)	5	17	29.4 (10.3 – 56.0)	0.23 (0.02 – 1.73)	0.113 §
Confirmed PTB	3	8	37.5 (8.5 – 75.5)	0	4	0.0 (0.0 – 60.2)	3	4	75.0 (19.4 – 99.4)	-	0.143 §
Bacteriologically-confirmed tuberculosis	10	48	20.8 (10.5 – 35.0)	2	27	7.4 (0.9 – 24.3)	8	21	38.1 (18.1 – 61.6)	0.13 (0.01 – 0.82)	0.014 §
Probable PTB	57	76	75.0 (63.7 – 84.2)	13	26	50.0 (29.9 – 70.1)	44	50	88.0 (75.7 – 95.5)	0.14 (0.04 – 0.49)	<0.001 §
Probable EPTB	4	8	50.0 (15.7 – 84.3)	1	5	20.0 (0.5 – 71.6)	3	3	100.0 (29.2 – 100.0)	-	0.143 §
Suspected tuberculosis	0	33	0.0 (0.0 – 10.6)	0	27	0.0 (0.0 – 12.8)	0	6	0.0 (0.0 – 45.9)	-	-
All tuberculosis	71	165	43.0 (35.4 – 51.0)	16	85	18.8 (11.2 – 28.8)	55	80	68.8 (57.4 – 78.7)	0.11 (0.05 – 0.23)	<0.001

Table 12: TST responses in HIV-infected and HIV-uninfected children by tuberculosis category in those who were scored using at least nine parameters

§ Fisher's exact test.

	HIV-infected					HIV-uninfected				
	Tuberculosis Score Chart		TST		P	Tuberculosis Score Chart		TST		P
	Score ≥ 7	Sensitivity (%)	TST Pos	Sensitivity (%)		Score ≥ 7	Sensitivity (%)	TST Pos	Sensitivity (%)	
Definite tuberculosis	8 / 23	34.8	2 / 23	8.7	0.071 §	7 / 17	41.2	5 / 17	29.4	0.473
Confirmed PTB	1 / 4	25.0	0 / 4	0.0	1.000 §	1 / 4	25.0	3 / 4	75.0	0.486 §
Bacteriologically-confirmed tuberculosis	9 / 27	33.3	2 / 27	7.4	0.039 §	8 / 21	38.1	8 / 21	38.1	1.000
Probable PTB	17 / 26	65.4	13 / 26	50.0	0.262	21 / 50	42.0	44 / 50	88.0	< 0.001
Probable EPTB	3 / 5	60.0	1 / 5	20.0	0.524 §	3 / 3	100.0	3 / 3	100.0	-
Suspected tuberculosis	6 / 27	22.2	0 / 27	0.0	0.023 §	0 / 6	0.0	0 / 6	0.0	-
All tuberculosis	35 / 85	41.2	16 / 85	18.8	0.002	32 / 80	40.0	55 / 80	68.8	< 0.001

Table 13: Comparison between sensitivities of the tuberculosis score chart and TST by HIV status

§ Fisher's exact test.

3.1.6 Cases with an untreated episode of bacteriologically-confirmed tuberculosis

Anti-tuberculous therapy was not started in 62 (44.0%) of all bacteriologically-confirmed (n=141) episodes of tuberculosis, as evidenced by the finding of culture- or smear-positive disease in instances where children had been discharged from CHBH without a diagnosis of tuberculosis and ancillary data sources failed to confirm that the child had been started on appropriate therapy. These episodes occurred in 61 children, one of whom (an HIV-infected male) had a second episode of culture-confirmed tuberculosis in which treatment was delayed, 13 months after the initial untreated episode; he appears not to have been treated for tuberculosis in the period intervening between these tuberculosis episodes.

Fifty-four (87.1%) of the 62 episodes with untreated bacteriologically-proven tuberculosis had culture-proven disease and 8 (12.9%) had smear-positive disease without culture-confirmation.

Instances where cases with bacteriologically-confirmed disease were not started on anti-tuberculous therapy occurred with equal frequency in the HIV-infected and -uninfected children: 44 (16.1%) of the 274 HIV-infected children ever diagnosed with tuberculosis and 17 (11.8%) of the 144 HIV-uninfected children with tuberculosis had an untreated episode of bacteriologically-confirmed disease (OR 1.43; 95% CI, 0.76 – 2.78), P=0.242. The results of this analysis held firm when defining the relative proportions of untreated bacteriologically-confirmed tuberculosis by HIV status, where 45 (45.0%) of the 100 HIV-infected children with bacteriologically-confirmed episodes of tuberculosis and 17 (42.5%)

of the 40 HIV-uninfected children with bacteriologically-confirmed episodes remained untreated for their condition (OR 1.11; 95% CI, 0.50 – 2.50), P=0.788.

Vaccination status also did not differ significantly amongst children who had untreated bacteriologically-confirmed disease, with 25 (44.6%) of the 56 PCV recipients with bacteriologically-confirmed disease and 37 (43.5%) of the 85 placebo recipients who were diagnosed with bacteriologically-confirmed tuberculosis remaining untreated for their condition (OR 1.05; 95% CI, 0.50 – 2.18), P=0.896. When limiting the analysis by vaccination and HIV status to untreated children who had bacteriologically-confirmed PTB (n=56), 14 (28.6%) of the 49 bacteriologically-confirmed PTB episodes in HIV-infected children who had received PCV and 27 (54.0%) of the 50 such PTB episodes in HIV-infected children who had received placebo had untreated bacteriologically-confirmed PTB (OR 0.34; 95% CI, 0.14 – 0.85), P=0.010, indicating that HIV-infected PCV recipients were significantly less likely than placebo recipients to remain untreated for bacteriologically-confirmed PTB. Contrary to this finding, HIV-uninfected PCV recipients had significantly more episodes of untreated bacteriologically-confirmed PTB than did the HIV-uninfected placebo recipients, with 10 (62.5%) of 16 bacteriologically-confirmed PTB episodes remaining untreated compared to 5 (23.8%) of 21 such PTB episodes amongst placebo recipients (OR 5.33; 95% CI, 1.06 – 28.35), P=0.018.

Seven (11.5%) of the 61 children who had bacteriologically-proven tuberculosis but who were not started on anti-tuberculous therapy died during the admission episode (**Table 14**): 5 were HIV-infected (4 had CDC Category C disease and the remaining child was classified as having CDC Category B disease) and 2 were HIV-uninfected. Median age at

admission in the children who died was 10.0 months (IQR 2.5 – 20.7) with no significant difference in age at admission between HIV-infected (5.7 months, IQR 2.5 – 15.4) and -uninfected children (15.3 months, IQR 10.0 – 20.7), P=0.439. These children presented with mean cough duration of 3.9 days (SD 1.8). HIV-infected children who died with untreated bacteriologically-confirmed tuberculosis had significantly shorter duration of cough (3.0 days, SD 1.0) compared to the HIV-uninfected children (6.0 days, SD 1.4), P=0.022. Median duration of admission to time of death was 4.0 days (IQR 1.0 – 13.0). Diagnosis at death in 5 of the children was pneumonia (4 of these children were HIV-infected with suspected *P. jiroveci* pneumonia: none of these had microbiological evidence to support that diagnosis). Diagnoses at death in the two remaining children were meningitis (in the HIV-infected child) and lymphoma with tumour lysis syndrome (in the HIV-uninfected child).

Twenty-one (34.4%) of the 61 children with untreated bacteriologically-confirmed tuberculosis were never readmitted to CHBH, and have unknown outcomes. Eleven (52.4%) of these children were HIV-infected (14.5 months, IQR 3.4 – 29.4) and 10 (47.6%) were HIV-uninfected (5.1 months, IQR 3.1 – 12.3), P=0.073. Ten (90.9%) of the HIV-infected children had been assessed by CDC categorisation of their HIV disease: 7 were CDC Category C patients, and 1 each was classified as having CDC Category N, A and B HIV disease. Median duration of cough in these patients was 3.0 days (IQR 2.0 – 6.0): shorter in the HIV-uninfected children (2.0 days, IQR 1.0 – 3.5) than in the HIV-infected children (3.0 days, IQR 3.0 – 7.0), P=0.045.

		All untreated episodes [n=62]	Untreated episodes that died [n=7]	Untreated episodes that were lost to follow-up [n=21]	Untreated episodes that were followed up [n=34]
HIV status, n (%)	HIV-infected	45 / 100 (45.0) *	5 / 45 (11.1) [+]	11 / 45 (24.4) [+]	29 / 45 (64.4) [+]
	HIV-uninfected	17 / 40 (42.5) *	2 / 17 (11.8) [+]	10 / 17 (58.8) [+]	5 / 17 (29.4) [+]
	OR (95% CI)	1.11 (0.50 – 2.50)	0.94 (0.13 – 10.86)	0.23 (0.06 – 0.86)	4.35 (1.14 – 18.30)
	P	0.788	1.000 [§]	0.011	0.013
Age at admission (months)	All	14.1 (IQR 5.4 – 39.1)	10.0 (IQR 2.5 – 20.7)	8.1 (IQR 3.4 – 14.5)	24.7 (IQR 7.6 – 52.2)
	HIV-infected	22.6 (IQR 5.6 – 45.1)	5.7 (IQR 2.5 – 15.4)	14.5 (IQR 3.4 – 29.4)	31.3 (IQR 6.3 – 52.7)
	HIV-uninfected	10.0 (IQR 3.5 – 14.0)	15.3 (IQR 10.0 – 20.7)	5.1 (IQR 3.1 – 12.3)	14.2 (IQR 10.2 – 18.8)
	P	0.028	0.439	0.073	0.395
Cough duration (days)	All	3.5 (IQR 2.0 – 7.0)	3.9 (SD ± 1.8)	3.0 (IQR 2.0 – 6.0)	4.0 (IQR 2.0 – 7.0)
	HIV-infected	4.0 (IQR 2.0 – 7.0)	3.0 (SD ± 1.0)	3.0 (IQR 3.0 – 7.0)	4.0 (IQR 2.0 – 7.0)
	HIV-uninfected	3.0 (IQR 1.0 – 5.0)	6.0 (SD ± 1.4)	2.0 (IQR 1.0 – 3.5)	4.0 (IQR 1.0 – 7.0)
	P	0.174	0.022	0.045	0.691

Table 14: Analysis of episodes with untreated bacteriologically-confirmed tuberculosis

* Expressed as percentage of all bacteriologically-confirmed tuberculosis in each HIV category.

[+] Expressed as a percentage of episodes with missed microbiologically-confirmed tuberculosis in each HIV category.

[§] Fisher's exact test.

		All untreated episodes [n=62]	Untreated episodes that died [n=7]	Untreated episodes that were lost to follow-up [n=21]	Untreated episodes that were followed up [n=34]
Algorithm score ≥ 7, n (%)	HIV-infected	16 / 39 (41.0)	2 / 5 (40.0)	3 / 10 (30.0)	11 / 24 (45.8)
	HIV-uninfected	1 / 17 (5.9)	0 / 2 (0.0)	0 / 10 (0.0)	1 / 5 (20.0)
	OR (95% CI)	11.13 (1.39 – 495.29)	-	-	3.38 (0.27 – 181.55)
	P	0.009	0.222 §	0.211 §	0.370 §
Length of admission (days)	All	6.0 (IQR 4.0 – 11.0)	4.0 (IQR 1.0 – 13.0)	7.0 (IQR 4.5 – 9.5)	6.0 (IQR 5.0 – 12.0)
	HIV-infected	7.0 (IQR 5.0 – 13.0)	4.0 (IQR 3.0 – 5.0)	7.0 (IQR 6.0 – 14.0)	7.5 (IQR 5.0 – 13.0)
	HIV-uninfected	5.0 (IQR 4.0 – 8.0)	7.0 (IQR 1.0 –13.0)	7.0 (IQR 4.0 – 9.0)	5.0 (IQR 3.0 – 5.0)
	P	0.074	0.845	0.381	0.025
Discharge diagnoses with respiratory illness, n (%)	HIV-infected	30 / 45 (66.7)	4 / 5 (80.0)	7 / 11 (63.6)	19 / 29 (65.5)
	HIV-uninfected	11 / 17 (64.7)	1 / 2 (50.0)	7 / 10 (70.0)	3 / 5 (60.0)
	OR (95% CI)	1.09 (0.27 – 4.01)	4.0 (0.03 – 390.99)	0.75 (0.08 – 6.54)	1.27 (0.91 – 12.99)
	P	0.884	1.000 §	1.000 §	1.000 §

Table 14, continued: Analysis of episodes with untreated bacteriologically-confirmed tuberculosis

§ Fisher's exact test.

Thirty-four (54.8%) of the 62 episodes that had unrecognised bacteriologically-confirmed tuberculosis were followed up at CHBH: 29 of these episodes occurred in 28 HIV-infected children and 5 occurred in HIV-uninfected children. Twenty-four (70.6%) of these children presented with cough with a median cough duration in the HIV-infected children of 4.0 days (IQR 2.0 – 7.0), equivalent to the median duration of cough in the HIV-uninfected children (4.0 days; IQR 1.0 – 7.0), P=0.691. Information regarding whether anti-tuberculous therapy was started in this sub-group of patients once the positive test was recognised, is scanty: only 16 (48.5%) of these 33 children (all of them HIV-infected) appear from the available data to have been started on anti-tuberculous treatment on admission episodes subsequent to the one in which the positive mycobacterial result occurred. Mean elapsed time between the admission episode when a diagnosis of bacteriologically confirmed tuberculosis went unrecognised, and subsequent admission when the tuberculosis diagnosis was identified and the child was started on anti-tuberculous therapy, was 44.2 days (SD 25.3).

3.1.7 Co-infections in the context of tuberculosis

3.1.7.1 Co-infections regardless of tuberculosis categorisation

Four hundred and thirty (88.7%) of the 485 episodes where tuberculosis was diagnosed or identified in children with defined HIV status were investigated for multiple pathogens during the diagnostic work-up for tuberculosis. One hundred and twelve (26.0%) of these 430 tuberculosis episodes were confirmed to have viral or *P. jiroveci* co-infections, or infection with significant bacterial isolates on blood culture. These investigations were conducted in 390 children.

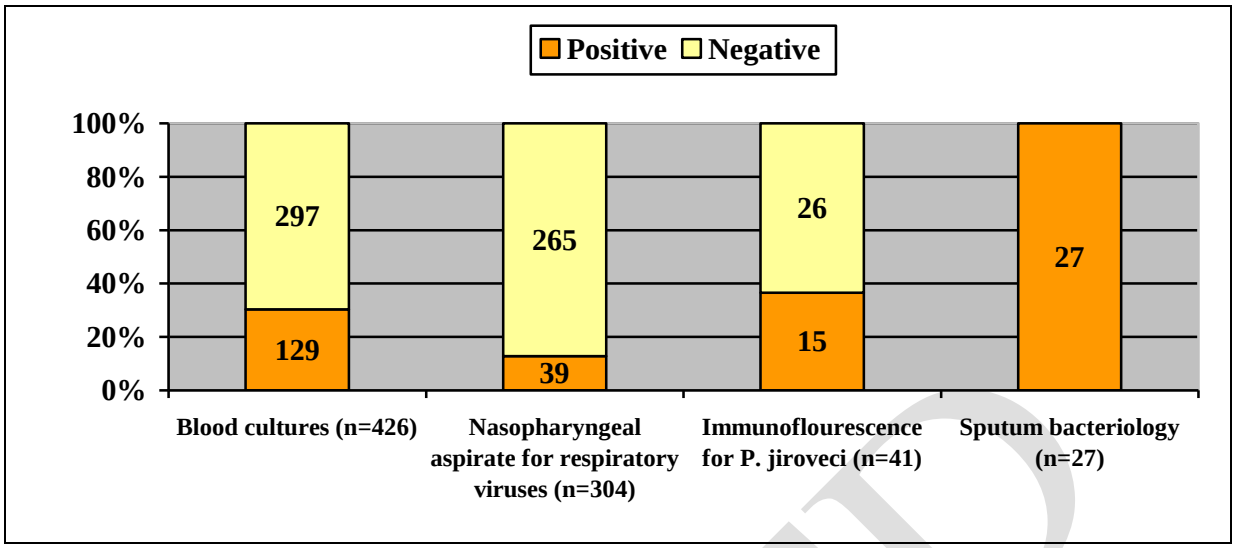


Figure 4: Microbiological investigations and yield for co-infections in tuberculosis episodes (n=430) amongst children with defined HIV status

Note: The total number of positive specimens (n=210) differs from the number of tuberculosis episodes with co-infection (n=112) as some of the tuberculosis episodes had co-infection with more than one pathogen.

Blood cultures were performed in 426, nasopharyngeal aspirates for respiratory viruses in 304 and *P. jiroveci* immunofluorescence in 41 episodes in which tuberculosis was diagnosed or identified. Results of 27 positive sputum bacterial cultures were also recorded in the RMPRU database (**Figure 4**). Hence, a total of 798 microbiological investigations were sent in 430 episodes of tuberculosis from 390 children that were screened for co-infection at the tuberculosis index episode.

One-hundred and twenty-nine (30.3%) of the 426 blood cultures were positive for bacterial organisms, however only 57 (13.4%) of all blood cultures isolated pathogenic bacteria (other positive cultures yielded likely contaminating organisms such as *Corynebacterium* spp, *Micrococcus* spp and Coagulase negative *Staphylococcus* spp). Three of the episodes with significant organisms on blood culture isolated two bacterial species during the hospitalisation: an HIV-infected child cultured methicillin-resistant *Staphylococcus aureus*

(MRSA) on an admission blood culture and *Enterococcus* sp on a subsequent blood culture during the same admission episode; 2 HIV-uninfected children isolated co-pathogens in their blood cultures – the first isolated MRSA and *Streptococcus pyogenes* and the second isolated *S. pneumoniae* and non-typable *H. influenzae*.

Information regarding sputum bacterial isolates in the cohort is incomplete: all of the 27 children with tuberculosis who had sputum screened for bacterial co-infections had evidence of bacterial infection on sputum culture. Eight (29.6%) of these children had dual bacterial isolates on sputum culture; 7 (87.5 percent of the children with sputum isolation of more than one bacterial species) were HIV-infected. Combinations of dual bacterial infection as isolated on sputum culture in these children were: non-typable *H. influenzae* and *S. aureus* (3 patients, one of whom was the only HIV-uninfected child with dual bacterial pathogens in sputum); pneumococcus and non-typable *H. influenzae* (3 children); pneumococcus and MRSA (1 child); and *E. coli* and *Klebsiella* sp (1 child).

Thirty-nine (12.8%) of 304 nasopharyngeal aspirates performed in the children with tuberculosis revealed evidence of concomitant respiratory virus infection, and two HIV-uninfected children isolated dual influenza A and respiratory syncytial virus (RSV) in nasopharyngeal aspirates taken during the admission episode in which tuberculosis was diagnosed.

Fifteen children (3.8 percent of the 390 children who had been screened for dual infection in the context of their tuberculosis index admission episode) had confirmed *P. jiroveci* pneumonia: 12 (80.0%) of these arose in HIV-infected children.

From **Table 15**, it can be appreciated that proportions of co-infections identified in individuals with confirmed, probable and suspected tuberculosis were 26.5, 19.6 and 31.9 percent, respectively. Whereas significantly fewer co-infections were identified in children with probable tuberculosis compared to those with suspected tuberculosis (OR 0.52; 95% CI, 0.30 – 0.90), P=0.013, there was no significant difference in the proportions of children with confirmed co-infection comparing those who had definite tuberculosis and those with suspected disease (OR 0.77; 95% CI, 0.44 – 1.35), P=0.333.

	Bacteriologically-confirmed tuberculosis [n=117]	Probable tuberculosis [n=153]	Suspected tuberculosis [n=160]
Individuals with polymicrobial infection (%)	31 / 117 (26.5)	30 / 153 (19.6)	51 / 160 (31.9)
Comparison of proportions	OR (95% CI)		P
Probable versus Bacteriologically-confirmed tuberculosis	0.68 (0.37 – 1.25)		0.180
Probable versus Suspected tuberculosis	0.52 (0.30 – 0.90)		0.013
Bacteriologically-confirmed versus Suspected tuberculosis	0.77 (0.44 – 1.35)		0.333

Table 15: Comparison of co-infection rates according to tuberculosis disease category, irrespective of HIV status

Table 16 explores the co-infections according to the children's HIV status and the strength of evidence of the tuberculosis diagnosis. HIV-infected children with tuberculosis were not significantly more likely to have dual infection than were -uninfected children: this was true even in HIV-infected children with a diagnosis of suspected tuberculosis (OR 1.39; 95% CI, 0.56 – 3.69), P=0.446.

