Aspects of Tuberculosis Case Management at Red Cross Children's Hospital

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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 Philosophy in Paediatrics in the University of Cape Town, South Africa. It has not been submitted before for any degree or examination at this or any other University.

……………………………......

David Paul Moore

27th day of May, 2010

DEDICATION

This work is warmly dedicated to my parents, David and Anne-Marie Moore, and siblings John and Elizabeth, for their support and encouragement during the period of my sub-specialist training in Paediatric Infectious Diseases at Red Cross War Memorial Children's Hospital, from 01 April 2007 to 31 March 2009.

I further dedicate this work to the children of Cape Town from whom clinicians working in child health care settings in the city continually gain clinical experience and expertise; hopefully, information obtained from this study will assist in enhancing the health care of children by informing doctors and nurses about strengths and potential weaknesses which exist in current clinical practice.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Oral Presentation

Moore DP, Barnabas S, Finlayson H, Nuttall J, Rinquest C, Whitelaw A, Eley B. Culture-confirmed Tuberculosis at Red Cross Children's Hospital. University of Cape Town School of Child and Adolescent Health Annual Research Day, Cape Town, 22 October 2008.

ABSTRACT

Aim

To describe the spectrum of tuberculosis in children <15 years of age attending Red Cross War Memorial Children's Hospital between January 2006 and December 2008.

Methods

A retrospective review of a paper-based Notifications Register and a database of culture-confirmed tuberculosis were undertaken. Laboratory and clinical data were analysed using standard statistical methods.

Results

1 314 episodes of tuberculosis were identified amongst 1 300 children, representing 12% of the Cape Town Metropole's child tuberculosis case load. 433 (33.0%) of all cases were culture-confirmed; however, 120 (27.7%) of all culture-confirmed cases were not recorded in the paper-based Notifications Register. 44% of children with culture-confirmed pulmonary tuberculosis presented with cough of less than 10 days' duration, and 40% of children with culture-confirmed tuberculosis were treated with regimens that were not widely available at down-referral clinics.

629 (48.4%) of the 1 300 children with tuberculosis had undefined HIV status. Factors associated with lack of HIV testing in tuberculosis suspects included outpatient management (adjusted Odds Ratio [AOR] 0.22; 95% CI, 0.17 – 0.29), and management by the surgical disciplines (AOR 0.23; 95% CI, 0.14 – 0.40).

70% of the ART-naïve HIV-tuberculosis co-infected children were tested for HIV during clinical evaluation for tuberculosis. Access to ART was achieved in 60% of HIV-tuberculosis co-infected children, with excellent immunologic and virologic response to

ART. Poorer virologic response to ART was observed in children treated with PI-based ART, compared to those treated with NNRTI-based ART (adjusted Hazard Ratio 8.20 [95% CI, 1.12 – 59.88]), P=0.038. Possible unmasking tuberculosis immune reconstitution inflammatory syndrome (IRIS) was associated with poorer virologic outcome compared to episodes with no IRIS (follow-up viral load: 5 100 vs. 25 RNA copies/ml), P=0.020.

HIV-infected compared to -uninfected children were more likely to be diagnosed with multidrug-resistant tuberculosis (AOR 4.03; 95% CI, 1.01 – 16.13), P=0.049.

Conclusions

To improve the clinical service, detection of HIV co-infection in children undergoing evaluation for tuberculosis should be enhanced and strategies adopted to ensure that all children with culture-confirmed disease are notified and access antituberculosis therapy.

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I am most grateful to Professors George Swingler and Heather Zar for their encouragement and support during my training period at RCWMCH, and particularly to Professor Zar for permitting me to explore the area of child tuberculosis at the institution from which she has contributed so effectively to the global understanding of this disease entity.

Finally, I thank Professor John Pettifor and my colleagues at the Department of Paediatrics and Child Health at Chris Hani Baragwanath Hospital, Johannesburg, for permitting me to take two years' leave of absence in order to participate in the Infectious Diseases sub-speciality training programme in Cape Town.

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NOMENCLATURE

ADA	adenosine deaminase
ART	antiretroviral therapy
BCG	bacillus Calmette-Guérin
CDC	Centers for Disease Control and Prevention
CHBH	Chris Hani Baragwanath Hospital
CI	confidence interval
CRP	C-reactive protein
ELISA	enzyme linked immunosorbent assay
ESR	erythrocyte sedimentation rate
GSH	Groote Schuur Hospital
HIV	human immunodeficiency virus
HR	Hazard Ratio
IDC	Infectious Diseases Clinics
IQR	interquartile range
IRIS	immune reconstitution inflammatory syndrome
NHLS	National Health Laboratory Service
OR	Odds Ratio
PCR	polymerase chain reaction
PMTCT	prevention of mother-to-child transmission (of HIV)
RCWMCH	Red Cross War Memorial Children's Hospital
RR	Risk Ratio
SANTCP	South African National Tuberculosis Control Programme
SD	standard deviation
TCH	Tygerberg Children's Hospital
WHO	World Health Organization

1.0 INTRODUCTION

1.1 Tuberculosis In South Africa

It is of considerable concern that, at current disease incidence rates, the sixth Millennium Development Goal parameter relating to tuberculosis, which aims to halve the 1990 tuberculosis prevalence and death rates by 2015, is unlikely to be met in sub-Saharan Africa. (1, 2)

South Africa is currently ranked 3rd after India and China in terms of the burden of numbers of new tuberculosis cases detected, and 2nd after Swaziland in terms of tuberculosis incidence, with an estimated 480 000 new cases detected and a tuberculosis incidence of 960 per 100 000 population in 2008. (3, 4) This burden of disease has variously been attributed to high human immunodeficiency virus (HIV) co-infection rates, (5) socio-economic factors such as overcrowding which perpetuate the risk of becoming infected with tuberculosis, (6) and poorly functioning and over-burdened health care services. (7, 8)

The implications which such a high burden of disease places on public health infrastructures are profound, and even well-functioning tuberculosis control programmes are threatened to be overwhelmed by the sheer case load of patients accessing care in such settings. (5, 9) With the erosion of public health capacity to succeed in the management of these tuberculosis case loads, the threat of sub-optimal case finding and support for directly-observed therapy ensues; adult tuberculosis cases may have prolonged periods of undiagnosed sputum smear-positive or -negative pulmonary

tuberculosis, during which time vulnerable close contacts are at particular risk for becoming infected with *Mycobacterium tuberculosis*. (10)

1.2 The Burden Of Tuberculosis In The Western Cape Province

The Western Cape Province has traditionally been viewed as the epicentre of the tuberculosis epidemic in South Africa, and studies conducted in impoverished communities in the Province have demonstrated that tuberculosis disease incidence is amongst the highest in the world in those settings. (6, 11) Furthermore, the HIV prevalence rates in peri-urban communities around Cape Town have increased markedly since the late 1990s, (5) indicating a rapidly maturing HIV epidemic, which fuels the tuberculosis incidence rates in these communities.

The annual risk of tuberculosis infection in children residing in the Cape Town suburbs of Ravensmead and Uitsig failed to decrease between 1998/9 and 2005, over which time period it was observed to range from 3.7% (95% CI, 3.1 – 3.9%) to 4.1% (95% CI, 3.8 – 4.5%). (12) An identical annual risk of infection (4.1%) was observed in a predominantly Xhosa-speaking peri-urban informal settlement in Cape Town in 2006/7. (13) Middelkoop et al. (13) observe that these annual risks of tuberculosis infection are markedly higher than those observed in other HIV-endemic sub-Saharan countries.

According to the South African Department of Health, the incidence of tuberculosis (all cases) in the Western Cape Province was 1 030.7 per 100 000 population in 2006 and 1 005.7 per 100 000 population in 2007, with a smear-positive pulmonary tuberculosis incidence of 369.3 and 343.4 per 100 000 population in 2006 and 2007, respectively. (14) Extrapulmonary tuberculosis accounted for 12% of all tuberculosis cases in the Province in

2006, and 11% of all cases in 2007. (14) Children under 15 years of age accounted for 13.6% of all tuberculosis cases in the Cape Town Metropole from 2006 to 2008, and 11 145 children under 15 years of age were reported to the City Health Department for the time-period 01 January 2006 until 31 December 2008 (**Table 1**). (15) Using population estimates for the City of Cape Town, (16) this tuberculosis case load translates into considerable tuberculosis incidence rates for children residing in the city (**Table 2**).

1.3 Tuberculosis In Children

Children under five years of age are at particular risk for progressing to tuberculosis disease after infection with *M. tuberculosis* (17, 18) in view of their relative immune immaturity and the fact that they are vulnerable to factors such as malnutrition which enhance the progression to tuberculosis disease in individuals who are latently infected with *M. tuberculosis*.

Children under five years of age also represent the house-bound age group, who may constantly be exposed to a family member or care-giver with tuberculosis from whom they may contract the infection. Factors affecting the transmissibility of *M. tuberculosis* to small children include not only the degree of smear positivity of the source case's sputum, but also the length of time over which a child is in contact with the source case; therefore, even smear-negative source cases have been demonstrated to transmit infection to small children. (10, 19, 20)

Most child tuberculosis occurs in the form of non-cavitating pulmonary disease, and is sputum smear negative, or 'paucibacillary' in nature; (21) consequently, microbiological

Year	Numbers of tuberculosis cases reported to Cape Town Health Department (15)				Population estimates for Cape Town (16)		
	0 – 4 years	5 – 14 years	All child TB cases (% of total)	All TB cases (adult and child)	0 – 4 years	5 – 14 years	Total Population
2006	2 444	1 186	3 630 (13.7)	26 427	283 774	585 558	3 230 098
2007	2 486	1 080	3 566 (13.4)	26 635	296 691	587 146	3 335 159
2008	2 829	1 120	3 949 (13.7)	28 880	373 829	668 459	3 620 326
2006 – 2008	7 759	3 386	11 145 (13.6)	81 942	-	-	-

Table 1: Tuberculosis cases reported to the City of Cape Town Health Department with estimated population of the Cape Town Metropole, 2006 to 2008

Year	Tuberculosis incidence in the Cape Town Metropole (cases per 100 000 population)			
	0 – 4 years	5 – 14 years	All child TB cases	All TB cases (adult and child)
2006	861 (827 – 896)	203 (191 – 214)	418 (404 – 431)	818 (808 – 828)
2007	838 (805 – 872)	184 (173 – 195)	403 (390 – 417)	799 (789 – 808)
2008	757 (729 – 785)	168 (158 – 178)	379 (367 – 391)	798 (789 – 807)

Table 2: Tuberculosis incidence rates for children residing in the Cape Town Metropole, 2006 to 2008

TB = tuberculosis

yields from children with pulmonary tuberculosis are poor, with culture positivity rates between 20-40% in most settings. (22, 23) Childhood tuberculosis is generally diagnosed on a presumptive basis, taking into consideration factors on patient history and clinical examination.

1.4 Tuberculosis In The Era Of HIV

HIV infection has been recognised as being one of the most potent risk factors for promoting progression to disease after infection with *M. tuberculosis*, (24, 25) and HIV-infected children in South Africa are at 24- to 29-fold greater risk for culture-confirmed tuberculosis compared to immunocompetent children. (26, 27)

It is difficult to diagnose tuberculosis in children in the era of HIV infection as many clinical characteristics, e.g. weight loss, reticulo-endothelial system activation as manifested by lymphadenopathy, hepato- and splenomegaly, and pneumonia presenting with respiratory symptoms, are shared by both infections. (28-30)

Commonly-used diagnostic modalities, such as the tuberculin skin test (31) and chest radiographs, (32-34) may be less sensitive and specific, respectively, in HIV-infected children with tuberculosis compared to immunocompetent children with tuberculosis, and, although the diagnostic yield from microbiological tests has been found to be equivalent in HIV-infected and -uninfected children, (23, 27, 35) access to microbiologic laboratories with capacity to perform mycobacterial cultures is restricted in many high-burdened settings in the developing world.

1.4.1 Tuberculosis immune reconstitution inflammatory syndrome

An additional aspect of the interplay between *M. tuberculosis* and HIV is that HIV-infected individuals are at risk for developing aggravation of tuberculosis-related symptomatology shortly after commencing antiretroviral therapy (ART), either through worsening of symptoms in those who had been undiagnosed with tuberculosis prior to ART initiation (unmasking immune reconstitution inflammatory syndrome [IRIS]), or through exacerbation of clinical symptoms or clinical deterioration in those who had been started on antituberculosis therapy prior to commencement of ART and had initially exhibited clinical improvement on antituberculosis treatment (paradoxical IRIS). (36, 37)

Tuberculosis IRIS in HIV-infected individuals that have commenced ART has been attributed to recovery of CD4 T-lymphocyte function (38) and number in response to ART-induced declines in HIV viral load (RNA copies per millilitre) levels, with consequent development of dysregulated immunologic activation against previously undetected *M. tuberculosis* antigens. (36, 39)

Tuberculosis IRIS is yet another clinical entity which may complicate the diagnosis of tuberculosis in HIV-infected children, because an unexpected deterioration in the clinical condition of children who had been responding well to a course of antituberculosis therapy prior to ART initiation may lead clinicians to question their earlier diagnosis of tuberculosis in such patients, or because illness occurring within a few months of ART initiation may be attributed to aetiologies other than tuberculosis.

1.5 Tuberculosis Diagnostic Algorithms

In response to the concerns regarding difficulty in establishing a diagnosis of tuberculosis in children, clinical scoring systems such as the modified Edwards score chart (40, 41) advocated for use in the South African National Tuberculosis Control Programme (SANCTP) Guidelines (2004), (42) (**Appendix 1**, page 140) were developed. These scoring systems are a means of coalescing clinical data in children with suspected tuberculosis in order to assist in diagnosing the disease process when microbiological evidence is lacking. These systems are problematic for numerous reasons, and have not been widely validated in HIV-infected children. (41, 43, 44)

When used in settings with a high burden of HIV, the sensitivity of these scoring systems may be eroded because many of the features included for scoring (e.g. loss of weight, lymphadenopathy) are commonly seen in HIV-infected children who may not have tuberculosis. (29, 41, 44) Additionally, parameters used for scoring may bias the user away from diagnosing tuberculosis in children who may present with acute pulmonary tuberculosis, as many of the parameters place emphasis on clinical features which indicate long-standing or extrapulmonary forms of the disease.

1.6 Acute Presentations Of Childhood Pulmonary Tuberculosis

That pulmonary tuberculosis can present acutely has been demonstrated in three South African studies which investigated the aetiology of acute respiratory tract infection in children under five years of age: 8-15% of these children had culture-confirmed tuberculosis, which was mainly diagnosed using gastric aspirates. (45-47) Taking into consideration the limited sensitivity of gastric aspirates for diagnosing childhood pulmonary

tuberculosis, (22, 23) it is likely that *M. tuberculosis* may contribute to a greater proportion of cases presenting with acute respiratory infection in high-burdened settings.

Acute clinical presentations in children with pulmonary tuberculosis may be precipitated by co-infection with bacterial pathogens such as *Streptococcus pneumoniae*. (27)

1.7 Tuberculosis Case Management At Two Teaching Hospitals in South Africa

The spectrum of active tuberculosis and prevalence of HIV co-infection amongst children with a hospital-based diagnosis of tuberculosis differ between and within countries, and comparisons made between child tuberculosis cohorts treated at different centres aid in informing epidemiological aspects of childhood tuberculosis as well as reforming strategies so as to optimise case management.

Tertiary centres in South Africa are at a considerable advantage over peripheral health care facilities in terms of their capacity to investigate suspected childhood tuberculosis cases by submission of samples for microbiologic testing, and descriptions of cohorts of children diagnosed with tuberculosis at these facilities assist in defining the spectrum of tuberculosis disease encountered in sick children requiring hospitalisation.

In a study conducted at Chris Hani Baragwanath Hospital (CHBH), a large teaching hospital in Soweto, Gauteng Province, data relating to 2 456 children who had been diagnosed with tuberculosis between 2003 and 2006 were analysed. (48) This retrospective observational study highlighted deficiencies in the management of children with tuberculosis at that hospital: only 72% of all children diagnosed with tuberculosis had been tested for HIV; 68% of those who were found to be HIV-infected were referred to HIV

clinics at the hospital; and only 50% had been started on ART at the time of data analysis. The study also revealed how efficacious ART is in terms of preventing mortality in tuberculosis and HIV co-infected children: mortality amongst HIV-infected children not on ART was significantly greater than mortality amongst those on ART (Risk Ratio [RR] 2.56; 95% CI, 1.07 – 6.13), P=0.023. (48)

A similar audit was conducted at Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town, Western Cape Province, by assessment of the 2006 child tuberculosis cohort (as derived from review of the paper-based Notifications Register kept by the hospital Infection Control nurse), and was presented to the Paediatric Department at RCWMCH in October 2007. (49) This audit drew interesting comparisons between RCWMCH and CHBH in terms of the numbers of children diagnosed with tuberculosis and how they were investigated. At RCWMCH, for example, only 41% of children diagnosed with tuberculosis were tested for HIV; however, as policy at RCWMCH is to involve the Infectious Diseases Clinics (IDC) clinicians in the management of all children diagnosed with HIV, there is potential for all children with dual infection to be started on ART at RCWMCH.

1.8 Culture-confirmed Tuberculosis In Paediatric Cohorts

Occasionally, a diagnosis of culture-confirmed tuberculosis can be ascribed to paediatric patients on the basis of specimens that are positive for *M. tuberculosis* on mycobacterial culture, but few studies have reported on culture-confirmed tuberculosis in children. (26, 50-59)

Schaaf et al. (58) describe one of the largest recent cohorts of children with culture-confirmed tuberculosis (n=596) treated at two academic centres in Cape Town between March 2003 and February 2005. This descriptive study identified an HIV prevalence of 22% (or 42% amongst those whose HIV infection status was determined) in children with culture-confirmed tuberculosis and resistance to isoniazid and/or rifampicin in 11% of the *M. tuberculosis* isolates. (58) It has now become policy in the Western Cape to routinely perform drug susceptibility testing against isoniazid and rifampicin in *M. tuberculosis* isolates obtained from paediatric patients, as these isolates serve as a barometer of currently-circulating *M. tuberculosis* strain drug susceptibility in high-burdened communities. (55)

1.8.1 Missed opportunities for treating children with culture-confirmed tuberculosis

Unfortunately, as has been demonstrated in three studies from CHBH, (27, 60, 61) the occurrence of culture-confirmation in children who had been investigated for tuberculosis in inpatient or outpatient settings by submission of material for mycobacterial culture, does not always translate into appropriate case management: 36-49% of children diagnosed with culture-confirmed disease were not referred for registration at a TB Care Centre affiliated with CHBH, (62) and were likely never started on appropriate antituberculosis therapy for their disease. Delays in initiating antituberculosis therapy in children with culture-confirmed disease have also been described at facilities in Durban (53) and Cape Town. (63)

Non-commencement of appropriate antituberculosis therapy in these children relates to the fact that there may be a delay of up to eight weeks before tuberculosis culture results become available, (63, 64) by which time children have usually been discharged from

hospital; these children may have responded to empiric antibiotic therapy for community-acquired bacterial pneumonia, which has been determined to be an important concomitant illness in children hospitalised with culture-confirmed pulmonary tuberculosis. (27)

Outpatient follow-up or the ability to recall patients is often hampered by inaccurate recording of patient contact details in hospital records, (61) and rapid turn-over of medical staff at busy public health facilities. (63)

1.8.2 Strategies for optimising the identification and initiation of appropriate therapy of children with untreated culture-confirmed tuberculosis

In 2006, medical personnel at the University of Cape Town anticipated the impact which loss to follow-up of children with culture-confirmed tuberculosis who were not started on antituberculosis treatment during the hospitalisation in which specimens were submitted for mycobacterial culture, would have on those patients. An algorithm was established which sought to improve the links between clinicians and the tuberculosis laboratory so as to achieve earlier notification of those cases with positive tuberculosis cultures. This algorithm was adopted in late 2006, and has been rigorously implemented at RCWMCH since 2007.

Briefly, this system depends upon the centralised microbiology laboratory based at Groote Schuur Hospital (GSH) sending weekly updates of results of positive paediatric mycobacterial cultures to the IDC clinicians at RCWMCH. Mycobacterial cultures at the GSH laboratory are routinely conducted using liquid-based techniques, using the Mycobacterial Growth Indicator Tube system (Becton-Dickinson, Sparks, Maryland, USA). Once information regarding positive mycobacterial cultures has been sent to the IDC Unit

at RCWMCH, the clinical records of these children are retrieved for data extraction; those children who are found not to be on antituberculosis treatment are actively traced either by telephonic interview or by contacting the Primary Health Care Clinic nearest to the child's place of residence, in order to initiate antituberculosis treatment.

1.9 Treatment Of Tuberculosis In Children

Children under eight years of age with uncomplicated ('paucibacilliary') forms of tuberculosis can effectively be treated utilising three first-line antituberculosis agents (rifampicin, isoniazid and pyrazinamide) in the two-month intensive phase of therapy, followed by a four-month continuation phase utilising isoniazid and rifampicin. The SANTCP Guidelines refer to this regimen as Regimen III.

The World Health Organization (WHO) has defined certain settings in which child tuberculosis cases should be treated using the four-drug (rifampicin, isoniazid, pyrazinamide and ethambutol) intensive phase of therapy utilised in adult patients. (65) The SANTCP Guidelines refer to this treatment regimen as Regimen I. Clinical scenarios in which Regimen I should be used include:

1. Treatment of children with smear-positive pulmonary tuberculosis;
2. Treatment of children with smear-negative pulmonary tuberculosis, whose chest radiographs reveal the presence of extensive pulmonary disease;
3. Treatment of children with clinically advanced HIV infection;
4. Treatment of severe forms of extrapulmonary tuberculosis, e.g. tuberculous pericarditis, tuberculous peritonitis, osteoarticular tuberculosis.

The continuation phase of antituberculosis therapy in children treated with Regimen I, consists of four months of rifampicin and isoniazid.

In South Africa, children with miliary tuberculosis and those with tuberculous meningitis are managed with high doses of rifampicin, isoniazid, pyrazinamide and ethionamide, (42) for six to 12 months' duration, depending on clinical response to therapy, and should ideally be managed in dedicated tuberculosis treatment facilities in order to ensure adherence to therapy for best outcomes.

The SANTCP does not readily accommodate the management of children using regimens other than Regimen III; therefore, children with severe forms of the disease may not be immediately down-referable for continued management at Primary Health Care facilities after a hospital-based diagnosis of their condition.

1.10 Aims And Objectives

1.10.1 Primary objectives

1. To describe demographic and laboratory-extracted clinical characteristics (including HIV infection status and degree of HIV-related immunosuppression) and spectrum of tuberculosis encountered in children who had been recorded in the paper-based Notifications Register at RCWMCH between 01 January 2006 and 31 December 2008.
2. To describe, by folder review, the demographic and clinical characteristics of children with culture-confirmed tuberculosis who had been identified either through review of the paper-based Notifications Register between 01 January 2006 and 31 December 2008, or through GSH microbiology laboratory feedback to RCWMCH

IDC clinicians over a 25-month period (14 November 2006 and 17 December 2008).

1.10.2 Secondary objectives

1. To describe the spectrum of clinical features and laboratory investigations used to diagnose tuberculosis in children at RCWMCH.
2. To determine the sensitivity of the tuberculosis score chart recommended by the SANTCP Guidelines (2004) (42) for the clinical diagnosis of tuberculosis in children, by scoring features obtained by record review in children who had culture-confirmed tuberculosis.
3. To describe the immunologic and virologic parameters of HIV-infected children with tuberculosis and to estimate the number of unmasking tuberculosis IRIS events that were encountered in HIV-infected children who had been initiated on ART prior to tuberculosis diagnosis.
4. To describe the antituberculosis treatment regimens adopted to treat children with culture-confirmed tuberculosis.
5. To describe tuberculosis-attributable mortality outcomes according to HIV infection status.
6. To estimate the burden of child tuberculosis managed at RCWMCH by comparison with total child tuberculosis cases as registered in the Cape Town Health Department database in 2006, 2007 and 2008.