	HIV-infected			HIV-uninfected		
	Confirmed TB * [n=80]	Probable TB [n=92]	Suspected TB [n=126]	Confirmed TB [n=37]	Probable TB [n=61]	Suspected TB [n=34]
Blood cultures						
S. pneumoniae	3	5	14	1	0	2
Strep spp	1	0	1	1	0	0
H. influenzae	0	0	5	1	0	0
S. aureus	1	2	1	1	0	2
Gram Negatives	2	2	11	1	0	0
Bacterial sputum cultures						
S. pneumoniae	0	1	3	0	0	0
H. influenzae	3	1	3	1	2	0
S. aureus	4	0	2	1	0	0
Gram Negatives	1	0	2	0	1	0
Respiratory virus isolated						
Influenza A	3	4	3	3	4	4
RSV	0	2	2	2	1	0
Adenovirus	1	1	3	0	1	0
Parainfluenza	0	1	1	0	0	1
Influenza + RSV	0	0	0	1	1	0
Other						
P. jiroveci	6	3	3	1	1	1
Total isolates	25	22	54	14	11	10
Individuals with polymicrobial infection, n (%)	21 (26.3)	19 (20.7)	42 (33.3)	10 (27.0)	11 (18.0)	9 (26.5)
Comparison of proportions with co-infection in each TB Category by HIV status: OR (95% CI), P		Confirmed TB		0.96 (0.37 – 2.61)		0.929
		Probable TB		1.18 (0.48 – 3.00)		0.690
		Suspected TB		1.39 (0.56 – 3.69)		0.446

Table 16: Co-infections in children diagnosed with tuberculosis, by HIV status

* 'TB' represents 'tuberculosis': 'Confirmed TB' represents cases with smear- and/or culture-positive disease.

HIV-infected children with tuberculosis had an overall increased burden of co-infection in terms of blood culture positivity for significant pathogens compared to HIV-uninfected children (**Table 17**). Forty-seven (18.8%) significant bacterial blood culture isolates were identified in 250 tuberculosis episodes amongst the HIV-infected children, compared to 7 (6.0%) significant blood culture isolates amongst 116 tuberculosis episodes in HIV-uninfected children (OR 3.61; 95% CI, 1.55 – 9.75), P=0.001. In contrast to this finding, HIV-uninfected children had a significantly greater burden of confirmed respiratory viral co-infection than did HIV-infected children (OR 2.57; 95% CI, 1.20 – 5.43), P=0.006. The overall burden of co-infection between HIV-infected and -uninfected children with bacteriologically-confirmed tuberculosis were equivalent (**Table 17**).

Table 18 reveals equivalent rates of co-infection across tuberculosis disease categories according to vaccination status of the children.

Table 19 explores the tuberculosis co-infection rates in the HIV-infected children according to PCV vaccination status and reveals no difference in co-infection amongst the children with bacteriologically-confirmed and probable tuberculosis; however, children who received PCV showed a trend towards fewer co-infection episodes in the context of suspected tuberculosis compared to those who received placebo (OR 0.51; 95% CI, 0.22 – 1.15), P=0.078.

There was no significant difference in co-infection rates amongst the HIV-uninfected children with tuberculosis according to vaccination status (**Table 20**).

		Tuberculosis episodes screened for co-infections *		Odds Ratio (95% CI)	P
		HIV-infected	HIV-uninfected		
All tuberculosis	Blood culture positive**, n (%)	47 / 250 (18.8)	7 / 116 (6.0)	3.61 (1.55 – 9.75)	0.001
	Sputum culture positive, n (%)	13 / 30 (43.3)	4 / 6 (66.7)	0.38 (0.03 – 3.24)	0.391 §
	Respiratory virus positive, n (%)	20 / 217 (9.2)	18 / 87 (20.7)	0.39 (0.18 – 0.83)	0.006
	P. jiroveci positive, n (%)	12 / 35 (34.3)	3 / 6 (50.0)	0.52 (0.06 – 4.58)	0.651 §
	All co-pathogens, n (%)	82 / 298 (27.5)	30 / 132 (22.7)	1.29 (0.78 – 2.17)	0. 297
Bacteriologically-confirmed tuberculosis +	Blood culture positive**, n (%)	7 / 72 (9.7)	3 / 34 (8.8)	1.11 (0.23 – 7.11)	1.000 §
	Sputum culture positive, n (%)	7 / 7 (100.0)	1 / 1 (100.0)	-	-
	Respiratory virus positive, n (%)	4 / 58 (6.9)	6 / 27 (22.2)	0.26 (0.05 – 1.24)	0.067 §
	P. jiroveci positive, n (%)	6 / 9 (66.7)	1 / 3 (33.3)	4.00 (0.13 – 274.49)	0.523 §
	All co-pathogens, n (%)	21 / 80 (26.3)	10 / 37 (27.0)	0.96 (0.37 – 2.61)	0.929

Table 17: Co-infection categories in children with tuberculosis, according to HIV status

* Numerators in each category represent numbers of individual children with positive results; denominators reflect the sum of the specimens with significant isolates and those that were negative.

** Blood cultures positive for significant pathogens – likely contaminating organisms excluded.

+ Bacteriologically-confirmed tuberculosis includes 'Definite TB' and 'Confirmed PTB' cases.

§ Fisher's exact test.

	PCV			Placebo		
	Confirmed TB * [n=46]	Probable TB [n=77]	Suspected TB [n=81]	Confirmed TB [n=71]	Probable TB [n=76]	Suspected TB [n=79]
Blood cultures						
S. pneumoniae	1	3	8	3	2	8
Strep spp	1	0	1	1	0	0
H. influenzae	1	0	0	0	0	5
S. aureus	1	1	2	1	1	1
Gram Negatives	1	0	5	2	2	6
Bacterial sputum cultures						
S. pneumoniae	0	1	0	0	0	3
H. influenzae	1	2	1	3	1	2
S. aureus	3	0	1	2	0	1
Gram Negatives	0	0	2	1	1	0
Respiratory virus isolated						
Influenza A	3	4	4	3	4	3
RSV	1	0	1	1	3	1
Adenovirus	0	1	1	1	1	2
Parainfluenza	0	0	2	0	1	0
Influenza + RSV	1	1	0	0	0	0
Other						
P. jiroveci	1	2	0	6	2	4
Total isolates	15	15	28	24	18	36
Individuals with polymicrobial infection, n (%)	11 (23.9)	13 (16.9)	22 (27.2)	20 (28.2)	17 (22.4)	29 (36.7)
Comparison of proportions with co-infection in each TB Category by vaccination status: OR (95% CI), P	Confirmed TB			0.80 (0.31 – 2.02)		0.610
	Probable TB			0.70 (0.29 – 1.69)		0.393
	Suspected TB			0.64 (0.31 – 1.32)		0.195

Table 18: Co-infections in children diagnosed with tuberculosis, by vaccination status

* 'TB' represents 'tuberculosis': 'Confirmed TB' represents cases with smear- and/or culture-positive disease.

HIV-infected	PCV			Placebo		
	Confirmed TB * [n=32]	Probable TB [n=45]	Suspected TB [n=62]	Confirmed TB [n=48]	Probable TB [n=47]	Suspected TB [n=64]
Blood cultures						
S. pneumoniae	0	3	6	3	2	8
Strep spp	0	0	1	1	0	0
H. influenzae	0	0	0	0	0	5
S. aureus	0	1	1	1	1	0
Gram Negatives	1	0	5	1	2	6
Bacterial sputum cultures						
S. pneumoniae	0	1	0	0	0	3
H. influenzae	1	1	1	2	0	2
S. aureus	3	0	1	1	0	1
Gram Negatives	0	0	2	1	0	0
Respiratory virus isolated						
Influenza A	1	2	1	2	2	2
RSV	0	0	1	0	2	1
Adenovirus	0	1	1	1	0	2
Parainfluenza	0	0	1	0	1	0
Influenza + RSV	0	0	0	0	0	0
Other						
P. jiroveci	1	2	0	5	1	3
Total isolates	7	11	21	18	11	33
Individuals with polymicrobial infection, n (%)	6 (18.8)	9 (20.0)	16 (25.8)	15 (31.3)	10 (21.3)	26 (40.6)
Comparison of proportions with co-infection in each TB Category by vaccination status in HIV-infected children: OR (95% CI), P	Confirmed TB			0.51 (0.14 – 1.64)		0.213
	Probable TB			0.93 (0.29 – 2.87)		0.880
	Suspected TB			0.51 (0.22 – 1.15)		0.078

Table 19: Co-infections in HIV-infected children, according to PCV vaccination status

* 'TB' represents 'tuberculosis': 'Confirmed TB' represents cases with smear- and/or culture-positive disease.

HIV-uninfected	PCV			Placebo		
	Confirmed TB [n=14]	Probable TB [n=32]	Suspected TB [n=19]	Confirmed TB [n=23]	Probable TB [n=29]	Suspected TB [n=15]
Blood cultures						
S. pneumoniae	1	0	2	0	0	0
Strep spp	1	0	0	0	0	0
H. influenzae	1	0	0	0	0	0
S. aureus	1	0	1	0	0	1
Gram Negatives	0	0	0	1	0	0
Bacterial sputum cultures						
S. pneumoniae	0	0	0	0	0	0
H. influenzae	0	1	0	1	1	0
S. aureus	0	0	0	1	0	0
Gram Negatives	0	0	0	0	1	0
Respiratory virus isolated						
Influenza A	2	2	3	1	2	1
RSV	1	0	0	1	1	0
Adenovirus	0	0	0	0	1	0
Parainfluenza	0	0	1	0	0	0
Influenza + RSV	1	1	0	0	0	0
Other						
P. jiroveci	0	0	0	1	1	1
Total isolates	8	4	7	6	7	3
Individuals with polymicrobial infection, n (%)	5 (35.7)	4 (12.5)	6 (31.6)	5 (21.7)	7 (24.1)	3 (20.0)
Comparison of proportions with co-infection in each TB Category by vaccination status in HIV-uninfected children: OR(95% CI), P	Confirmed TB			2.00 (0.35 – 11.19)		0.454 §
	Probable TB			0.45 (0.09 – 2.06)		0.323 §
	Suspected TB			1.85 (0.30 – 13.77)		0.697 §

Table 20: Co-infections in HIV-uninfected children, according to PCV vaccination status

* 'TB' represents 'tuberculosis': 'Confirmed TB' represents cases with smear- and/or culture-positive disease.

§ Fisher's exact test.

3.1.7.2 Co-infections in children with culture-confirmed tuberculosis

Analysis of co-infections encountered in children with culture-confirmed tuberculosis was conducted in order to achieve an appreciation as to which co-pathogens were encountered in this group of children (**Table 21**). Twenty-two (23.4%) of the 94 culture-positive tuberculosis episodes that were investigated for co-infections, had documented polymicrobial infections.

A wide variety of micro-organisms were identified in co-infection with culture-confirmed tuberculosis (**Table 21**): *Pneumocystis jiroveci* was encountered in 4 (50.0%) of 8 placebo-related culture-confirmed tuberculosis episodes and 1 (50.0%) of the 2 PCV-related episodes that were investigated for pneumocystis pneumonia, P=1.000 (Fisher's exact test). Viral pathogens were isolated in 4 (17.4%) of 23 PCV-vaccinated patients and 4 (8.0%) of 50 placebo patients investigated for viral co-infection in the context of their culture-confirmed tuberculosis, P=0.251 (Fisher's exact test).

Clinically significant bacterial sputum isolates were encountered in one (2.9%) of 34 PCV recipients and 5 (8.3%) of 60 placebo recipients with culture-confirmed tuberculosis who were investigated for co-infections (OR 0.33; 95% CI, 0.01 – 3.19), P=0.413 (Fisher's exact test), **Table 21**.

Similarly, clinically significant bacterial blood culture isolates were identified in 4 (11.8%) of 34 culture-confirmed tuberculosis episodes in which bacterial blood culture results were obtainable amongst PCV recipients, compared to 5 (9.6%) of 52 cultures amongst the placebo group, P=0.735 (Fisher's exact test).

Whilst **Table 21** illustrates co-infection events amongst episodes of culture-confirmed tuberculosis in the PCV study cohort according to vaccination and HIV status, it does not detail the types of co-infections encountered in individual trial participants. A detailed account of the co-infections encountered in children with bacteriologically-confirmed tuberculosis is presented in **Table 22**.

Three (12.5%) of 24 episodes of culture-proven tuberculosis in HIV-infected children who received PCV and were investigated for co-infections, had confirmed co-infections at the tuberculosis index admission, compared to 10 (25.0%) of 40 similarly-investigated episodes in HIV-infected placebo recipients, P=0.339 (Fisher's exact test). Amongst HIV-uninfected children, 4 (40.0%) of 10 culture-confirmed tuberculosis episodes in PCV recipients were associated with co-infections, compared to 5 (25.0%) of 20 culture-confirmed tuberculosis episodes amongst placebo recipients, P=0.431 (Fisher's exact test), **Table 22**.

As mentioned above, 22 children with culture-confirmed tuberculosis were identified as having co-infections with significant bacterial or viral pathogens, or *P. jiroveci* in the context of their tuberculosis. Thirteen (59.1%) of these children were HIV-infected, and 15 (68.2%) were placebo recipients. Neither HIV-infected children who received PCV, nor those who were HIV-uninfected, were more likely than placebo recipients to develop co-infections in the context of culture-confirmed tuberculosis (**Table 22**).

		PCV		Placebo	
		HIV-infected [n=24]	HIV-uninfected [n=10]	HIV-infected [n=40]	HIV-uninfected [n=20]
Blood Cultures	Negative	22	8	31	16
	S. pneumoniae	0	1	3	0
	Streptococcus spp	0	1	0	0
	H. influenzae	0	1	0	0
	S. aureus	0	1	1	0
	Gram Negatives	0	0	0	1
	OR (95% CI), P *	1.25 (0.23 – 6.33)			0.735 §
Sputum cultures	*S. pneumoniae*	0	0	0	0
	H. influenzae	0	0	1	1
	S. aureus	1	0	0	1
	Gram Negatives	0	0	1	0
	C. albicans	0	0	1	0
	OR (95% CI), P *	0.33 (0.01 – 3.19)			0.413 §
Respiratory viruses	Negative	16	3	31	15
	Influenza A	1	2	1	1
	RSV	0	0	0	1
	Adenovirus	0	0	1	0
	Influenza + RSV	0	1	0	0
	OR (95% CI), P *	2.42 (0.40 – 14.27)			0.251 §
P. jiroveci	Negative	1	0	2	2
	Positive	1	0	3	1
	OR (95% CI), P *	1.00 (0.10 – 98.00)			1.000 §
Individuals with polymicrobial infection, n (%)		3 (12.5)	4 (40.0)	10 (25.0)	5 (25.0)
		7 (20.6)		15 (25.0)	
OR (95% CI), P *		0.78 (0.24 – 2.35)			0.627

Table 21: Microbiological investigations sent on children with culture-proven tuberculosis: all episodes

* P- value compares co-infections in children according to vaccination status.

§ Fisher's exact test.

	HIV-infected		HIV-uninfected	
	PCV [n=24]	Placebo [n=40]	PCV [n=10]	Placebo [n=20]
One significant organism on blood culture	-	4	-	1
Two significant organisms on blood culture	-	-	1	-
Two significant organisms on blood culture and two significant respiratory viruses	-	-	1	-
One significant organism on sputum culture	1	2	-	-
Two significant organisms on sputum culture	-	-	-	1
One significant respiratory virus	1	1	2	2
P. jiroveci and one significant respiratory virus	-	1	-	-
P. jiroveci	1	2	-	1
Total, n (%)	3 (12.5)	10 (25.0)	4 (40.0)	5 (25.0)
P *	0.339 §		0.431 §	

Table 22: Profile of significant co-pathogens identified in the context of culture-confirmed tuberculosis, according to HIV and vaccination status

* P-value compares proportions of children with co-infections by vaccination status, within HIV infection status categories.

§ Fisher's exact test.

3.1.8 Adverse events to anti-tuberculous therapy

Three children (all of them HIV-infected) were noted to have drug reactions that were attributable to anti-tuberculous therapy, and required hospitalisation to manage these adverse events: 2 had hepatitis and 1 had a skin reaction. As 446 tuberculosis episodes (305 HIV-infected, 134 HIV-uninfected and 7 with unknown HIV status) were known to have been initiated on anti-tuberculous therapy in this cohort, this represents an adverse event rate of 0.7 percent (95% CI, 0.1 – 2.0) for the cohort, or a rate of 1.0 percent (95% CI, 0.2 – 3.0) for HIV-infected children, P=0.555 (Fisher's exact test).

3.1.9 Tuberculosis-related deaths

Fifty-one (12.0%) of the 425 children ever identified with tuberculosis died at CHBH: 40 (78.4%) of these deaths occurred in the HIV-infected children, 10 (19.6%) in HIV-uninfected children, and 1 (2.0%) in a child who had no definitive HIV result: 48 (94.1%) of the deaths occurred during the tuberculosis index admission episode. Fifteen (29.4%) of the children who died with tuberculosis at the hospital had bacteriologically-confirmed disease. As has been discussed in **Section 3.1.6**, seven of these cases had not been started on anti-tuberculous therapy.

Eight children (6 HIV-infected and 2 HIV-uninfected) died in the context of bacteriologically-confirmed tuberculosis and had been started on anti-tuberculous therapy prior to their demise. Mean cough duration in these children was 4.7 days (SD 2.3), this cough duration being similar to that in the group of children with untreated tuberculosis (see **Table 14**) who died with bacteriologically-confirmed disease (3.9 days, SD 1.8), P=0.483.

Median length of hospitalisation in the children who died on anti-tuberculous therapy was 12.0 days (IQR 4.5 – 40.0), not significantly longer than that encountered in the children with untreated bacteriologically-confirmed disease who died (4.0 days, IQR 1.0 – 13.0), P=0.201.

Seven of these children were scored using the diagnostic algorithm (all of the scores utilised at least 9 of the 12 parameters enumerated in the scoring system), and only one of

these seven (an HIV-infected child) had a total score which was compatible with a diagnosis of tuberculosis according to the algorithm. The yield of score positivity in this group of children compared to those who died with undiagnosed bacteriologically-confirmed tuberculosis (where 2 out of 7 children had a total score compatible with the algorithm diagnosis of tuberculosis) was equivalent, P=1.000 (Fisher's exact test).

	HIV-infected [n=274]	HIV-uninfected [n=144]	Odds Ratio (95% CI)	P
Bacteriologically-confirmed tuberculosis, n (%)	11 (4.0)	4 (2.8)	1.46 (0.42 – 6.41)	0.593 §
Probable tuberculosis, n (%)	4 (1.5)	2 (1.4)	1.04 (0.15 – 11.67)	1.000 §
Suspected tuberculosis, n (%)	23 (8.4)	3 (2.1)	4.31 (1.26 – 22.74)	0.011
Same episode tuberculosis, n (%)	2 (0.7)	1 (0.7)	1.05 (0.05 – 62.2)	1.000 §
All tuberculosis, n (%)	40 (14.6)	10 (6.9)	2.29 (1.08 – 5.30)	0.022
Tuberculosis with confirmed co-infection, n (%) *	9 / 38 (23.7)	2 / 9 (22.2)	1.09 (0.16 – 12.52)	1.000 §

Table 23: Deaths in study participants, according to HIV status and category of tuberculosis

* Expressed as a percentage of those children who died who were investigated for co-infections.

Case fatality rates amongst children who died during an admission in which tuberculosis was diagnosed (see **Table 23**) were similar in HIV-infected and -uninfected children for all categories of tuberculosis besides that of 'suspected tuberculosis' (OR 4.31; 95% CI, 1.26 – 22.74), P=0.011. Overall, HIV-infected children had a greater tuberculosis-related case fatality rate (OR 2.29; 95% CI, 1.08 – 5.30), P=0.022.

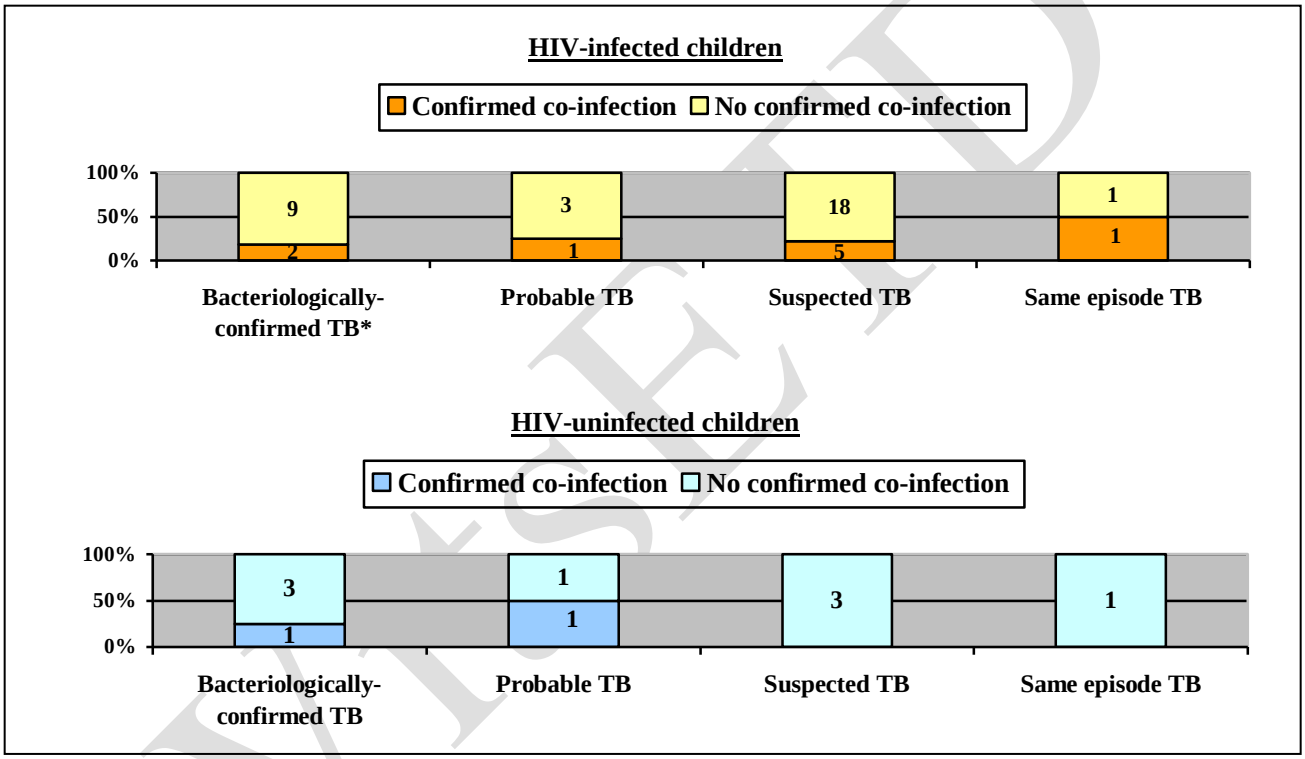


Figure 5: Proportions of children dying with confirmed co-infection in the context of their tuberculosis, by HIV status

* 'TB' represents 'tuberculosis'.

Forty-seven (92.2%) of the tuberculosis episodes in which children died were investigated for bacterial, viral or *P. jiroveci* co-infections and 11 (23.4%) of these revealed evidence of polymicrobial infection. From **Figure 5**, it can be appreciated that 9 HIV-infected children died with confirmed co-infections compared to 2 HIV-uninfected children (OR 1.09; 95% CI, 0.16 – 12.52), P=1.000 (Fisher's exact test).

A diverse spectrum of co-pathogens was identified in children dying with a diagnosis of tuberculosis: these are tabulated in **Table 24** (below).

Specimen type	Organisms	HIV-infected	HIV-uninfected	Category of Tuberculosis		
				Confirmed *	Probable	Suspected
Blood culture isolates	*S. pneumoniae*	3	-	1	1	1
	Pseudomonas spp	-	1	1	-	-
Sputum isolates	*Enterobacter* spp	-	1	-	1	-
Blood culture and sputum isolates	*Klebsiella* spp and *E. coli*	1	-	-	-	1
Nasopharyngeal aspirates	Adenovirus	1	-	-	-	1
	RSV	1		-	-	1
Other isolates	*P. jiroveci*	1	-	1	-	-
Mixed isolates	*H. influenzae* and *P. jiroveci*	1	-	-	-	1
	Neisseria spp and RSV	1	-	-	-	1

Table 24: Organisms identified in children dying with tuberculosis, according to HIV status and category of tuberculosis disease

* 'Confirmed' represents tuberculosis cases with smear- and/or culture-positive disease.

3.2 The Role of Pneumococcal Co-infection as a Cause for Hospitalisation in Children with Pulmonary Tuberculosis

By using the 9-valent PCV as a probe, it was possible to estimate the proportion of hospitalised tuberculosis cases which ordinarily arise in the context of an intercurrent pneumococcal co-infection. We postulated that, if pneumococcal co-infections do play a significant role in co-infection amongst children who require hospitalisation for PTB, then children who had received the vaccine would have a lower burden of tuberculosis compared to those who had received placebo.

Tables 25 and **26**, which illustrate the initial analysis comparing all forms (definite, probable and suspected) of PTB as diagnosed in the cohort, and stratified according to age at which the tuberculous episode was diagnosed and vaccination status, showed no difference in the overall incidence of tuberculosis between PCV and placebo groups (Incidence Rate Ratio [IRR] 0.90; 95% CI, 0.73 – 1.10), P=0.292 for first episode tuberculosis (**Table 25** and **Figure 6**) and IRR 0.87 (95% CI, 0.72 – 1.05), P=0.146, for all PTB episodes (**Table 26**).

When using the more specific diagnosis of culture-confirmed PTB (excluding recurrent episodes of culture-confirmed disease), 31 cases of all culture-confirmed PTB occurred in the vaccinated group compared to 55 cases amongst the placebo recipients (IRR 0.56; 95% CI, 0.35 – 0.89), P=0.010, representing a 44 percent (95% CI, 11 – 65) risk reduction in culture-confirmed PTB amongst PCV recipients (**Table 27** and **Figure 7**).

Age group (months)	Hospital child-years of follow-up		Number of PTB cases diagnosed *		Tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	38	43	516 (365 – 708)	585 (423 – 787)	0.88 (0.56 – 1.40)	0.577
6.1 – 12.0	9841	9827	33	46	325 (222 – 459)	468 (343 – 624)	0.72 (0.44 – 1.14)	0.144
1.5 – 12.0	17 206	17 183	71	89	413 (322 – 521)	518 (416 – 637)	0.80 (0.74 – 1.10)	0.153
12.1 – 24.0	19 675	19 647	43	43	219 (158 – 294)	219 (158 – 295)	1.00 (0.64 – 1.56)	0.995
24.1 – 36.0	19 666	19 639	20	26	101 (62 – 157)	132 (86 – 194)	0.77 (0.41 – 1.43)	0.379
36.1 – 48.0	19 664	19 637	18	17	92 (54 – 145)	87 (50 – 139)	1.06 (0.51 – 2.18)	0.871
48.1 – 60.0	21 596	21 685	17	13	79 (46 – 126)	60 (32 – 103)	1.31 (0.60 – 2.94)	0.466
>60.0	52 478	52 595	16	18	30 (17 – 50)	34 (20 – 54)	0.89 (0.43 – 1.85)	0.741
All ages	150 285	150 386	185	206	123 (106 – 142)	137 (119 – 157)	0.90 (0.73 – 1.10)	0.292

Table 25: Incidence of PTB as diagnosed at CHBH, according to vaccination status: incident tuberculosis

* All PTB cases excluding cases with recurrent tuberculosis episodes.

Age group (months)	Hospital child-years of follow-up		Number of PTB cases diagnosed [+]		Tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	38	43	516 (365 – 708)	585 (423 – 787)	0.88 (0.56 – 1.40)	0.577
6.1 – 12.0	9841	9827	35	47	356 (248 – 495)	478 (351 – 636)	0.74 (0.47 – 1.18)	0.185
1.5 – 12.0	17 206	17 183	72	90	418 (327 – 527)	524 (421 – 644)	0.80 (0.58 – 1.10)	0.156
12.1 – 24.0	19 675	19 647	48	46	244 (180 – 323)	234 (171 – 312)	1.04 (0.68 – 1.60)	0.843
24.1 – 36.0	19 666	19 639	24	29	122 (78 – 182)	148 (99 – 212)	0.83 (0.46 – 1.47)	0.494
36.1 – 48.0	19 664	19 637	18	27	92 (54 – 145)	138 (91 – 200)	0.67 (0.35 – 1.25)	0.182
48.1 – 60.0	21 596	21 685	22	20	102 (64 – 154)	92 (56 – 142)	1.10 (0.58 – 2.13)	0.751
>60.0	52 478	52 595	24	28	46 (29 – 68)	53 (35 – 77)	0.86 (0.48 – 1.54)	0.589
All ages	150 285	150 386	209	240	139 (121 – 159)	160 (140 – 181)	0.87 (0.72 – 1.05)	0.146

Table 26: Incidence of PTB as diagnosed at CHBH, according to vaccination status: all tuberculosis episodes

[+] All PTB cases (including recurrent episodes).

Age group (months)	Hospital child-years of follow-up		Number of culture-positive PTB cases diagnosed *		Definite tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	6	12	81 (30 – 177)	163 (84 – 285)	0.50 (0.15 – 1.44)	0.166
6.1 – 12.0	9841	9827	4	9	41 (11 – 104)	92 (42 – 174)	0.44 (0.10 – 1.59)	0.179
1.5 – 12.0	**17 206**	**17 183**	**10**	**21**	**58 (28 – 107)**	**122 (76 – 187)**	**0.48 (0.20 – 1.06)**	**0.050**
12.1 – 24.0	19 675	19 647	9	12	46 (21 – 87)	61 (32 – 107)	0.75 (0.28 – 1.94)	0.521
24.1 – 36.0	**19 666**	**19 639**	**0**	**7**	**0 (0 – 19)**	**36 (14 – 73)**	**0.0 (0.0 – 0.69)**	**0.008**
36.1 – 48.0	19 664	19 637	7	6	36 (14 – 73)	31 (11 – 67)	1.17 (0.34 – 4.20)	0.792
48.1 – 60.0	21 596	21 685	2	4	9 (1 – 33)	18 (5 – 47)	0.50 (0.05 – 3.50)	0.456
>60.0	52 478	52 595	3	5	6 (1 – 17)	10 (3 – 22)	0.60 (0.09 – 3.09)	0.510
All ages	**150 285**	**150 386**	**31**	**55**	**21 (14 – 29)**	**37 (28 – 48)**	**0.56 (0.35 – 0.89)**	**0.010**

Table 27: Incidence of definite PTB, according to vaccination status: incident tuberculosis

* Culture-confirmed PTB cases excluding cases with recurrent tuberculosis episodes.

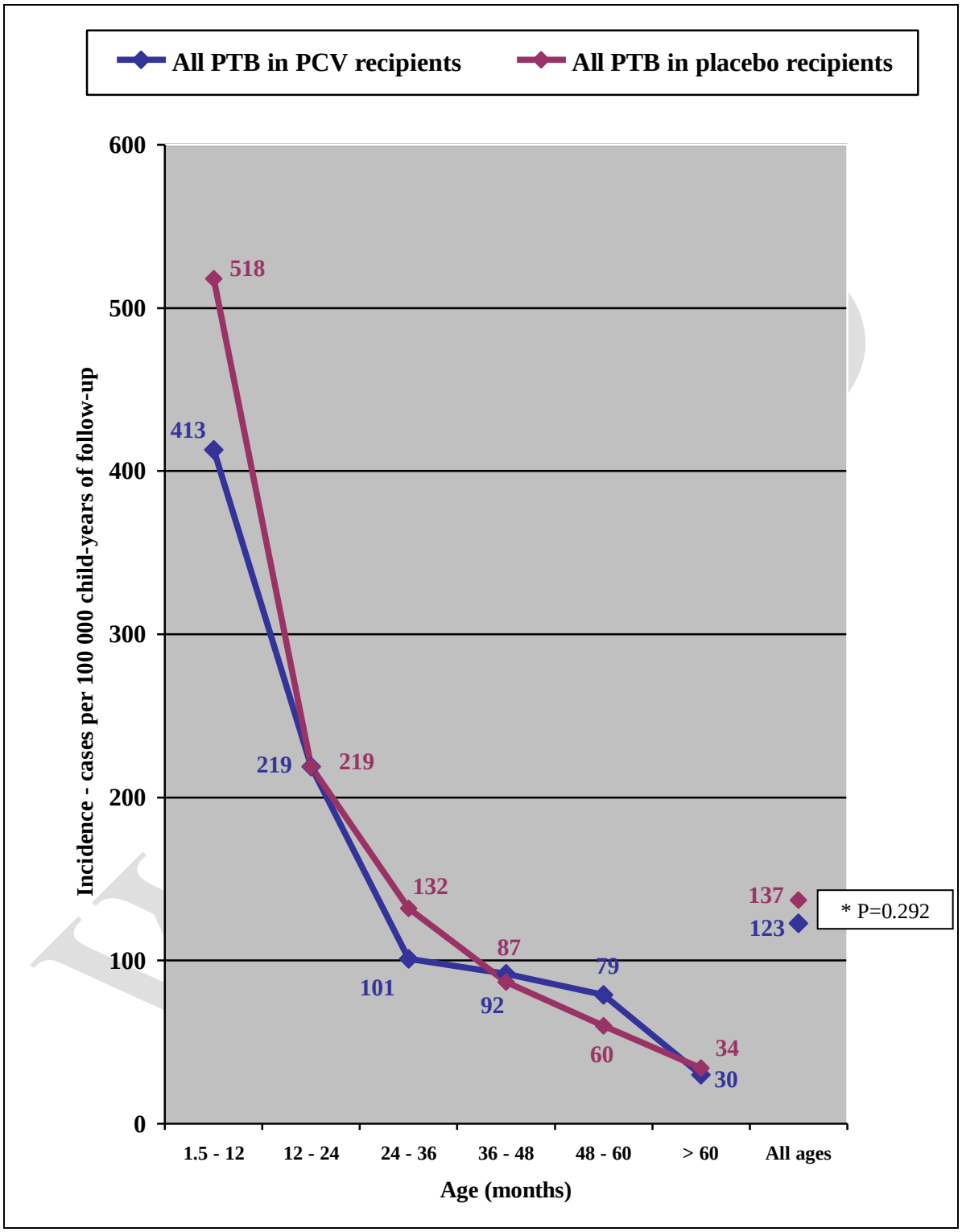


Figure 6: Incidence of PTB according to PCV vaccination status

* This Figure is derived from **Table 25**: P-value describes the difference in incidence of PTB between PCV and placebo recipients in the 'All ages' category.

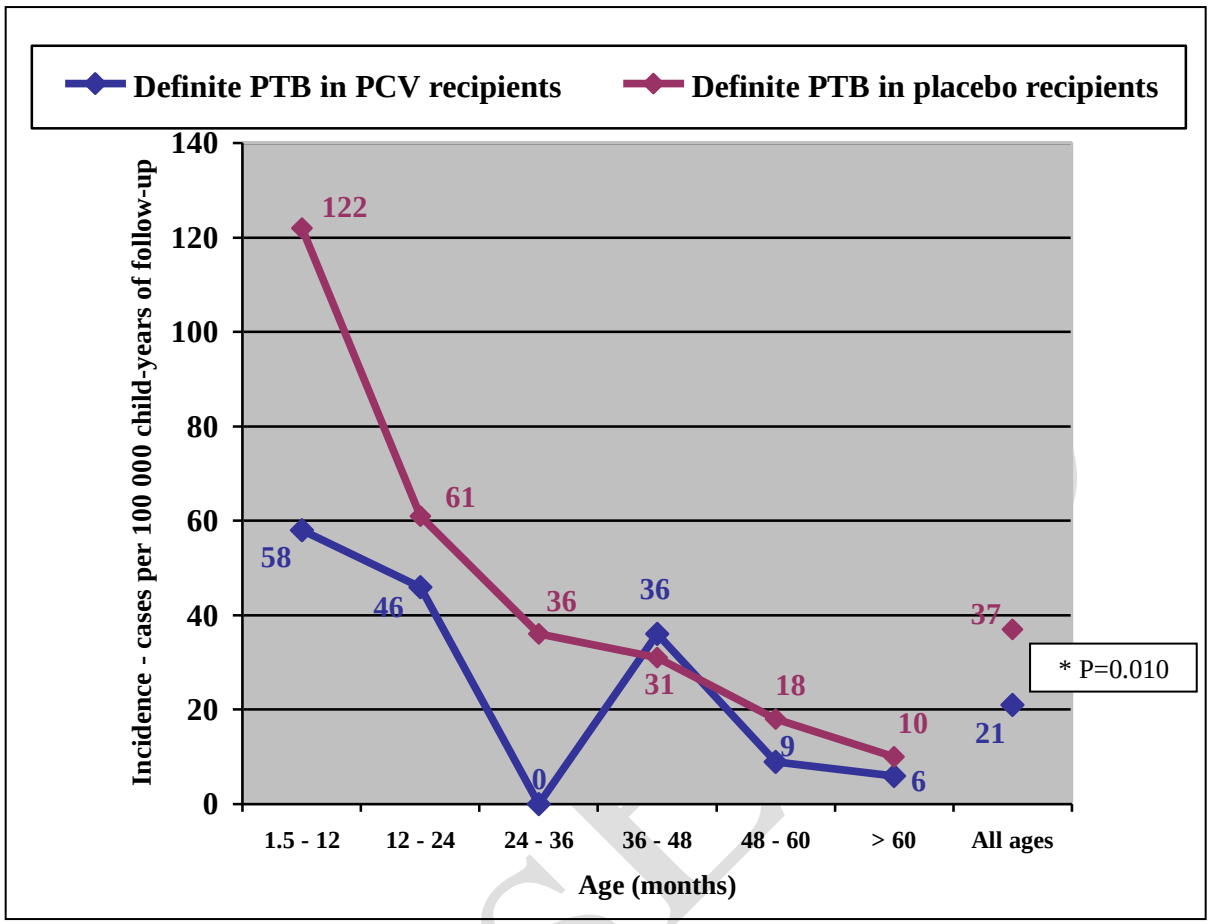


Figure 7: Incidence of culture-confirmed PTB according to PCV vaccination status

* This Figure is derived from **Table 27**: P-value describes the difference in rates of culture-confirmed PTB between PCV and placebo recipients in the 'All ages' category.

Although significant reductions in culture-confirmed tuberculosis could not be demonstrated in the PCV recipients by age group (on the basis of small numbers of tuberculosis cases and relatively short cumulative follow-up periods), the overall effect of the PCV was to appreciably reduce the burden of culture-positive disease in vaccinated over placebo recipients until 3 years of age (**Table 25** and **Figure 7**). This indicates that a substantial proportion of children below five years of age hospitalised for culture-confirmed PTB in Soweto may have concomitant pneumococcal co-infection precipitating the acute illness.

A similar reduction in culture-confirmed PTB amongst PCV recipients was demonstrated when analysing for all definite PTB episodes (**Table 28**), with an overall PCV effect giving rise to an apparent reduction in culture-confirmed PTB of 43 percent (95% CI, 13 – 63), P=0.007, compared to placebo recipients.

The cumulative PCV effect of revealing likely pneumococcal co-infection in children hospitalised with PTB could also be demonstrated for children in the vaccinated cohort when the analysis was extended to include all cases with bacteriologically-confirmed (culture- and microscopy-positive PTB), as in **Table 29,** which illustrates that 45 cases of bacteriologically-confirmed tuberculosis occurred in the vaccinated group compared to 65 in the placebo group (IRR 0.69; 95% CI, 0.46 – 1.03), P=0.058. Extending the analysis to include all episodes of bacteriologically-confirmed PTB (**Table 30**) enhanced the statistical significance of the findings in **Table 29**, and revealed significant reductions of up to 31 percent (95% CI, 1 – 52) amongst PCV recipients, P=0.037.

The apparent PCV-induced reductions in the case load of bacteriologically-confirmed PTB in the cohort was due to effects on culture-proven disease only, as can be appreciated from **Table 31** which indicates that there was a tendency for PCV recipients to have more episodes of smear-positive PTB without culture confirmation than did the placebo recipients (OR 1.20; 95% CI, 0.57 – 2.56), P=0.606. That this tendency failed to erode the statistical significance of the analysis of the combined bacteriologically-confirmed cases (**Table 30**) highlights the significance of the analysis as conducted for culture-confirmed disease.

Age group (months)	Hospital child-years of follow-up		Number of culture-positive PTB cases diagnosed [+]		Definite tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	6	12	81 (30 – 177)	163 (84 – 285)	0.50 (0.15 – 1.44)	0.166
6.1 – 12.0	9841	9827	4	9	41 (11 – 104)	92 (42 – 174)	0.44 (0.10 – 1.59)	0.179
1.5 – 12.0	**17 206**	**17 183**	**10**	**21**	**58 (28 – 107)**	**122 (76 – 187)**	**0.48 (0.20 – 1.06)**	**0.050**
12.1 – 24.0	19 675	19 647	10	12	51 (24 – 93)	61 (32 – 107)	0.83 (0.32 – 2.10)	0.675
24.1 – 36.0	**19 666**	**19 639**	**1**	**8**	**5 (0.1 – 28)**	**41 (18 – 80)**	**0.12 (0.00 – 0.93)**	**0.021**
36.1 – 48.0	19 664	19 637	7	7	36 (14 – 73)	36 (14 – 73)	1.00 (0.30 – 3.34)	0.998
48.1 – 60.0	21 596	21 685	4	7	19 (5 – 47)	32 (13 – 67)	0.57 (0.12 – 2.26)	0.391
>60.0	52 478	52 595	4	8	8 (2 – 20)	15 (7 – 30)	0.50 (0.11 – 1.87)	0.269
All ages	**150 285**	**150 386**	**36**	**63**	**24 (17 – 33)**	**42 (32 – 54)**	**0.57 (0.37 – 0.87)**	**0.007**

Table 28: Incidence of definite PTB, according to vaccination status: all tuberculosis episodes

[+]All culture-confirmed PTB cases (including recurrent episodes).