2.0 MATERIALS AND METHODS

This observational study entailed the retrospective review of all paediatric patients (children under the age of 180 months, i.e. under 15 years of age) notified for tuberculosis at RCWMCH from 01 January 2006 to 31 December 2008 by review of a paper-based Notifications Register. Additionally, retrospective analysis of data extracted by folder review for children identified as having culture-confirmed tuberculosis was undertaken.

Two databases were created in order to achieve the study objectives:

1. An electronic Notifications Database into which all children who had been entered into a paper-based Notifications Register between 01 January 2006 and 31 December 2008 were recorded;
2. A Culture-confirmed Tuberculosis Database in which all culture-confirmed tuberculosis cases identified between 14 November 2006 and 17 December 2008 by feedback from the centralised microbiology laboratory at GSH to IDC clinicians at RCWMCH were recorded.

The Culture-confirmed Tuberculosis Database was initiated in late 2006, as a means of identifying children with culture-confirmed tuberculosis who may have missed access to antituberculosis therapy (as described in **Section 1.8.1**), and therefore does not include information relating to all children treated at RCWMCH who had culture-confirmed tuberculosis prior to November 2006.

The Notifications Database and Culture-confirmed Tuberculosis Database were merged after data collection and cleaning (which included removal of duplicate entries) to synthesise a Combined Database, which was utilised for data analysis.

2.1 Ethics And Consent

Ethics approvals for commencement of data collection in the two aspects of this study were obtained through the University of Cape Town Health Sciences Faculty Research Ethics Committee in 2008: reference numbers **REC REF: 234/2008** (**Appendix 2**, page 141) and **REC REF: 233/2008** (**Appendix 3**, page 143).

2.2 The Notifications Database

Details of children notified for tuberculosis and other notifiable conditions were obtained from the paper-based Notifications Register, which is routinely updated by the RCWMCH Infection Control nurse. Patient names and hospital numbers (which were used for data retrieval off the National Health Laboratory Service [NHLS] database) were entered into a study Microsoft Access (Microsoft, Redmond, Washington, USA) database. Data from the paper-based Notifications Register were captured by a clinician (DM) and two data capturers affiliated with the IDC.

All data entered into the Notifications Database after transcription of patient identifiers from the paper-based system were captured by the principal investigator (DM); these parameters are tabulated in **Appendix 4** (page 145).

Data pertaining to the clinical presentation of children entered into the Notifications Database, who were found on review of the NHLS Database to have culture-confirmed

tuberculosis, were obtained by folder review and extracted onto data collection forms which included parameters listed in **Appendix 5** (page 147). All culture-confirmed cases were identified through the NHLS database. Children whose mycobacterial cultures remained negative, or who were diagnosed with tuberculosis without submission of specimens for mycobacterial culture, were classified as being 'other than culture-confirmed' cases (66) (see **Section 2.5**, below) and folder reviews were not conducted for these patients.

2.3 The Culture-confirmed Tuberculosis Database

Data pertaining to children treated at RCWMCH who were identified as having culture-confirmed tuberculosis (by feedback from the GSH Microbiology Laboratory, as mentioned in **Section 1.8.2**) were collected retrospectively, in order to trace children who may potentially not have been started on anti-tuberculosis therapy during the course of the RCWMCH admission in which specimens were submitted for mycobacterial culture. These data were assimilated in a separate Microsoft Access database, designated the Culture-confirmed Tuberculosis Database.

Folder reviews were conducted in order to ascertain the clinical characteristics at presentation in children with culture-confirmed tuberculosis. Parameters entered into the Culture-confirmed Tuberculosis Database are tabulated in **Appendix 5**, page 147.

2.4 The Combined Database

After completion of data collection of the electronic Notifications and Culture-confirmed Tuberculosis Databases, the databases were merged to synthesise a Combined Database. Duplicate entries (identified as being hospitalisation events occurring in the

same child [identified using first name, surname, date of birth, and hospital identification number] on the same date of admission) were excluded and only the first of these entries was used for analysis, so as not to overestimate the number of tuberculosis cases managed at the hospital.

The Combine Database formed the foundation from which data analyses were conducted.

2.5 Categorisation Of Children With Tuberculosis

Children identified as having been diagnosed with tuberculosis were classified using a modification of the WHO definitions for tuberculosis in children (28, 67) according to the strength of evidence available in arriving at a diagnosis of active tuberculosis in each case (**Appendix 6**, page 149).

As a dedicated folder review was not conducted for all children who had been identified as having tuberculosis through the Notifications Database, but only for those who had culture-confirmed disease, only two categories of tuberculosis could be attributed in this cohort; namely, ‘culture-confirmed tuberculosis’ and ‘other than culture-confirmed tuberculosis’. (66) As all children entered into the Culture-confirmed Tuberculosis Database had bacteriologic evidence of their diagnosis, all of these children had ‘culture-confirmed tuberculosis’.

Additional tuberculosis categories attributed to children included in the Notifications Database and Culture-confirmed Tuberculosis Database included:

1. Pulmonary tuberculosis: attributed in cases in which respiratory symptomatology predominated, chest radiographs were suggestive, and/or *M. tuberculosis* was cultured from respiratory samples (broncho-alveolar lavage, gastric aspirates, nasopharyngeal aspirates, expectorated or induced sputum, or tracheal aspirates).

Children who had extrapulmonary tuberculosis, and from whom respiratory samples cultured *M. tuberculosis*, were classified as pulmonary tuberculosis cases, according to WHO definitions; (68)

2. Extrapulmonary tuberculosis: attributed in cases in which tuberculosis involving extrapulmonary sites was diagnosed. Extrapulmonary tuberculosis cases were further stratified as being:
    a) Mild extrapulmonary tuberculosis where tuberculous adenitis or pleural effusion were identified, or
    b) Severe extrapulmonary tuberculosis in children who had tuberculous pericarditis, tuberculous peritonitis, or osteoarticular tuberculosis.

3. Miliary tuberculosis or tuberculous meningitis: attributed in children with culture-confirmed disease who had been noted (on folder review) to have a miliary pattern on chest radiograph, or those in whom *M. tuberculosis* was cultured from blood, bone marrow or cerebrospinal fluid. The term ‘disseminated tuberculosis’ has also been used to describe miliary tuberculosis. (30) Children with ‘other than culture-confirmed’ tuberculosis who were notified as having miliary tuberculosis or tuberculous meningitis were also included in this category of patients.

4. Recurrent tuberculosis: attributed in children who had a previous diagnosis of tuberculosis (more than six months previously*) and who were subsequently diagnosed with tuberculosis, regardless of HIV-infection status.

Children from whom non-tuberculous mycobacteria or *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) were cultured from clinical specimens, were classified as 'not tuberculosis' cases and were not included in the analysis.

2.6 Determination Of HIV Infection Status

HIV infection status in the children was identified by review of the NHLS database (and hospital records for children with culture-confirmed tuberculosis). A diagnosis of HIV infection was attributed in instances where a positive HIV enzyme linked immunosorbent assay (ELISA) result was recorded in children older than 18 months of age, or a positive HIV polymerase chain reaction (PCR) result was retrieved in children less than 18 months of age. For the purposes of this study, a single positive result was deemed sufficient evidence to attribute a diagnosis of HIV infection. Children with no retrievable HIV result, or those who were younger than 18 months of age with a positive ELISA but no follow-on PCR result, were labelled as having undefined HIV status.

The immunologic classification of HIV-infected children whose baseline CD4 T-lymphocyte cell parameters were available was determined using current WHO guidelines (**Appendix 7**, page 150). (69) Data reflecting whether HIV-infected children were on ART, timing of

* The exception to this rule was an HIV-infected child with culture-confirmed drug susceptible tuberculosis who cultured multidrug-resistant tuberculosis 4.9 months into the course of therapy for the initial illness; as these isolates differed markedly in terms of their drug susceptibility profiles, the two episodes were considered as being separate events.

ART in relation to the tuberculosis diagnosis, and which ART regimen was used, were obtained from the IDC Database.

2.7 Patient Demographics And Hospital Outcome Data

Patient demographics (gender, date of birth) and details of each hospitalisation episode (dates of admission or death) for children included in both the Notifications Database and the Culture-confirmed Tuberculosis Database were obtained using the RCWMCH electronic patient management system (CliniCom™ Application Manager, version 2.12.3.1, Siemens Medical Solutions, Cape Town), routine RCWMCH mortality data (these data were obtained using a mortality database which is regularly updated by a principal paediatrician working at RCWMCH) and by folder review (for children with culture-confirmed tuberculosis only).

Age of children at admission was calculated by subtracting the date of birth from the date of admission. As the hospital discharge date was not routinely recorded in the paper-based Notifications Register, the length of hospital stay (date of discharge minus date of admission) could not be calculated for all children. Age at death in children who died was calculated by subtracting the date of birth from the date of death.

In children who were noted to have died (through either the use of the electronic patient management system, the mortality database mentioned above, or by folder review), only deaths which occurred during the course of antituberculosis therapy were deemed to be tuberculosis-attributable deaths. Case fatality rates for tuberculosis were calculated using tuberculosis-attributable deaths only.

2.8 Possible Unmasking Tuberculosis IRIS

Definitions proposed for the diagnosis of unmasking tuberculosis IRIS events in adult patients require that a diagnosis of tuberculosis occurs within three months of initiation of ART in individuals who had not been diagnosed with tuberculosis prior to ART initiation; (36, 37) although this time frame is less applicable to paediatric practice, principally because it is difficult to distinguish between unmasking tuberculosis IRIS and incident tuberculosis in young children who have recently been started on ART, (37) for the purposes of this study children diagnosed with tuberculosis within the first three months of ART were regarded as having possible unmasking tuberculosis IRIS. (70)

As the date of initiation of antituberculosis treatment was not known in all cases (because this information was not recorded in the paper-based Notifications Register), time to onset of possible unmasking IRIS was calculated by subtracting the date of admission during which a child was investigated for tuberculosis from the date of initiation of ART: children with negative time-values calculated in this way were deemed to have started ART prior to the admission episode in which the child was investigated for tuberculosis. A child with culture-confirmed tuberculosis was initiated onto ART during the admission episode during which specimens were submitted for mycobacterial culture, and started ART prior to the date of submission of specimens for mycobacterial culture or commencement of antituberculosis therapy: this child was also regarded as being at risk for possible unmasking tuberculosis IRIS.

As date of initiation of antituberculosis treatment was unknown in most cases (as mentioned above), an estimate of the number of paradoxical tuberculosis IRIS events (worsening of tuberculosis-related symptoms and clinical signs shortly after

commencement of ART in children who had started antituberculosis treatment before ART initiation) was not attempted in this study.

2.9 Assessment Of The SANTCP Tuberculosis Scoring System

The SANTCP Guidelines (2004) (42) recommend the use of a score chart to guide clinicians in achieving a diagnosis of tuberculosis in children. The system, which scores 'General' and 'Local' features, is tabulated in **Appendix 1**, page 140. Children who were identified as having culture-confirmed tuberculosis were scored using this score chart by retrospective review of their medical records in order to estimate the sensitivity of the algorithm.

The retrospective nature of this study was problematic, in that not all score chart parameters were recorded in clinical notes in all children with culture-confirmed tuberculosis; in instances where information pertaining to particular parameters was not recorded in clinical notes, these were omitted in the score assessment. The number of parameters used to score each child (using criteria included in the score chart that had been noted in clinical records) were recorded as the 'score basis' on which an algorithm score could be calculated. In an attempt not to underestimate the sensitivity of the score chart sensitivity in detecting cases of culture-confirmed tuberculosis, the performance of the score chart was undertaken only in children who could be evaluated with the use of at least 10 of the 12 parameters stipulated in the score chart.

Additionally, the score chart sensitivity (with exclusion of the chest radiograph parameter) was assessed in order to compare score performance in the RCWMCH cohort with that observed in a study conducted in Zambian children. (41) In order to achieve more accurate

score chart sensitivities in the latter approach, only children who had been fully scored (using 11 parameters) were evaluated in this way.

2.10 Statistical Analysis

Data were analysed using STATA™ version 11.0 (StataCorp, College Station, Texas, USA). Means and medians of continuous variables were analysed using the unpaired Student's *t* test or appropriate non-parametric tests, respectively; paired statistical tests (the Paired *t* test and Wilcoxon sign-rank test) were applied for the comparison of means and medians of baseline and follow-up CD4 T-lymphocyte and HIV viral load determinations in HIV-infected children in whom these data were available.

Categorical variables were analysed using Pearson's χ^2 test; Fisher's exact test was used if there were five or fewer anticipated observations in any cell of generated contingency tables.

Multivariate analyses using logistic regression models were adopted to explore which factors were significantly associated with HIV testing at RCWMCH, positive tuberculosis score chart outcomes, multidrug-resistant tuberculosis, and tuberculosis-attributable death within the cohort. Parameters which had been identified as having P-values of ≤ 0.2 in univariate analyses were included for evaluation in each of these multiple logistic regression models.

A time-series analysis and Cox proportional hazards methods were used to analyse factors which were associated with virologic failure in children treated with ART and antituberculosis therapy concomitantly. Time on ART to potential virologic failure was

calculated by subtracting the date of initiation of ART from the date of follow-up HIV viral load testing. Virologic failure was defined as a follow-up viral load of ≥ 400 RNA copies per millilitre; (71) the analysis was also conducted using the higher viral load cut-off of ≥ 1 000 RNA copies per millilitre. Follow-up viral load was defined as being an HIV viral load determination that was obtained during or after completion of antituberculosis therapy. Baseline HIV viral loads were defined as tests that had been conducted either prior to or at the admission episode during which an HIV-infected child was investigated for tuberculosis, and subsequently diagnosed with the disease.

An alpha ≤ 0.05 was considered as being significant in both the univariate and multivariate analyses; all quoted P-values are two-tailed.

Agreement between the Mantoux test and SANTCP Guidelines (2004) tuberculosis modified Edwards score chart in predicting tuberculosis in children with culture-confirmed disease was assessed using weighted kappa statistics. (44)

For children with culture-confirmed tuberculosis in whom nutritional status (weight in kilograms) were recorded at the time of clinical evaluation for tuberculosis, Epi Info version 6.04d (Centers for Disease Control and Prevention [CDC], Atlanta, Georgia, USA) was used to calculate weight-for-age z-scores (using 1978 CDC/WHO Growth Reference Curves) for the assessment of nutritional status at the admission episode in which *M. tuberculosis* was cultured. As the lengths and/or heights of children identified as having culture-confirmed tuberculosis were not recorded in the data collection phase of this study (**Appendices 4** and **5**, pages 145 and 147), nutritional status of children using the 2006 WHO Child Growth Standards (72) could not be calculated. The Wellcome Classification (73) was utilised to describe the children's nutritional status, as this system is included in

the nutritional evaluation of children in the modified Edwards tuberculosis score chart (40) recommended for use by the SANTCP Guidelines (2004) (**Appendix 1**, page 140).

Burden of disease estimates were undertaken by comparison of the proportion of tuberculosis cases managed at RCWMCH to the total child tuberculosis case load reported to the Cape Town City Health Department during the study period. Proportions and their 95% confidence intervals for tuberculosis incidence rates were derived by using the Poisson distribution.

3.0 RESULTS

3.1 The Notifications Database

One thousand six hundred and fifty-three individuals were recorded in the Notifications Database between 01 January 2006 and 31 December 2008; 1 607 (97.2%) were under the age of 15 years. Of the 1 607 children, 856 (53.3%) were male, and 1 188 (73.9%) had at least one episode of tuberculosis. Of these tuberculosis cases, 580 (48.8%) had no defined HIV result, 245 (20.6%) were HIV-infected and 363 (30.6%) were HIV-uninfected. Hence, 40.3% (245/608) of all children with tuberculosis who were tested for their HIV infection status were confirmed to be HIV-infected.

There were 1 669 notification episode events entered into the Notifications Database over the study period: 15 repeat notification events (12 of which were attributable to tuberculosis) occurred in children under 15 years of age, and one repeat notification event (tuberculosis) occurred in a child over 15 years of age. Median time between first and subsequent tuberculosis notifications in the 13 individuals who had recurrent tuberculosis episodes, was 12.8 months (Interquartile Range [IQR] 9.6 – 15.2); HIV-infection status did not impact on time to repeat notification for tuberculosis, $P=0.886$.

The spectrum of disease (including recurrent notification events) for which individuals were recorded in the Notifications Database is tabulated below (**Table 3**); tuberculosis accounted for the majority (n=1 220, 73.1%) of notifications within the cohort. Of the 1 220 tuberculosis episodes, 1 200 (98.4%) occurred in children under 15 years of age; children under the age of five years where more frequently diagnosed with tuberculosis than were children older than five years of age, $P<0.001$ (**Table 3**).

Notifiable Condition	Age Group (months)		P-values[*]	Age Group (months)		P-values[†]
	<60	60–180		<180	≥180	
Tuberculosis	875 (78.9)[‡]	325 (63.4)	<0.001	1 200 (74.0)	20 (42.6)[§]	<0.001
Acute Flaccid Paralysis	13 (1.2)	7 (1.4)	0.744	20 (1.2)	0 (0.0)	1.000[**]
Acute Rheumatic Fever	2 (0.2)	17 (3.3)	<0.001	19 (1.2)	1 (2.1)	0.437[**]
Diphtheria	0 (0.0)	1 (0.2)	0.316[**]	1 (0.1)	0 (0.0)	1.000[**]
Food Poisoning	3 (0.3)	1 (0.2)	1.000[**]	4 (0.3)	0 (0.0)	1.000[**]
Haemophilus influenzae type b	2 (0.2)	0 (0.0)	1.000[**]	2 (0.1)	1 (2.1)	0.082[**]
Hepatitis A	27 (2.4)	56 (10.8)	<0.001	83 (5.1)	5 (10.6)	0.097[**]
Hepatitis (unspecified)	32 (2.9)	55 (10.7)	<0.001	87 (5.4)	7 (14.9)	0.014[**]
Malaria	4 (0.4)	1 (0.2)	1.000[**]	5 (0.3)	0 (0.0)	1.000[**]
Measles	4 (0.4)	0 (0.0)	0.314[**]	4 (0.3)	1 (2.1)	0.133[**]
Mumps	4 (0.4)	0 (0.0)	0.314[**]	4 (0.3)	0 (0.0)	1.000[**]
Neisseria meningitidis	19 (1.7)	6 (1.2)	0.409	25 (1.5)	1 (2.1)	0.527[**]
Organophosphate poisoning	32 (2.9)	8 (1.6)	0.109	40 (2.5)	3 (6.5)	0.118[**]
Pertussis	6 (0.5)	0 (0.0)	0.185[**]	6 (0.4)	0 (0.0)	1.000[**]
Congenital syphilis	11 (1.0)	0 (0.0)	Not applicable	11 (0.7)	0 (0.0)	Not applicable
Neonatal tetanus	1 (0.1)	0 (0.0)	Not applicable	1 (0.1)	0 (0.0)	Not applicable
Typhoid fever	0 (0.0)	4 (0.8)	0.010[**]	4 (0.3)	1 (2.1)	0.133[**]
Other	10 (0.9)	1 (0.2)	0.190[**]	11 (0.7)	0 (0.0)	1.000[**]
Unspecified conditions††	64 (5.8)	31 (6.0)	0.828	95 (5.9)	7 (14.9)	0.021[**]
Total	1 109 (100.0)	513 (100.0)	-	1 622 (100.0)	47 (100.0)	-

Table 3: Conditions for which individuals were registered in the Notifications Database

* P-values compare the proportion of children under 180 months of age who were diagnosed with each notifiable condition, according to age group (<60 months and 60–180 months).

† P-values compare the proportion of children (individuals <180 months of age) compared to adults who were diagnosed with each notifiable condition.

‡ Numbers in brackets are column percentages of notified individuals in each age group.

§ The paucity of tuberculosis cases in individuals ≥ 180 months of age is explained by the fact that RCWMCH serves a predominantly childhood patient population.

** Fisher's exact test.

†† Individuals notified with 'unspecified conditions' had insufficient data recorded in the paper-based Notifications Register to attribute a specific diagnosis.

3.2 The Culture-confirmed Tuberculosis Database

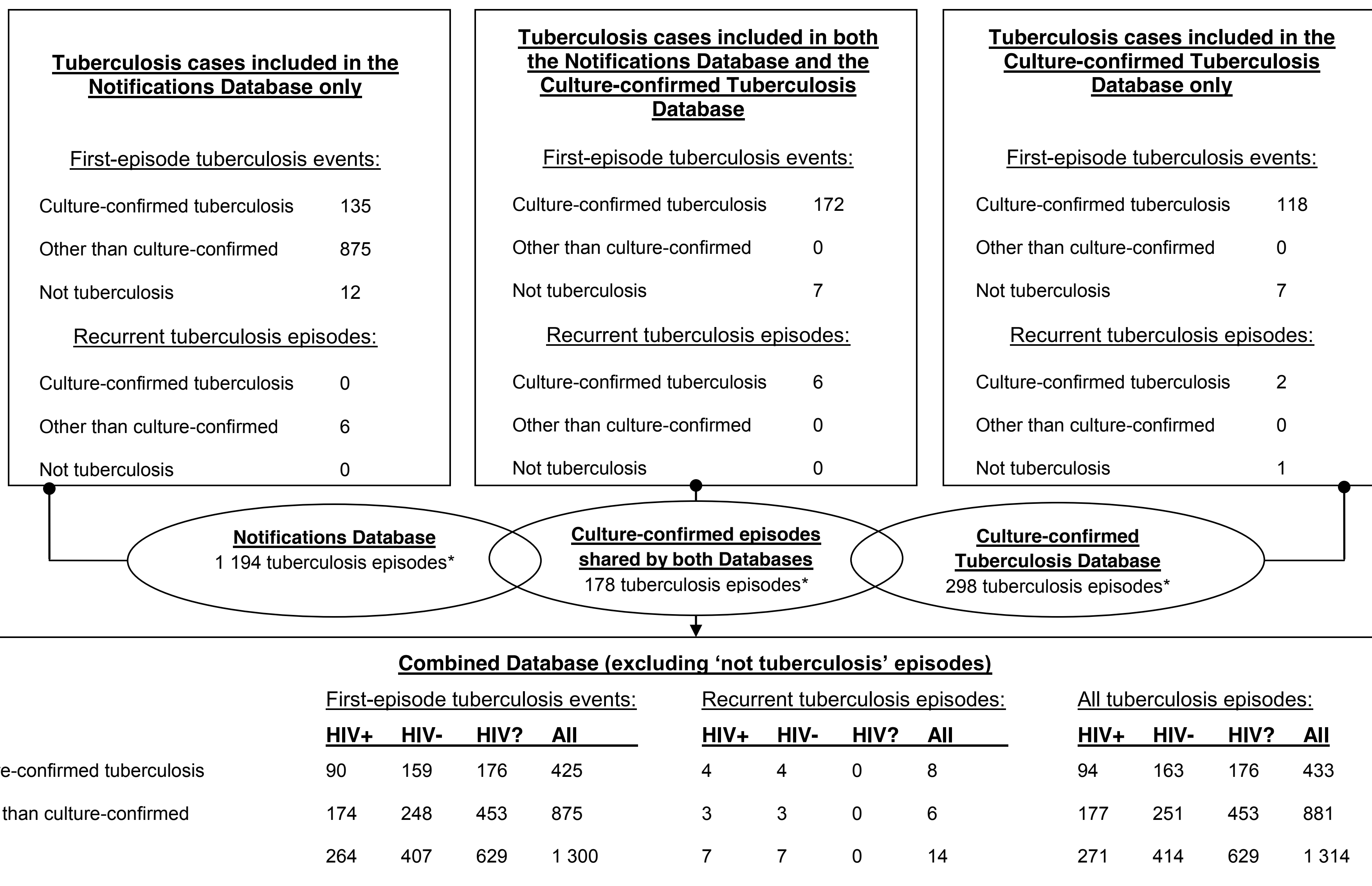
Three-hundred and four children under 15 years of age (174 [57.2%] of which were males) were registered in the Culture-confirmed Tuberculosis Database between 14 November 2006 and 17 December 2008.