Age group (months)	Hospital child-years of follow-up		Number of culture- and smear-positive PTB cases diagnosed *		Confirmed tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	9	16	122 (56 – 232)	218 (124 – 353)	0.56 (0.22 – 1.35)	0.168
6.1 – 12.0	9841	9827	6	11	61 (22 – 133)	112 (56 – 200)	0.54 (0.17 – 1.61)	0.237
1.5 – 12.0	17 206	17 183	15	27	87 (49 – 144)	157 (104 – 229)	0.55 (0.27 – 1.08)	0.065
12.1 – 24.0	19 675	19 647	11	14	56 (28 – 100)	71 (39 – 120)	0.78 (0.32 – 1.86)	0.555
24.1 – 36.0	19 666	19 639	2	8	10 (1 – 37)	41 (18 – 80)	0.25 (0.03 – 1.25)	0.065
36.1 – 48.0	19 664	19 637	7	6	36 (14 – 73)	31 (11 – 67)	1.17 (0.34 – 4.20)	0.792
48.1 – 60.0	21 596	21 685	4	5	19 (5 – 47)	23 (7 – 54)	0.80 (0.16 – 3.73)	0.759
>60.0	52 478	52 595	6	5	11 (4– 25)	10 (3 – 22)	1.20 (0.31 – 4.98)	0.772
All ages	150 285	150 386	45	65	30 (22 – 40)	43 (33 – 55)	0.69 (0.46 – 1.03)	0.058

Table 29: Incidence of bacteriologically-confirmed PTB, according to vaccination status: incident tuberculosis

* Bacteriologically-confirmed PTB (smear and culture-positive), excluding recurrent cases.

Age group (months)	Hospital child-years of follow-up		Number of culture- and smear-positive PTB cases diagnosed [+]		Confirmed tuberculosis incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	9	16	122 (56 – 232)	218 (124 – 353)	0.56 (0.22 – 1.35)	0.168
6.1 – 12.0	9841	9827	6	11	61 (22 – 133)	112 (56 – 200)	0.54 (0.17 – 1.61)	0.237
1.5 – 12.0	17 206	17 183	15	27	87 (49 – 144)	157 (104 – 229)	0.55 (0.27 – 1.08)	0.065
12.1 – 24.0	19 675	19 647	12	14	61 (32 – 107)	71 (39 – 120)	0.86 (0.36 – 1.99)	0.698
24.1 – 36.0	19 666	19 639	3	9	15 (3 – 45)	46 (21 – 87)	0.33 (0.06 – 1.33)	0.092
36.1 – 48.0	19 664	19 637	7	9	36 (14 – 73)	46 (21 – 87)	0.78 (0.25 – 2.34)	0.627
48.1 – 60.0	21 596	21 685	6	8	28 (10 – 60)	37 (16 – 73)	0.75 (0.22 – 2.48)	0.613
>60.0	52 478	52 595	11	11	21 (10 – 38)	21 (10 – 37)	1.00 (0.39 – 2.55)	0.996
All ages	**150 285**	**150 386**	**54**	**78**	**36 (27 – 47)**	**52 (41 – 65)**	**0.69 (0.48 – 0.99)**	**0.037**

Table 30: Incidence of bacteriologically-confirmed PTB, according to vaccination status: all tuberculosis episodes

[+] All bacteriologically-confirmed PTB (smear and culture-positive cases), including recurrences.

Age group (months)	Hospital child-years of follow-up		Number of smear-positive PTB cases diagnosed *		Confirmed PTB incidence (cases per 100 000 hospital child-years of follow-up, 95% CI)		Incidence Rate Ratio (95% CI)	P
	PCV	Placebo	PCV	Placebo	PCV	Placebo		
1.5 – 6.0	7365	7356	3	4	41 (8 – 119)	54 (15 – 139)	0.75 (0.11 – 4.43)	0.725
6.1 – 12.0	9841	9827	2	2	20 (2 – 73)	20 (2 – 74)	1.00 (0.07 – 13.78)	0.999
1.5 – 12.0	17 206	17 183	5	6	29 (9 – 68)	35 (13 – 76)	0.83 (0.20 – 3.27)	0.773
12.1 – 24.0	19 675	19 647	2	2	10 (1 – 37)	10 (1 – 37)	1.00 (0.07 – 13.78)	0.999
24.1 – 36.0	19 666	19 639	2	1	10 (1 – 37)	5 (0.1 – 28)	2.00 (0.10 – 117.83)	0.626
36.1 – 48.0	19 664	19 637	0	2	0 (0 – 19)	10 (1 – 37)	0 (0 – 5.32)	0.250
48.1 – 60.0	21 596	21 685	2	1	9 (1 – 33)	5 (0.1 – 26)	2.01 (0.10 – 118.48)	0.623
>60.0	52 478	52 595	7	3	13 (5 – 27)	6 (1 – 17)	2.33 (0.53 – 14.01)	0.225
All ages	150 285	150 386	18	15	12 (7 – 19)	10 (6 – 16)	1.20 (0.57 – 2.56)	0.606

Table 31: Incidence of confirmed PTB, according to vaccination status: all tuberculosis episodes

[+] All smear-positive PTB cases, including recurrences.

When exploring the relationship between vaccine and culture-proven tuberculosis by HIV status, significant differences in the incidences of culture-confirmed PTB could be demonstrated in the HIV-infected children, and a similar (although non-significant) trend was observed in the HIV-uninfected children. HIV-infected children who received PCV exhibited a 47 percent (95% CI, 10 – 69) reduction in hospitalisation with culture positive PTB compared to those who received placebo, P=0.017 (**Table 32**).

When children who were diagnosed with recurrent tuberculosis episodes were included in the analysis (**Table 33**), this effect was again demonstrated amongst the culture-confirmed cases that were HIV-infected, with a calculated 44 percent decrease in culture-confirmed tuberculosis cases (95% CI, 10 – 66), P=0.015, amongst PCV recipients compared to placebo recipients.

These data suggest that up to 47 percent of culture-confirmed PTB in HIV-infected children occurs in the context of concurrent *S. pneumoniae* infection. As proportions of HIV-infected children in the definite PTB group according to vaccine status (20 [64.5%] of 31 cases in PCV recipients versus 38 [69.1%] of 55 cases in placebo recipients, **Table 32**) did not differ significantly, P=0.664, these findings are likely to represent a true PCV effect, and not merely a chance finding following selection bias between the vaccination groups.

The intention-to-treat analysis (as conducted in **Table 34**) failed to erode the statistical significance of the reduction in culture-confirmed tuberculosis amongst PCV-vaccinated HIV-infected children compared to those who received placebo (RR 0.53; 95% CI, 0.31 – 0.90), P=0.017.

	All PTB *			Confirmed PTB *			Culture-confirmed PTB *		
	All cases	HIV-infected	HIV-uninfected	All cases	HIV-infected	HIV-uninfected	All cases	HIV-infected	HIV-uninfected
Vaccinated group (%)	185 / 19 922 (0.93)	119 / 1289 (9.23)	66 / 18 633 (0.35)	14 / 19 922 (0.07)	9 / 1289 (0.70)	5 / 18 633 (0.03)	**31 / 19 922 (0.16)**	**20 / 1289 (1.55)**	11 / 18 633 (0.06)
Placebo group (%)	204 / 19 914 (1.02)	138 / 1288 (10.71)	66 / 18 626 (0.35)	9 / 19 914 (0.05)	6 / 1288 (0.47)	3 / 18 626 (0.02)	**55 / 19 914 (0.28)**	**38 / 1288 (2.95)**	17 / 18 626 (0.09)
Risk Ratio	0.91	0.86	1.00	1.55	1.50	1.67	**0.56**	**0.53**	0.65
95% Confidence Interval	0.74 – 1.11	0.68 – 1.09	0.71 – 1.41	0.67 – 3.59	0.54 – 4.20	0.40 – 6.97	**0.36 – 0.87**	**0.31 – 0.90**	0.30 – 1.38
P	0.331	0.209	0.998	0.298	0.438	0.727 §	**0.010**	**0.017**	0.256

Table 32: PTB cases according to HIV and vaccination status: incident tuberculosis

* Excluding recurrent PTB episodes. Denominators for each group by HIV and vaccination status are derived from **Table 1**.

§ Fisher's exact test.

	All PTB [+]			Number Confirmed PTB cases diagnosed [+]			Culture-confirmed PTB [+]		
	All cases	HIV-infected	HIV-uninfected	All cases	HIV-infected	HIV-uninfected	All cases	HIV-infected	HIV-uninfected
Vaccinated group (%)	211 / 19 922 (1.06)	143 / 1289 (11.09)	68 / 18 633 (0.36)	18 / 19 922 (0.09)	13 / 1289 (1.01)	5 / 18 633 (0.03)	**36 / 19 922 (0.18)**	**25 / 1289 (1.94)**	11 / 18 633 (0.06)
Placebo group (%)	239 / 19 914 (1.20)	168 / 1288 (13.04)	71 / 18 626 (0.38)	14 / 19 914 (0.07)	11 / 1288 (0.85)	3 / 18 626 (0.02)	**63 / 19 914 (0.32)**	**45 / 1288 (3.49)**	18 / 18 626 (0.10)
Risk Ratio	0.88	0.85	0.96	1.81	1.18	1.67	**0.57**	**0.56**	0.61
95% Confidence Interval	0.73 – 1.06	0.69 – 1.05	0.69 – 1.33	0.53 – 2.63	0.53 – 2.63	0.40 – 6.97	**0.38 – 0.86**	**0.34 – 0.90**	0.29 – 1.29
P	0.183	0.129	0.797	0.683	0.683	0.727 [§]	**0.007**	**0.015**	0.193

Table 33: PTB cases according to HIV and vaccination status: all tuberculosis episodes

[+] Including recurrent PTB cases. Denominators for each group by HIV and vaccination status are derived from **Table 1**.

[§] Fisher's exact test.

		All PTB*		Culture-confirmed PTB*	
		Intention-to-treat [+]	Per protocol [++]	Intention-to-treat [+]	Per protocol [++]
HIV-infected	Vaccinated group (%)	118 / 1289 (9.2)	104 / 1201 (8.7)	**20 / 1289 (1.6)**	**17 / 1201 (1.4)**
	Placebo group (%)	138 / 1288 (10.7)	132 / 1200 (11.0)	**38 / 1288 (3.0)**	**34 / 1200 (2.8)**
	Risk Ratio	0.85	0.79	**0.53**	**0.50**
	95% Confidence Interval	0.68 – 1.08	0.62 – 1.00	**0.31 – 0.90**	**0.28 – 0.89**
	P	0.186	0.054	**0.017**	**0.016**
HIV-uninfected	Vaccinated group (%)	63 / 18633 (0.3)	55 / 17356 (0.3)	11 / 18633 (0.06)	11 / 17356 (0.06)
	Placebo group (%)	66 / 18626 (0.4)	62 / 17350 (0.4)	17 / 18626 (0.09)	16 / 17350 (0.09)
	Risk Ratio	0.95	0.89	0.68	0.69
	95% Confidence Interval	0.68 – 1.35	0.62 – 1.27	0.32 – 1.45	0.32 – 1.48
	P	0.790	0.516	0.319	0.335

Table 34: Risk for PTB amongst children by HIV and vaccination status

* Excluding recurrent PTB cases.

[+] Intention-to-treat analysis includes children who received fewer than 3 doses of PCV or placebo.

[++] Per protocol analysis: children that received 3 doses of PCV or placebo as per study schedule and in whom vaccine efficacy-related events occurred more than 14 days following the third dose of study vaccine.

3.3 The Burden of Tuberculosis Amongst PCV Study Participants

3.3.1 Incidence rates of tuberculosis for the cohort by HIV status

The incidence rate for PTB, of which 391 cases were identified in the PCV Study cohort, was 982 per 100 000 (95% CI, 887 – 1084). Three hundred and eighty-six incident cases of PTB arose in children with defined HIV status: 255 (66.1%) of these arose amongst HIV-infected children, yielding an incidence rate for PTB in these children of 9895 per 100 000 (95% CI, 8718 – 11 187), whilst 131 (33.9%) incident PTB cases were identified in HIV-uninfected children (352 cases per 100 000 children: 95% CI, 294 – 417). HIV-infected children had a 28-fold increased risk for incident PTB (95% CI, 22.9 – 34.6) compared to HIV-uninfected children, P<0.001 (**Table 35**).

The tuberculosis incidence rate for all forms of the disease (425 incident cases in the study cohort of 39 836 children) was 1067 cases per 100 000 (95% CI, 968 – 1173): 296 per 100 000 (95% CI, 245 – 355) for bacteriologically-confirmed tuberculosis (**Table 35**). HIV-infected children carried an incidence rate of 10 633 (95% CI, 9411 – 11 969) per 100 000 for all forms of tuberculosis, and a rate of 3027 (95% CI, 2393 – 3778) per 100 000 for bacteriologically-confirmed disease. HIV-uninfected children carried incidence rates of 386 (95% CI, 326 – 455) per 100 000, and 105 (95% CI, 74 – 143) per 100 000 for all forms of tuberculosis and bacteriologically-confirmed tuberculosis, respectively (**Table 35** and **Figure 8**). This translated into a significantly greater risk of active tuberculosis amongst the HIV-infected children compared to those who were HIV-uninfected (RR 27.5; 95% CI, 22.6 – 33.5), P<0.001, for all forms of tuberculosis, as well as for bacteriologically-confirmed tuberculosis (RR 28.9; 95% CI, 19.7 – 42.4), P<0.001.

	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (HIV-infected to HIV-uninfected, using baseline cohort size[+])	P
	All *	HIV-infected	HIV-uninfected	All	HIV-infected	HIV-uninfected		
Definite PTB	86	58	28	216 (173 – 267)	2251 (1709 – 2910)	75 (50 – 109)	29.9 (19.1 – 46.9)	< 0.001
Definite EPTB	8	5	3	20 (9 – 40)	194 (63 – 453)	8 (2 – 24)	24.1 (5.8 – 100.8)	< 0.001 [§]
Confirmed PTB	24	15	8	60 (39 – 90)	582 (326 – 960)	21 (9 – 42)	27.1 (11.5 – 63.9)	< 0.001 [§]
Bacteriologically-confirmed tuberculosis**	118	78	39	296 (245 – 355)	3027 (2393 – 3778)	105 (74 – 143)	28.9 (19.7 – 42.4)	< 0.001
Probable PTB	122	64	55	306 (254 – 366)	2484 (1913 – 3171)	148 (111 – 192)	16.8 (11.8 – 24.1)	< 0.001
Probable EPTB	25	13	10	63 (41 – 93)	504 (269 – 863)	27 (13 – 49)	18.7 (8.2 – 42.8)	< 0.001 [§]
Suspected tuberculosis	160	119	40	402 (342 – 469)	4618 (3825 – 5526)	107 (77 – 146)	43.0 (30.1 – 61.4)	< 0.001
All PTB	391	255	131	982 (887 – 1084)	9895 (8718 – 11 187)	352 (294 – 417)	28.1 (22.9 – 34.6)	<0.001
All tuberculosis	425	274	144	1067 (968 – 1173)	10 633 (9411 – 11 969)	386 (326 – 455)	27.5 (22.6 – 33.5)	< 0.001

Table 35: Incidence rates for tuberculosis in the PCV Study cohort: first episode tuberculosis

* Including cases with undefined HIV status.

** Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: HIV-infected (n=2577), HIV-uninfected (n=37 259). [§] Fisher's exact test.

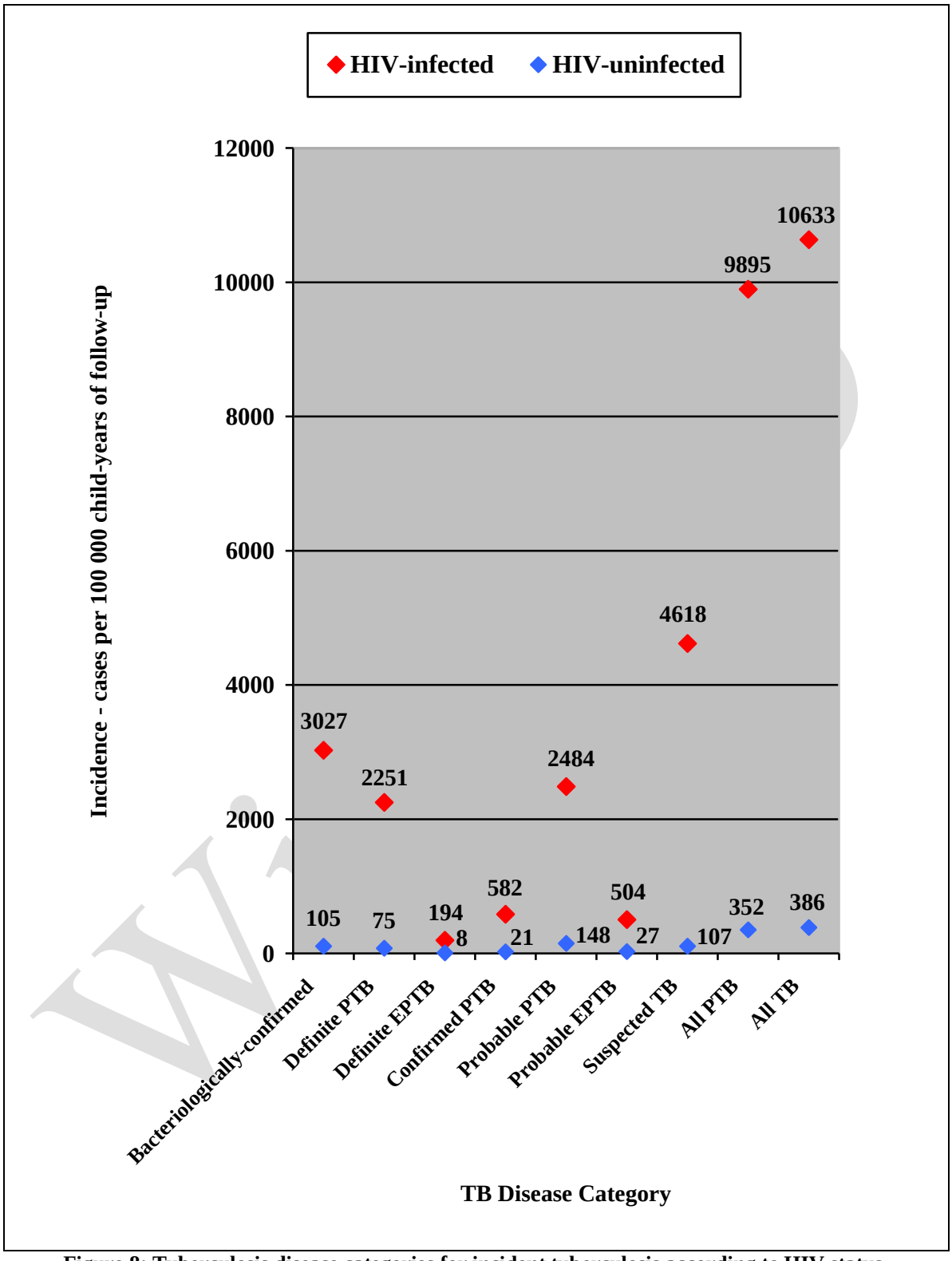


Figure 8: Tuberculosis disease categories for incident tuberculosis according to HIV status

This Figure is derived from **Table 35**.

	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (HIV-infected to HIV-uninfected, using baseline cohort size[+])	P
	All *	HIV-infected	HIV-uninfected	All	HIV-infected	HIV-uninfected		
Definite PTB	99	70	29	249 (202 – 303)	2716 (2118 – 3432)	78 (52 – 112)	34.9 (22.7 – 53.7)	< 0.001
Definite EPTB	9	6	3	23 (10 – 43)	233 (85 – 507)	8 (2 – 24)	28.9 (7.2 – 115.6)	< 0.001
Confirmed PTB	33	24	8	83 (57 – 116)	931 (597 – 1386)	21 (9 – 42)	43.4 (19.5 – 96.5)	< 0.001 [§]
Bacteriologically-confirmed tuberculosis**	141	100	40	354 (298 – 417)	3880 (3157 – 4720)	107 (77 – 146)	36.1 (5.1 – 52.0)	< 0.001
Probable PTB	136	75	58	341 (286 – 404)	2910 (2289 – 3648)	156 (118 – 201)	18.7 (13.3 – 26.3)	< 0.001
Probable EPTB	33	21	10	83 (57 – 116)	815 (504 – 1246)	27 (13 – 49)	30.4 (14.3 – 64.4)	< 0.001 [§]
Suspected tuberculosis	182	138	43	457 (393 – 528)	5355 (4499 – 6327)	115 (84 – 155)	46.4 (33.0 – 65.2)	< 0.001
All PTB	449	306	138	1127 (1025 – 1236)	11 874 (10 581 – 13 282)	370 (311 - 438)	32.1 (26.3 – 39.0)	<0.001
All tuberculosis	492	334	151	1235 (1128 – 1349)	12 961 (11 608 – 14 428)	405 (343 – 475)	32.0 (26.5 – 38.6)	< 0.001

Table 36: Incidence rates for tuberculosis in the PCV Study cohort: all episodes

* Including cases with undefined HIV status.

** Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: HIV-infected (n=2577), HIV-uninfected (n=37 259). [§] Fisher's exact test.

Table 36 explores the Public Health impact of hospital-diagnosed tuberculosis in the PCV study cohort according to HIV status by including all tuberculosis episodes in the analysis. This analysis is important, as the hospital-based diagnosis of tuberculosis in these children would have necessitated referral to primary health care clinics for on-going treatment of their disease. It is therefore useful to estimate the additional burden which recurrent tuberculosis episodes would have on the Primary Health Care System.

3.3.2 HIV-uninfected status as a proxy for 'tuberculosis vaccination'

An alternative means of confirming the calculated relative risk for active tuberculosis disease in HIV-infected compared to HIV-uninfected children, is to apply a 'vaccine efficacy' calculation using the proportions of cases of tuberculosis arising within a cohort according to the HIV status of the participants[48 49]. As all of the study participants received BCG at birth regardless of HIV-infection status, some degree of BCG-derived background immunity against tuberculosis would be anticipated in the cohort.

In the context of tuberculosis, one could consider HIV infection (with its adverse impact on immune status) as diminishing the BCG-primed, intact immune system's ability to maintain tuberculosis infection in its latent state; consequently, HIV-infected individuals are at greater risk for progression to active tuberculosis disease after primary infection with MTB. BCG-vaccinated, HIV-uninfected children are essentially protected (or 'vaccinated') against active tuberculosis compared to HIV-infected children by virtue of their intact immune surveillance mechanisms. The calculation is highlighted in **Appendix 6**, page 133.

When comparing the relative burden of tuberculosis in the HIV-infected children to that in the HIV-uninfected children, the IRR of 27.5 using the 'vaccine efficacy' calculation was equivalent to the measured Risk Ratio of 27.5 which was derived from the statistical analysis of the cohort disease burden in **Section 3.3.1**. It is noteworthy that, by using this method, it can also be shown that HIV-infected children were 29.4 times more likely to develop culture-confirmed tuberculosis compared to HIV-uninfected children (see **Appendix 6**, page 133). An additional important finding of this approach is that the immune response to BCG in an HIV-uninfected child appears to be 96.4 percent relatively more effective in preventing progression to active tuberculosis compared to the immune system of a BCG-vaccinated HIV-infected child.

3.3.3 Incidence rates of tuberculosis for the cohort by vaccination status

As has been demonstrated in **Section 3.2**, PCV played a significant role in limiting the incidence of culture-confirmed PTB amongst HIV-infected children.

When analysing the tuberculosis incidence rates for different categories of tuberculosis disease in the cohort according to vaccination status, children who received PCV had a significantly lower burden of culture-confirmed PTB episodes (RR 0.56; 95% CI, 0.36 – 0.87), P=0.010, compared to the placebo recipients (**Table 37**).

In the incidence rate calculation of all tuberculosis cases in children with defined HIV status (**Table 38**), PCV was associated with a significant reduction in culture-confirmed PTB (RR 0.57; 95% CI, 0.38 – 0.86), P=0.007.

	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	**86**	**31**	**55**	**216 (173 – 267)**	**156 (106 – 221)**	**276 (208 – 360)**	**0.56 (0.36 – 0.87)**	**0.010**
Definite EPTB	8	2	6	201 (87 – 396)	10 (1 – 36)	30 (11 – 66)	0.33 (0.07 – 1.65)	0.180 [§]
Confirmed PTB	24	14	10	60 (39 – 90)	70 (38 – 118)	50 (24 – 92)	1.40 (0.62 – 3.15)	0.415
Bacteriologically-confirmed tuberculosis*	**118**	**47**	**71**	**296 (245 – 355)**	**236 (173 – 314)**	**357 (278 – 450)**	**0.66 (0.46 – 0.96)**	**0.027**
Probable PTB	122	60	62	306 (254 – 366)	301 (230 – 388)	311 (239 – 399)	0.97 (0.68 – 1.38)	0.854
Probable EPTB	25	17	8	63 (41 – 93)	85 (50 – 137)	40 (17 – 79)	2.12 (0.92 – 4.92)	0.072
Suspected tuberculosis	160	81	79	402 (342 – 469)	407 (323 – 505)	397 (314 – 494)	1.02 (0.75 – 1.40)	0.876
All PTB	391	185	206	1067 (968 – 1173)	1029 (893 – 1180)	1034 (898 – 1156)	0.90 (0.74 – 1.09)	0.284
All tuberculosis	425	205	220	1067 (968 – 11 733)	1029 (893 – 1180)	1105 (964 – 1261)	0.93 (0.77 – 1.13)	0.462

Table 37: Incidence of tuberculosis according to disease category and vaccination status: first episode tuberculosis

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=19 922), Placebo (n=19 914). [§] Fisher's exact test.

	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	**99**	**36**	**63**	**249 (202 – 303)**	**181 (127 – 250)**	**316 (243 – 405)**	**0.57 (0.38 – 0.86)**	**0.007**
Definite EPTB	9	2	7	23 (10 – 43)	10 (1 – 36)	35 (14 – 72)	0.29 (0.06 – 1.37)	0.109 [§]
Confirmed PTB	33	18	15	83 (57 – 116)	90 (54 – 143)	75 (42 – 124)	1.20 (0.60 – 2.38)	0.602
Bacteriologically-confirmed tuberculosis*	**141**	**56**	**85**	**354 (298 – 417)**	**281 (212 – 365)**	**427 (341 – 528)**	**0.66 (0.47 – 0.92)**	**0.014**
Probable PTB	136	65	71	341 (286 – 404)	326 (252 – 416)	357 (278 – 450)	0.92 (0.65 – 1.28)	0.605
Probable EPTB	33	21	12	83 (57 – 116)	105 (65 – 161)	60 (31 – 105)	1.75 (0.86 – 3.55)	0.117
Suspected tuberculosis	182	91	91	457 (393 – 528)	457 (368 – 561)	457 (368 – 561)	1.00 (0.75 – 1.34)	1.000
All PTB	449	209	240	1127 (1025 – 1236)	1049 (912 – 1201)	1205 (1058 – 1368)	0.87 (0.72 – 1.05)	0.140
All tuberculosis	492	233	259	1235 (1128 – 1349)	1170 (1024 – 1330)	1301 (1147 – 1469)	0.90 (0.75 – 1.07)	0.236

Table 38: Incidence of tuberculosis according to disease category and vaccination status: all episodes

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=19 922), Placebo (n=19 914). [§] Fisher's exact test.

3.3.4 Incidence rates of tuberculosis for the cohort by vaccination and HIV status

Tables 39 through **42** illustrate the tuberculosis incidence rates by category of tuberculosis disease according to vaccination and HIV status, and highlight the fact that PCV recipients who were HIV-infected had a significant reduction in incident and recurrent culture-confirmed PTB.

PCV-vaccinated HIV-infected children with incident tuberculosis exhibited a 47 percent (95% CI, 10 – 69) reduction in culture confirmed PTB compared to the placebo recipients, P=0.017 (**Table 39**); there was a 44 percent (95% CI, 10 – 66), P=0.015, reduction in culture-confirmed PTB in HIV-infected PCV recipients compared to the placebo recipients when analysing for all tuberculosis episodes (**Table 40**).

This effect was not readily appreciated in PCV-vaccinated HIV-uninfected children in whom relative risks for culture-confirmed PTB were not statistically diminished compared to those in the placebo group (**Tables 41** and **42**). The inability to demonstrate a reduction in culture-confirmed tuberculosis amongst HIV-uninfected PCV recipients, whereas significance was demonstrated in the HIV-infected children, may be because the study was not adequately powered to explore this effect amongst HIV-uninfected children.

HIV-infected	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	**58**	**20**	**38**	**2251 (1709 – 2910)**	**1552 (948 – 2396)**	**2950 (2088 – 4050)**	**0.53 (0.31 – 0.90)**	**0.017**
Definite EPTB	5	2	3	194 (63 – 453)	155 (19 – 560)	233 (48 – 681)	0.67 (0.11 – 3.98)	0.687 [§]
Confirmed PTB	15	9	6	582 (326 – 960)	698 (319 – 1325)	466 (171 – 1014)	1.50 (0.54 – 4.20)	0.438
Bacteriologically-confirmed tuberculosis*	78	31	47	3027 (2393 – 3778)	2405 (1634 – 3414)	3649 (2681 – 4852)	0.66 (0.42 – 1.03)	0.065
Probable PTB	64	32	32	2484 (1913 – 3171)	2483 (1698 – 3505)	2484 (1699 – 3507)	1.00 (0.62 – 1.62)	1.000
Probable EPTB	13	7	6	504 (269 – 863)	543 (218 – 112)	466 (171 – 1014)	1.17 (0.39 – 3.46)	0.782
Suspected tuberculosis	119	58	61	4618 (3825 – 5526)	4500 (3417 – 5817)	4736 (3623 – 6084)	0.95 (0.67 – 1.35)	0.775
All PTB	255	118	137	9895 (872 – 11 187))	9154 (7577 – 10 963)	10 637 (893 – 12 574)	0.86 (0.68 – 1.09)	0.208
All tuberculosis	274	128	146	10 633 (9411 – 11 969)	9930 (8285 – 11 807)	11 335 (9571 – 13 330)	0.88 (0.70 – 1.10)	0.247

Table 39: Incidence of tuberculosis according to vaccination status in HIV-infected children: first episode tuberculosis

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=1289), Placebo (n=1288). [§] Fisher's exact test.

HIV-infected	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	**70**	**25**	**45**	**2716 (2118 – 3432)**	**1939 (1255 – 2863)**	**3494 (2548 – 4675)**	**0.56 (0.34 – 0.90)**	**0.015**
Definite EPTB	6	2	4	233 (85 – 507)	155 (19 – 560)	311 (85 – 795)	0.50 (0.09 – 2.72)	0.453 [§]
Confirmed PTB	24	13	11	931 (597 – 1386)	1009 (537 – 1725)	854 (426 – 1528)	1.18 (0.53 – 2.63)	0.683
Bacteriologically-confirmed tuberculosis*	**100**	**40**	**60**	**3880 (3157 – 4720)**	**3103 (2217 – 4226)**	**4658 (3555 – 5996)**	**0.67 (0.45 – 0.99)**	**0.041**
Probable PTB	75	36	39	2910 (2289 – 3648)	2793 (1956 – 3867)	3028 (2153 – 4139)	0.92 (0.59 – 1.44)	0.723
Probable EPTB	21	11	10	815 (504 – 1246)	853 (426 – 1527)	776 (372 – 1428)	1.10 (0.47 – 2.58)	0.828
Suspected tuberculosis	138	67	71	5355 (4499 – 6327)	5198 (4028 – 6601)	5512 (4305 – 6953)	0.94 (0.68 – 1.30)	0.723
All PTB	306	140	166	11 874 (10 581 – 13 282)	10 861 (9137 – 12 817)	12 888 (11 002 – 15 005)	0.84 (0.68 – 1.04)	0.112
All tuberculosis	334	154	180	12 961 (11 608 – 14 428)	11 947 (10 135 – 13 990)	13 975 (12 008 – 16 173)	0.85 (0.70 – 1.05)	0.125

Table 40: Incidence of tuberculosis according to vaccination status in HIV-infected children: all episodes

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=1289), Placebo (n=1288). [§] Fisher's exact test.

HIV-uninfected	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	28	11	17	75 (50 – 109)	59 (29 – 106)	91 (53 – 146)	0.65 (0.30 – 1.38)	0.256
Definite EPTB	3	0	3	8 (2 – 24)	0 (0 – 20)	16 (3 – 47)	-	0.125 [§]
Confirmed PTB	8	5	3	21 (9 – 42)	27 (9 – 63)	16 (3 – 47)	1.67 (0.40 – 6.97)	0.727 [§]
Bacteriologically-confirmed tuberculosis*	39	16	23	105 (74 – 143)	86 (49 – 139)	123 (78 – 185)	0.70 (0.37 – 1.32)	0.262
Probable PTB	55	27	28	148 (111 – 192)	145 (95 – 211)	150 (100 – 217)	0.96 (0.57 – 1.63)	0.892
Probable EPTB	10	8	2	27 (13 – 49)	43 (19 – 85)	11 (1 – 39)	4.00 (0.85 – 18.83)	0.109 [§]
Suspected tuberculosis	40	22	18	107 (77 – 146)	118 (74 – 179)	97 (57 – 153)	1.22 (0.66 – 2.28)	0.528
All PTB	131	65	66	352 (294 – 417)	349 (269 – 445)	354 (274 – 251)	0.98 (0.70 – 1.39)	0.929
All tuberculosis	144	73	71	386 (326 – 455)	392 (307 – 493)	381 (298 – 481)	1.03 (0.74 – 1.42)	0.869

Table 41: Incidence of tuberculosis according to vaccination status in HIV-uninfected children: fist episode tuberculosis

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=18 633), Placebo (n=18 626). [§] Fisher's exact test.

HIV-uninfected	Tuberculosis cases identified			Tuberculosis Incidence Rates (cases per 100 000 PCV participants), 95% CI			Risk Ratio (PCV and Placebo groups, using baseline cohort size[+])	P
	All	PCV	Placebo	All	PCV	Placebo		
Definite PTB	29	11	18	78 (52 – 112)	59 (29 – 106)	97 (57 – 153)	0.61 (0.29 – 1.29)	0.193
Definite EPTB	3	0	3	8 (2 – 24)	0 (0 – 20)	16 (3 – 47)	-	0.125 [§]
Confirmed PTB	8	5	3	21 (9 – 42)	27 (9 – 63)	16 (3 – 47)	1.69 (0.40 – 6.97)	0.727 [§]
Bacteriologically-confirmed tuberculosis*	40	16	24	107 (77 – 146)	86 (49 – 139)	129 (83 – 192)	0.67 (0.35 – 1.25)	0.205
Probable PTB	58	28	30	156 (118 – 201)	150 (100 – 217)	161 (109 – 230)	0.93 (0.56 – 1.56)	0.792
Probable EPTB	10	8	2	27 (13 – 49)	43 (19 – 85)	11 (1 – 39)	4.00 (0.85 – 18.83)	0.109 [§]
Suspected tuberculosis	43	23	20	115 (84 – 155)	123 (78 – 185)	107 (66 – 166)	1.15 (0.63 – 2.09)	0.648
All PTB	138	67	71	370 (311 – 438)	360 (279 – 457)	381 (298 – 481)	0.94 (0.68 – 1.32)	0.731
All tuberculosis	151	75	76	405 (343 – 475)	403 (317 – 505)	408 (321 – 511)	0.99 (0.72 – 1.36)	0.933

Table 42: Incidence of tuberculosis according to vaccination status in HIV-uninfected children: all episodes

* Bacteriologically-confirmed tuberculosis consists of 'Definite PTB', 'Definite EPTB' and 'Confirmed PTB' cases.

[+] Baseline cohort size: PCV (n=18 633), Placebo (n=18 626). [§] Fisher's exact test.

4.0 DISCUSSION

4.1 General Comments

This retrospective analysis of secondary endpoints of an important PCV efficacy trial which was conducted in Soweto from 1998 to 2006 elucidates some intriguing information regarding the burden and spectrum of tuberculosis in children below the age of six years that were resident in the study setting.

A large proportion (64.5%) of the children with tuberculosis, and 66 percent of all incident culture-proven disease in this cohort, had confirmed HIV infection, as has been demonstrated in a number of descriptive studies from Southern Africa which have revealed that 11 to 64 percent of childhood tuberculosis cases in the region are HIV-infected [15 51 52].

As this study was largely conducted before April 2004, when antiretroviral therapy was first made widely available to patients in the public health sector of South Africa, these findings represent the natural history of HIV and tuberculosis (HIV-TB) co-infection in high HIV-TB burdened settings in the absence of antiretroviral therapy.

The finding that the median age of children diagnosed with tuberculosis was significantly older in HIV-infected children (19.8 months; IQR 7.7 – 44.3) compared to HIV-uninfected children (12.3 months; IQR 6.0 – 26.3), $P<0.001$, indicates that these individuals remain persistently vulnerable to progression of LTBI to active disease. This was further illustrated by the finding of significantly higher tuberculosis incidence rates amongst the HIV-infected children in the cohort, these children having a 27.5-fold greater burden of tuberculosis

(95% CI, 22.6 – 33.5), P<0.001, compared to HIV-uninfected children (**Table 35** and **Figure 8**). This relative risk for tuberculosis corresponds well with that which was estimated to have occurred in a cohort of paediatric patients with pneumonia that was investigated by Madhi *et al.* at CHBH in 1997 and 1998, where HIV-infected children were found to have a 22.5-fold increased risk for active tuberculosis compared to HIV-uninfected children[23]; additionally, a recent study reporting on the incidence of culture-confirmed tuberculosis in an infant cohort in Cape Town identified a relative risk of tuberculosis amongst HIV-infected compared to HIV-uninfected infants of 24.2 (95% CI, 17 – 34)[53].

A novel finding in this study, through the application of the 'vaccine efficacy' calculation in order to derive the degree of protection which a BCG-vaccinated, immunocompetent (i.e. HIV-uninfected) immune system confers in limiting progression to active tuberculosis, is that BCG vaccine in HIV-uninfected children is 96 percent relatively more effective in protecting against active disease (all forms, as well as culture-confirmed cases) compared to BCG-vaccinated HIV-infected children in children hospitalised with tuberculosis (see **Section 3.3.2**, page 93 and **Appendix 6**, page 133). This finding adds further motivation to strengthen prevention of mother to child transmission (PMTCT) programmes in high-burdened settings, in an effort to prevent vertical transmission of HIV to infants[54].

Although there is evidence to support a reduction in vulnerability to tuberculosis in HIV-infected children who have been initiated on highly active antiretroviral therapy (HAART)[55], this effect could not be adequately explored in this cohort because data

relating to which of the children were initiated on HAART during the study period were not captured for analysis.

Not only do the results of this study offer a window onto the natural history of HIV-TB co-infection in the pre-HAART era as mentioned above, but it also offers a unique perspective on the interplay between *S. pneumoniae*, the PCV and MTB, highlighting the fact that a substantial proportion of culture-confirmed tuberculosis in hospitalised children in a sub-Saharan African setting arises in the context of pneumococcal co-infection. Demonstration of the latter effect may be difficult to reproduce in South Africa because the National Department of Health has recently included the PCV in the Expanded Programme on Immunisation (EPI) schedule and nation-wide coverage of infants is anticipated to commence in April 2009[56].

4.2 Effect of the PCV on Tuberculosis Incidence in the Study Cohort

Receipt of the 9-valent PCV was demonstrated to be associated with a significant reduction in culture-confirmed tuberculosis in the study cohort for incident tuberculosis episodes (**Table 4**), with an apparent 40 percent (95% CI, 10 – 59) risk reduction in incident culture-confirmed PTB, P=0.011, in vaccine compared to placebo recipients, suggesting that pneumococcal co-infection frequently plays a role in co-infection in paediatric cases with culture-confirmed tuberculosis.

The incidence of PTB was demonstrably lower in vaccine recipients compared to those who received placebo up to the age of 36 months in the analysis of incident cases, although

numbers of cases and cohort size constrained demonstration of statistical significance in this analysis (**Table 25**).

Reductions in the burden of culture- and bacteriologically (i.e. microscopy and/or culture positive) confirmed PTB could be demonstrated in the combined analysis, where case numbers and the magnitude of the follow-up time over which cases were detected combined to secure statistical significance (**Tables 27**, **28** and **30**): PCV recipients exhibited apparent reductions of incident culture-confirmed tuberculosis of up to 44 percent (95% CI, 11 – 65), P=0.010. PCV recipients exhibited a trend towards reduction in incident bacteriologically-confirmed disease (RR 0.69; 95% CI, 0.46 – 1.03), P=0.058, compared to placebo recipients and a more striking reduction when incident and recurrent bacteriologically-confirmed disease episodes were combined (RR 0.69; 95% CI, 0.48 – 0.99), P=0.037.

The apparent reduction in bacteriologically-confirmed tuberculosis, indicating that culture-confirmed disease frequently arises in the context of pneumococcal co-infection, was attributable mainly to the disease burden experienced by HIV-infected children, as is demonstrated in **Tables 32** and **33**. The risk ratio for incident culture-confirmed PTB comparing HIV-infected children who had received the PCV and those who received placebo was 0.53, indicating a 47 percent risk reduction (95% CI, 10 – 69), P=0.017, in vaccinees; therefore, up to 47 percent of culture-confirmed tuberculosis may arise in the context of pneumococcal co-infection in HIV-infected children.

An intention-to-treat analysis as represented in **Table 34** suggests that the benefit of the apparent PCV effect on culture-confirmed tuberculosis in HIV-infected children was experienced in partially-vaccinated children as well as those who had completed a full course (3 doses, given four weeks apart) of PCV. HIV-infected children who completed a full course of PCV exhibited a 50 percent (95% CI, 11 – 72) reduced burden of culture-confirmed tuberculosis compared to placebo recipients, P=0.016; this was finding was only marginally eroded by the intention-to-treat analysis.

Three postulated PCV-related effects may explain why the incidence of culture-confirmed tuberculosis was significantly lower in the vaccinated children compared to those who received placebo. The first, and most intuitive, effect is that in high-burdened settings the pneumococcus is frequently implicated as a coincidental co-infecting agent in childhood tuberculosis and that in settings where an effective vaccine which protects children from pneumococcal infection can be administered, less co-infection with tuberculosis is appreciated.

The second postulated effect relies on the assumption that co-infection with the pneumococcus is a significant immune regulatory disruption event and that children who are infected with *S. pneumoniae* and have LTBI are at significant risk of acutely reactivating their tuberculosis, with subsequent development of active tuberculosis disease; hence the propensity for LTBI to reactivate in the presence of an acute pneumococcal infection would be diminished in PCV-vaccinated compared to -unvaccinated individuals.