One-hundred and eight (35.5%) of these children had undefined HIV infection status, 72 (23.7%) were HIV-infected and 124 (40.8%) were HIV-uninfected; 36.7% (72/196) of the children entered into the Culture-confirmed Tuberculosis Database who had been tested for their HIV infection status, were confirmed to be HIV-infected.

There were 313 episodes in which mycobacterial species were cultured amongst the 304 children who were entered into the Culture-confirmed Tuberculosis Database: five repeat episodes in HIV-infected children and four repeat episodes in HIV-uninfected children. Mean time to repeat mycobacterial species culture was 15.1 months (Standard Deviation [SD] 7.3), with no significant difference in time to recurrence according to HIV infection status, P=0.413. Only 185 (59.1%) of these 313 episodes were registered in the hospital Notifications Database (**Figure 1**).

3.3 Adjustments To The Number Of Tuberculosis Cases Identified At RCWMCH: The Combined Database

Closer scrutiny of the Notifications Database revealed that 13 children who had been notified with conditions other than tuberculosis had *M. tuberculosis* cultured from clinical specimens submitted during the course of the admission. Alternative diagnoses made in these children included: acute flaccid paralysis (n=4), unspecified viral hepatitis (n=3), hepatitis A (n=2), infection with *Neisseria meningitidis* (n=1), unspecified bacterial



	First-episode tuberculosis events:				Recurrent tuberculosis episodes:				All tuberculosis episodes:			
	HIV+	**HIV-**	**HIV?**	**All**	**HIV+**	**HIV-**	**HIV?**	**All**	**HIV+**	**HIV-**	**HIV?**	**All**
Culture-confirmed tuberculosis	90	159	176	425	4	4	0	8	94	163	176	433
Other than culture-confirmed	174	248	453	875	3	3	0	6	177	251	453	881
Total	264	407	629	1 300	7	7	0	14	271	414	629	1 314

Figure 1: Flow diagram of numbers of children identified with tuberculosis at RCWMCH from 01 January 2006 till 31 December 2008

* Total numbers quoted for the Notifications and Culture-confirmed Tuberculosis Databases include recurrent tuberculosis episodes, but exclude 'not tuberculosis' cases. **HIV+** = HIV-infected; **HIV-** = HIV-uninfected; **HIV?** = HIV status undefined; **All** = all children, regardless of HIV status.

meningitis (n=1), kwashiorkor (n=1) and organophosphate poisoning (n=1). Additionally, 19 children who had been notified as having tuberculosis cultured either *M. bovis* BCG (n=11) or non-tuberculous mycobacteria (n=8) and so did not qualify to be classified as tuberculosis cases; hence, there were 1 194 tuberculosis cases overall (in the Notifications Database) when taking into account these adjustments (**Figure 1**).

Similarly, there were 15 instances of culture-confirmed *M. bovis* BCG disease in the cohort of children registered in the Culture-confirmed Tuberculosis Database, necessitating their reclassification as ‘not tuberculosis’ cases. *Mycobacterium bovis* BCG was cultured from 10 HIV-infected children, three HIV-uninfected children and one child with undefined HIV infection status who were registered in the Culture-confirmed Tuberculosis Database. One HIV-infected child had a recurrent episode of culture-confirmed *M. bovis* BCG 16.1 months after the initial culture-confirmed *M. bovis* BCG episode.

Taking into consideration the reclassifications of disease as mentioned above, the adjusted number of children under 15 years of age who were identified as having at least one episode of tuberculosis at RCWMCH between 01 January 2006 and 31 December 2008, was 1 300 children (**Figure 1**), 705 (54.2%) of which were boys; in the analysis of first-episode tuberculosis events, 425 (32.7%) of these children had culture-confirmed tuberculosis, and 875 (67.3%) had ‘other than culture-confirmed’ tuberculosis. HIV infection status was undetermined in 629 (48.4%) of the children identified with tuberculosis, 264 (20.3%) were HIV-infected and 407 (31.3%) were HIV-uninfected; hence, 39.3% (264/671) of the children identified with tuberculosis who were investigated for HIV infection, were HIV-infected. Of all 433 culture-confirmed tuberculosis cases identified, 120 (27.7%; 95% CI, 23.5 – 32.2) episodes were not included in the Notifications Database, and were likely not notified (**Figure 1**).

3.3.1 Recurrent tuberculosis episodes

Fourteen children with defined HIV status developed tuberculosis on two occasions during the study period (**Figure 1**); no children who had undefined HIV infection status had recurrent tuberculosis. Seven (2.7%) of the 264 HIV-infected children ever diagnosed with tuberculosis had a recurrent tuberculosis episode, compared to seven (1.7%) of the 407 HIV-uninfected children ever diagnosed with tuberculosis (Odds Ratio [OR] 1.56; 95% CI, 0.50 – 5.26), P=0.420. Mean time to recurrent tuberculosis episode was 15.5 months (SD 7.8), and did not differ according to HIV infection status, P=0.286. The recurrent tuberculosis episodes within the cohort augmented the total number of tuberculosis episodes registered in the Combined Database from 1 300 to 1 314 (**Figure 1**).

Whereas only first-episode tuberculosis events were utilised in the description of the demographic parameters of the cohort, all tuberculosis episodes (first-episode as well as recurrent episodes) were considered for other analyses, including clinical disciplines in which cases were investigated and managed, clinical findings, and investigative procedures undertaken in children identified with tuberculosis in the cohort.

3.4 General Demographics Of Children With Tuberculosis

3.4.1 Age at tuberculosis diagnosis

The median age of the 1 300 children under 15 years of age who were identified as having first-episode tuberculosis, was 26.8 months (IQR 12.3 – 69.4); 31.6 months (IQR 12.4 – 74.2) in HIV-infected children compared to 23.1 months (IQR 10.7 – 54.8) in HIV-uninfected children, P=0.021 (**Figure 2A**). Similarly, the median age at tuberculosis diagnosis in children with culture-confirmed disease was significantly older in HIV-infected children (n=90: 60.8 months; IQR 23.5 – 105.6) compared to HIV-uninfected children

(n=159: 25.2 months; IQR 10.7 – 63.6), P<0.001 (**Figure 2B**). The age distribution at clinical evaluation for tuberculosis demonstrated the classical early age-peak described in other paediatric tuberculosis cohorts, as illustrated in the histograms below for the children with defined HIV infection status.

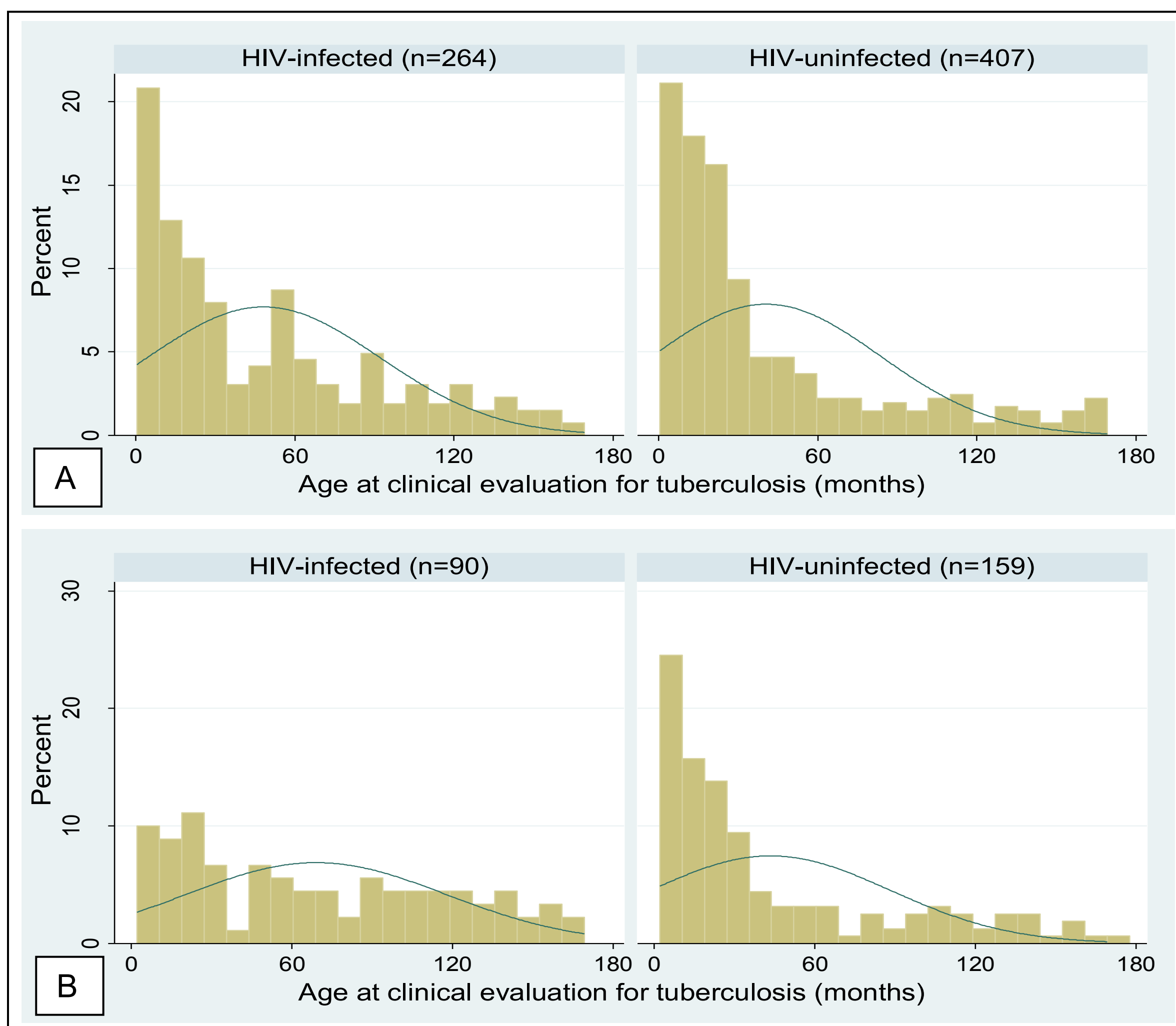


Figure 2: Age distribution (months) at work-up for first-episode tuberculosis*, according to HIV infection status

* **Figure 2A** depicts the age distribution at clinical evaluation for tuberculosis in children whose HIV infection status was defined; **Figure 2B** depicts the age distribution at admission with culture-confirmed tuberculosis in children whose HIV infection status was defined.

3.4.2 Area of residence of children with tuberculosis

Detail regarding area of residence was available in 1 246 (95.8%) of the 1 300 children identified as having tuberculosis. Although there was a wide geographic distribution in which these children resided, 927 (74.4%) came from six locations within the Cape Town Metropole: Khayelitsha (n=366; 29.4%), Gugulethu (n=169; 13.6%), Mitchell's Plain (n=162; 13.0%), Nyanga (n=103; 8.3%), Phillipi (n=78; 6.3%) and Langa (n=49; 3.9%).

3.4.3 Clinical setting in which children were investigated for tuberculosis

The majority (n=1 144; 87.1%) of the 1 314 (first- and recurrent) episodes of tuberculosis in children registered in the Combined Database, were assessed by Paediatric Medical disciplines (including the Paediatric Intensive Care Unit) at RCWMCH; 79 (6.0%) were identified through the Paediatric Surgical disciplines. Most (n=792; 60.3%) of the children were investigated for tuberculosis in the medical outpatient setting (**Figure 3**).

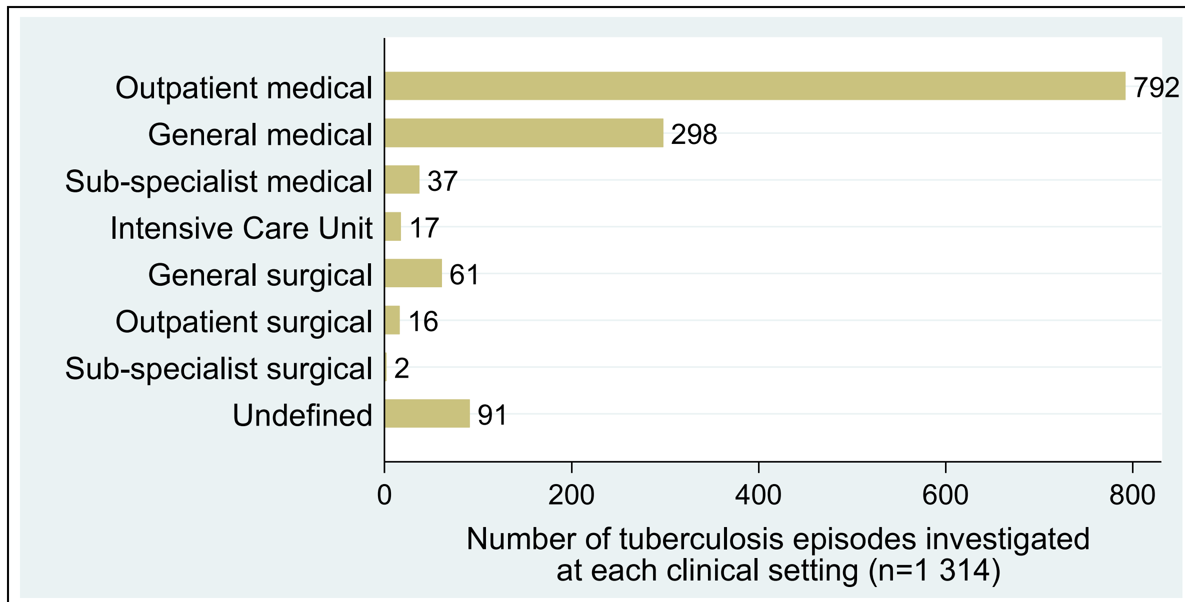


Figure 3: Clinical settings in which tuberculosis episodes in children attending RCWMCH between 01 January 2006 and 31 December 2008 were identified

3.5 Factors Impacting On HIV Testing Of Children With Tuberculosis At RCWMCH

3.5.1 Year in which children were investigated for tuberculosis

The proportion of children with tuberculosis who were tested for their HIV infection status improved over time, with 55.4% of children who were worked up for tuberculosis remaining untested for their HIV infection status in 2006, 49.8% remaining untested in 2007 and 41.7% remaining untested in 2008 (**Figure 4**), chi-squared test for trend, P<0.001.

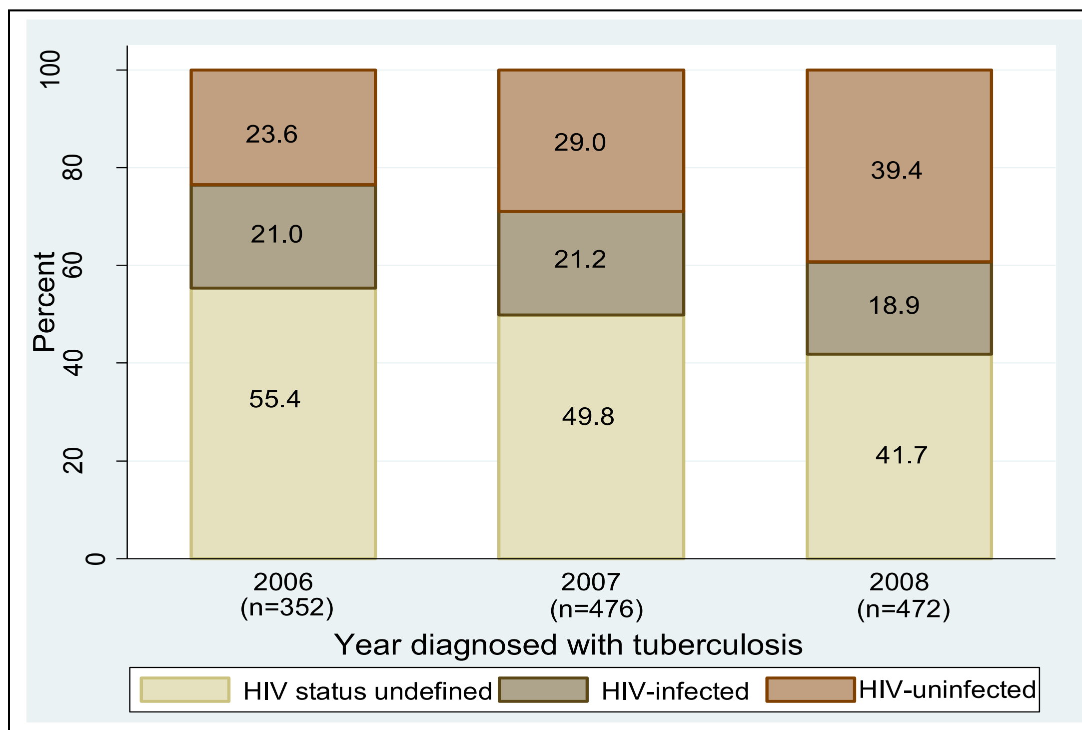


Figure 4: Proportions of children worked up for tuberculosis who were investigated for HIV infection status, stratified according to year in which tuberculosis work-up was conducted

3.5.2 Age of children at investigation for tuberculosis

Four-hundred and ninety-eight (74.2%) of 671 children under five years of age had retrievable HIV results compared to 433 (68.8%) of 629 children between the ages of five and 15 years (OR 1.30; 95% CI, 1.02 – 1.67), P=0.032.

3.5.3 Clinical discipline in which children were investigated for tuberculosis

HIV-infection was more prevalent in children diagnosed with tuberculosis in the medical wards compared to those investigated for tuberculosis by the surgical disciplines, **Table 4**. Children investigated for tuberculosis in the Surgical Outpatient and Inpatient settings were investigated for their HIV infection status significantly less frequently compared with those investigated for tuberculosis in the Medical Outpatient and Inpatient settings (**Table 4** and **Table 5**).

HIV status	Medical	Surgical	Undefined*	Total	P-values†
HIV-infected	239 (21.1)‡	7 (9.0)	18 (20.5)	264 (20.3)	0.010
HIV-uninfected	345 (30.4)	21 (26.9)	41 (46.5)	407 (31.3)	0.515
HIV status undefined	550 (48.5)	50 (64.1)	29 (33.0)	629 (48.4)	0.008
Total	1 134 (100.0)	78 (100.0)	88 (100.0)	1 300 (100.0)	-

Table 4: Proportions of children with tuberculosis according to HIV infection status in each clinical discipline

HIV status	Inpatient		P-values§	Outpatient		P-values§
	Medical	Surgical		Medical	Surgical	
HIV-infected	95 (27.4)‡	6 (9.5)	0.002	144 (18.2)	1 (6.7)	0.494**
HIV-uninfected	168 (48.6)	20 (31.8)	0.014	177 (22.5)	1 (6.7)	0.212**
HIV status undefined	83 (24.0)	37 (58.7)	<0.001	467 (59.3)	13 (86.6)	0.032
Total	346 (100.0)	63 (100.0)	-	788 (100.0)	15 (100.0)	-

Table 5: Proportion of children with available HIV results according to clinical discipline in which they were investigated, and whether investigated in the inpatient or outpatient setting

* 'Undefined' reflects children in whom ward allocations were not obtainable from source databases.
† P-values calculated in the comparison of proportions of children who were identified with tuberculosis by Medical and Surgical disciplines, according to HIV infection status.
‡ Numbers in brackets reflect column percentages by HIV infection status for each clinical discipline.
§ P-values calculated in the comparison of proportions of children identified with tuberculosis by Medical and Surgical disciplines in the Inpatient and Outpatient settings, respectively, by HIV infection status.
** Fisher's exact test.

3.5.4 Multivariate analysis to assess factors relating to adequacy of HIV infection status testing in children with tuberculosis at RCWMCH, 2006 – 2008

Year in which HIV infection status was assessed, age at investigation for tuberculosis, clinical discipline in which the children were assessed for tuberculosis and strength of evidence of tuberculosis were included in a multivariate model which explored factors relating to completeness of tuberculosis work-up in terms of HIV infection status testing; results of the multivariate analysis are tabulated below (**Table 6**).

		Univariate Analysis		**Multivariate Analysis***	
		Odds Ratio (95% CI)	**P-values**	**Adjusted Odds Ratio (95% CI)**	**P-values**
Year of study	2007 versus 2006	1.25 (0.95 – 1.65)	0.110	1.21 (0.83 – 1.51)	0.454
	2008 versus 2006	1.73 (1.31 – 2.29)	<0.001	1.54 (1.13 – 2.10)	0.006
Age (months)		1.00 (0.99 – 1.00)	0.020	1.00 (0.99 – 1.00)	0.295
Age group <60 months versus 60–180 months		1.30 (1.02 – 1.66)	0.032	1.08 (0.62 – 1.89)	0.792
Outpatient versus inpatient setting		0.28 (0.22 – 0.36)	<0.001	0.22 (0.17 – 0.29)	<0.001
Surgical versus medical care		0.53 (0.33 – 0.85)	0.009	0.23 (0.14 – 0.40)	<0.001
Culture-confirmed tuberculosis versus other than culture-confirmed		1.52 (1.20 – 1.92)	<0.001	1.41 (1.08 – 1.83)	0.012

Table 6: Multiple logistic regression analysis of factors which influenced testing for HIV infection status in children investigated for tuberculosis at RCWHCH

* The multivariate analysis utilised each of the parameters investigated in the univariate analysis, as all of the P-values observed in the univariate analysis were ≤ 0.20.

3.6 Categorisation Of Tuberculosis Cases By Age Group And HIV Infection Status

Four hundred and thirty-three (33.0%) of the 1 314 (first- and recurrent) tuberculosis episodes in children under 15 years of age were culture-confirmed (**Figure 1**). When stratifying by age-group according to WHO recommendations for reporting on childhood tuberculosis cases, children under five years of age were significantly less likely to have culture confirmation of their disease compared to those between five and 15 years of age (**Table 7**).

Tuberculosis Classification	Age Group (months)			Odds Ratio (95% CI)*	P-value[†]
	<60	60–180	All children		
Culture-confirmed	271 (28.8)[‡]	162 (43.3)	433 (33.0)	0.53 (0.41 – 0.69)	<0.001
Other than culture-confirmed	669 (71.2)	212 (56.7)	881 (67.0)	1.89 (1.46 – 2.44)	
Total	940 (100.0)	374 (100.0)	1 314 (100.0)	-	-

Table 7: Categorisation of tuberculosis (all episodes) according to age group

In the analysis of first- and recurrent tuberculosis episodes, 94 (34.7%) of the 271 episodes in HIV-infected children were culture-confirmed, compared to 163 (39.4%) of the 414 tuberculosis episodes in HIV-uninfected children, P=0.216. Children with undefined HIV infection status were significantly less likely to have culture-confirmed tuberculosis compared to HIV-uninfected children, P<0.001, and HIV-infected children, P=0.044 (**Table 8**).

* Odds ratios explore the likelihood of having culture-confirmed or 'other than culture-confirmed' tuberculosis, according to age group (<60 months compared to 60–180 months of age).
[†] P-value compares proportions of children according to the strength of evidence for a diagnosis of tuberculosis, by age group.
[‡] Numbers in brackets reflect column percentages, unless otherwise specified.

Tuberculosis Classification	HIV-infected		HIV-uninfected		HIV status undefined		Total	
Culture-confirmed	94 (34.7)*		163 (39.4)		176 (28.0)		433 (33.0)	
Other than culture-confirmed	177 (65.3)		251 (60.6)		453 (72.0)		881 (67.0)	
Age Group (months)	<60	60–180	<60	60–180	<60	60–180	<60	60–180
Culture-confirmed	48 [25.7][†]	46 [54.8]	120 [37.5]	43 [45.7]	103 [23.8]	73 [37.2]	271 [28.8]	162 [43.3]
Other than culture-confirmed	139 [74.3]	38 [45.2]	200 [62.5]	51 [54.3]	330 [76.2]	123 [62.8]	669 [71.2]	212 [56.7]
Total (by Age Group)	187 [100.0]	84 [100.0]	320 [100.0]	94 [100.0]	433 [100.0]	196 [100.0]	940 [100.0]	374 [100.0]
Total (regardless of Age Group)	271 (100.0)*		414 (100.0)		629 (100.0)		1 314 (100.0)	

Comparison of proportions with culture-confirmed tuberculosis	Odds Ratios (95% CI) and P-values[‡]					
	<60 months		60–180 months		All age groups	
HIV-infected compared to -uninfected	0.58 (0.38 – 0.87)	0.006	1.44 (0.76 – 2.71)	0.230	0.82 (0.59 – 1.14)	0.216
HIV-infected compared to HIV status undefined	1.11 (0.73 – 1.67)	0.617	2.04 (1.18 – 3.54)	0.007	1.37 (0.99 – 1.87)	0.044
HIV-uninfected compared to HIV status undefined	1.92 (1.38 – 2.67)	<0.001	1.42 (0.84 – 2.41)	0.167	1.67 (1.27 – 2.19)	<0.001

Table 8: Categorisation of tuberculosis (all episodes) by HIV infection status and age group

* Numbers in brackets reflect column percentages regardless of age group, unless otherwise specified.
† Numbers in square brackets reflect column percentages according to age group.
‡ P-values compare proportions of children according to strength of evidence for a diagnosis of tuberculosis, HIV infection status and age group.

HIV-uninfected children under five years of age were significantly more frequently found to have culture-confirmed tuberculosis than either HIV-infected children (P=0.006) or those with undefined HIV infection status (P<0.001) under five years of age, **Table 8**.

3.7 Clinical Characteristics Of Children With Culture-confirmed Tuberculosis

The clinical characteristics of 428 (98.8%) of the 433 culture-confirmed tuberculosis episodes in children under 15 years of age were obtained by folder review in order to assess the spectrum of clinical presentation of culture-confirmed tuberculosis in children attending RCWMCH. Eight of these episodes were recurrent culture-confirmed tuberculosis events, arising in children with defined HIV infection status (four HIV-infected children and four HIV-uninfected children); hence 420 (98.9%) of the 425 children ever identified with culture-confirmed tuberculosis were evaluated by folder review.

Two-hundred and thirty-six (56.2%) of the 420 children whose folders were reviewed, were boys. One hundred and seventy-five (41.7%) of these children had undefined HIV status, 89 (21.2%) were HIV-infected, and 156 (37.1%) were HIV-uninfected; hence, 89 (36.3%) of the 245 children with culture-confirmed tuberculosis with defined HIV infection status whose folders were reviewed, were HIV-infected.

Mean time to repeat culture-confirmed tuberculosis episode in the eight children whose folders were reviewed, was 15.0 months (SD 7.8), with no significant difference in time to recurrence according to HIV infection status, P=0.449.

The median age of children at clinical evaluation in culture-confirmed tuberculosis episodes (first- and recurrent) whose clinical characteristics were reviewed was 33.5

months (IQR 15.4 – 96.0), with boys being younger (30.2 months; IQR 14.5 – 80.3) than girls (46.5 months; IQR 16.9 – 113.0), P=0.013. Culture-confirmed tuberculosis episodes occurred at a median age of 57.0 months (IQR 23.0 – 105.4) in HIV-infected children, compared to 25.2 months (IQR 10.7 – 63.7) in HIV-uninfected children, P<0.001.

3.7.1 The presenting complaint in children with culture-confirmed tuberculosis

Constitutional symptoms were present in 150 (35.0%) of the 428 culture-confirmed tuberculosis (first- and recurrent) episodes in which folder reviews were conducted. Four constitutional symptom clusters were reported by caregivers, including: night sweats and loss of appetite (n=57; 38.0%); fever (n=48; 32.0%); loss of appetite, loss of weight and failure to thrive (n=42; 28.0%); and lethargy (n=3; 2.0%), **Table 9**. HIV-infected children with culture-confirmed tuberculosis presented with a history of constitutional symptoms significantly more frequently than did children with undefined HIV infection status (OR 2.06; 95% CI, 1.18 – 3.59), P=0.007 (**Table 9**).

HIV-uninfected children (OR 1.98; 95% CI, 0.96 – 4.30), P=0.048, and those whose HIV infection status was undefined (OR 2.90; 95% CI, 1.44 – 6.14), P=0.001, tended to present with extrapulmonary symptoms (other than central nervous system symptoms) in which constitutional symptoms were not a prominent feature, more frequently than did HIV-infected children (**Table 10**).

Children with undefined HIV infection status presented with shorter duration of cough than did HIV-infected or -uninfected children (**Table 11**).

Presenting Complaint		HIV-infected	HIV-uninfected	HIV status undefined	All culture-confirmed tuberculosis episodes	Comparison of proportions: HIV-infected and HIV-uninfected (P-values)	Comparison of proportions: HIV-infected and HIV status undefined (P-values)	Comparison of proportions: HIV-uninfected and HIV status undefined (P-values)
Constitutional symptom clusters*	Night sweats and loss of appetite	15 (16.1)	24 (15.0)	18 (10.2)	57 (13.3)	0.810	0.166	0.193
	Fever	14 (15.1)	15 (9.4)	19 (10.9)	48 (11.2)	0.172	0.320	0.654
	Loss of appetite, weight loss and failure to thrive	12 (12.9)	18 (11.2)	12 (6.9)	42 (9.8)	0.695	0.099	0.160
	Lethargy	1 (1.1)	1 (0.6)	1 (0.6)	3 (0.7)	1.000†	1.000‡	1.000‡
	No constitutional symptoms	51 (54.8)‡	102 (63.8)	125 (71.4)	278 (65.0)	0.162	0.007	0.133
Total		93 (100.0)	160 (100.0)	175 (100.0)	428 (100.0)	-	-	-

Table 9: Constitutional symptoms at clinical evaluation in children who were subsequently proven to have culture-confirmed tuberculosis

* Constitutional symptom clusters as tabulated may or may not have presented with other symptom clusters as tabulated in **Table 10**, below.
† Fisher's exact test.
‡ Numbers in brackets represent column percentages of children presenting with constitutional symptoms, according to HIV infection status.

Presenting Complaint		HIV-infected	HIV-uninfected	HIV status undefined	Comparison of proportions		
					HIV-infected and HIV-uninfected	HIV-infected and HIV status undefined	HIV-uninfected and HIV status undefined
					P-values		
Constitutional symptoms*		8 (8.6)[†]	11 (6.9)	7 (4.0)	0.615	0.161[‡]	0.244
Cough	With constitutional symptoms	30 (32.3)	46 (28.8)	40 (22.9)	0.557	0.095	0.217
	Without constitutional symptoms	25 (26.8)	52 (32.5)	53 (30.2)	0.349	0.383	0.663
	Total	55 (59.1)	98 (61.3)	93 (53.1)	0.741	0.626	0.134
Central nervous system symptoms	With constitutional symptoms	0 (0.0)	1 (0.6)	0 (0.0)	1.000[‡]	-	0.478[‡]
	Without constitutional symptoms	7 (7.5)	6 (3.8)	7 (4.0)	0.239[‡]	0.253[‡]	0.906
	Total	7 (7.5)	7 (4.4)	7 (4.0)	0.393[‡]	0.253[‡]	0.864
Extrapulmonary other than central nervous system	With constitutional symptoms	2 (2.1)	0 (0.0)	2 (1.1)	0.134[‡]	0.611[‡]	0.500[‡]
	Without constitutional symptoms	13 (14.0)	39 (24.4)	56 (32.0)	0.048	0.001	0.122
	Total	15 (16.1)	39 (24.4)	58 (33.1)	0.123	0.003	0.077
Unrelated to tuberculosis diagnosis[§]		2 (2.1)	1 (0.6)	1 (0.6)	0.556[‡]	0.277[‡]	1.000[‡]
Undefined		6 (6.5)	4 (2.5)	9 (5.1)	0.178[‡]	0.781[‡]	0.211
Total		93 (100.0)	160 (100.0)	175 (100.0)	-	-	-

Table 10: Presenting symptoms at clinical evaluation in children who were subsequently proven to have culture-confirmed tuberculosis

* Children presenting solely with constitutional symptoms as the chief complaint, without cough or other symptom clusters as tabulated in this Table.

[†] Numbers in brackets represent column percentages of children presenting according to each symptom cluster.

[‡] Fisher's exact test.

[§] Children presenting with complaints unrelated to their tuberculosis diagnosis included two children with acute gastroenteritis and two with febrile convulsions; all of these children were subsequently confirmed to have pulmonary tuberculosis from specimens submitted during the admission episode in which they presented with 'unrelated complaints'. Both of the HIV-infected children, and the child with undefined HIV infection status had concomitant constitutional symptoms.

Presenting Complaint		Median Symptom Duration in days (IQR)			Comparison of proportions		
		HIV-infected	HIV-uninfected	HIV status undefined	HIV-infected and HIV-uninfected	HIV-infected and HIV status undefined	HIV-uninfected and HIV status undefined
					P-values*		
Constitutional symptoms	Night sweats and loss of appetite	21 (14 – 90)	30 (14 – 60)	14 (5 – 60)	0.798	0.190	0.109
	Fever	7 (2 – 25)	14 (7 – 21)	14 (3 – 14)	0.241	0.652	0.250
	Loss of appetite, weight loss and failure to thrive	7 (5 – 45)	21 (7 – 60)	14 (7 – 26)	0.753	0.975	0.538
	Lethargy	7	1	14	-	-	-
	All cases	14 (7 – 30)	21 (7 – 60)	14 (6 – 21)	0.323	0.393	0.025
Cough	With constitutional symptoms	18 (7 – 30)	21 (7 – 60)	14 (6 – 21)	0.451	0.226	0.015
	Without constitutional symptoms	14 (7 – 30)	14 (7 – 30)	7 (3 – 21)	0.770	0.095	0.075
	All cases	15 (7 – 30)	14 (7 – 30)	7 (4 – 21)	0.823	0.029	0.003
Central nervous system symptoms	With constitutional symptoms	-	3	-	-	-	-
	Without constitutional symptoms	4 (1 – 16)	2 (1 – 14)	2 (1 – 7)	0.377	0.339	1.000
	All cases	4 (1 – 21)	2 (1 – 14)	2 (1 – 7)	0.359	0.339	0.880
Extrapulmonary other than central nervous system	With constitutional symptoms	5 (2 – 7)	-	11 (7 – 14)	-	0.221	-
	Without constitutional symptoms	5 (2 – 21)	14 (5 – 21)	21 (7 – 42)	0.207	0.109	0.277
	All cases	5 (2 – 14)	14 (5 – 21)	14 (7 – 42)	0.106	0.050	0.303
Unrelated to tuberculosis diagnosis†		4 (3 – 5)	5	1	-	-	-

Table 11: Duration of symptoms in children who were subsequently proven to have culture-confirmed tuberculosis

IQR = interquartile range.
* P-values are derived using the Mann-Whitney test by comparison of medians between groups, according to HIV infection status.
† Children presenting with complaints unrelated to their tuberculosis diagnosis included two children with acute gastroenteritis and two with febrile convulsions who were found incidentally to have pulmonary tuberculosis.

A history of cough was recorded in 209 (66.3%) of 315 children with culture-confirmed pulmonary tuberculosis*, and duration of cough was recorded in 195 (93.3%) of these episodes; 86 (44.1%) of the children for whom cough duration was recorded at clinical evaluation, presented with cough of less than 10 days' duration. HIV-infected (OR 2.20; 95% CI, 0.96 – 5.09), P=0.042, and -uninfected children (OR 1.90; 95% CI, 0.94 – 3.83), P=0.052, tended to present with longer cough duration than did those with undefined HIV infection status (**Table 12**); however, children with undefined HIV infection status were twice as likely as those with defined HIV infection status (HIV-infected and -uninfected) to present with a history of acute cough (OR 2.00; 95% CI, 1.07 – 3.77), P=0.020.

Cough duration	**HIV-infected**	**HIV-uninfected**	**HIV status undefined**	**Total**	**Comparison of proportions**		
					HIV-infected and HIV-uninfected	**HIV-infected and HIV status undefined**	**HIV-uninfected and HIV status undefined**
					P-values[†]		
<10 days	16 (35.6)[‡]	30 (39.0)	40 (54.8)	86 (44.1)	0.708	0.042	0.052
≥10 days	29 (64.4)	47 (61.0)	33 (45.2)	109 (55.9)			
Total	45 (100.0)	77 (100.0)	73 (100.0)	195 (100.0)	-	-	-

Table 12: Proportions of tuberculosis episodes that presented with acute onset cough, stratified by HIV infection status

3.7.2 History of contact with a source case of tuberculosis

Information regarding the tuberculosis contact history was available from 420 (98.1%) of the 428 culture-confirmed tuberculosis episodes in which hospital records were reviewed;

* Pulmonary tuberculosis episodes include extrapulmonary tuberculosis episodes in which respiratory symptoms were recorded and *M. tuberculosis* was cultured from respiratory specimens.

[†] P-values compare proportions of culture-confirmed tuberculosis episodes in which children presented with short (<10 days) or long (≥10 days) cough duration, according to HIV infection status.

[‡] Numbers in brackets represent column percentages, by HIV infection status.

in 39 (9.3%) of these 420 episodes, a history relating to a possible source case for tuberculosis was not enquired about. Therefore, the potential tuberculosis source case was recorded in 381 (89.0%) of the 428 culture-confirmed tuberculosis cases in which folder reviews were conducted (**Table 13**).

Source case	**HIV-infected**		**HIV-uninfected**		**HIV status undefined**	**All children**	
	First-episode	**All episodes[*]**	**First-episode**	**All episodes**	**First-episode[†]**	**First-episode**	**All episodes**
No known contact	22 (28.2)[‡]	23 (28.1)	70 (48.3)	70 (47.0)	72 (48.0)	164 (44.0)	165 (43.3)
Parent	23 (29.5)	25 (30.5)	26 (17.9)	27 (18.1)	28 (18.7)	77 (20.6)	80 (21.0)
Aunt or uncle	5 (6.4)	5 (6.1)	19 (13.1)	19 (12.8)	19 (12.7)	43 (11.5)	43 (11.3)
Grandparent	4 (5.1)	4 (4.9)	3 (2.1)	3 (2.0)	3 (2.0)	10 (2.7)	10 (2.6)
Sibling or cousin	5 (6.4)	5 (6.1)	6 (4.1)	6 (4.0)	8 (5.3)	19 (5.1)	19 (5.0)
Neighbour	2 (2.6)	2 (2.4)	3 (2.1)	3 (2.0)	7 (4.7)	12 (3.2)	12 (3.2)
Previous tuberculosis in the index case	8 (10.3)	9 (11.0)	0 (0.0)	3 (2.0)	1 (0.7)	9 (2.4)	13 (3.4)
Unspecified	9 (11.5)	9 (11.0)	18 (12.4)	18 (12.1)	12 (8.0)	39 (10.5)	39 (10.2)
Total	78 (100.0)	82 (100.0)	145 (100.0)	149 (100.0)	150 (100.0)	373 (100.0)	381 (100.0)

Table 13: Potential tuberculosis source cases in children with culture-confirmed tuberculosis

* ‘All episodes’ of culture-confirmed tuberculosis include first- and recurrent episodes.
† There were no recurrent tuberculosis episodes in children with undefined HIV infection status; therefore, only ‘first episode’ events are recorded for this group of children.
‡ Numbers in brackets represent column percentages.

In the analysis of first-episode tuberculosis events (as recorded in **Table 13**), both HIV-uninfected children (OR 2.38; 95% CI, 1.27 – 4.52), P=0.004, and those with undefined HIV infection status (OR 2.35; 95% CI, 1.26 – 4.45), P=0.004, were significantly more likely to have no known tuberculosis contact compared to HIV-infected children. There was also a non-significant trend for HIV-infected children to have a parent reported as being the tuberculosis source case compared to HIV-uninfected children (OR 1.91; 95% CI, 0.95 – 3.83), P=0.047, and those with undefined HIV infection status (OR 1.82; 95% CI, 0.91 – 3.61), P=0.063.

In the analysis of all tuberculosis episodes, HIV-infected children were significantly more likely to have had a previous history of tuberculosis (n=9) compared to HIV-uninfected children (n=3: OR 5.84; 95% CI, 1.39 – 34.24), P=0.006, and those with undefined HIV infection status (n=1: OR 18.37; 95% CI, 2.44 – 810.26), P=0.001.

A history of isoniazid preventive therapy (IPT) use was obtained in 44 (12.0%) of the 368 culture-confirmed tuberculosis episodes in which information regarding a possible source case was obtainable, and in whom the tuberculosis source case was not attributed as being the child being worked up for tuberculosis (on the basis of a previous tuberculosis treatment history, n=13: **Table 13**); in 31 (70.5%) of these episodes, IPT had not been used despite a history of there being a tuberculosis contact. In 13 (29.5%) of these 44 culture-confirmed tuberculosis episodes, a documented history of IPT exposure was obtained, and the tuberculosis isolates cultured in 12 (92.3%) of these episodes were susceptible to isoniazid and rifampicin; one *M. tuberculosis* isolate in a child who had received IPT was multidrug-resistant.

3.7.3 Nutritional status in children with culture-confirmed tuberculosis

The nutritional status of 425 (99.3%) of the 428 culture-confirmed tuberculosis episodes in which hospital records were reviewed, could be ascertained from case notes. When classifying nutritional status in children with culture-confirmed tuberculosis according to the Wellcome Classification, (73) children with undefined HIV infection status were more likely than both HIV-infected children (OR 3.75; 95% CI, 2.05 – 6.85), P<0.001, and HIV-uninfected children (OR 4.66; 95% CI, 2.76 – 7.94), P<0.001, to have normal nutritional status (defined as being over 80% of expected weight for age), **Table 14**.

Nutritional status (% expected weight for age)	**HIV-infected**	**HIV-uninfected**	**HIV status undefined**	**Total**
> 80%*	51 (54.8)[†]	79 (49.4)	141 (82.0)	271 (63.8)
> 60 to ≤ 80%*	20 (21.5)	48 (30.0)	17 (9.9)	85 (20.0)
≤ 60%*	22 (23.7)	33 (20.6)	14 (8.1)	69 (16.2)
Total	93 (100.0)	160 (100.0)	172 (100.0)	425 (100.0)

Table 14: Nutritional status of children with culture-confirmed tuberculosis

HIV-infected children were no more likely than HIV-uninfected children to be underweight (P=0.142) or marasmic (P=0.573) at clinical evaluation for tuberculosis; however, HIV-infected children were more likely than those with undefined HIV infection status to be underweight for age (OR 2.50; 95% CI, 1.16 – 5.39), P<0.001, or marasmic (OR 3.50; 95% CI, 1.60 – 7.81), P<0.001. Similarly, HIV-uninfected children were more likely than those

* >80% expected weight for age denotes normal nutritional status; 60–80% expected weight for age denotes being underweight for age (or kwashiorkor in the presence of pedal oedema); ≤60% expected weight for age denotes marasmus (or marasmic-kwashiorkor in the presence of pedal oedema).

[†] Numbers in brackets denote column percentages according to HIV infection status.

with undefined HIV infection status to be underweight (OR 3.91; 95% CI, 2.07 – 7.62), P<0.001, or marasmic (OR 2.93; 95% CI, 1.45 – 6.18), P=0.001.

Culture-confirmed tuberculosis episodes in which children presented with constitutional symptoms were associated with a lower mean weight-for-age z-score (-1.79, SD 1.47) compared to episodes in which constitutional symptoms were not a prominent feature (mean weight-for-age z-score [-1.37, SD 1.47]), P=0.005. There was, however, no significant difference in weight-for-age z-score at presentation in the comparison between groups that presented with constitutional symptoms and without constitutional symptoms, according to HIV infection status (**Figure 5**).

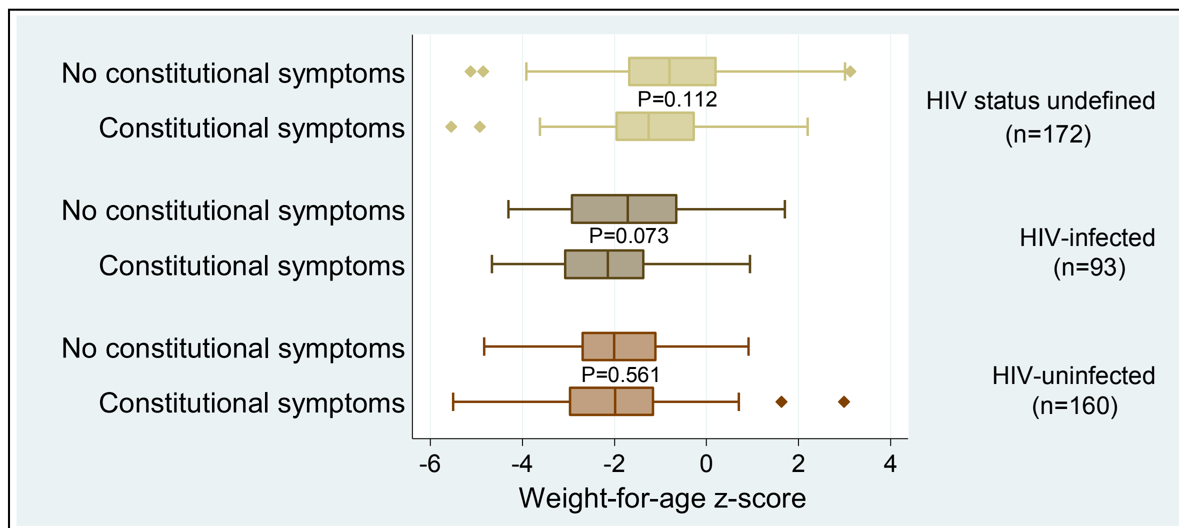


Figure 5: Weight-for-age z-scores in children with culture-confirmed tuberculosis, according to HIV infection status and presence or absence of constitutional symptoms at presentation

Mean weight-for-age z-scores at clinical evaluation in children with culture-confirmed tuberculosis were significantly lower in HIV-infected children (-1.85, SD 1.32) and HIV-uninfected children (-1.94, SD 1.36) compared to children whose HIV infection status was undefined (-0.94, SD 1.50), P<0.001.

3.7.4 Adenopathy in children with culture-confirmed tuberculosis

Adenopathy was more frequently encountered in culture-confirmed tuberculosis episodes in HIV-infected children compared to those who were HIV-uninfected or those whose HIV status remained undetermined. Sites of adenopathy observed in these children are tabulated below (**Table 15**).

Site of adenopathy	HIV-infected	HIV-uninfected	HIV status undetermined	Comparison of proportions		
				HIV-infected and HIV-uninfected	HIV-infected and HIV status undetermined	HIV-uninfected and HIV status undetermined
				P-values*		
Generalised	41 (53.2)[†]	32 (24.6)	15 (11.5)	<0.001	<0.001	0.006
Head and neck	17 (22.1)	35 (26.9)	52 (39.7)	0.437	0.009	0.029
Axillary	4 (5.2)	8 (6.2)	6 (4.6)	1.000[‡]	1.000[‡]	0.573
Inguinal	2 (2.6)	1 (0.8)	1 (0.7)	0.557[‡]	0.556[‡]	1.000[‡]
No adenopathy	13 (16.9)	54 (41.5)	57 (43.5)	<0.001	<0.001	0.747
Total	77 (100.0)	130 (100.0)	131 (100.0)	-	-	-
All adenopathy[§]	23 (63.9)	44 (44.9)	59 (50.9)	0.051	0.171	0.384
No adenopathy	13 (36.1)	54 (55.1)	57 (49.1)			
Total	36 (100.0)	98 (100.0)	116 (100.0)	-	-	-

Table 15: Sites of adenopathy in culture-confirmed tuberculosis episodes, according to HIV infection status

* P-values derived by comparison of proportions with lymphadenopathy at different sites, according to HIV infection status.
[†] Numbers in brackets reflect column percentages for each HIV infection status group.
[‡] Fisher's exact test.
[§] All adenopathy with exclusion of tuberculosis episodes that were associated with 'generalised adenopathy'.