Thirdly, the corollary to the above-mentioned postulated effect may also apply: namely, that infection with MTB causes immune dysregulation which increases childhood susceptibility to infection with *S. pneumoniae*; in this context, children who had received PCV (which is known to be highly effective in protecting against invasive disease caused by *S. pneumoniae* in both HIV-infected and -uninfected children[41]) would be relatively better protected against secondary infection with the pneumococcus.

The fact that HIV-infected children were demonstrated to have the most significant PCV-related reductions in pneumococcal-tuberculosis co-infections (as mentioned above) may support either of the last two proposed explanations, as HIV-infected children invariably have background immune surveillance dysregulation which would be further compromised with the additional 'hit' of infection with the pneumococcus and/or MTB.

It has been noted that "[a]ntecedent or current infections can alter the immunopathologic outcome of a subsequent unrelated infection."[57] Our current understanding as to how pathogens interact with the host immune system, potentially creating an immunological milieu which may predispose to infection with other pathogens, is constantly being improved. Whilst the specific interactions between MTB and *S. pneumoniae* have not been studied to date, numerous experiments conducted using the murine model[58 59 60 61 62] highlight potential effects on both the innate and adaptive arms of the immune response which may impact on infection caused by either of these organisms. These immunological interactions are summarised in **Figure 9**.

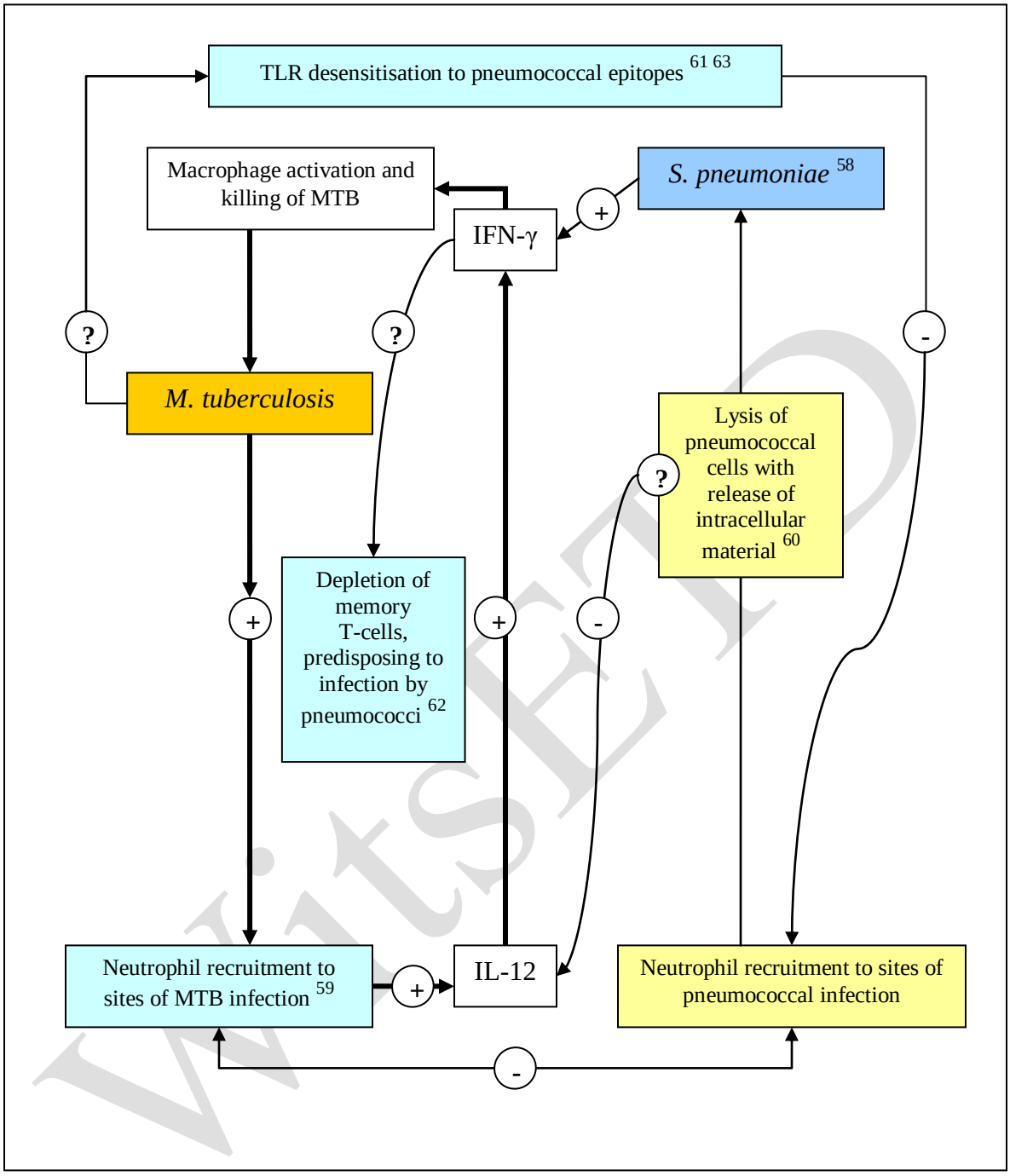


Figure 9: Possible immunological interactions between *M. tuberculosis* and *S. pneumoniae* as demonstrated in the mouse model

(+) Positive/stimulatory influence on downstream events.

(-) Negative/inhibitory influence on downstream events.

(?) Putative influence on downstream events.

Light yellow blocks represent immunological influences exerted *by S. pneumoniae* which may predispose to active tuberculosis.

Light blue blocks represent immunological influences exerted by *M. tuberculosis* which may predispose to pneumococcal infection.

Experiments conducted by Rubins *et al.* demonstrate that serum concentrations of gamma interferon (IFN-γ) have been found to increase dramatically within 48 hours of intranasal infection of mice with virulent strains of *S. pneumoniae*, the magnitude of the rise in IFN-γ correlating proportionately with the organism's virulence[58]. This finding is intriguing, as it highlights the fact that similar cytokine responses are elicited in host defense against pneumococci and MTB (IFN- γ is a pivotal cytokine in the immune response against tuberculosis[12]).

Neutrophils, traditionally thought to be involved in defense against bacterial pathogens rather than mycobacteria *per se*, have been found to be essential in containing MTB infection in mice through nonphagocytic mechanisms, probably by immunomodulatory activity through the production of interleukin-12 (IL-12) at the sites of bacillary deposition in infected organs[59]. IL-12, which is an important immunomodulatory cytokine in the immune response against tuberculosis, tips the inflammatory cytokine response in favour of early IFN-γ production. Additionally, it has been demonstrated that neutropaenia at the time of infection with MTB predisposes to wide-spread dissemination of tuberculous bacilli and disseminated tuberculosis in mice[59].

Intracellular components of Gram positive bacteria, such as would be encountered as a consequence of a successful immune response to infection caused by these organisms (through destruction of bacterial cells by the actions of the neutrophil oxidative burst or by the actions of complement, for example) down-regulate the production of IL-12[60]; hence, recovering Gram positive infection could potentially dampen immune responses which control LTBI.

Prior influenza infection has been found to have long-term effects on Toll-like receptor (TLR) function in the lung, causing their desensitisation to bacterial molecular patterns with consequent lack of neutrophil recruitment to sites of bacterial colonisation in the lungs, followed by bacterial overgrowth and secondary pneumonia[61]. There is mounting evidence that MTB or its antigenic components can dampen TLR signaling in the lung[63], and it is feasible that chronic pulmonary infection with MTB could predispose individuals to secondary bacterial pneumonias by similar mechanisms seen to those observed after recovery from influenza.

Infection caused by intracellular bacteria such as *Mycobacterium bovis* BCG, *Listeria monocytogenes*, and *Salmonella typhimurium* have been found to irreversibly deplete pre-existing CD8 memory T-cells in mice, this effect being mediated through the production of IFN-γ[62]. The effect that tuberculosis has on immunological memory has not yet been studied, however it is feasible that infection caused by MTB could deplete memory CD8 T-cell populations to previously-encountered pathogens in a similar manner to that observed for BCG.

4.3 Strength of Evidence for Tuberculosis in the PCV Study Cohort

The WHO categorisation of childhood tuberculosis was adopted for use in this study in order to attribute the strength of evidence for a diagnosis of tuberculosis in the PCV efficacy trial cohort.

The gold standard for a diagnosis of active tuberculosis in childhood subjects is demonstration of *M. tuberculosis* by culture from clinical specimens; however, current methodologies of microbiological isolation remain insensitive despite recent advances in culture techniques[64], so children in whom the organism is not isolated cannot with certainty be classified as being tuberculosis disease free. The frequently made statement that a diagnosis of tuberculosis is difficult to confirm in children, rests on the fact that the methodologies of suitable specimen collection are labour intensive (and so frequently not performed), and that the yield is generally perceived to be too low to be worth the effort of subjecting paediatric patients to these invasive procedures.

This study revealed that 26 percent of all tuberculosis episodes (and 23.4 percent of those with culture-confirmed disease) that were worked up for the presence of co-pathogens, had co-infections with significant viral and bacterial pathogens, or *P. jiroveci* at the tuberculosis index admission. This further confounding factor to accurate diagnosis of tuberculosis in children raises concern that children with tuberculosis may have their illness misattributed to identified co-pathogens rather than to tuberculosis, should microbiological tests for MTB remain negative.

In contrast to studies in adult tuberculosis patients, where HIV-infected status tends to confer consistently lower yields of microbiological testing[65], there was no difference in bacteriological yield between HIV-infected and -uninfected children with bacteriologically-confirmed tuberculosis in this cohort: 78 (28.5%) of 274 HIV-infected children with tuberculosis had smear- and/or culture-proven disease compared to 39 (27.1%) of 144 HIV-uninfected children, P=0.765. This phenomenon has previously been demonstrated amongst

children hospitalised with tuberculosis at CHBH[52 66], and is most likely due to the fact that paucibacillary forms of disease (i.e. disease manifestations where a robust CMI response has not been directed against the infective focus, with consequent lack of development of tissue-destructive lesions such as pulmonary cavitation which tends to yield higher smear- and culture-positive microbiological samples) predominate in both immunocompromised and immunocompetent children with primary tuberculosis, whilst immunocompetent adults with PTB frequently present with cavitary disease (frequently smear-positive) whereas adults with advanced HIV disease tend to present with paucibacillary forms of disease. Children will therefore tend to have lower microbiological yields regardless of HIV status, whilst HIV-uninfected adults will have higher yields compared to HIV-infected adults because of this phenomenon.

As the WHO classification system (**Appendix 5**, page 132) stipulates a positive TST reaction as being one of the criteria by which paediatric patients can be labelled as having probable tuberculosis, and TST reactivity is known to be impaired in HIV-infected children[15 67], the finding that HIV-uninfected children were more frequently classified as having probable tuberculosis than were HIV-infected children (OR 2.11; 95% CI, 1.35 – 3.28), P<0.001, was expected. Conversely, in situations where the clinical picture is consistent with a diagnosis of tuberculosis based on history, clinical examination and radiological findings but without the supportive evidence of TST reactivity or bacteriological confirmation, HIV-infected children (8.6 times [95%CI, 3.83 – 19.44], P<0.001, more likely to exhibit TST anergy than HIV-uninfected children in this analysis) are significantly more likely to be classified as suspected tuberculosis cases (OR 2.00; 95% CI, 1.27 – 3.17), P=0.002.

TST using the Mantoux method generally performed poorly in this cohort, with negative responses occurring in 25 (92.6%) of 27 HIV-infected children with bacteriologically-confirmed tuberculosis and 13 (61.9%) of 21 HIV-uninfected children with the same disease classification (OR 7.69; 95% CI, 1.22 – 81.00), 0=0.014 (Fisher's exact test), **Table 12**. Although this may be an unrepresentative result, attributable to the small numbers of children tested, it is worth noting that the observed under-performance of the TST in bacteriologically-confirmed cases in this cohort is concerning as this is the most widely-utilised screening tool for tuberculosis in low-income, high tuberculosis burdened settings. TST anergy in HIV-infected children with bacteriologically-confirmed disease was 3-fold (95% CI, 0.49 – 22.46), P=0.235 (Fisher's exact test) more frequent than in HIV-uninfected children with the same disease classification, although small sample size limited the demonstration of statistical significance in this analysis.

It is interesting to note that, in this cohort, the score chart proposed for use by the SANTP Guidelines (2004)[19] was demonstrated to have improved sensitivity (33.3 versus 7.4 percent) in diagnosing bacteriologically-confirmed disease in HIV-infected children compared to the TST alone (**Table 13**); this would indicate that the scoring system may be useful when evaluating HIV-infected children presenting with a disease process suggestive of tuberculosis, albeit only with moderate sensitivity.

HIV-infected children with the most tenuous evidence for active tuberculosis (those with suspected disease) were no more likely to have polymicrobial infections than were HIV-uninfected children with suspected tuberculosis (OR 1.39; 95% CI, 0.56 – 3.69), P=0.446

(**Table 16**). This finding suggests that HIV-infected children classified as having suspected disease were not merely misdiagnosed with tuberculosis on the basis of an intercurrent infection which took some time to respond to broad-spectrum therapy.

Similarly, **Table 15** illustrates that although the proportion of co-infections identified in children with probable tuberculosis were significantly smaller than that encountered in children with suspected tuberculosis (OR 0.52; 95% CI, 0.30 – 0.90), P=0.013, there was no significant difference in the proportions of children with confirmed co-infection comparing those who had bacteriologically-confirmed tuberculosis and those with suspected disease (OR 0.77; 95% CI, 0.44 – 1.35), P=0.333. This analysis is important as it likewise supports the argument that disease categorisation in instances where there was less robust evidence of causation by MTB, was not merely attributed on the basis of misdiagnosis of a disease process caused by a co-pathogen as 'tuberculosis' because of lengthy time to clinical resolution.

It must be noted that a proportion of the children diagnosed with tuberculosis in this cohort may not truly have had active MTB disease, although clinical presentation was suggestive enough to warrant a discharge diagnosis of tuberculosis in these children. Children with smear-positive but culture-negative disease may have had colonisation or infection with non-tuberculous mycobacterial species, and those with suspected disease may not have had tuberculosis at all. This is a commonly-occurring paradox in clinical practice, which nevertheless results in the notification and referral of paediatric cases to the Primary Health Care system for directly observed treatment of tuberculosis.

The public health burden of childhood tuberculosis inevitably accommodates the management of misdiagnosed cases in National Tuberculosis Programmes (NTPs) globally, and it is therefore important to document the impact of tuberculosis diagnoses regardless of strength of evidence for tuberculosis because these cases add to the case load which primary level care needs to manage. It is striking that HIV-infected children carried an overall tuberculosis incidence (once recurrent tuberculosis cases were included in the analysis) of 12 961 per 100 000 (95% CI, 11 608 – 14 428), or 3880 per 100 000 (95% CI, 3157 – 4720) for bacteriologically-confirmed disease (**Table 36**), suggesting that HIV-TB co-infection has a substantial impact on the Primary Health Care infrastructure in the study setting.

In the era of HIV, and in settings with high dual burdens of HIV and tuberculosis, over-diagnosis (e.g. in HIV-infected children with clinical signs such as chronic weight loss and lymphadenopathy which may be misattributed to tuberculosis) and under-diagnosis of tuberculosis (e.g. in those who have HIV-related lung disease such as lymphocytic interstitial pneumonitis who are not investigated for the presence of dual tuberculosis infection) in children who lack bacterial confirmation of active disease caused by MTB is a possibility[68]. When considering how vulnerable immunocompromised children are to the infection, it has been suggested that children presenting with pneumonia in developing countries with high tuberculosis and HIV prevalence rates, should be suspected as having tuberculosis and that anti-tuberculous therapy should not be withheld because of a lack of bacteriological evidence of infection caused by MTB[66].

4.4 Recurrent Tuberculosis Episodes

HIV-infected children had a 3-fold (95% CI, 1.6 – 6.7), P<0.001, increased risk of developing recurrent tuberculosis compared to HIV-uninfected children, equivalent to the risk of recurrence observed in HIV-infected compared to -uninfected patients with tuberculosis described by Driver *et al.* (RR 3.6; 95% CI, 1.9 – 6.7) in New York in 2001[69].

Comparing the HIV-infected and -uninfected groups, HIV-infected children tended to manifest recurrent tuberculosis after a previous episode of bacteriologically-confirmed disease (P=0.016, Fisher's exact test) whereas HIV-uninfected children who developed recurrences, tended to do so after an episode of previous probable tuberculosis (P=0.030, Fisher's exact test), **Table 6**. Twenty-two percent of the HIV-infected children with recurrent tuberculosis had more than one recurrence of disease, whereas no HIV-uninfected child had more than one recurrence. Time to first recurrence was similar regardless of HIV status (14.7 months in HIV-infected children compared to 15.9 months in the HIV-uninfected, P=0.793). However, bacterial burden of disease demonstrably shortened time to recurrence in the HIV-infected children (10.0 months in those with previous smear-positive disease compared to 19.0 months in those with previous smear-negative but culture-confirmed disease, P=0.039).

Clinicians caring for children have raised concern about the efficacy of short-course anti-tuberculous chemotherapy in HIV-infected children, questioning whether a prolonged continuation phase of therapy should not be adopted in order to prevent recurrence of disease in these patients[15 70]; this concern is also becoming more widely appreciated in the

general medical literature[71]. Recurrent tuberculosis in high-burdened settings frequently arises as a result of exogenous re-infection[72] but may also arise as a result of endogenous reactivation of dormant foci of bacilli which failed to respond to a previous course of anti-tuberculous therapy; HIV-infection as a cause of severe immunosuppression may cause an increase in both exogenous re-infection and endogenous reactivation of disease[73].

The tuberculosis recurrence rate amongst HIV-uninfected children was 5.6 percent (8 cases amongst 144 HIV-uninfected children who were ever diagnosed with tuberculosis during the study duration), which is commensurate with the expected 2 to 7 percent recurrence rate of disease described in HIV-uninfected patients with active drug-susceptible tuberculosis, treated with six months of short-course antituberculous therapy[74 75 76 77]. The overall recurrence rate in HIV-infected children was 18.2 percent (50 cases amongst 274 HIV-infected children ever diagnosed with tuberculosis over the study time period).

Schaaf *et al.* calculated a relapse rate of 19.6 percent for culture-confirmed tuberculosis in HIV-infected children who had had previous definite tuberculosis[73]. Similarly, in the CHBH PCV Study cohort, amongst 21 HIV-infected children who had had previous definite tuberculosis, 5 (23.8%) culture-confirmed relapses were identified. It is noteworthy that, in the CHBH cohort, 31 recurrent tuberculosis episodes arose amongst HIV-infected children who had had previous bacteriologically-confirmed (smear- and/or culture-positive) disease, and 13 (41.9%) of these recurrent episodes were themselves bacteriologically-confirmed.

The findings of enhanced recurrence of disease in HIV-infected children who had prior bacteriologically-confirmed disease and shorter time to recurrence in those with previous smear-positive disease, may suggest that an extension of the continuation phase of therapy would be of benefit in treating HIV-infected children with bacteriologically-confirmed tuberculosis. An important adjunct to monitor disease response to therapy in children with bacteriologically-confirmed tuberculosis would be to submit repeat samples for bacteriological evaluation at the end of the intensive phase of therapy in order to demonstrate reversion to smear- and/or culture negativity, as is the practice in adult patients. Radiological follow-up in order to demonstrate improvement in parenchymal lung disease during antituberculous therapy is another important strategy by which efficacy of therapy can be determined[73].

4.5 Untreated Tuberculosis Episodes

This is the third study which has demonstrated that substantial proportions of children investigated for tuberculosis at CHBH do not access appropriate care for their bacteriologically-confirmed disease, highlighting deficiencies in the follow-up care of children that are investigated for active tuberculosis at the facility.

The first study that highlighted deficiencies in this aspect of tuberculosis management at the hospital was conducted by Ntwaza by retrospective review of children admitted to the general paediatric wards from February 1997 to February 1999[28]. Seven hundred and forty-five children had culture-confirmed tuberculosis during the study period, 342 (45.9%) of whom were included for review in that study: 173 (50.6%) of these children had not been

notified for tuberculosis. A firm recommendation was made in response to the study findings, to adopt strategies that would improve the notification and treatment of paediatric bacteriologically-confirmed tuberculosis at the hospital[28].

Edginton *et al.* describe the outcome of a prospective study conducted at CHBH which sought to critically analyse the practice of tuberculosis culture submission by clinicians caring for patients with suspected tuberculosis at the facility[78]. The study was conducted from January to March 2006, during which time period 5404 samples were submitted from 3909 patients with suspected tuberculosis; 1160 (29.7%) of the patients were children under 15 years of age. Of all children investigated for tuberculosis, 122 (10.5%) had positive culture results and, by review of all available data sources, 44 (36.1%) of these children may not have been on initiated on antituberculous therapy[78].