Generalised adenopathy was significantly more frequently encountered in tuberculosis episodes amongst HIV-infected children; however, when analysing for localised adenitis only, there was no significant difference in proportions of children with localised lymphadenitis when stratifying according to HIV infection status (**Table 15**).

Episodes of tuberculosis in which HIV infection status was not defined presented more frequently with localised head and neck adenitis than did episodes arising in HIV-infected (RR 1.80; 95% CI, 1.12 – 2.88), P=0.009, and -uninfected children (RR 1.47; 95% CI, 1.04 – 2.10), P=0.029, respectively (**Table 15**).

3.7.5 Abdominal tuberculosis in children with culture-confirmed tuberculosis

Forty-three abdominal tuberculosis episodes were encountered in children with culture-confirmed tuberculosis, and were encountered more frequently in children with defined HIV status (n=38) than in those with undefined HIV status (n=5; RR 5.05; 95% CI, 2.03 – 12.56), P<0.001. Thirty-nine (90.7%) of the 43 abdominal tuberculosis episodes were diagnosed based on ultrasonographic findings, three (7.0%) on operative findings and one (2.3%) on culture of peritoneal fluid.

Ultrasonographic findings in children with abdominal tuberculosis included visualisation of: intra-abdominal lymph nodes (n=18); ascites (n=8); splenic microabscesses (n=7); lymph nodes, ascites and splenic microabscesses (n=5); and thickened bowel loops (n=1). *Mycobacterium tuberculosis* was cultured from numerous specimen types in abdominal tuberculosis episodes: gastric aspirates and sputum (expectorated and induced) in 31 (72.1%) of the 43 abdominal tuberculosis episodes, tissue (n=7), peritoneal fluid (n=1), pleural fluid (n=1), pus (n=1), urine (n=1) and vaginal exudate (n=1).

3.7.6 Chest radiographic findings in culture-confirmed tuberculosis episodes

Three-hundred and thirty-five (78.3%) of the 428 culture-confirmed tuberculosis episodes in which folder reviews were conducted, were assessed by evaluation of a chest radiograph, the findings of which (as described in hospital records) are tabulated below. HIV-uninfected children were less likely than either HIV-infected children or those with undefined HIV infection status to have a normal chest radiograph (**Table 16**).

Pertinent chest radiographic findings	HIV-infected	HIV-uninfected	HIV status undefined	Comparison of proportions: HIV-infected and HIV-uninfected	HIV-infected and HIV status undefined	HIV-uninfected and HIV status undefined
				P-values*		
Normal film	7 (8.7)[†]	2 (1.6)	10 (7.8)	0.035[‡]	0.992	0.019
Intrathoracic adenopathy	39 (48.7)	63 (50.4)	61 (48.4)	0.904	0.878	0.755
Pleural effusion	8 (10.0)	14 (10.2)	18 (13.3)	0.816	0.389	0.464
Cavitation	3 (3.8)	8 (6.3)	2 (1.6)	0.535[‡]	0.375[‡]	0.060[‡]
Miliary pattern	3 (3.8)	9 (7.1)	2 (1.6)	0.377[‡]	0.375[‡]	0.034[‡]
Pulmonary infiltrates	18 (22.4)	25 (19.7)	31 (24.2)	0.627	0.776	0.382
Pneumothorax	1 (1.3)	1 (0.8)	2 (1.6)	1.000[‡]	1.000[‡]	1.000[‡]
Pericardial effusion	1 (1.3)	3 (2.4)	1 (0.8)	1.000[‡]	1.000[‡]	0.370[‡]
'Suggestive film'[§]	0 (0.0)	2 (1.6)	1 (0.8)	0.523[‡]	1.000[‡]	0.622[‡]
Total	80 (100.0)	127 (100.0)	128 (100.0)	-	-	-

Table 16: Chest radiographic findings in culture-confirmed tuberculosis, according to HIV infection status

* P-values are derived by comparison of proportions of children with each chest radiographic finding, stratified according to HIV infection status.
[†] Numbers in brackets represent column percentages, according to each HIV infection group.
[‡] Fisher's exact test.
[§] Tuberculosis episodes in which hospital notes merely recorded the chest radiographic changes as being 'suggestive of tuberculosis' without further describing the radiographic findings, are categorised as having 'suggestive films' in this Table.

3.7.7 Tuberculin skin test results in children with culture-confirmed tuberculosis

Three-hundred and twenty-four (75.7%) of the 428 culture-confirmed tuberculosis episodes in which folder reviews were conducted, were tested using the Mantoux test at clinical evaluation for tuberculosis; the results of 40 (12.3%) of these tests were not recorded in clinical notes. Of the 284 Mantoux reactions for which results were recorded, 211 (74.3%) were positive (**Table 17**).

Mantoux test sensitivity according to HIV infection status is tabulated below (**Table 17**); HIV-infected children were more likely than HIV-uninfected children (OR 1.95; 95% CI, 0.94 – 4.02), P=0.049, or children with undefined HIV infection status (OR 6.47; 95% CI, 2.79 – 15.14), P<0.001, to have a negative Mantoux response.

Mantoux result*	**HIV-infected**	**HIV-uninfected**	**HIV status undefined**	**All episodes**
Positive Mantoux response	29 (53.7)[†]	77 (69.4)	105 (88.2)	211 (74.3)
Negative Mantoux response	25 (46.3)	34 (30.6)	14 (11.8)	73 (25.7)
Total	54 (100.0)	111 (100.0)	119 (100.0)	284 (100.0)
Comparison of proportions with positive Mantoux results			**Odds Ratio (95% CI)**	**P-values[‡]**
HIV-infected and -uninfected			0.51 (0.25 – 1.06)	0.049
HIV-infected and HIV status undefined			0.15 (0.07 – 0.36)	<0.001
HIV-uninfected and HIV status undefined			0.30 (0.14 – 0.63)	<0.001

Table 17: Mantoux test sensitivity in children with culture-confirmed tuberculosis, stratified by HIV infection status

* In HIV-infected children, a positive Mantoux response was defined as being ≥5 mm of induration, as measured in the transverse diameter; in HIV-uninfected children, and those whose HIV infection status was undefined, a positive Mantoux response was defined as being ≥10 mm of induration, as measured in the transverse diameter.

[†] Numbers in brackets reflect column percentages, according to HIV infection status, unless otherwise specified.

[‡] P-values calculated in the comparison of proportions of children who had a positive Mantoux response, stratified by HIV infection status.

The diameter of induration was recorded in only 119 (41.9%) of the 284 culture-confirmed tuberculosis episodes in which Mantoux reactions were recorded in patient folders. Median tuberculin skin test induration was 15 mm (IQR 0 – 20) overall: 10 mm (IQR 0 – 21) in HIV-infected children (n=26); 15 mm (IQR 0 – 20) in HIV-uninfected children (n=45) and 15 mm (IQR 14 – 20) in tuberculosis episodes amongst children with no defined HIV result (n=48), P=0.127 (**Figure 6**). When excluding tuberculosis episodes in which the tuberculin skin test reaction was recorded as being anergic, median tuberculin skin test results were similar between groups, irrespective of HIV infection status: 21 mm (IQR 15 – 23) in tuberculosis episodes in HIV-infected children (n=14); 20 mm (IQR 15 – 20) in tuberculosis episodes in HIV-uninfected children (n=30), and 17 mm (IQR 15 – 22) in episodes amongst children with no defined HIV status (n=41), P=0.753 (**Figure 6**).

Anergy occurred in 25 (64.1%) of 39 culture-confirmed tuberculosis episodes in HIV-infected children whose Mantoux results were recorded, 31 (50.8%) of 61 culture-confirmed tuberculosis episodes in HIV-uninfected children whose Mantoux results were recorded, and 14 (25.5%) of 55 such tuberculosis episodes in children with undefined HIV infection status. Hence, HIV-infected children were no more likely than HIV-uninfected children to exhibit tuberculin skin test anergy (RR 1.26; 95% CI, 0.90 – 1.77), P=0.192, but were 2.5-fold (95% CI, 1.51 – 4.19) more likely to exhibit tuberculin skin test anergy compared to children with undefined HIV infection status, P<0.001.

Baseline absolute CD4 T-lymphocyte counts were determined in 46 (85.2%) of the 54 HIV-infected children with culture-confirmed tuberculosis whose Mantoux responses were recorded in clinical notes, and were significantly greater in those whose Mantoux responses were positive (n=23: 595 × 10^6 cells/l; IQR 310 – 1 151) compared to those

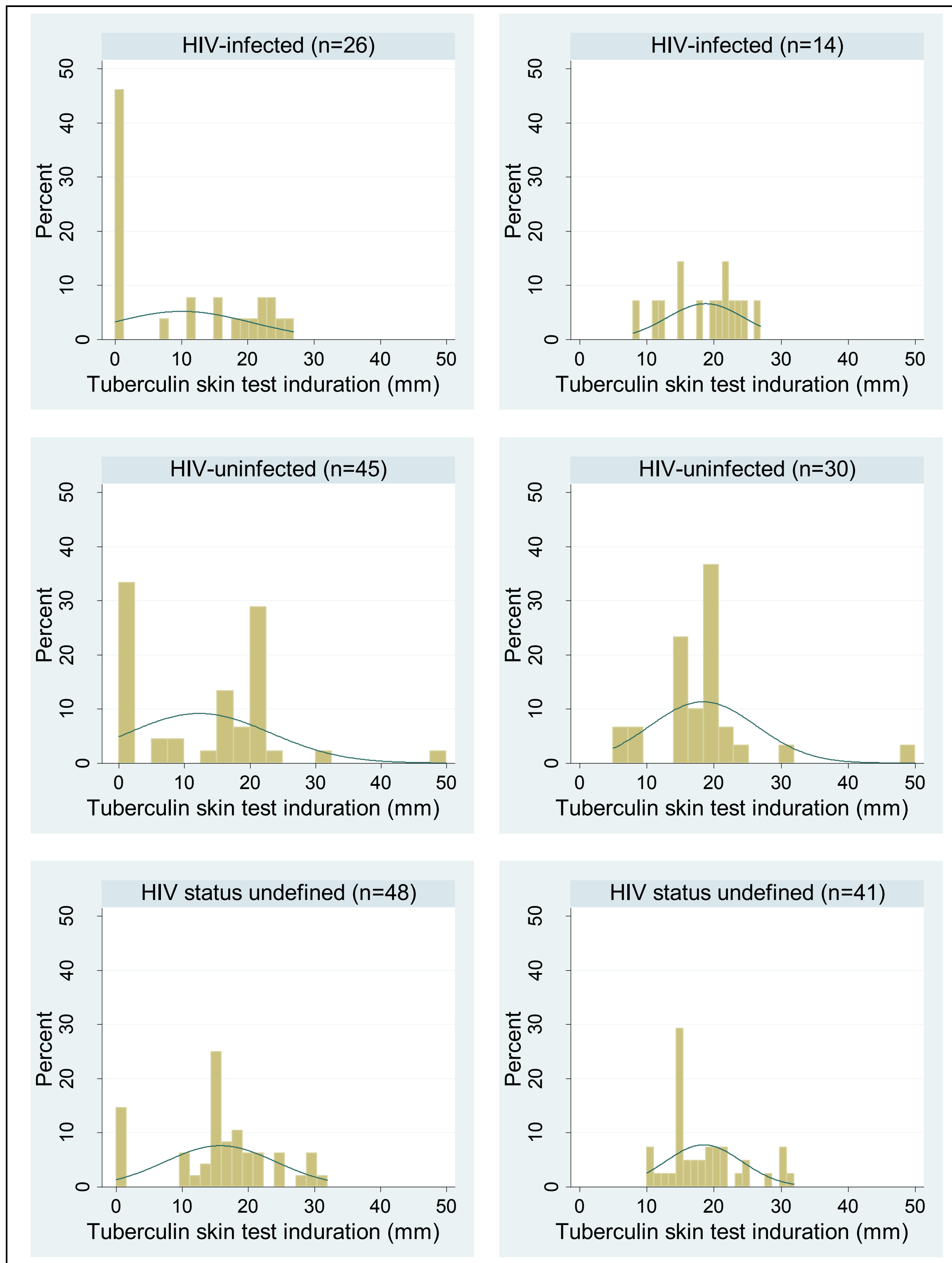


Figure 6: Tuberculin skin test response* in children with culture-confirmed tuberculosis, according to HIV status

* Histograms in the left column represent tuberculin skin test indurations (mm) with inclusion of anergic responses; those in the right hand column reflect distribution of induration in culture-confirmed tuberculosis episodes amongst the children in whom there were no anergic responses.

whose Mantoux responses were recorded as being negative (n=23: 131 × 10^6 cells/l; IQR 97 – 492), P=0.008 (**Figure 7**). Baseline mean CD4 T-lymphocyte percentages did not differ significantly between HIV-infected children whose Mantoux responses were reactive (n=23: 20.5%, SD 10.9) and those with negative Mantoux responses (n=23: 16.1%, SD 8.4), P=0.134 (**Figure 7**).

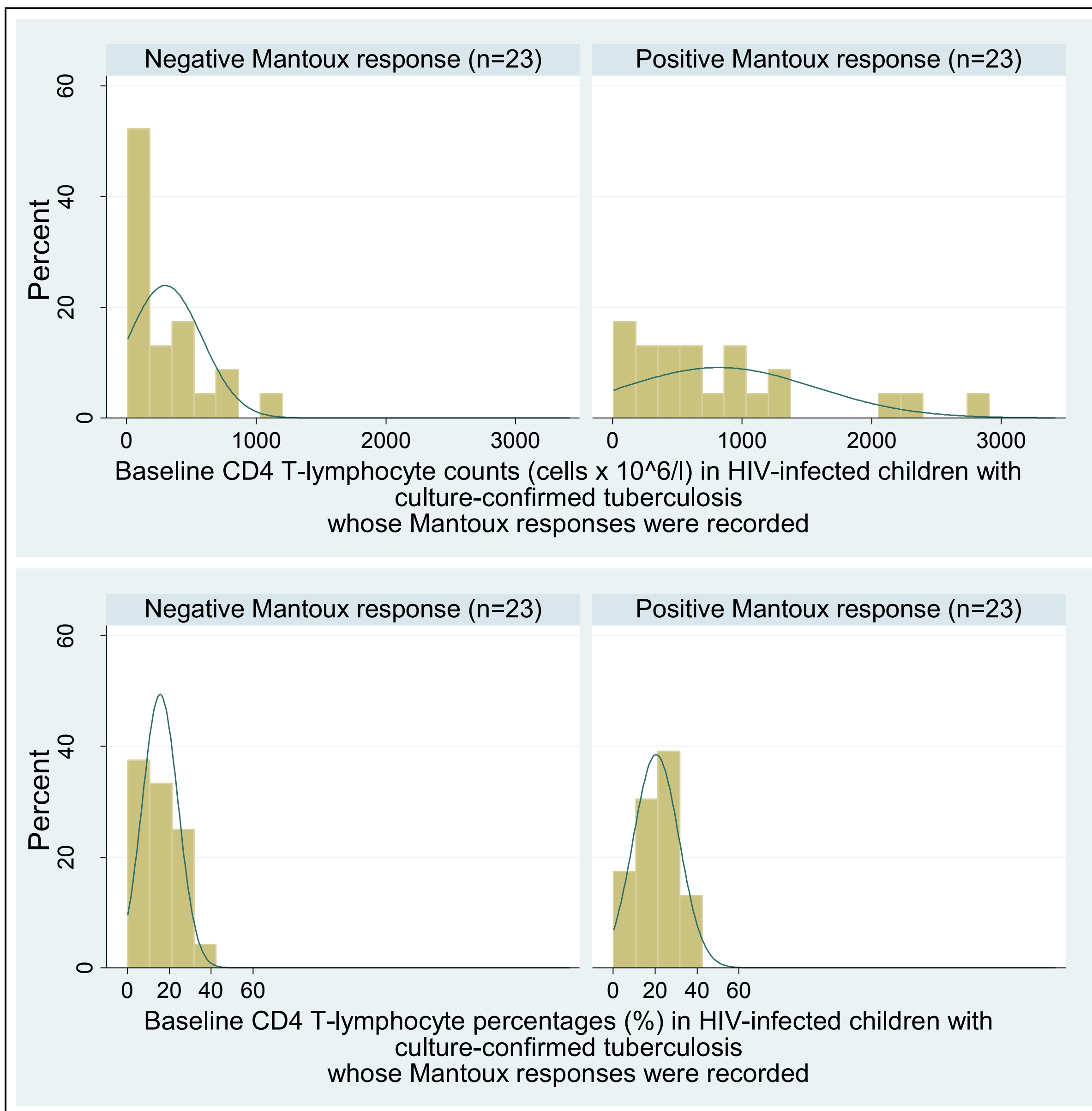


Figure 7: Immunologic parameters at baseline in HIV-infected children with culture-confirmed tuberculosis whose Mantoux responses were recorded in hospital folders

3.7.8 Screening for tuberculosis using the diagnostic algorithm in children with culture-confirmed tuberculosis

Four-hundred and twenty-eight (98.8%) of the 433 culture-confirmed tuberculosis episodes were scored using the diagnostic algorithm used in the SANTCP Guidelines (2004), **Appendix 1** (page 140). (42) Overall, diagnostic algorithm scores were based upon the assessment of a median of 11 (IQR 10 – 12) of the 12 parameters stipulated for scoring by the algorithm, with no significant difference in completeness of scoring between HIV infection status groups, P=0.193. Three-hundred and forty-one (79.7%) of the scored culture-confirmed tuberculosis episodes were scored using at least 10 of the 12 parameters, and were used to estimate the sensitivity of the algorithm in establishing a diagnosis of tuberculosis in children (**Table 18**). Parameters used to score children according to the algorithm are tabulated in **Table 19** and **Table 20**.

	HIV-infected	**HIV-uninfected**	**HIV status undefined**	**All episodes**
Positive score	55 (72.4)*	96 (72.2)	82 (62.1)	233 (68.3)
Negative score	21 (27.6)	37 (27.8)	50 (37.9)	108 (31.7)
Total	76 (100.0)	133 (100.0)	132 (100.0)	341 (100.0)†

Comparison of proportions with positive algorithm scores	**Odds Ratio (95% CI)**	**P-values‡**
HIV-infected compared to -uninfected	1.01 (0.52 – 2.00)	0.977
HIV-infected compared to HIV status undefined	1.60 (0.83 – 3.12)	0.133
HIV-uninfected compared to HIV status undefined	1.58 (0.91 – 2.75)	0.081

Table 18: Sensitivity of the tuberculosis diagnostic algorithm in culture-confirmed tuberculosis episodes, according to HIV infection status

* Numbers in brackets reflect column percentages, according to HIV infection status, unless otherwise specified.
† Only children who were scored using at least 10 of the 12 parameters stipulated by the algorithm (**Appendix 1**, page 140) were included in the analysis of score chart sensitivity.
‡ P-values calculated in the comparison of proportions of children who attained a positive score, stratified by HIV infection status.

Parameter	HIV-infected	HIV-uninfected	HIV status undefined	Comparison of proportions: HIV-infected and HIV-uninfected	HIV-infected and HIV status undefined	HIV-uninfected and HIV status undefined
				P values*		
Duration of illness	40 (55.6)[†] / 72[‡]	73 (59.4) / 123	62 (47.7) / 130	0.604	0.284	0.063
Malnutrition	34 (44.7) / 76	66 (49.6) / 133	26 (19.7) / 132	0.496	<0.001	<0.001
Family history of tuberculosis	41 (56.9) / 72	66 (51.6) / 128	65 (50.8) / 128	0.464	0.402	0.900
Tuberculin skin test	28 (57.1) / 49	75 (70.8) / 106	96 (88.1) / 109	0.095	<0.001	0.002
No weight gain	20 (39.2) / 51	39 (45.4) / 86	12 (16.0) / 75	0.483	0.003	<0.001
Fever	9 (12.2) / 74	16 (12.0) / 133	13 (10.0) / 130	0.978	0.632	0.599

Table 19: Tuberculosis algorithm scores (General Features) for children with culture-confirmed tuberculosis, according to HIV infection status

Children with undefined HIV infection status attained positive scores for malnutrition less frequently, exhibited poor weight gain trends less frequently, and had significantly greater tuberculin skin test reactivity than either HIV-infected or -uninfected children (**Table 19**).

* P-values calculated in the comparison of proportions of children who attained a positive score for each parameter, stratified by HIV infection status.

[†] Numbers in brackets reflect the percentage of children who were scored and who attained a positive score for each parameter, stratified by HIV infection status.

[‡] Denominators reflect total number of children who were scored for each parameter, stratified by HIV infection status.

Parameter	HIV-infected	HIV-uninfected	HIV status undefined	Comparison of proportions		
				HIV-infected and HIV-uninfected	HIV-infected and HIV status undefined	HIV-uninfected and HIV status undefined
				P values[*]		
Adenopathy	59 (84.3)[†] / 70[‡]	69 (58.0) / 119	58 (51.8) / 112	<0.001	<0.001	0.344
Orthopaedic tuberculosis	1 (1.3) / 75	4 (3.0) / 133	6 (4.6) / 131	0.656[§]	0.426[§]	0.539[§]
Abdominal tuberculosis	14 (18.4) / 76	20 (15.0) / 133	4 (3.0) / 132	0.524	<0.001	0.001
Central nervous system tuberculosis	8 (10.5) / 76	18 (13.5) / 133	7 (5.3) / 132	0.526	0.174[§]	0.022
Intrathoracic adenopathy	36 (50.0) / 72	59 (49.2) / 120	58 (49.2) / 118	0.911	0.910	0.998
Gibbus	0 (0.0) / 76	1 (0.8) / 133	3 (2.3) / 131	1.000[§]	0.300[§]	0.368[§]

Table 20: Tuberculosis algorithm scores (Local Features) for children with culture-confirmed tuberculosis, according to HIV infection status

A significantly greater proportion of HIV-infected children with culture-confirmed tuberculosis presented with adenopathy compared to HIV-uninfected children and those with undefined HIV status. Children who had abdominal tuberculosis were more frequently investigated for HIV infection status (**Table 20**).

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* P-values calculated in the comparison of proportions of children who attained a positive score for each parameter, stratified by HIV infection status.

† Numbers in brackets reflect percentage of children who were scored who attained a positive score for each parameter, stratified by HIV infection status.

‡ Denominators reflect total number of children who were scored for each parameter, stratified by HIV infection status.

§ Fisher's exact test.

3.7.9 Comparison of the score chart sensitivity observed in the RCWMCH cohort, with those observed in Van Rheenen's Zambian cohort

Van Rheenen (41) utilised an adapted form of the Edwards score chart, (40) in order to evaluate the sensitivity of the chart in assisting in the diagnosis of a cohort of Zambian children with suspected tuberculosis; the format of the score chart used by Van Rheenen differs from that recommended for use by the SANTCP Guidelines (2004) (42) in that it does not include chest radiographic features as one of the scoring parameters. By adopting Van Rheenen's approach for the analysis of score sensitivity in the cohort of children managed for culture-confirmed tuberculosis at RCWMCH (by exclusion of the chest radiograph parameter), and using only scores which had been fully evaluated (using all 11 parameters required for scoring according to Van Rheenen's methodology), score chart sensitivity was 68% overall, and 77% in HIV-infected children (**Table 21**).

	HIV-infected	**HIV-uninfected**	**HIV status undefined**	**All episodes**
Positive score	20 (76.9)*	33 (73.3)	27 (58.7)	80 (68.4)
Negative score	6 (23.1)	12 (26.7)	19 (41.3)	37 (31.6)
Total	26 (100.0)	45 (100.0)	46 (100.0)	117 (100.0)†

Comparison of proportions with positive algorithm scores	**Odds Ratio (95% CI)**	**P-values‡**
HIV-infected compared to –uninfected	1.21 (0.35 – 4.58)	0.738
HIV-infected compared to HIV status undefined	2.35 (0.72 – 8.43)	0.119
HIV-uninfected compared to HIV status undefined	1.94 (0.73 – 5.18)	0.141

Table 21: Adjusted score chart sensitivities, with exclusion of the chest radiograph parameter (as utilised in Van Rheenen's analysis) (41)

* Numbers in brackets reflect column percentages, according to HIV infection status, unless otherwise specified.

† Only children who were scored using all 11 parameters stipulated by the algorithm utilised by Van Rheenen (41) were included in the analysis of score chart sensitivity.

‡ P-values calculated in the comparison of proportions of children who attained a positive score, stratified by HIV infection status.

3.7.10 Comparison of the Mantoux test and the score chart in children with culture-confirmed tuberculosis

The sensitivities of the Mantoux test and score charts in indicating the presence of tuberculosis infection in children with culture-confirmed tuberculosis are explored in **Table 22** (below); all children who had Mantoux results in this analysis were scored using the algorithm.

	HIV-infected		HIV-uninfected		HIV status undefined	
		Sensitivity (%)		Sensitivity (%)		Sensitivity (%)
Mantoux tests*	29/54†	53.7	77/111	69.4	105/119	88.2
Algorithm scores‡	55/76§	72.4	96/133	72.2	82/132	62.1
P-values**	0.028		0.630		<0.001	

Table 22: Comparison of the Mantoux test and tuberculosis score chart sensitivities, according to HIV infection status

Weighted Kappa statistics for assessment of the degree of agreement in indicating a diagnosis of culture-confirmed tuberculosis were calculated for the cohort of children in whom both Mantoux test results and algorithm scores were available (n=284): overall, there was fair agreement between Mantoux tests and the algorithm scores (κ=0.294). Strength of agreement between the tuberculosis score sheet and the Mantoux test was at best only moderate, as observed in HIV-uninfected children (κ=0.447), (**Table 23**).

* Mantoux test sensitivities are derived from **Table 17**.
† Row numerators describe the number of positive Mantoux responses; denominators describe the total number of Mantoux tests performed, stratified by HIV infection status.
‡ Score chart sensitivities are derived from **Table 18** (only instances where the algorithm was scored using at least 10 of the 12 parameters are included).
§ Row numerators describe the number of positive scores; denominators describe the total number of tuberculosis episodes that were scored stratified by HIV infection status.
** P-values compare the sensitivities of the Mantoux test and the tuberculosis score chart in each group, stratified by HIV infection status.

		Negative Mantoux	Positive Mantoux	Total	Agreement (%)		Kappa statistic
					Observed	Expected	
HIV-infected children	**Negative algorithm score**	9	4	13	63.0	51.9	0.230
	Positive algorithm score	16	25	41			
	Total	25	29	54			
HIV-uninfected children	**Negative algorithm score**	19	10	29	77.5	59.3	0.447
	Positive algorithm score	15	67	82			
	Total	34	77	111			
Children with undefined HIV status	**Negative algorithm score**	10	31	41	70.6	61.9	0.228
	Positive algorithm score	4	74	78			
	Total	14	105	119			
All children	**Negative algorithm score**	38	45	83	71.8	60.1	0.294
	Positive algorithm score	35	166	201			
	Total	73	211	284			

Table 23: Agreement between the tuberculosis score chart and Mantoux tests in corroborating a diagnosis of tuberculosis in children who were subsequently identified as having culture-confirmed tuberculosis

3.7.11 Multivariate analysis of factors which contributed to a positive tuberculosis score

A multiple logistic regression model utilising patient age, year of investigation for tuberculosis as well as the clinical discipline in which the child was assessed, HIV infection status, completeness of scoring, and Mantoux response was adopted to assess which

Shaded blocks represent the culture-confirmed tuberculosis episodes investigated with the use of the score chart and Mantoux test in which there was agreement between the two tests.

factors contributed to a positive score in children with culture-confirmed tuberculosis (first- and recurrent) episodes, **Table 24**.

		Univariate Analysis		Multivariate Analysis*	
		Odds Ratio (95% CI)	**P-value**	**Adjusted Odds Ratio (95% CI)**	**P-value**
Year of study	2007 versus 2006	1.32 (0.79 – 2.20)	0.293	-	-
	2008 versus 2006	1.32 (0.79 – 2.22)	0.287	-	-
Age (months)		0.99 (0.99 – 1.00)	0.002	1.01 (0.99 – 1.02)	0.296
Age group <60 months versus 60–180 months		2.01 (1.35 – 3.01)	0.001	3.15 (0.72 – 13.75)	0.127
Outpatient versus inpatient setting		0.94 (0.63 – 1.40)	0.758	-	-
Surgical versus medical care		0.31 (0.16 – 0.60)	0.001	1.71 (0.52 – 5.64)	0.377
HIV infection status	HIV-infected versus HIV-uninfected	0.77 (0.45 – 1.32)	0.346	-	-
	HIV-infected versus HIV status undefined	1.46 (0.87 – 2.45)	0.155	3.99 (1.44 – 11.00)	0.008
	HIV-uninfected versus HIV status undefined	1.89 (1.21 – 2.95)	0.005	2.08 (1.01 – 4.27)	0.045
Completeness of scoring†		2.12 (1.74 – 2.58)	<0.001	2.24 (1.58 – 3.19)	<0.001
Positive Mantoux score versus negative Mantoux score		3.86 (2.18 – 6.84)	<0.001	6.98 (3.28 – 14.86)	<0.001

Table 24: Multiple logistic regression analysis of factors which influenced tuberculosis score chart outcomes in children investigated for tuberculosis at RCWHCH who were subsequently found to have culture-confirmed tuberculosis

* The multivariate analysis utilised only the parameters which had achieved P-values of ≤ 0.20 in the univariate analyses.

† ‘Completeness of scoring’ indicates the number of score chart parameters that were recorded in hospital notes for each culture-confirmed tuberculosis episode in which folder reviews were available for review: children with one parameter recorded in hospital records were the least completely scored patients whilst those who had 12 scored parameters were completely scored. The coefficient attributable to each ‘unit rise’ in completeness of scoring was 0.81 (95% CI, 0.46 – 1.16) in the adjusted analysis.

3.8 Specimens Submitted For Bacteriologic Confirmation Of Tuberculosis

A total of 1 053 (80.1%) of the 1 314 tuberculosis episodes were investigated by submission of specimens for mycobacterial microscopy and culture (**Figure 8**).

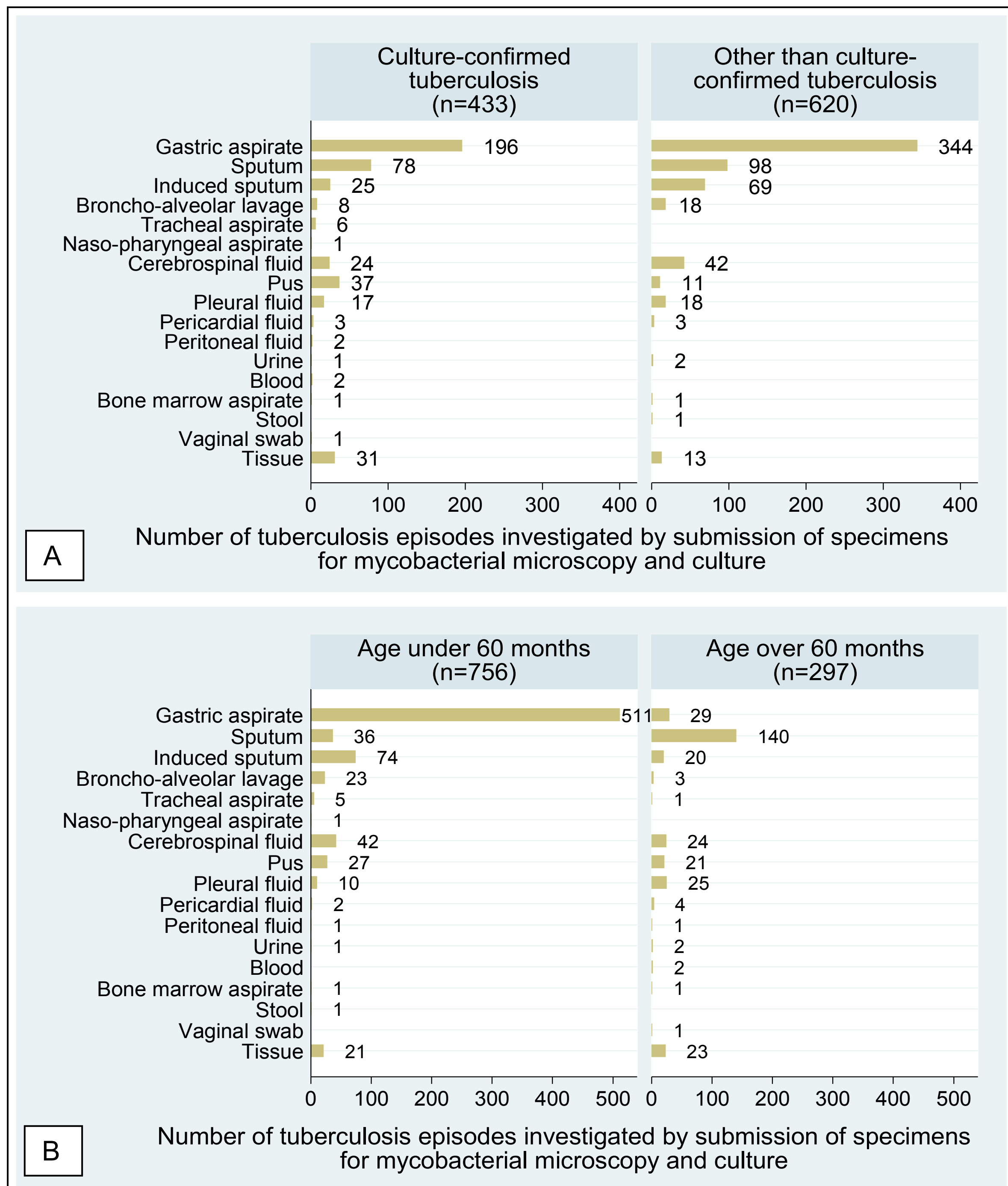

Figure 8: Specimens submitted for tuberculosis investigation in children diagnosed with tuberculosis, by disease category [A] and age category [B]

All 433 of the culture-confirmed tuberculosis episodes were investigated by submission of specimens for microbiological identification of *M. tuberculosis*, compared to 620 (70.4%) of the 881 'other than culture-confirmed' tuberculosis episodes, P<0.001. There was no significant difference in the proportion of tuberculosis cases investigated by submission of specimens for bacteriological confirmation according to age group (P=0.678) or HIV infection status (249 [91.9%] of the 271 tuberculosis episodes in HIV-infected children were investigated by submission of samples for microscopy and mycobacterial culture, compared to 369 [89.1%] of the 414 episodes in HIV-uninfected children, P=0.236).

3.8.1 Microscopy and culture for mycobacteria

The majority (n=843; 80.1%) of the 1 053 tuberculosis episodes in which samples were submitted for microbiological testing, were investigated by submission of samples derived from the respiratory tract, **Table 25**; microscopy yields improved substantially when evaluating only the tuberculosis episodes which were culture-confirmed (**Table 26**).

Three-hundred and four (70.4%) of the 432 specimens from which time to culture positivity could be calculated, flagged positive within three weeks of submission. Smear positive samples had a significantly shorter time to culture positivity (11 days; IQR 9 – 15) compared to smear-negative specimens (19 days; IQR 15 – 25), P<0.001.

Microscopy yields (n=34: 7.8%) in children with undefined HIV infection status (n=435: not shown in **Tables 25** to **27**) were significantly lower compared to yields in both HIV-infected (16.5%), P<0.001, and -uninfected children (12.7%), P=0.021; however, culture yields (n=176: 40.5%) in children with undefined HIV infection status did not differ significantly from those in HIV-infected (37.8%), P=0.486, or -uninfected children (44.2%), P=0.288.

Specimen type		All tuberculosis episodes (n=1 053)			HIV-infected (n=249)			HIV-uninfected (n=369)			P-values*
		Total	Pos	%	Total	Pos	%	Total	Pos	%	
Respiratory specimens	Gastric aspirate	540	56	10.4	115	17	14.8	212	22	10.4	0.241
	Sputum	176	29	16.5	59	14	23.7	38	8	21.1	0.759
	Induced sputum	94	4	4.3	37	2	5.4	28	2	7.1	1.000†
	Other‡	33	9	27.3	8	1	12.5	18	5	27.8	0.628†
	All respiratory specimens	843	98	11.6	219	34	15.5	296	37	12.5	0.325
Body fluids	CSF§	66	3	4.5	9	0	0.0	26	2	7.7	1.000†
	Pleural fluid	35	0	0.0	3	0	0.0	11	0	0.0	-
	Pericardial fluid	6	2	33.3	1	1	100.0	3	1	33.3	1.000†
	Peritoneal fluid	2	0	0.0	0	-	-	1	0	0.0	-
	Pus	48	7	14.6	9	2	22.2	11	3	27.3	1.000†
	Blood culture	2	0	0.0	0	-	-	1	0	0.0	-
	Bone marrow	2	1	50.0	1	1	100.0	0	-	-	-
	Urine	3	0	0.0	2	0	0.0	1	0	0.0	-
	Stool	1	1	100.0	0	-	-	0	-	-	-
	Vaginal swab	1	0	0.0	0	-	-	1	0	0.0	-
	All body fluids	166	14	8.4	25	4	16.0	55	6	10.9	0.717†
Tissue		44	10	22.7	5	3	60.0	18	4	22.2	0.142†
All specimens		1 053	122	11.6	249	41	16.5	369	47	12.7	0.193

Table 25: Proportion of childhood tuberculosis episodes in which positive microscopy from submitted specimens were obtained, by HIV infection status

* P-values compare the positive mycobacterial microscopy (percentage) yields from different sources, according to HIV-infection status: children with no defined HIV result are excluded from this analysis.

† Fisher's exact test.

‡ Other respiratory specimens included broncho-alveolar lavage fluid, tracheal aspirates and nasopharyngeal aspirates.

§ CSF = cerebrospinal fluid.

Specimen type		Culture-confirmed tuberculosis episodes (n=433)			HIV-infected (n=94)			HIV-uninfected (n=163)			P-values*
		Total	Pos	%	Total	Pos	%	Total	Pos	%	
Respiratory specimens	Gastric aspirate	196	54	27.6	39	16	41.0	84	22	26.2	0.098
	Sputum	78	28	35.9	31	13	41.9	19	8	42.1	0.991
	Induced sputum	25	4	16.0	7	2	28.6	10	2	20.0	1.000†
	Other‡	15	6	40.0	2	1	50.0	11	3	27.3	1.000†
	All respiratory specimens	314	92	29.3	79	32	40.5	124	35	28.2	0.070
Body fluids	CSF§	24	2	8.3	4	0	0.0	8	2	25.0	0.515†
	Pleural fluid	17	0	0.0	2	0	0.0	6	0	0.0	-
	Pericardial fluid	3	2	66.7	1	1	100.0	1	1	100.0	-
	Peritoneal fluid	2	0	0.0	0	-	-	1	0	0.0	-
	Pus	37	6	16.2	4	2	50.0	9	2	22.2	0.530†
	Blood culture	2	0	0.0	0	-	-	1	0	0.0	-
	Bone marrow	1	1	100.0	1	1	100.0	0	-	-	-
	Urine	1	0	0.0	1	0	0.0	0	-	-	-
	Vaginal swab	1	0	0.0	0	-	-	1	0	0.0	-
	All body fluids	88	11	12.5	13	4	30.8	27	5	18.5	0.437†
Tissue		31	7	22.6	2	1	50.0	12	3	25.0	0.505†
All specimens		433	110	25.4	94	31	33.0	163	43	26.4	0.261

Table 26: Proportion of culture-confirmed childhood tuberculosis episodes in which positive microscopy from submitted specimens were obtained, by HIV infection status

* P-values compare the positive mycobacterial microscopy (percentage) yields from different sources, according to HIV-infection status in children with culture-confirmed tuberculosis: children with no defined HIV result are excluded from this analysis.

† Fisher's exact test.

‡ Other respiratory specimens included broncho-alveolar lavage fluid, tracheal aspirates and nasopharyngeal aspirates.

§ CSF = cerebrospinal fluid.

Specimen type		All tuberculosis episodes (n=1 053)			HIV-infected (n=249)			HIV-uninfected (n=369)			P-values*
		Total	Pos	%	Total	Pos	%	Total	Pos	%	
Respiratory specimens	Gastric aspirate	540	196	36.3	115	39	33.9	212	84	39.6	0.309
	Sputum	176	78	44.3	59	31	52.5	38	19	50.0	0.807
	Induced sputum	94	25	26.6	37	7	18.9	28	10	35.7	0.127
	Other†	33	15	45.5	8	2	25.0	18	11	61.1	0.202‡
	All respiratory specimens	843	314	37.2	219	79	36.1	296	124	41.9	0.182
Body fluids	CSF§	66	24	36.4	9	4	44.4	26	8	30.8	0.685‡
	Pleural fluid	35	17	48.6	3	2	66.7	11	6	54.5	1.000‡
	Pericardial fluid	6	3	50.0	1	1	100.0	3	1	33.3	1.000‡
	Peritoneal fluid	2	2	100.0	0	-	-	1	1	100.0	-
	Pus	48	37	77.1	9	4	44.4	11	9	81.8	0.160‡
	Blood culture	2	2	100.0	0	-	-	1	1	100.0	-
	Bone marrow	2	1	50.0	1	1	100.0	0	-	-	-
	Urine	3	1	33.3	2	1	50.0	1	0	0.0	1.000‡
	Stool	1	0	0.0	0	-	-	0	-	-	-
	Vaginal swab	1	1	100.0	0	-	-	1	1	100.0	-
	All body fluids	166	88	53.0	25	13	52.0	55	27	49.1	0.809
Tissue		44	31	70.5	5	2	40.0	18	12	66.7	0.343‡
All specimens		1 053	433	41.1	249	94	37.8	369	163	44.2	0.112

Table 27: Proportion of childhood tuberculosis episodes in which positive mycobacterial cultures from submitted specimens were obtained, by HIV infection status

* P-values compare the positive mycobacterial culture (percentage) yields from different sources, according to HIV-infection status: children with no defined HIV result are excluded from this analysis.

† Other respiratory specimens included broncho-alveolar lavage fluid, tracheal aspirates and nasopharyngeal aspirates.

‡ Fisher's exact test.

§ CSF = cerebrospinal fluid.

3.8.2 Histology

Specimens for histologic confirmation of tuberculosis were submitted in 135 (10.3%) of the 1 314 tuberculosis (initial and recurrent) episodes that were registered amongst children in the Combined Database. **Figure 9** illustrates the types of tissue specimens submitted from children in this cohort.

Of the 433 culture-confirmed tuberculosis episodes, 80 (18.5%) had tissue specimens submitted for histologic confirmation of the disease, whilst only 55 (6.2%) of the 881 'other than culture-confirmed' tuberculosis episodes were investigated by submission of tissue specimens (OR 3.40; 95% CI, 2.33 – 5.00), $P<0.001$. Histology was suggestive of tuberculosis in 66 (82.5%) of the 80 tissue specimens submitted in tuberculosis episodes that were subsequently classified as being culture-confirmed.

Fourteen (14.9%) of the 94 culture-confirmed tuberculosis episodes in HIV-infected children were investigated with submission of tissue for histology, compared to 31 (19.0%) of the 163 culture-confirmed tuberculosis episodes in HIV-uninfected children, $P=0.402$. Histologic examination of tissue specimens tended to be less reliable in HIV-infected children, where nine (64.3%) of the 14 specimens submitted in children with culture-confirmed tuberculosis had suggestive histology, compared to 27 (87.1%) of the 31 histology specimens submitted in HIV-uninfected children with culture-confirmed tuberculosis (OR 0.27; 95% CI, 0.04 – 1.59), $P=0.111$. There was no statistically significant disparity in baseline CD4 T-lymphocyte counts between HIV-infected children with culture-confirmed tuberculosis whose histology was suggestive of tuberculosis (n=9: 31×10^6 cells/l; IQR 24 – 492) and those whose histology was not suggestive of tuberculosis (n=5: 242×10^6 cells/l; IQR 57 – 494), $P=0.865$.

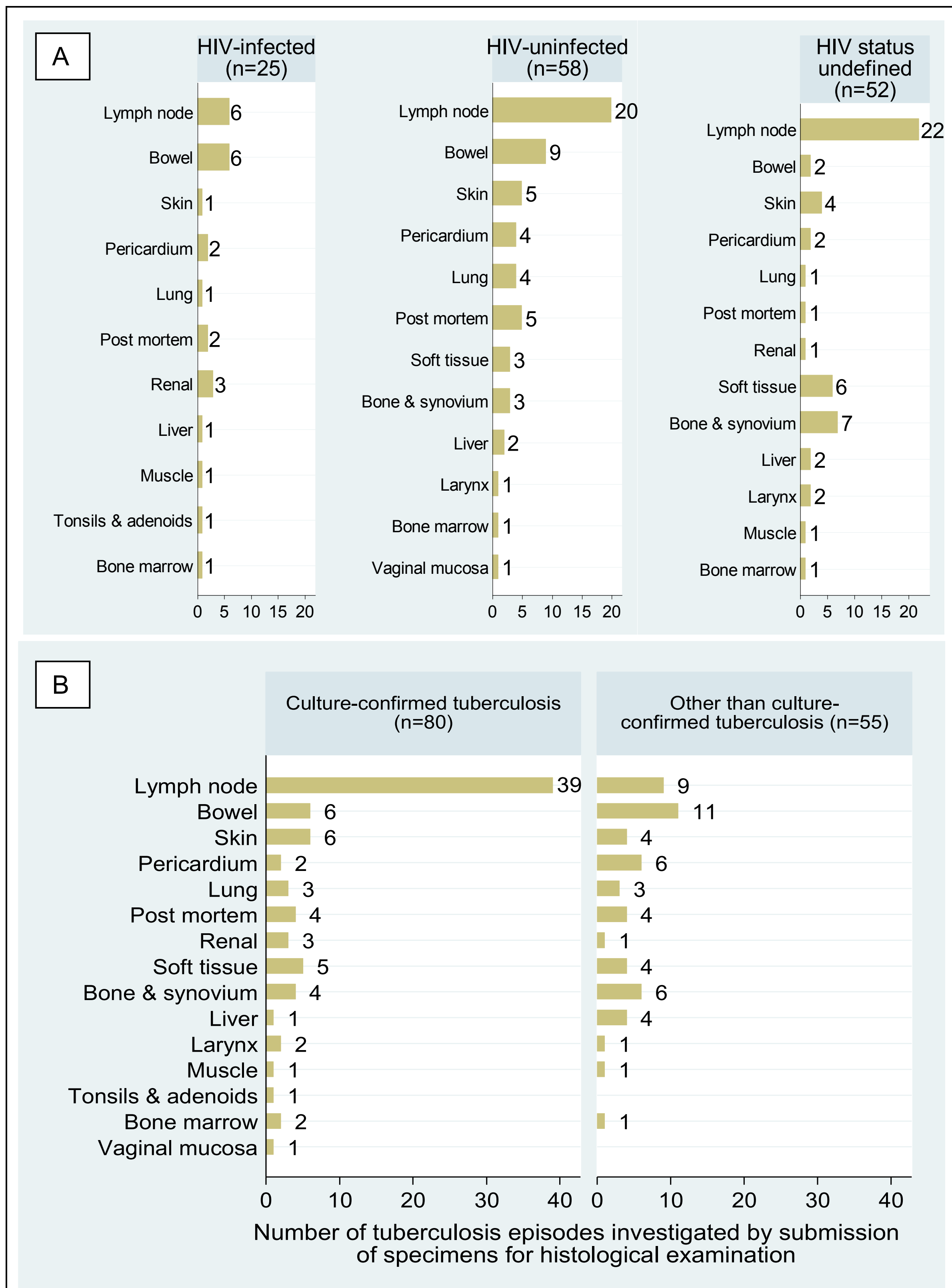

Figure 9: Tissue specimens submitted in the investigation for tuberculosis in children who were subsequently diagnosed with tuberculosis, according to HIV status [A] and strength of evidence for the diagnosis [B]

3.8.3 Pulmonary and extrapulmonary tuberculosis according to age group and HIV infection status

One thousand and twenty-nine (78.3%) of all 1 314 tuberculosis episodes, which included 63 episodes in children with extrapulmonary tuberculosis and concomitant pulmonary tuberculosis, (68) were classified as pulmonary tuberculosis episodes; 152 (11.6%) were extrapulmonary tuberculosis cases without pulmonary involvement; 122 (9.3%) were miliary tuberculosis and/or tuberculosis meningitis cases. Eleven (0.8%) children who had been notified for tuberculosis were notified for tuberculosis infection, and initiated on isoniazid preventive therapy (**Table 28**).