The analysis in the PCV Study cohort as presented in this dissertation, intermediate in time sequence between the previous two studies, identified that 62 (44.0%) of all bacteriologically-proven tuberculosis episodes had not been started on anti-tuberculous therapy. When assessing untreated episodes in HIV-infected and -uninfected children according to culture-positivity, 38 (50.0%) of the 76 culture-confirmed episodes in HIV-infected children and 16 (50.0%) of the 32 culture-confirmed episodes in HIV-uninfected children were not started on appropriate treatment, P=1.000. A similar analysis, focussing on smear-positive episodes without culture-confirmation that were not started on appropriate therapy, reveals that amongst HIV-infected children 7 (29.2%) of 24 episodes with confirmed PTB compared to 1 (12.5%) of 8 such episodes in HIV-uninfected children were not started on appropriate therapy, P=0.642 (Fisher's exact test).

Neither HIV-infected children with culture-proven tuberculosis (OR 2.42; 95% CI, 0.83 – 7.69), P=0.074, nor those with culture-confirmed disease who were HIV-uninfected (OR 7.0; 95% CI, 0.72 – 334.74), P=0.107 (Fisher's exact test) were significantly more likely to have remained untreated for their disease compared to those with smear-positive disease.

Median cough duration in these children was 3.5 days (IQR 2.0 – 7.0) lending feasibility to the hypothesis that, as these children presented with an acute illness, the diagnosis of tuberculosis was not considered likely, although clinical suspicion was sufficient to submit tests to investigate for active disease caused by MTB.

The median duration of admission in the children with bacteriologically-confirmed disease who remained untreated and survived was 6.0 days (IQR 4.0 – 11.0): comparing this to the duration of admission amongst the bacteriologically-confirmed tuberculosis cases who survived (9.0 days [IQR 4.0 – 21.0]), P=0.222, fails to clarify the possible reason for omission of antituberculous therapy as being a perceived lack of clinical severity of the untreated cases.

It is likely that omission in commencing appropriate therapy in children with culture-proven tuberculosis is a challenge in other centres in high tuberculosis-burdened settings, and that CHBH does not stand alone in this regard. A diagnosis of tuberculosis was missed in 25 (21.2%) of 118 children with culture-confirmed tuberculosis at a Durban hospital in 1998 and 1999[15]. Possible reasons for omission of commencement of therapy in the Durban

cohort, such as overburdened hospital settings with pressure on clinicians to discharge patients before tuberculosis culture results become available, as well as frequent acute presentations (in 43 percent) of tuberculosis in this cohort which may have prompted clinicians not to consider the diagnosis of tuberculosis[15], would apply to the clinical environment at CHBH and other large hospitals in under-resourced settings.

Other factors which impair initiation of bacteriologically-confirmed tuberculosis cases on appropriate therapy may result from inability to trace patients once results become available because of inaccurate or incomplete patient records in which addresses and/or contact numbers have been erroneously recorded[78], or erratic retrieval of laboratory results because of rapid turn-over of medical staff in busy teaching hospitals[79].

A recent critical analysis of the diagnostic value of culture-confirmation of tuberculosis in children, conducted in a tertiary centre whose staff complement includes experienced paediatric tuberculosis clinicians, highlighted that 127 (80.4%) of 158 children with culture-confirmed tuberculosis had been diagnosed on clinical grounds and had been started on antituberculous therapy prior to tuberculosis culture result retrieval. Thirty-one (19.6%) of the children were diagnosed subsequent to culture result availability, with 17 of these (9.2 percent of the cohort) not being started on appropriate therapy by hospital clinicians[79]. The authors commented that, although tuberculosis culture results take 2 to 8 weeks to achieve positivity and as such these investigations add very little to the acute management decisions surrounding children admitted with and investigated for tuberculosis, culture is important in order to obtain isolates for drug susceptibility testing[79]. Paediatric tuberculosis is a sentinel public health event, which often reflects recent infection acquired from adults

in the child's immediate environment, and drug susceptibility profiles of paediatric isolates are a useful indicator of current drug-resistant tuberculosis isolates in circulation in that environment[80].

4.6 The Burden of Tuberculosis in Hospitalised Children Under 5.3 Years of Age in Soweto

Few studies have described the tuberculosis incidence in a cohort of children enrolled in early infancy over time using a longitudinal approach, because active tuberculosis is seen as being a 'rare event' in many settings, and this approach would necessitate the enrolment of very large baseline cohorts with careful follow-up of the cohort over time in order to identify those who develop active tuberculosis[81].

Cross-sectional approaches to disease incidence estimation offer a reasonable compromise to the longitudinal approach; however the cross-sectional approach is not without its inherent difficulties, chiefly the fact that it relies on population estimates which are used as denominators in the disease incidence calculations.

Using the longitudinal approach to disease incidence estimation, this study demonstrates that the Soweto paediatric tuberculosis incidence is 425 incident cases and 118 incident bacteriologically-confirmed cases in a cohort of 39 836 children that were followed up for a period of 5.3 years. These figures translate into a tuberculosis incidence rate of 1067 per 100 000 children (95% CI, 968 – 1173), or 296 per 100 000 (95% CI, 245 – 355) for bacteriologically-confirmed disease (**Table 35**).

It is interesting to note that, even if all incident cases of suspected tuberculosis (n=160) were omitted from the incidence analysis in this cohort, on the basis of insufficient grounds for a diagnosis of the disease because of the lack of robust clinical, radiological or bacteriological evidence, 265 incident cases (of bacteriologically-confirmed and probable disease) were identified in the hospitalised cohort: 665 cases per 100 000 (95% CI, 588 – 750).

As mentioned above, the incidence rate calculations highlighted that HIV-infected children bore the brunt of the tuberculosis burden: 10 633 cases per 100 000 HIV-infected children compared to 386 per 100 000 HIV-uninfected children (RR 27.5; 95% CI, 22.6 – 33.5).

5.0 STUDY LIMITATIONS

Although a rigorous strategy was adopted in order to identify all cases of tuberculosis amongst PCV Study participants that had been admitted to CHBH, areas of uncertainty remain as to the true burden of tuberculosis in the study cohort. The inevitable disadvantages of a retrospective data collection strategy were experienced during the course of this study, and it is possible that children who participated in the PCV Study and had a hospital-based diagnosis of tuberculosis were unintentionally omitted in this analysis, especially when considering that many of the data sources used were incomplete. For example, the change-over in NHLS data-recording and retrieval systems in 2000 may have led to an under-appreciation of bacteriologically-confirmed cases prior to 2000, and there were no notification booklets retrievable for the period before 2002 coupled with the fact that systematic recording of tuberculosis notification data was only adopted at CHBH in 2003. These sampling errors could have impacted on the yield of tuberculosis cases in this cohort quite substantially, as they would have diminished the detection of tuberculosis cases at a time when the study cohort would have been at its youngest, and consequently most vulnerable to developing active tuberculosis[7].

Mitigating against the above-mentioned tendency to under-estimate the true tuberculosis burden in the study cohort, numerous sources of bias arise when assessing tuberculosis disease burdens from hospitalised cohorts, some of which would tend to inflate the true tuberculosis burden in a cohort, as well as potentially exaggerating the negative effects which HIV-infection has on children co-infected with tuberculosis.

Compared to children diagnosed with tuberculosis in the community, hospitalised children usually have more severe or advanced disease, and as such may have more bacteriologically-confirmed tuberculosis; there may be higher proportions of HIV-infected children in hospital-derived cohorts, as these patients tend to present with more severe disease; mortality rates are also greater in hospitalised tuberculosis cohorts. It is also possible that there is a lower threshold for making a diagnosis of tuberculosis in hospitalised children, with a propensity to over-diagnose the condition in very ill children responding poorly to treatment of community-acquired pneumonia.

It is anticipated that the dual effects of under-estimating tuberculosis burden through the retrospective approach to case-finding, as was adopted in this study, would be counter-balanced by the potential to over-diagnose tuberculosis in ill hospitalised children where bacteriological confirmation of disease is not available.

Whilst incidence rates derived from hospitalised cohorts may give a biased perspective of disease, such analyses are useful in order to gauge trends of disease burden over time, and baseline analyses such as this serve as reference points for comparison of incidence rates in future incidence analyses.

6.0 CONCLUSIONS

In summary, this longitudinal study has demonstrated a high burden of tuberculosis in a cohort of 39 836 children that were enrolled in a PCV efficacy trial and followed up over 5.3 years in Soweto, with the greatest disease burden occurring in HIV-infected children.

By using a vaccine-probe approach in comparing the relative incidences of PTB amongst PCV and placebo recipients, it has been possible to demonstrate that co-infection with *S. pneumoniae* occurs in up to 44 percent (95% CI, 11 – 65) of children hospitalised with culture-confirmed tuberculosis, this effect being most marked in HIV-infected children in whom a 47 percent (95% CI, 10 – 69) apparent reduction in culture-confirmed tuberculosis was demonstrated in vaccinees, P=0.017. The occurrence of tuberculosis and pneumococcal co-infections in settings with a high prevalence of both HIV and tuberculosis may be explained by heterologous immune interactions, although studies designed to analyse the specific immunologic interactions between these two organisms have not been conducted.

A substantial proportion of children with bacteriologically-confirmed disease remained untreated for their condition, highlighting operational barriers to translating laboratory results into meaningful management outcomes at CHBH. This failure of management is likely not unique to the study setting, and probably reflects intrinsic deficiencies which occur in many high HIV-TB burdened settings. Laboratory and clinical feedback mechanisms need to be strengthened and maintained in order to secure favourable outcomes in paediatric patients investigated for active tuberculosis in hospitals in high-burdened settings.

APPENDICES

Appendix 1: Childhood Tuberculosis Diagnostic Score Chart Recommended for Use by the SANTP (2004)

General Feature	**0**	**1**	**2**	**3**	**4**	**Score**
Weeks of illness	< 2	2 – 4		> 4		
Nutrition (% weight for age)	> 80%	60 – 80%		< 60%		
Family history of tuberculosis	None	Reported by family		Proved sputum positive		
Tuberculin skin test				Positive		
Malnutrition				Not improving after four weeks		
Unexplained fever			No response to treatment			
Local Feature				**3**	**4**	**Score**
Findings on clinical examination				Lymph-adenopathy		
				Joint or bone swelling		
				Abdominal mass or ascites		
				CNS signs, CSF abnormal		
Radiological findings				Broad mediastinum due to enlarged hilar glands	Angle deformity of spine	
Total (any score ≥7 is suggestive of tuberculosis)						

Appendix 2: Data Collection Sheet Utilised in the PCV Study

Vaccine patient data form:

1. Admission Date:_____ 2. Unit:____ 3. Ex-unit:_____
4. Date of birth:_____ 5. Sex: M / F 6. Hosp No:_____
7. Surname:_____
8. Study number:_____
9. Dates of doses: 1._____2._____3._____

History:

10. Cough duration (days):_____
11. Fever: Y / N 12. Fever duration (days):_____
13. Refusing feeds: Y / N
14. Vomiting: Y / N
15. Diarrhoea: Y / N 16. Frequency: ____/day 17. Duration (days):____
18. Loss of weight: Y / N 19. Duration (days):____
20. Night sweats: Y / N
21. Seizures: Y / N
22. Irritable / Excessive crying: Y / N 23. Duration (days):_____
24. Hospitalization in previous 2 weeks: Y / N 25. Diagnoses:_____
26. > 2 admissions for pneumonia: Y / N 27. Number of pneumonia admissions:___
28. On oral antibiotics: Y / N 29. Name:_____30. Date started:_____
31. Bactrim prophylaxis: Y / N 32. Date started:_____
33. TB contact: Y / N 34. Who:_____35. When:_____
36. Predisposing medical cause for pneumonia: Y / N
37. Diagnosis: 1=Ex-prem 2=Cardiac condition 3=RVD 4=Chronic lung 5=TB
37a. Neonatal history: Birth weight (g) _____ Gestational age (weeks): _____
Ventilated: Y / N

Examination details:

38. Weight (kg):_____ 39. Weight centile:_____
40. Height (cm):_____ 41. Height centile:_____
42. Head circumference (cm):_____ 43. Head circumference centile:_____
44. Temperature (thermoscan):_____ 45. Temperature (axillary):_____
46. Respiratory rate (per min):_____
47. Room air sats (%):_____ 48. Sats on O_2 (%):_____
49. Pulse rate (per min):_____
50. Kwashiorkor: Y / N
51. Cyanosis: Y / N 52. Clubbing: Y / N
53. Intercostal recession: Y / N 54. Subcostal retractions: Y / N
55. Crepitations: Y / N 56. Wheezing: Y / N
57. Stridor: Y / N 58. Bronchial breathing: Y / N
59. Oral candidiasis: Y / N 60. Perineal candidiasis: Y / N
61. Chronic ear discharge: Y / N 62. Chronic sinusitis: Y / N
63. Acute otitis media: Y / N
64. Hepar (exclude hyperinflation): Y / N 65. Splenomegaly: Y / N
66. LN: Y / N 67. Dermatitis: Y / N
68. CDC HIV Class: N A B C
69. Neck stiffness: Y / N 70. Bulging fontanelle: Y / N
71. GCS:_____{E:___M:___V:___} 72. Dehydration (%):_____ 73. Other:_____

74. **Outcome:** 1=Discharged 2=Demised 3=RHT
75. Date of discharge:_____
76. ICU admission: 1=No 2=Yes 3=Not considered re: HIV 4=no bed 5=in 36
Discharge diagnosis: 77. Primary Dx:_____ 78. Secondary Dx:_____ 79. Underlying Dx:___

Result sheet:
FBC: 78. WCC_____ 79. Hb____ 80. MCV____ 81. Plt_____
82. Neut_____ 83. Lymph____ 84. Mono_____ 85. Eosino____
86. **CRP:_____**
87. **LDH:_____**

88. **HIV ELISA:** 1=Pos 2=Neg 3=Not done
89. **HIV PCR:** 1=Pos 2 =Neg 3=Not done

90. **Blood cultures:** 1=Neg 2=Pneumococcus 3=Hib 4=*S. aureus*
5=Not done 6=Other

CSF: 91. Neut___ 92.Lymph___ 93.RBC_____
94.Tot prot____ 95.Gluc ____ 96.Cl____ 97.LDH_____
98. **Gram stain:** 1=GPC 2=GNB 3=GNDC 4=Other 5=Neg
99. **Latex:** 1=Pn 2=Hib 3=Nmen 4=Neg 5=Not done
100. **Culture:** 1=Pn 2=Hib 3=Nmen 4=Neg 5=Not done

Other fluids: 101. 1=Pleural 2=Other:_____
Results: 102.Neut____ 103.Lymph____ 104.RBC_____
105.Tot prot____ 106.Gluc____ 107.Cl _____ 108. LDH_____
109. **Gram stain:** 1=GPC 2=GNB 3=GNDC 4=Other 5=Neg
110. **Culture:** 1=Pn 2=Hib 3=Nmen 4=Neg 5=Not done

111. **PPD (mm):_____**
112. **Gastric washings:** 1=1 done 2=2 done 3=3 done 4=>3 done 5=Not done
113. **Ao Results:** 1=Neg 2=Pos 3=Not done
114. **Bactec Result:** 1=Neg 2=Pos 3=Not done

115. **Nasopharyngeal aspirates:** 1=Done 2=Not done
116. Result: 1=Neg 2=RSV 3=Inf A 4=Inf B 5=Para I 6=Adeno
117. PCP: Y / N
118. PCP results: 1=Pos 2=Neg 3=Not done

Chest Radiographic Changes:
Parenchymal changes: 117._____118._____
1=Patchy infiltrate 2=Interstitial infiltrate 3=Dense non-lobar 4=Lobar/multi-
5=Poor quality 6=Normal 7=Not done
Secondary changes: 119._____120._____
1=Effusion 2=Cavitation 3=Atelectasis 4=Hilar LN
5=Hyperinflation

Appendix 3: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Moore

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M060344**
PROJECT	Defining the Burden of Pulmonary Tuberculosis & Probing the Prevalence of Pneumococcal Bacterial Co-Infections.....
INVESTIGATORS	Dr DP Moore
DEPARTMENT	Dept of Paediatrics & Child Health
DATE CONSIDERED	06.03.31
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06.04.03 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

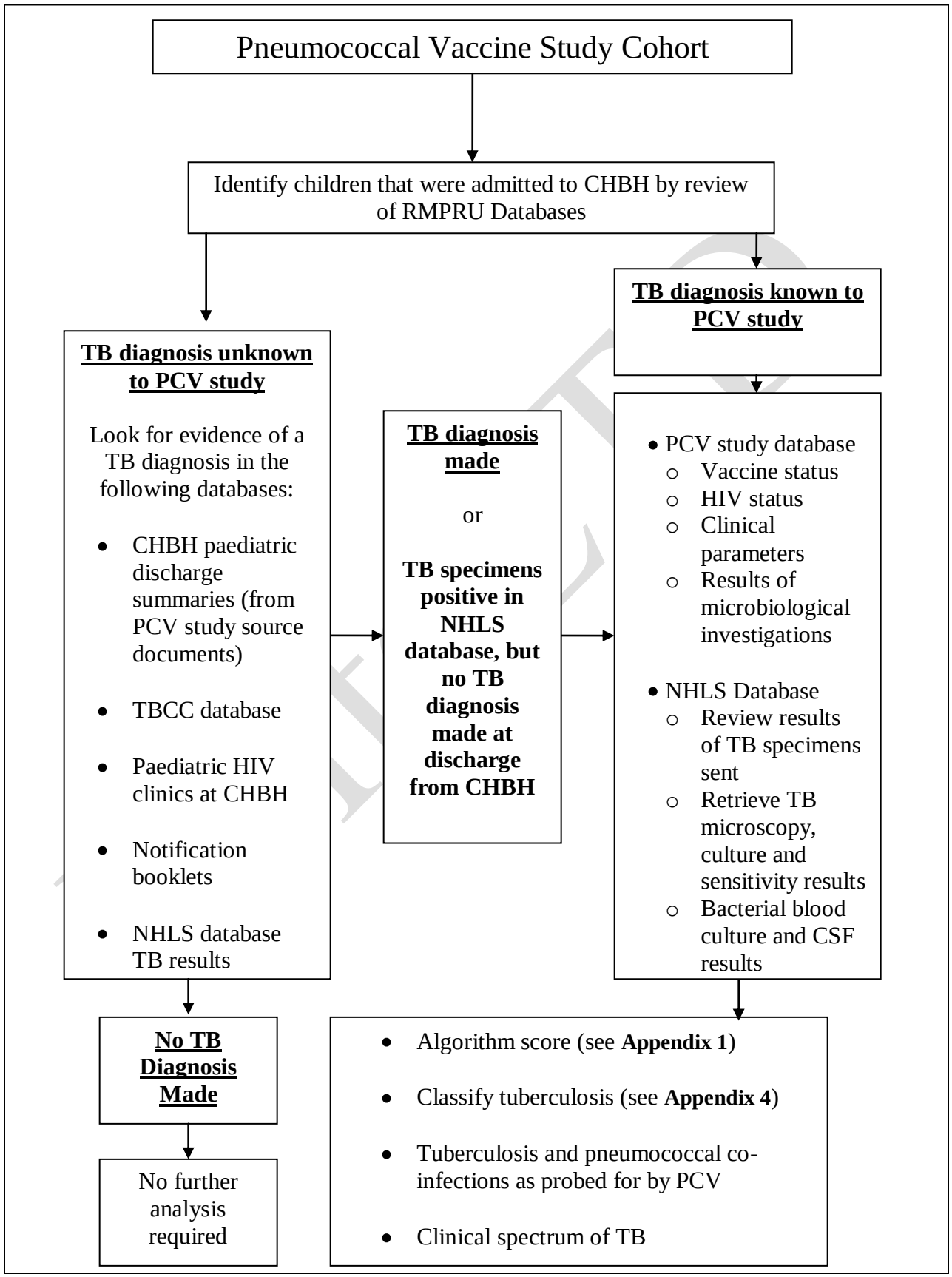
cc: Supervisor : Dr SA Madhi

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4: Strategy of Data Collection Utilised in this MMed Project



Appendix 5: Categorisation of Childhood Tuberculosis

<table>
<tr><th colspan="3">Bacteriologically-confirmed tuberculosis [47]</th><th colspan="2" rowspan="2">Probable tuberculosis [47]</th><th rowspan="2">Suspected tuberculosis [47]</th><th rowspan="2">Same-episode tuberculosis</th></tr>
<tr><td colspan="3">MTB detected on smear or culture from secretions or tissues</td></tr>
<tr><td>Definite tuberculosis</td><td>Confirmed PTB</td><td>Untreated tuberculosis</td><td colspan="2" rowspan="2">A suspected case (see alongside) with any of the following:

1. Positive TST
2. Suggestive chest radiograph
3. Suggestive histology
4. Favourable response to anti-tuberculous therapy</td><td rowspan="3">Ill child with a history of contact with a confirmed case of PTB

or

Any child:

1. Not regaining normal health after measles or whooping cough

2. With weight loss, cough or wheeze not responding to antibiotic therapy for respiratory disease

3. With painless swelling in a group of superficial nodes</td><td rowspan="3">For the purposes of this study

Cases diagnosed with tuberculosis who required subsequent hospitalisation within the six months of anti-tuberculous treatment (these cases were often listed in the PCV databases as having a discharge diagnosis of tuberculosis)</td></tr>
<tr><td>For the purposes of this study *

Disease with cultures positive for MTB:

1. PTB if culture-positive on respiratory specimens

2. EPTB if culture-positive on samples from extrapulmonary sites</td><td>For the purposes of this study

Respiratory illness with specimens positive on microscopy for AFB, without culture-confirmation</td><td>For the purposes of this study

Instances of bacteriologically-confirmed disease where a discharge diagnosis of tuberculosis was not made according to PCV source documents</td><td>Probable PTB</td><td>Probable EPTB</td></tr>
<tr><td colspan="3"></td><td>For the purposes of this study

Compatible history, clinical features and chest radiograph; no bacteriological confirmation</td><td>For the purposes of this study

Compatible history, clinical features and radiological investigations ± histology; no bacteriological confirmation</td></tr>
</table>

* Shaded columns, labelled "For the purposes of this study" reflect definitions devised for analysis in this dissertation; other definitions are derived from Reference 47.