Children under five years of age presented more frequently with pulmonary tuberculosis (82.1%; 772 of 940 tuberculosis episodes) compared to those between the ages of five and fifteen years (68.7%; 257 of 374 tuberculosis episodes: OR 2.09; 95% CI, 1.57 – 2.78), P<0.001. Additionally, children under five years of age presented more frequently with culture-confirmed pulmonary tuberculosis compared to children between five and fifteen years of age (212 [78.2%] pulmonary tuberculosis episodes amongst 271 culture-confirmed tuberculosis episodes in children under five years, compared to 103 [63.6%] of 162 such episodes in older children: OR 2.06; 95%CI, 1.31 – 3.24), P=0.001.

Children older than five years of age had a higher burden of extrapulmonary tuberculosis than did younger children: 80 (21.4%) of 374 episodes in older children were classified as being extrapulmonary in nature, compared to 72 (7.7%) of 940 tuberculosis episodes in children under five (OR 3.28; 95% CI, 2.29 – 4.70), P<0.001. This finding was also borne out in the analysis of culture-confirmed extrapulmonary tuberculosis episodes: 45 (27.8%) of 162 culture-confirmed tuberculosis episodes in children over five were classified as being extrapulmonary in nature, compared to 30 (11.1%) of 271 culture-confirmed

	All tuberculosis episodes				Culture-confirmed tuberculosis episodes			
	HIV-infected	HIV-uninfected	HIV status undefined	All cases	HIV-infected	HIV-uninfected	HIV status undefined	All cases
Pulmonary tuberculosis*	226 (83.4)†	307 (74.2)	496 (78.9)	1 029 (78.3)	80 (85.0)	121 (74.2)	114 (64.7)	315 (72.8)
Mild extrapulmonary tuberculosis	4 (1.5)	22 (5.3)	53 (8.4)	79 (6.0)	3 (3.2)	10 (6.1)	33 (18.8)	46 (10.6)
Severe extrapulmonary tuberculosis	21 (7.8)	28 (6.8)	24 (3.8)	73 (5.6)	4 (4.3)	11 (6.8)	14 (8.0)	29 (6.7)
All extrapulmonary tuberculosis	25 (9.3)	50 (12.1)	77 (12.2)	152 (11.6)	7 (7.5)	21 (12.9)	47 (26.8)	75 (17.3)
Miliary tuberculosis & tuberculous meningitis	20 (7.3)	54 (13.0)	48 (7.6)	122 (9.3)	7 (7.5)	21 (12.9)	15 (8.5)	43 (9.9)
Isoniazid preventive therapy	0 (0.0)	3 (0.7)	8 (1.3)	11 (0.8)	-	-	-	-
Total	271 (100.0)	414 (100.0)	629 (100.0)	1 314 (100.0)	94 (100.0)	163 (100.0)	176 (100.0)	433 (100.0)

		Comparison of proportions					
		Pulmonary tuberculosis		All extrapulmonary tuberculosis		Miliary tuberculosis & tuberculous meningitis	
		Odds Ratio (95% CI)	P-value‡	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
HIV-infected versus HIV-uninfected	**All episodes**	1.75 (1.17 – 2.64)	0.004	0.74 (0.43 – 1.26)	0.242	0.53 (0.29 – 0.93)	0.020
	Culture-confirmed	1.92 (0.98 – 4.19)	0.042	0.54 (0.19 – 1.40)	0.178	0.54 (0.19 – 1.40)	0.178
HIV-infected versus HIV status undefined	**All episodes**	1.35 (0.92 – 2.00)	0.117	0.73 (0.43 – 1.19)	0.190	0.96 (0.53 – 1.70)	0.896
	Culture-confirmed	3.11 (1.58 – 6.42)	<0.001	0.22 (0.08 – 0.52)	<0.001	0.86 (0.27 – 2.36)	0.758
HIV-uninfected versus HIV status undefined	**All episodes**	0.77 (0.57 – 1.04)	0.078	0.98 (0.66 – 1.46)	0.937	1.82 (1.18 – 2.80)	0.004
	Culture-confirmed	1.57 (0.96 – 2.58)	0.059	0.41 (0.22 – 0.74)	0.002	1.59 (0.75 – 3.44)	0.193

Table 28: Tuberculosis clinical categorisation according to HIV infection status

* Pulmonary tuberculosis episodes include extrapulmonary episodes (n=14 in HIV-infected children; n=27 in HIV-uninfected children; n=22 in children with undefined HIV infection status) in which *M. tuberculosis* was either cultured from respiratory specimens, or extrapulmonary disease was associated with a clinical suspicion of pulmonary tuberculosis. (68)

† Numbers in brackets reflect column percentages of each tuberculosis disease category, according to HIV infection status, unless otherwise specified.

‡ P-values are calculated by comparison of proportions in each HIV infection status group that were diagnosed as having pulmonary tuberculosis, extrapulmonary tuberculosis, or miliary tuberculosis/tuberculous meningitis.

tuberculosis episodes in children under five (OR 3.09; 95% CI, 1.80 – 5.34), P<0.001. There was no demonstrable age predilection for miliary tuberculosis or tuberculous meningitis according to age group when considering all notified cases (P=0.954) or culture-confirmed cases (P=0.488).

In the analysis of all tuberculosis episodes, HIV-infected children were more likely to have pulmonary tuberculosis (P=0.004), but less likely to have tuberculous meningitis or miliary tuberculosis compared to HIV-uninfected children (P=0.020); however, these findings did not retain statistical significance in the analysis of culture-confirmed disease, **Table 28**.

HIV-infected children had significantly more episodes of culture-confirmed pulmonary tuberculosis (P<0.001), and significantly fewer episodes of culture-confirmed extrapulmonary tuberculosis (P<0.001), compared to children whose HIV status remained untested, **Table 28**. Similarly, HIV-uninfected children had fewer episodes of culture-confirmed extrapulmonary tuberculosis compared to children with undefined HIV infection status (P=0.002), **Table 28**.

3.9 Antituberculosis Regimens Adopted To Manage Children With Tuberculosis At RCWMCH

3.9.1 Estimation of antituberculosis regimens used to treat children identified as having tuberculosis at RCWMCH between 01 January 2006 and 31 December 2008

By derivation from the clinical spectrum of tuberculosis encountered in the RCWMCH cohort (as explored in **Section 3.8.3**), an estimation of the types of antituberculosis therapy which would have been used in these children could be made (**Table 29**).

	Regimen I*				Regimen III†			Miliary tuberculosis and tuberculous meningitis regimen‡	Total
	Smear-positive pulmonary tuberculosis in children < 8 years of age	All pulmonary tuberculosis, or mild extrapulmonary forms of tuberculosis in children ≥ 8 years of age	Severe forms of extrapulmonary tuberculosis, irrespective of age	Pulmonary tuberculosis with concomitant severe form of extrapulmonary tuberculosis	Smear-negative pulmonary tuberculosis in children < 8 years of age	Mild forms of extrapulmonary tuberculosis in children < 8 years of age	Pulmonary tuberculosis with concomitant mild form of extrapulmonary tuberculosis in children < 8 years of age		
HIV-infected	22	30	21	14	161	3	0	20	271
HIV-uninfected	19	41	28	20	227	15	7	54	411
HIV status undefined	16	92	24	12	388	31	10	48	621
All patients	57	163	73	46	776	49	17	122	1 303§ (100.0)
Total numbers treated using each regimen	339 (26.0)**				842 (64.6)			122 (9.4)	

Table 29: Treatment regimens utilised in children, inferred according to clinical category of tuberculosis

* Regimen I as prescribed by the SANTCP consists of a two-month intensive phase of treatment utilising four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol), followed by a four-month continuation phase using isoniazid and rifampicin.

† Regimen III as prescribed by the SANTCP consists of a two-month intensive phase of treatment utilising three drugs (isoniazid, rifampicin and pyrazinamide), followed by a four-month continuation phase using isoniazid and rifampicin.

‡ Miliary tuberculosis and tuberculous meningitis are treated with a six- to 12-month course of high-dose isoniazid, rifampicin, pyrazinamide and ethionamide.

§ A total of 1 303 tuberculosis episodes are listed here, as children identified with tuberculosis infection who were treated with isoniazid preventive therapy (n=11, **Table 28**) are excluded from the Table.

** Numbers in brackets represent the row percentages of children who were treated with each antituberculosis regimen.

By extrapolation, 461 (35.4%) of the 1 303 children identified with tuberculosis who were treated with regimes other than IPT required treatment using regimens (Regimen I and the miliary tuberculosis/tuberculous meningitis regimen) that are not readily available at Primary Health Care facilities catering for the care of children in the community (**Table 29**).

3.9.2 Antituberculosis treatment regimens used to treat children with culture-confirmed tuberculosis

Information regarding antituberculosis regimens chosen to treat children with culture-confirmed tuberculosis during hospitalisation or at hospital discharge was obtained by folder review in 421 (97.2%) of the 433 culture-confirmed tuberculosis episodes. The timing of initiation of antituberculosis treatment was evident in 371 (86.7%) of the 428 culture-confirmed tuberculosis episodes in which folder reviews were conducted; median time to antituberculosis treatment initiation was one day after admission (IQR 0 – 5), and did not differ significantly according to HIV infection status, P=0.104.

Forty-two (11.3%) tuberculosis episodes in children with culture-confirmed disease were commenced on antituberculosis treatment prior to the date of admission at RCWMCH in which specimens were submitted for mycobacterial culture, at a median of four days (IQR 1 – 15) before admission; 197 (53.1%) tuberculosis episodes in children with culture-confirmed tuberculosis were commenced on antituberculosis therapy during the course of the admission episode in which specimens were submitted for mycobacterial culture, at a median of one day (IQR 0 – 3) after admission; and antituberculosis therapy was commenced after discharge from RCWMCH in 132 (35.6%) of the culture-confirmed tuberculosis episodes, at a median of four days (IQR 2 – 17) post discharge.

The majority of cases (206/421; 48.9%) were treated using Regimen III (**Table 30**); however, 169 (those initiated on Regimen I, 'liver-friendly' treatment regimens, and multidrug-resistant treatment regimens: 40.1%) were treated with regimens that are not widely available in down-referral Primary Health Care facilities.

Forty-six (10.9%) children were never issued antituberculosis therapy at RCWMCH (**Table 30**); of these, 16 (34.8%) had been notified for tuberculosis, and were down-referred to the Primary Health Care Clinics for commencement of antituberculosis therapy, whilst 30 (65.2%) were not recorded in the Notifications Database, and appear not to have been notified for their condition. Consequently, 30 (7.1%) of the 421 episodes of culture-confirmed tuberculosis in which information regarding the type of antituberculosis therapy prescribed was obtained by folder review, may not have been initiated on appropriate therapy.

Children with undefined HIV infection status were commenced on Regimen III more frequently than were those with defined HIV infection status (OR 2.21; 95% CI, 1.46 – 3.35), $P<0.001$, whereas children with defined HIV infection status were significantly more likely to be treated for miliary tuberculosis or tuberculous meningitis than were those whose HIV infection status remained undefined (OR 2.83; 95% CI, 1.55 – 5.38), $P<0.001$.

HIV-infected children were significantly more likely to be treated for multidrug-resistant tuberculosis compared to HIV-uninfected children (OR 4.20; 95% CI, 1.12 – 19.13), $P=0.017$ or those with undefined HIV infection status (OR 18.33; 95% CI, 2.44 – 807.68), $P<0.001$, **Table 30**.

Anti-tuberculosis treatment regimens*	HIV-infected	HIV-uninfected	HIV status undefined	Total	Comparison of proportions		
					HIV-infected versus HIV-uninfected	HIV-infected versus HIV status undefined	HIV-uninfected versus HIV status undefined
					P-values[†]		
Anti-tuberculosis treatment not issued at RCWMCH	9 (9.8)	14 (8.8)	23 (13.5)	46 (10.9)	0.796	0.377	0.175
Regimen I	21 (22.8)	27 (17.0)	24 (14.1)	72 (17.1)	0.257	0.074	0.473
Regimen III	33 (35.9)	70 (44.0)	103 (60.6)	206 (48.9)	0.206	<0.001	0.003
Miliary TB & TB meningitis regimen	20 (21.7)	40 (25.2)	17 (10.0)	77 (18.3)	0.541	0.009	<0.001
Liver-friendly treatment	0 (0.0)	4 (2.5)	2 (1.2)	6 (1.4)	0.300[‡]	0.543[‡]	0.435[‡]
Multidrug-resistant tuberculosis treatment	9 (9.8)	4 (2.5)	1 (0.6)	14 (3.3)	0.017[‡]	<0.001[‡]	0.201[‡]
Regimens other than Regimen III	50 (54.3)	75 (47.2)	44 (25.9)	169 (40.1)	0.273	<0.001	<0.001
Total	92 (100.0)	159 (100.0)	170 (100.0)	421 (100.0)	-	-	-

Table 30: Antituberculosis treatment regimens utilised in children with culture-confirmed tuberculosis, according to HIV infection status

* Antituberculosis treatment regimens prescribed by the SANTCP include: Regimen I (rifampicin, isoniazid, pyrazinamide and ethambutol in the intensive phase of therapy [2 months' duration]; rifampicin and isoniazid in the continuation phase [4 months' duration]); Regimen III (rifampicin, isoniazid and pyrazinamide in the intensive phase of therapy [2 months' duration]; rifampicin and isoniazid in the continuation phase [4 months' duration]); Miliary TB & TB meningitis regimen (high doses of rifampicin, isoniazid, pyrazinamide and ethionamide for the duration of anti-tuberculosis therapy [6 to 12 months' duration]); 'Liver-friendly regimen' (amikacin, ciprofloxacin and ethambutol) changed over to conventional therapy once transaminitis resolved, where possible; Multidrug-resistant regimen usually individualised according to isolate's drug susceptibility pattern, and administered for 18 months after the first negative mycobacterial culture.

[†] P-values calculated by comparison of proportions treated with each antituberculosis regimen, according to HIV infection status.

[‡] Fisher's exact test.

Amongst the 421 children with culture-confirmed tuberculosis whose treatment regimen data were available, Regimen I was utilised in children over five years of age (48 [30.6%] of 157 children) significantly more frequently than it was used to treat children under five years of age (24 [9.1%] of 264 children), OR 4.40 (95% CI, 2.49 – 7.90) P<0.001. Whereas the SANTCP Guidelines endorse the use of Regimen I in children over eight years of age, of the 105 children over eight years of age in our cohort whose treatment regimen data were assessed, a substantial number (n=34; 32.4%) were treated using Regimen III.

One-hundred and forty-two (53.8%) of the 264 children under five years of age with culture-confirmed tuberculosis were treated using Regimen III, compared to 64 (40.8%) of those older than five years of age at tuberculosis diagnosis (OR 1.69; 95% CI, 1.11 – 2.58), P=0.010. Children under five years (n=56: 21.2%) of age were also more likely to have been treated using the miliary tuberculosis and tuberculous meningitis regimen compared to older children (n=21: 13.4%), OR 1.74 (95% CI, 0.99 – 3.17), P=0.044.

There was no significant age predilection for the use of ‘liver-friendly’ antituberculosis treatment and multidrug-resistant tuberculosis treatment (data not shown).

In the analysis only of the children who had been prescribed antituberculosis treatment at RCWMCH (by exclusion of the 46 children who were not treated at the facility, as mentioned above), older children (78 [54.9%] of 142 children) were significantly more likely than those under five years of age (91 [39.1%] of 233 children) to have been prescribed treatment regimens other than Regimen III overall, OR 1.90 (95% CI, 1.22 – 2.97), P=0.003.

3.10 *Mycobacterium tuberculosis* Drug Susceptibility Profiles

Four-hundred and six (95.5%) of the 425 first-episode *M. tuberculosis* complex isolates cultured underwent drug susceptibility testing: 356 (87.7%) were isoniazid and rifampicin susceptible and 50 (12.3%) were resistant to varying combinations of the first-line antituberculosis drugs (**Table 31**). In the analysis of all culture-confirmed tuberculosis episodes (n=433), 414 (95.6%) underwent drug-susceptibility testing and 53 (12.8%) isolates were resistant to first-line antituberculosis agents (**Table 31**).

All *M. tuberculosis* isolates obtained from the eight recurrent culture-confirmed tuberculosis episodes which occurred in the cohort, were subjected to drug-susceptibility testing. Four children (one HIV-infected and three HIV-uninfected) had recurrent episodes of culture-confirmed tuberculosis in which drug susceptibility testing had been conducted on both the initial and subsequent isolates. The HIV-infected child cultured fully-susceptible *M. tuberculosis* 4.9 months prior to the isolation of a multidrug-resistant strain, after having defaulted the initial course of antituberculosis treatment. The three HIV-uninfected children cultured *M. tuberculosis* with identical susceptibility patterns when comparing the initial and recurrent isolates: two cultured isoniazid mono-resistant *M. tuberculosis* on both occasions (time interval between cultures in these two children were 6.8 months and 15.3 months, respectively), the other cultured fully-susceptible *M. tuberculosis* on both occasions (time interval between cultures was 13.7 months).

In the analysis of first-episode tuberculosis, HIV-infected children were significantly more likely to have multidrug-resistant tuberculosis compared to HIV-uninfected children (OR 5.19; 95% CI, 1.19 – 31.02), P=0.019; this finding was recapitulated in the analysis of all tuberculosis episodes (**Table 31**), and in multivariate analyses when controlling for age at investigation for tuberculosis and repeat tuberculosis episode (**Table 32**).

Susceptibility pattern	First-episode tuberculosis				Recurrent tuberculosis episodes		All episodes			
	HIV-infected	HIV-uninfected	HIV status undefined	All children	HIV-infected	HIV-uninfected	HIV-infected	HIV-uninfected	HIV status undefined	All children
Isoniazid and rifampicin susceptible	68 (81.0)*	135 (89.4)	153 (89.5)	356 (87.7)	3 (75.0)	2 (50.0)	71 (80.7)	137 (88.4)	153 (89.5)	361 (87.2)
Isoniazid mono-resistant	5 (6.0)	12 (8.0)	8 (4.7)	25 (6.2)	0 (0.0)	2 (50.0)	5 (5.7)	14 (9.0)	8 (4.7)	27 (6.5)
Rifampicin mono-resistant	3 (3.6)	1 (0.7)	4 (2.3)	8 (2.0)[†]	0 (0.0)	0 (0.0)	3 (3.4)	1 (0.7)	4 (2.3)	8 (1.9)[†]
Multidrug-resistant	8 (9.5)	3 (2.0)	6 (4.0)	17 (4.2)	1 (25.0)	0 (0.0)	9 (10.2)	3 (1.9)	6 (3.5)	18 (4.3)
Total isolates with any resistance	16 (19.0)	16 (10.6)	18 (11.9)	50 (12.3)	1 (25.0)	2 (50.0)	17 (19.3)	18 (11.0)	18 (10.5)	53 (12.8)
Total isolates tested	84 (100.0)	151 (100.0)	171 (100.0)	406 (100.0)	4 (100.0)	4 (100.0)	88 (100.0)	155 (100.0)	171 (100.0)	414 (100.0)

Susceptibility pattern	Comparison of proportions (all culture-confirmed tuberculosis episodes)					
	HIV-infected versus -uninfected		HIV-infected versus HIV status undefined		HIV-uninfected versus HIV status undefined	
	Odds Ratio (95% CI)	P-value[‡]	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
Isoniazid and rifampicin susceptible	0.55 (0.25 – 1.21)	0.100	0.49 (0.22 – 1.08)	0.050	0.90 (0.42 – 1.91)	0.755
Isoniazid mono-resistant	0.61 (0.17 – 1.87)	0.350	1.23 (0.31 – 4.41)	0.768[§]	2.02 (0.76 – 5.73)	0.118
Rifampicin mono-resistant	5.44 (0.43 – 287.11)	0.137[§]	1.47 (0.21 – 8.91)	0.692[§]	0.27 (0.01 – 2.79)	0.374[§]
Multidrug-resistant	5.77 (1.38 – 33.82)	0.010[§] **	3.13 (0.95 – 11.04)	0.046[§]	0.54 (0.09 – 2.60)	0.507[§]

Table 31: Drug susceptibility patterns of *M. tuberculosis* complex isolates (first- and recurrent tuberculosis episodes)

* Numbers in brackets reflect column percentages of drug susceptibility profiles according to HIV infection status, unless otherwise specified.
[†] Of the eight rifampicin monoresistant *M. tuberculosis* isolates, two were isolated in 2006, four in 2007 and two in 2008.
[‡] P-values are derived by comparison of proportions of *M. tuberculosis* isolates according to susceptibility pattern and HIV infection status.
[§] Fisher's exact test.
** For first-episode multidrug-resistant tuberculosis episode comparison between HIV-infected and -uninfected children: OR 5.19 (95% CI, 1.19 – 31.02), P=0.019.

		Univariate Analysis		Multivariate Analysis*	
		Odds Ratio (95% CI)	P-values	Adjusted Odds Ratio (95% CI)	P-values
Year of study	2007 versus 2006	0.55 (0.16 – 1.96)	0.357	-	-
	2008 versus 2006	0.78 (0.24 – 2.52)	0.674	-	-
Duration of illness		1.00 (0.98 – 1.01)	0.305	-	-
Repeat TB episode		3.48 (0.40 – 30.02)	0.256	3.14 (0.33 – 30.12)	0.321
Age (months)		1.01 (1.00 – 1.01)	0.218	-	-
Age group <60 months versus 60–180 months		0.31 (0.11 – 0.87)	0.025	0.39 (0.11 – 1.45)	0.162
Gender		1.41 (0.53 – 3.74)	0.486	-	-
History of TB contact		1.15 (0.38 – 3.50)	0.800	-	-
IPT given†		1.04 (0.09 – 12.65)	0.974	-	-
HIV-infected versus HIV-uninfected		5.07 (1.31 – 19.63)	0.019	4.03 (1.01 – 16.13)	0.049

Table 32: Multiple logistic regression analysis of factors which influenced the occurrence of multidrug-resistant tuberculosis in children investigated for tuberculosis at RCWHCH (all tuberculosis episodes)

* The multivariate analysis utilised only the parameters which had achieved P-values of ≤ 0.20 in the univariate analyses; however, as repeat tuberculosis episode may be construed to be associated with an increased likelihood of drug-resistant tuberculosis, this parameter was factored into the analysis even though the P-value was > 0.20 in the univariate analysis.

† IPT = isoniazid preventive therapy

3.11 Tuberculosis In HIV-infected Children

Two-hundred and six (78.0%) of the 264 HIV-infected children ever diagnosed with tuberculosis (**Figure 1**) were ART-naïve at clinical work-up for first-episode tuberculosis. Timing of HIV testing in relation to the hospitalisation episode in which a diagnosis of first-episode tuberculosis was made, was available in 189 (91.7%) of these ART-naïve children; 132 (69.8%) of these 189 children were tested for HIV during or after the course of the hospitalisation episode in which tuberculosis was diagnosed, and appear to have had their HIV infection status confirmed during a sentinel diagnosis of tuberculosis.