Appendix 6: Vaccine Efficacy Calculation to Determine the Incidence Rate Ratio (IRR) for Tuberculosis Between HIV-infected and -uninfected Children

The following calculation, which adopts a 'vaccine efficacy' approach[48 49] to determine the degree of intrinsic protectivity which a BCG-vaccinated, intact immune system confers in protecting against active tuberculosis, is described below.

1. If:

Proportion of HIV-uninfected children with tuberculosis (pcv) =

1 – (Proportion of HIV-infected children with tuberculosis)

2. And:

Proportion of cohort which is HIV-uninfected (ppv) =

1 – (Proportion of cohort which is HIV-infected)

3. And:

Vaccine Efficacy (VE) = 1 – (pcv(1 – ppv)) / (ppv(1 – pcv)) [49]

VE = 1 – (tuberculosis incidence in HIV-uninfected / tuberculosis incidence in HIV-infected) [49]

4. From **Table 3**, 65.6 percent of first-episode tuberculosis cases where a definitive HIV result was available were identified in HIV-infected children:

$pcv = 1 - 0.656 = 0.344$

5. From population estimates [43 44], the estimated proportion of the PCV study cohort which is HIV-infected is 6.5 percent:

$$ppv = 1 - 0.065 = 0.935$$

6. Then,

$$VE = 1 - (0.344\ (1 - 0.935)) / (0.935\ (1 - 0.344))$$

$$VE = 1 - (0.344 \times 0.065) / (0.935 \times 0.656)$$

$$VE = 1 - (0.022 / 0.613)$$

$$VE = 1 - 0.036 = 0.964$$

7. BCG vaccination is therefore 96.4 percent relatively more effective in protecting against progression to active tuberculosis in HIV-uninfected compared to HIV-infected children. The Incidence Rate Ratio (IRR) of tuberculosis between HIV-infected and -uninfected children in this cohort (from the alternative form of the Vaccine Efficacy formula in point 3, above) is:

$$\textit{Incidence Rate Ratio (IRR) = incidence in HIV-infected / incidence in HIV-uninfected}$$

where

$$VE = 1 - 1 / (IRR)$$

hence

$$IRR = 1 / (1 - VE)$$

and

$$IRR = 1 / (1 - 0.964)$$

$$IRR = 1 / 0.036 = 27.5$$

The calculation (for all forms of incident tuberculosis as well as incident culture-confirmed disease) is tabulated below:

	Section 3.3.2	
	Efficacy of HIV-uninfected status in 'protecting' against incident tuberculosis	
	All forms of tuberculosis	Definite tuberculosis **
pcv (proportion of tuberculosis cases which were 'vaccinated'*)	0.344	0.330
1 - pcv (proportion of tuberculosis cases which were 'unvaccinated')	0.655	0.670
ppv (proportion of the population which was 'vaccinated'*)	0.935	0.935
VE [+] ('vaccine efficacy')	0.964	0.966
Efficacy of 'vaccine' in preventing tuberculosis in the cohort (%)	96.4	96.6
IRR [++] (incidence rate ratio)	27.5	29.4

'Vaccine efficacy' calculations for Section 3.3.2

* The term 'vaccinated' here refers to the 'immunological advantage' conferred to individuals in the analysis: 'vaccinated' individuals are those who are HIV-uninfected.

** "Definite tuberculosis" refers to incident cases with culture-confirmed PTB and EPTB (63 cases in HIV-infected children and 31 cases in HIV-uninfected children, **Table 3**).

[+] *Vaccine Efficacy (VE) = 1 – (pcv(1 – ppv)) / (ppv(1 – pcv)).*

[++] *Incidence Rate Ratio (IRR) = 1 / (1 – VE).*

REFERENCES

[1] Kochi A. The global tuberculosis situation and the new control strategy of the World Health Organization. Tubercle 1991; 72: 1 – 6.

[2] Van Rie A, Beyers N, Gie RP, Kunneke M, Zietsman L, Donald PR. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. Arch Dis Child 1999; 80: 433 – 437.<http:adc.bmj.com> [Accessed 05.04.2008]

[3] Lawn SD, Bekker L-G, Middelkoop K, Myer L, Wood R. Impact of HIV Infection on the Epidemiology of Tuberculosis in a Peri-Urban Community in South Africa: The Need for Age-Specific Interventions. Clin Infect Dis 2006; 42: 1040 – 1047.

[4] Global tuberculosis control: surveillance, planning, financing: WHO report 2008. Geneva, World Health Organization. <http:www.who.org>(WHO/HTM/TB/2008.393)[Accessed 05.04.2008]

[5] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global Burden of Tuberculosis: Estimated Incidence, Prevalence, and Mortality by Country. JAMA 1999; 282: 677 – 686.<www.jama.com> [Accessed 05.04.2008]

[6] Newton SM, Brent AJ, Anderson S, Whittaker E, Kampmann B. Paediatric tuberculosis. Lancet Infect Dis 2008; 8: 498 – 510. <www.thelancet.com/infection>[Accessed 05.08.2008]

[7] 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: 392 – 402.

[8] Bloch AB, Snider DE. How much tuberculosis in children must we accept? Am J Public Health 1986; 76: 14 – 15.

[9] Starke JR. Pediatric tuberculosis: time for a new approach. Tuberculosis (Edinb) 2003; 83: 208 – 212. <www.elsevierhealth.com/journals/tube> [Accessed 26.04.2008]

[10] Jeena PM, Mitha T, Bamber S, Wesley A, Coutsoudis A, Coovadia HM. Effects of the human immunodeficiency virus on tuberculosis in children. Tuber Lung Dis 1996; 77: 437 – 443.

[11] Swaminathan S. Tuberculosis in HIV-infected children. Paediatr Respir Rev 2004; 5: 225 – 230.

[12] Kampmann B, Young D. Childhood tuberculosis: advances in immunopathogenesis, treatment and prevention. Curr Opin Infect Dis 1998; 11: 331 – 335.

[13] Boom WH, Canaday DH, Fulton SA, Gehring AJ, Rojas RE, Torres M. Human immunity to *M. tuberculosis*: T cell subsets and antigen processing. Tuberculosis (Edinb) 2003; 83: 98 – 106.

[14] Flynn JL. Immunology of tuberculosis and implications in vaccine development. Tuberculosis (Edinb) 2004; 84: 93 – 101.

[15] 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: 672 – 678.

[16] Palme IG, 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. Pediatr Infect Dis J 2002; 21: 1053 – 1061.

[17] Lolekha R, Anuwatnonthakate A, Nateniyom S, Sumnapun S, Yamada N, Wattanaamornkiat W, et al. Childhood TB epidemiology and treatment outcomes in Thailand: a TB active surveillance network, 2004 to 2006. BMC Infect Dis 2008; 8: 94. <www.biomedcentral.com/1471-2334/8/94> [Accessed 02 January 2009]

[18] Hesseling AC, Schaaf HS, Gie RP, Starke JR, Beyers N. A critical review of diagnostic approaches used in the diagnosis of tuberculosis in children. Int J Tuberc Lung Dis 2002; 6: 1038 – 1045.

[19]South African Department of Health. The South African National Tuberculosis Control Programme Practical Guidelines 2004.<www.ecdoh.gov.za/uploads/files/150906152233.pdf>
[Accessed 22 January 2007]

[20] Edwards K. The Diagnosis of Childhood Tuberculosis. P N G Med J 1987; 30: 169 – 178.

[21] Van Rheenen P. The use of the paediatric tuberculosis score card in an HIV-endemic area. Trop Med Int Health 2002; 7: 435 – 441.

[22] 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: 263 – 269.

[23] 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: 170 – 176.

[24] Zar HJ, Apolles P, Argent A, Klein M, Burgess J, Hanslo D, et al. The etiology and outcome of pneumonia in human immunodeficiency virus-infected children admitted to intensive care in a developing country. Pediatr Crit Care Med 2001; 2: 108 – 112.

[25] 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: 1440 – 1451.

[26] Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005; 365: 130 – 134. <http:www.thelancet.com> [Accessed 26.04.2008]

[27] Graham SM, Mtitimila EI, Kamanga HS, Walsh AL, Hart CA, Molyneaux ME. Clinical presentation and outcome of *Pneumocystis carinii* pneumonia in Malawian children. Lancet 2000; 355: 369 – 373.

[28] Ntwaza MC. Evaluation of Treatment Adequacy of Culture Confirmed Pulmonary Tuberculosis in a Tertiary Hospital Chris Hani Baragwanath Hospital [research report]. Johannesburg (Gauteng): University of the Witwatersrand, 2004.

[29] Miller FJW. Tuberculosis in Children: Evolution, Epidemiology, Treatment, Prevention. 1st ed. Edinburgh: Churchill Livingstone, 1982: 11 – 13.

[30] Adegbola RA, Falade AG, Sam BE, Aidoo M, Baldeh I, Hazlett D, et al. The etiology of pneumonia in malnourished and well-nourished Gambian children. Pediatr Infect Dis J 1994; 13: 975 – 982.

[31] Echave P, Bille J, Audet C, Talla I, Vaudaux B, Gehri M. Percentage, Bacterial Etiology and Antibiotic Susceptibility of Acute Respiratory Infection and Pneumonia among Children in Rural Senegal. J Trop Pediatr 2003; 49: 28 – 32.

[32] 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: 985 – 990.

[33] Park DR, Sherbin VL, Goodman MS, Pacifico AD, Rubenfeld GD, Polissar NL, et al. The etiology of community-acquired pneumonia at an urban public hospital: influence of human immunodeficiency virus infection and initial severity of illness. J Infect Dis 2001; 184: 268 – 277.

[34] Scott JAG, Hall AJ, Muyodi C, Lowe B, Ross M, Chohan B, et al. Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. Lancet 2000; 355: 1225 – 1230.

[35] Nyamande K, Lalloo UG, John M. TB presenting as community-acquired pneumonia in a setting of high TB incidence and high HIV prevalence. Int J Tuberc Lung Dis 2007; 1308 – 1313.

[36] Schleicher GK, Feldman C. Dual infection with Streptococcus pneumoniae and Mycobacterium tuberculosis in HIV-seropositive patients with community-acquired pneumonia. Int J Tuberc Lung Dis 2003; 7: 1207 – 1208.

[37] Choi D, Lee KS, Suh GY, Kim TS, Kwon OJ, Rhee CH, et al. Pulmonary Tuberculosis Presenting as Acute Respiratory Failure: Radiologic Findings. J Comput Assist Tomogr 1999; 23: 107 – 113.

[38] Wei C-J, Tiu C-M, Chen J-D, Chou Y-H, Chang C-Y, Yu C. Computed Tomography Features of Acute Pulmonary Tuberculosis. Am J Emerg Med 2004; 22: 171 – 174.

[39] Mulholland K, Hilton S, Adegbola R, Usen S, Oparaugo A, Omosigho C, et al. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate vaccine [corrected] for prevention of pneumonia and meningitis in Gambian infants. Lancet 1997; 349: 1191 – 1197.

[40] Madhi SA, Klugman KP & The Vaccine Trialist Group. A role for *Streptococcus pneumoniae* in virus-associated pneumonia. Nat Med 2004; 10: 811 – 813.

[41] Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A Trial of a 9-Valent Pneumococcal Conjugate Vaccine in Children with and Those without HIV Infection. N Engl J Med 2003; 349: 1341 – 1348.

[42] Madhi SA, Kuwanda L, Cutland C, Klugman K. The Impact of a 9-Valent Pneumococcal Conjugate Vaccine on the Public Health Burden of Pneumonia in HIV-Infected and –Uninfected Children. Clin Infect Dis 2005; 40: 1511 – 1518.

[43] Health Systems Research and Epidemiology. Summary report: national HIV seroprevalence survey of women attending antenatal clinics in South Africa. Pretoria, South Africa: V & R Printing Works, 2001.

[44] Gray GE, McIntyre JA, Lyons SF. Effect of breastfeeding on vertical transmission of HIV-1 in Soweto, South Africa [abstract Th.C.413]. In: Programme and abstracts of the 11th International Conference on AIDS, Vancouver. 1997.

[45] Edginton ME, Wong ML, Phofa R, Mahlaba D, Hodkinson HJ. Tuberculosis at Chris Hani Baragwanath Hospital: numbers of patients diagnosed and outcomes of referrals to district clinics. Int J Tuberc Lung Dis 2005; 9: 398 – 402.

[46] Osborne CM. The challenge of diagnosing childhood tuberculosis in a developing country. Arch Dis Child 1995; 72: 369 – 374.

[47] Houwert KAF, 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: 1116 – 1120.

[48] Hilderbrand K, Boulle A, Coetzee D, Goemaere E, Van Cutsem G, Bedelu M. The impact of antiretroviral treatment on tuberculosis case-finding in a setting of extremely high tuberculosis/HIV co-morbidity. Proceedings of the 9th International Workshop on HIV Observational Databases; 2005 April; Budapest.

[49] 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: 1009 – 1021. <www.archinternmed.com> [Accessed 06.06.2008]

[50] Altman DG, Bland M. Diagnostic tests 1: sensitivity and specificity. Br Med J 1994; 308: 1552.

[51] Coovadia HM, Jeena P, Wilkinson D. Childhood human immunodeficiency virus and tuberculosis co-infections: reconciling conflicting data. Int J Tuberc Lung Dis 1998; 10: 844 – 851.

[52] Moore DP, Edginton ME, Moultrie HM, Meyers TM, Madhi SA, Yacoob S, et al. Analysis of 2456 Children Treated for Tuberculosis at Chris Hani Baragwanath Hospital, Soweto, South Africa. Int J Tuberc Lung Dis 2007; 11(1 Suppl): S155 – S156.

[53] Hesseling AC, Cotton MF, Jennings T, Whitelaw A, Johnson LF, Eley B, et al. High Incidence of Tuberculosis in Infants: Evidence From A South African Population-based Study Highlights The Need For Improved Tuberculosis Control Strategies. Clin Infect Dis 2009; 48: 108 - 114.

[54] Hesseling AC, Cotton MF, Fordham von Reyn C, Graham SM, Gie RP, Hussey GD. Consensus statement on the revised World Health Organization recommendations for BCG vaccination in HIV-infected infants. Int J Tuberc Lung Dis 2008; 12: 1376 – 1379.

[55] Walters E, Cotton MF, Rabie H, Schaaf HS, Walters LO, Marais BJ. Clinical presentation and outcome of Tuberculosis in Human Immunodeficiency Virus infected children on anti-retroviral therapy. BMC Pediatr 2008; 8. <www.biomedcentral.com/1471-2431/8/1> [Accessed 12.10.2008]

[56] The Government Communication and Information System. SA Milestone vaccine rollout to fight infant mortality. BuaNews Online 2008 Sept 12. <www.buanews.gov.za/news/08/08091213451003> [Accessed 12.10.2008]

[57] Page KR, Scott AL, Manabe YC. The expanding realm of heterologous immunity: friend or foe? Cell Microbiol 2006; 8: 185 – 196.

[58] Rubins JB, Pomeroy C. Role of Gamma Interferon In the Pathogenesis of Bacteremic Pneumococcal Pneumonia. Infect Immun 1997; 65: 2975 – 2977.

[59] Pedrosa J, Saunders BM, Appelberg R, Orme IM, Silva MT, Cooper AM. Neutrophils Play a Protective Nonphagocytic Role in Systemic *Mycobecterium tuberculosis* Infection of Mice. Infect Immun 2000; 68: 577 – 583.

[60] Barkman C, Martner A, Hessle C, Wold AE. Soluble bacterial constituents down-regulate secretion of IL-12 in response to intact Gram-positive bacteria. Microbes Infect 2008; 10: 1484 – 1493.

[61] Didierlaurent A, Goulding J, Patel S, Snelgrove R, Low L, Bebien M, et al. Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. J Exp Med 2008; 205; 323 – 329.

[62] Dudani R, Murali-Krishna K, Krishnan L, Sad S. IFN-γ Induces the Erosion of Preexisting CD8 T Cell Memory during Infection with a Heterologous Intracellular Bacterium. J Immunol 2008; 181: 1700 – 1709.

[63] Jo E-K. Mycobacterial interaction with innate receptors: TLRs, C-type lectins, and NLRs. Curr Opin Infect Dis 2008; 21: 279 – 286.

[64] Brittle W, Marais BJ, Hesseling AC, Schaaf HS, Kidd M, Wasserman E, et al. Improvement in mycobacterial yield and reduced time to detection in pediatric samples by use of a nutrient broth growth supplement. J Clin Microbiol 2009; 47: 1287 – 1289.

[65] Hargreaves NJ, Kadzakumanja O, Phiri S, Nyangulu DS, Salaniponi FML, Harries AD, et al. What causes smear-negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence? Int J Tuberc Lung Dis 2001; 5: 113 – 122.

[66] Madhi SA, Huebner RE, Doedens L, Aduc T, Wesley D, Cooper PA. HIV-1 co-infection in children hospitalized with tuberculosis in South Africa. Int J Tuberc Lung Dis 2000; 4: 448 – 454.

[67] Madhi SA, Gray G, Huebner RE, Sherman G, McKinnon D, Pettifor J. Correlation between $CD4^+$ lymphocyte counts, concurrent antigen skin test and tuberculin skin test reactivity in human immunodeficiency virus type 1-infected and –uninfected children with tuberculosis. Pediatr Infect Dis J 1999; 18: 800 – 805.

[68] Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis 2004; 8: 636 – 647.

[69] Driver CR, Munsiff SS, Li J, Kundamal N, Osahan SS. Relapse in Persons Treated for Drug-Susceptible Tuberculosis in a Population with High Coinfection with Human Immunodeficiency Virus in New York City. Clin Infect Dis 2001: 33: 1762 – 1769.

[70] Soeters M, de Vries AM, Kimpen JLL, Donald PR, Schaaf HS. Clinical features and outcome in children admitted to a TB hospital in the Western Cape – the influence of HIV infection and drug resistance. S Afr Med J 2005; 8: 602 – 606.

[71] Nahid P, Gonzalez LC, Rudoy I, De Jong BC, Unger A, Kawamura LM, et al. Treatment Outcomes of Patients with HIV and Tuberculosis. Am J Respir Crit Care Med 2007: 175; 1199 – 1206.

[72] Van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA, et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med 1999; 341: 1174 – 1179.

[73] Schaaf HS, Krook S, Hollemans DW, Warren RM, Donald PR, Hesseling AC. Recurrent Culture-Confirmed Tuberculosis in Human Immunodeficiency Virus-Infected Children. Pediatr Infect Dis J 2005; 24: 685 – 691.

[74] British Medical Research Council / Hong Kong Chest Services. Five-Year Follow-Up of a Controlled Trial of Five 6-Month Regimens of Chemotherapy for Pulmonary Tuberculosis. Am Rev Respir Dis 1987; 136: 1339 – 1342.

[75] British Medical Council / Hong Kong Chest Services. Controlled Trial of 2, 4 and 6 Months of Pyrazinamide in 6-Month, 3-Times-Weekly Regimens for Smear-positive Pulmonary Tuberculosis, Including an Assessment of a Combined Preparation of Isoniazid, Rifampicin and Pyrazinamide: results at 30 months. Am Rev Respir Dis 1991; 143; 700 – 706.

[76] Iukhimenko NV. Efficacy of shorter chemotherapy courses for intrathoracic tuberculosis in children [Abstract]. Probl Tuberk 2000; 3: 20 – 23.

[77] Tuberculosis Research Centre, Indian Council of Medical Research, Chennai, India. Low rate of emergence of drug resistance in sputum positive patients treated with short course chemotherapy. Int J Tuberc Lung Dis 2001; 5: 40 – 45.

[78] Edginton ME, Rakgokong L, Verver S, Madhi SA, Koornhof HJ, Wong ML, et al. Tuberculosis culture testing at a tertiary care hospital: options for improved management and use for treatment decisions. Int J Tuberc Lung Dis 2008; 12: 786 – 791.

[79] Engelbrecht AL, Marais BJ, Donald PR, Schaaf HS. A critical look at the diagnostic value of culture-confirmation in childhood tuberculosis. J Infect 2006; 53: 364 – 369.

[80] Schaaf HS, Marais BJ, Hesseling AC, Gie RP, Beyers N, Donald PR. Childhood drug-resistant tuberculosis in the Western Cape Province of South Africa. Acta Paediatr 2006; 95: 523 – 528.

[81] Dye C, Bassili A, Bierrenbach AL, Broekmans JF, Chadha VK, Glaziou P, et al. Measuring tuberculosis burden , trends, and the impact of control pogrammes. Lancet Infect Dis 2008; 8: 233 – 243.