3.11.1 Immunologic and virologic parameters in HIV-infected children with and without tuberculosis

Two-hundred and twenty-one (83.7%) of the 264 HIV-infected children identified with first-episode tuberculosis had retrievable baseline CD4 T-lymphocyte count determinations, compared to 20 (64.5%) of 31 HIV-infected children notified with other disease conditions, P=0.009.There were no significant differences in baseline CD4 T-lymphocyte counts or percentages amongst ART-naïve and ART-experienced children with and without tuberculosis (data not shown).

One hundred and seventy-nine (67.8%) of the 264 HIV-infected who were ever diagnosed with tuberculosis had baseline HIV viral load determinations at or before work-up for tuberculosis, compared to 20 (64.5%) of the 31 HIV-infected children notified for illness other than tuberculosis, P=0.712. The median baseline viral load in children notified for tuberculosis was 170 000 RNA copies per millilitre (IQR 18 000 – 1 100 000), compared to 1 950 000 RNA copies per millilitre (IQR 130 000 – 3 000 000) in those notified for other disease processes, P=0.022. Baseline median viral load $\log_{10}$ values were 5.30 copies per millilitre (IQR 4.26 – 6.08) in children notified with

tuberculosis compared to 6.28 copies per millilitre (IQR 4.97 – 6.69) in those with other disease conditions, P=0.024.

One-hundred and twenty-five (69.8%) of the 179 children with tuberculosis who had baseline HIV viral load determinations were ART-naïve, compared to 11 (55.0%) of the 20 children notified for other disease conditions, P=0.176. Baseline HIV viral load results were 430 000 RNA copies per millilitre (IQR 72 000 – 1 900 000) in ART-naïve children diagnosed with tuberculosis (n=125) compared to 3 000 000 (IQR 290 000 – 3 000 000) in those who were notified for illnesses other than tuberculosis (n=11), P=0.010.

Of the children who had retrievable baseline viral loads and were ART-experienced at diagnosis of tuberculosis or other notifiable condition, children with tuberculosis (n=54) tended to have lower HIV viral loads (1 045 RNA copies per millilitre [IQR 25 – 300 000]) compared to children notified for diseases other than tuberculosis (n=9: 580 000 RNA copies per millilitre [IQR 140 – 2 500 000]), P=0.113. ART-experienced children notified for diseases other than tuberculosis had been on antiretroviral therapy for 3.1 months
(IQR 2.4 – 25.7), whilst ART-experienced children notified for tuberculosis had been on antiretroviral therapy for 10.1 months (IQR 4.4 – 27.3), P=0.396.

3.11.2 Baseline immunologic parameters in HIV-infected children diagnosed with tuberculosis

Immunologic status of HIV-infected children could be classified according to the WHO immunologic grading system (69) (**Appendix 7**, page 150) in 223 (84.5%) of all 264 first-episode tuberculosis events observed in HIV-infected children. There was no significant difference in stratification of degree of immunodeficiency according to the

strength of evidence for tuberculosis; however children under five years of age at clinical assessment were significantly less likely to have normal immunologic parameters compared to children over five years of age (OR 0.41; 95% CI, 0.21 – 0.81), P=0.005 (**Table 33**).

Degree of immunodeficiency	Strength of evidence for tuberculosis		P-values*
	Culture-confirmed tuberculosis	Other than culture-confirmed tuberculosis	
None	20 (28.2)[†]	40 (26.3)	0.771
Mild	4 (5.6)	8 (5.3)	1.000[‡]
Advanced	10 (14.1)	23 (15.1)	0.837
Severe	37 (52.1)	81 (53.3)	0.870
Total	71 (100.0)	152 (100.0)	-
Degree of immunodeficiency	**Age group**		**P-values[§]**
	<60 months	**60 – 180 months**	
None	34 [21.5]**	26 [40.0]	0.005
Mild	9 [5.7]	3 [4.6]	1.000[‡]
Advanced	28 [17.7]	5 [7.7]	0.055
Severe	87 [55.1]	31 [47.7]	0.316
Total	158 [100.0]	65 [100.0]	-

Table 33: Immunologic classification of HIV-infected children at clinical evaluation for first-episode tuberculosis

Sixty-nine (76.7%) of the 90 HIV-infected children with first-episode culture-confirmed tuberculosis had retrievable baseline CD4 T-lymphocyte results compared to 152 (87.4%) of 174 HIV-infected children with 'other than culture-confirmed' tuberculosis, P=0.026. HIV-infected children with culture-confirmed tuberculosis had significantly

* P-values are derived by comparison of proportions of tuberculosis cases presenting in each immunologic category according to the strength of evidence for tuberculosis.
[†] Numbers in brackets are column percentages according to strength of evidence for tuberculosis.
[‡] Fisher's exact test.
[§] P-values are derived by comparison of proportions of tuberculosis cases presenting in each immunologic category according to age group.
** Numbers in squared brackets are column percentages according to age group at tuberculosis diagnosis.

lower baseline median CD4 T-lymphocyte counts (386 × 10^6 cells/l [IQR 107 – 825]) compared to HIV-infected children with ‘other than culture-confirmed’ tuberculosis (603 × 10^6 cells/l [IQR 263 – 1 084]), P=0.013; however, CD4 T-lymphocyte percentages were not statistically divergent in HIV-infected children with culture-confirmed tuberculosis (19.6% [IQR 9.6 – 26.7]) compared to those with ‘other than culture-confirmed’ tuberculosis (17.4% [IQR 11.2 – 26.7]), P=0.850.

Baseline median CD4 T-lymphocyte counts (326 × 10^6 cells/l [IQR 97 – 633] compared to 896 × 10^6 cells/l [IQR 595 – 1 276], P=0.003) and mean CD4 T-lymphocyte percentages (17.1% [SD 10.3] compared to 23.9% [SD 10.2], P=0.031) were significantly lower in ART-naïve children compared to ART-experienced children with culture-confirmed tuberculosis.

No statistically significant differences in baseline immunologic parameters according to ART experience were observed in the children with ‘other than culture-confirmed’ tuberculosis; neither could differences in baseline immunologic status be demonstrated according to age group, regardless of the tuberculosis disease classification (data not shown).

3.11.3 Baseline virologic parameters in HIV-infected children diagnosed with tuberculosis

There was no statistically significant difference in baseline virologic parameters in the HIV-infected children according to the strength of evidence for tuberculosis. Children with culture-confirmed tuberculosis (n=62) had baseline HIV viral loads of 220 000 RNA copies per millilitre (IQR 4 800 – 1 100 000) compared to 160 000 RNA copies per millilitre

(IQR 24 000 – 1 200 000) in children with ‘other than culture-confirmed’ disease (n=117), P=0.607. Baseline HIV viral load $\log_{10}$ values were 5.34 copies per millilitre (IQR 3.68 – 6.04) and 5.23 copies per millilitre (IQR 4.38 – 6.08) in children with culture-confirmed tuberculosis and those with ‘other than culture-confirmed’ tuberculosis, respectively, P=0.594.

Baseline virologic parameters did not differ according to strength of evidence for the tuberculosis diagnosis when one took into consideration whether children were ART-naïve or -experienced at baseline (data not shown).

3.11.4 Highly active antiretroviral therapy in HIV-infected children with tuberculosis

Of the 264 HIV-infected children ever diagnosed with tuberculosis, children under five years of age were no more likely to be ART-naïve (143/182: 78.6%) at tuberculosis diagnosis compared to those who were older than five (63/82: 76.8%), P=0.752. In the analysis of all tuberculosis episodes (**Table 34**), there was a tendency for those under five years of age to be commenced on ART during the course of antituberculosis treatment more frequently than children who were over five years of age at tuberculosis diagnosis (OR 1.95; 95% CI, 0.92 – 4.17), P=0.057.

In the analysis of all tuberculosis episodes amongst ART-naïve HIV-infected children in whom immunologic classification according to the WHO classification system could be assigned (n=162), those under five years of age (97/117: 82.9%) at tuberculosis diagnosis were 2.7-fold (95% CI, 1.13 – 6.22), P=0.011 more likely to be classified as having any degree of immunodeficiency compared to those over five years of age (29/45: n=64.4%).

Timing of ART	Tuberculosis disease category			Age at tuberculosis diagnosis		
	Culture-confirmed tuberculosis	Other than culture-confirmed	P-values*	Under 60 months	60–180 months	P-values[†]
Before tuberculosis episode	18 (30.0)[‡]	44 (41.1)	0.154	41 (33.6)	21 (46.7)	0.121
During tuberculosis admission, prior to antituberculosis treatment	1 (1.7)	Unable to quantify[§]	-	1 (0.8)	0 (0.0)	1.000**
During antituberculosis treatment	32 (53.3)	55 (51.4)	0.811	69 (56.6)	18 (40.0)	0.057
After completion of antituberculosis treatment	6 (10.0)	8 (7.5)	0.573**	10 (8.2)	4 (8.9)	1.000**
Timing of ART uncertain[††]	3 (5.0)	0 (0.0)	0.045**	1 (0.8)	2 (4.4)	0.177**
Total who ever started ART	60 (100.0)	107 (100.0)	-	122 (100.0)	45 (100.0)	-
ART-naïve at tuberculosis diagnosis[‡‡]	75 [79.8][§§]	133 [75.1]	0.389	145 [77.5]	63 [75.0]	0.647
Never started ART	34 [36.2]	70 [39.5]	0.586	65 [34.8]	39 [46.4]	0.068
Ever started ART	60 [63.8]	107 [60.5]		122 [65.2]	45 [53.6]	
Total	94 [100.0]	177 [100.0]	-	187 [100.0]	84 [100.0]	-

Table 34: Timing of ART according to strength of evidence for tuberculosis and age category at tuberculosis diagnosis (all tuberculosis episodes)

* P-values are derived by comparison of proportions of cases, according to classification of tuberculosis disease and timing of initiation of ART.
[†] P-values are derived by comparison of proportions of cases, according to age group and timing of initiation of ART.
[‡] Numbers in rounded brackets reflect column percentages for tuberculosis cases that were ever commenced on ART.
[§] As no folder reviews were conducted for children with 'other than culture-confirmed' tuberculosis, date of initiation of antituberculosis treatment was not available in these cases, and timing of ART in relation to antituberculosis treatment could not be evaluated.
** Fisher's exact test.
[††] Timing of ART could not be determined in three children who were diagnosed with tuberculosis at RCWMCH, but who were commenced on ART (either during or after the course of anti-tuberculosis therapy) at down-referral centres.
[‡‡] Children classified as being ART-naïve at tuberculosis diagnosis included those who commenced ART during or after the course of antituberculosis treatment, as well as those who were never started on ART.
[§§] Numbers in square brackets reflect column percentages of all tuberculosis cases amongst HIV-infected children (n=271, **Figure 1**), regardless as to whether ART was commenced or not.

In the analysis of first-episode tuberculosis events only, 57 (63.3%) of the 90 HIV-infected children who were ever identified as having culture-confirmed tuberculosis were ever commenced on ART, compared to 105 (60.3%) of the 174 children ever diagnosed with 'other than culture-confirmed' tuberculosis, P=0.636.

In the analysis of all tuberculosis episodes amongst children who were initiated on ART before tuberculosis diagnosis (n=62, **Table 34**), median duration of ART in those under five years of age was 6.4 months (n=39: IQR 3.6 – 25.6), compared to 20.0 months (n=21: IQR 11.0 – 31.3) in those who were older than five, P=0.029. ART regimens used at tuberculosis diagnosis in these 62 episodes consisted of protease inhibitor (PI)-based regimens (n=34: 54.8%) and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens (n=27: 43.5%); the details of the ART regimen used to treat one child was unknown, **Figure 10**.

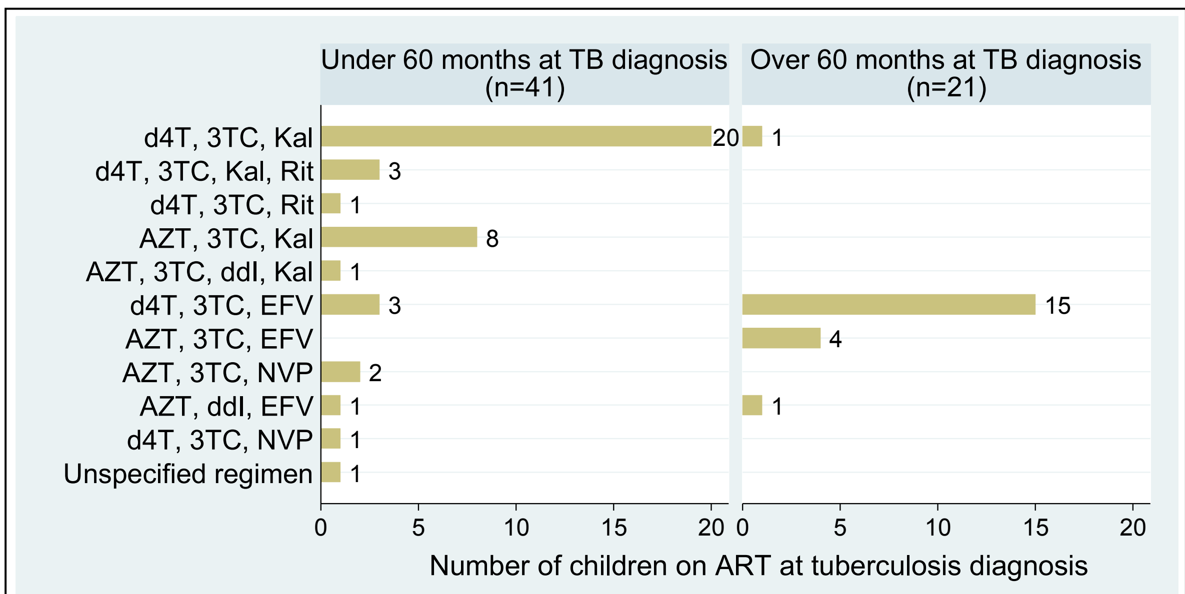

Figure 10: Antiretroviral drug regimens* utilised in children who had been established on ART prior to tuberculosis diagnosis

* 3TC = lamivudine; AZT = zidovudine; d4T = stavudine; ddI = didanosine; EFV = efavirenz; NVP = nevirapine; Kal = lopinavir/ritonavir; Rit = ritonavir.

There was no significant difference in the proportions of children being treated with second-line ART according to age group, amongst those who had been initiated on ART before the tuberculosis episode: five (12.2%) of the 41 tuberculosis episodes in children under five years of age were treated using second-line ART regimens, compared to two (9.5%) of the 21 tuberculosis episodes in children older than five years of age, P=1.000.

Time to ART initiation in 87 tuberculosis episodes in which children were started on ART during the course of anti-tuberculosis therapy (**Table 34**) was 1.0 month (IQR 0.6 – 1.8) in children under five years of age (n=69), and 0.6 month (IQR 0.3 – 1.6) in those older than five (n=18), P=0.043. ART therapy initiated during the course of antituberculosis therapy consisted of PI-based regimens in 52 (75.4%) of the 69 tuberculosis episodes in children under five years of age whilst NNRTI-based regimens were prescribed in 17 (24.6%) such episodes. The median age of the children under five years of age who were initiated on PI-based regimens during the course of antituberculosis therapy was 8.2 months (IQR 4.1 – 18.0) compared to 43.1 months (IQR 24.9 – 50.1) in those initiated on NNRTI-based regimens, P<0.001. NNRTI-based regimens were used in 17 (94.4%) of the 18 tuberculosis episodes in which ART was commenced during the course of antituberculosis treatment in children over five years of age.

In the 14 tuberculosis episodes in which ART was initiated after completion of anti-tuberculosis therapy (**Table 34**), this occurred at 9.0 months (IQR 8.3 – 14.3) after diagnosis with tuberculosis in those under five years of age (n=10), and at 7.4 months (IQR 6.1 – 18.0) in those older than five (n=4), P=0.480. Five of the 10 children under five years of age who were started on ART after completion of antituberculosis therapy, were initiated on PI-based regimens; the remaining nine children (five under five years of age and four over five years of age), were initiated on NNRTI-based regimens.

3.11.5 Immunologic parameters in HIV-infected children after recovery from tuberculosis

Follow-up CD4 T-lymphocyte results were available in 119 (45.1%) of the 264 HIV-infected children ever diagnosed with tuberculosis, in whom information regarding exposure to ART was available. One-hundred and eleven (50.2%) of the 221 HIV-infected children with tuberculosis who had baseline CD4 T-lymphocyte results and had a history regarding exposure to ART, had retrievable repeat CD4 T-lymphocyte determinations; comparisons between baseline and follow-up median CD4 T-lymphocyte counts in these 111 children are tabulated below (**Table 35**). The median time period between initial and subsequent CD4 T-lymphocyte determinations was 7.9 months (IQR 6.3 – 10.7).

The median follow-up CD4 T-lymphocyte count in HIV-infected children with culture-confirmed tuberculosis was 679 × 10^6 cells/l (IQR 302 – 985), and the median follow-up CD4 T-lymphocyte count in children with ‘other than culture-confirmed’ tuberculosis was 991 × 10^6 cells/l (IQR 600 – 1 134), P=0.008. Overall, there were significant improvements in CD4 T-lymphocyte counts and percentages, in the comparison of baseline and follow-up results (**Table 35**).

In children never started on ART (n=14), the median baseline CD4 T-lymphocyte count was 694 × 10^6 cells/l (IQR 430 – 1 086), and the median follow-up CD4 T-lymphocyte count was 619 × 10^6 cells/l (IQR 309 – 991), P=0.638. In children who had been established on ART at the time of tuberculosis diagnosis, or commenced on ART during the course of antituberculosis therapy (n=97), the median baseline CD4 T-lymphocyte count was 508 × 10^6 cells/l (IQR 154 – 930), and the median follow-up CD4 T-lymphocyte count was 946 × 10^6 cells/l (IQR 569 – 1 284), P<0.001.

		Culture-confirmed tuberculosis*			Other than culture-confirmed tuberculosis[†]		
		Baseline	**Follow-up**	**P-values[‡]**	**Baseline**	**Follow-up**	**P-values[‡]**
CD4 T-lymphocyte counts (cells × 10^6/l)	**All children**	353 (105 – 854)[§]	679 (302 – 985)	0.008	574 (255 – 1 056)	991 (600 – 1 314)	<0.001
	Age < 60 months	417 (121 – 994)	819 (419 – 1 122)	0.134	624 (295 – 1 086)	1 055 (673 – 1 356)	<0.001
	Age 60 – 180 months	305 (33 – 729)	523 (285 – 762)	0.019	385 (175 – 713)	658 (251 – 1 094)	0.184
	ART-naïve**	276 (79 – 666)	570 (294 – 952)	0.022	556 (254 – 928)	769 (517 – 1 314)	0.004
	ART-experienced**	694 (SD 442)	869 (SD 480)	0.133	815 (SD 604)	1 198 (SD 540)	<0.001
CD4 T-lymphocyte percentages (%)	**All children**	17.7 (8.2 – 26.7)	21.4 (15.1 – 31.3)	0.002	16.7 (11.8 – 24.6)	22.1 (14.0 – 28.5)	0.004
	Age < 60 months	18.7 (SD 9.4)	24.2 (SD 10.5)	0.041	18.9 (SD10.1)	22.3 (SD 9.2)	0.012
	Age 60 – 180 months	15.4 (SD 11.4)	21.1 (SD 8.5)	0.022	16.5 (SD 10.6)	18.0 (SD 8.8)	0.436
	ART-naïve**	16.4 (SD 9.9)	22.1 (SD 9.7)	0.007	17.4 (SD 10.4)	19.7 (SD 9.0)	0.066
	ART-experienced**	23.3 (8.2 – 29.0)	27.5 (16.3 – 33.3)	0.116	20.1 (14.7 – 27.8)	26.2 (16.9 – 32.7)	0.032

Table 35: Comparison of baseline and follow-up immunological parameters in HIV-infected children treated for tuberculosis at RCWMCH

* There were 30 children with culture-confirmed tuberculosis in whom baseline and follow-up CD4 T-lymphocyte determinations were available: <60 months (n=16), 60-180 months (n=14); ART-naïve (n=24), ART-experienced (n=6).

[†] There were 81 children with 'other than culture-confirmed' tuberculosis in whom baseline and follow-up CD4 T-lymphocyte determinations were available: <60 months (n=63), 60-180 months (n=18); ART-naïve (n=55), ART-experienced (n=26).

[‡] P-values derived by comparison of median CD4 T-lymphocyte counts and percentages at baseline and follow-up determinations (using the Wilcoxon sign-rank test for skewed data, and the Paired *t* test for normally-distributed data).

[§] Numbers in brackets are interquartile ranges, unless otherwise specified. SD = standard deviation.

** ART-naïve reflects children who were not on ART at tuberculosis diagnosis, ART-experienced reflects children who were on ART at time of tuberculosis diagnosis.

Similar trends were observed in the comparison of baseline and follow-up CD4 T-lymphocyte percentages, stratified according to ART experience.

Twenty-eight children exhibited a fall in CD4 T-lymphocyte counts in the comparison between baseline and follow-up determinations: seven (50.0%) of the 14 children who were not commenced on ART exhibited CD4 T-lymphocyte count attrition, compared to 21 (21.6%) of 97 children who were either on ART at tuberculosis diagnosis, or started on ART in response to the tuberculosis diagnosis (RR 2.31; 95% CI, 1.21 – 4.41), P=0.043.

3.11.6 Virologic parameters in HIV-infected children after recovery from tuberculosis

Follow-up HIV viral load results were available in 108 (40.9%) of the 264 HIV-infected children ever diagnosed with tuberculosis. **Table 36** illustrates viral load determinations in 91 (50.8%) of the 179 HIV-infected children notified for tuberculosis who had baseline viral load results and whose follow-up viral load parameters were available: there were significant improvements in virologic parameters in the comparison of baseline and follow-up results overall. The median time period between initial and subsequent viral load determinations was 7.5 months (IQR 6.3 – 10.4).

3.11.7 Virologic response according to highly active antiretroviral therapy regimen in children who were on ART before or during antituberculosis treatment

The virologic response according to ART regimen was explored in 77 tuberculosis episodes amongst children who were initiated on ART either before or during antituberculosis treatment, in whom both baseline and follow-up viral load results were

		Culture-confirmed tuberculosis*			Other than culture-confirmed tuberculosis[†]		
		Baseline	**Follow-up**	**P-values[‡]**	**Baseline**	**Follow-up**	**P-values[‡]**
HIV viral load (RNA copies/ml)	**All children**	520 000 (14 000 – 1 400 000)[§]	25 (25 – 130)	<0.001	140 000 (26 500 – 1 100 000)	25 (25 – 2 200)	<0.001
	Age < 60 months	900 000 (160 000 – 1 800 000)	25 (25 – 7 600)	0.003	290 000 (24 000 – 2 200 000)	25 (25 – 2 500)	<0.001
	Age 60 – 180 months	37 000 (25 – 690 000)	25 (25 – 25)	0.059	94 500 (29 000 – 360 000)	25 (25 – 170)	0.038
	ART-naïve**	725 000 (109 000 – 1 650 000)	25 (25 – 155)	<0.001	360 000 (89 000 – 2 200 000)	25 (25 – 920)	<0.001
	ART-experienced**	3 200 (25 – 56 000)	25 (25 – 25)	0.476	45 000 (25 – 680 000)	25 (25 – 6 800)	0.001
HIV viral load log_{10} values	**All children**	5.72 (4.15 – 6.15)	1.38 (1.38 – 2.11)	<0.001	5.28 (4.49 – 6.06)	1.38 (1.38 – 3.34)	<0.001
	Age < 60 months	5.95 (5.20 – 6.26)	1.38 (1.38 – 3.88)	0.001	5.47 (4.38 – 6.34)	1.38 (1.38 – 3.40)	<0.001
	Age 60 – 180 months	4.57 (1.38 – 5.84)	1.38 (1.38 – 1.38)	0.036	4.98 (4.64 – 5.56)	1.38 (1.38 – 2.23)	0.014
	ART-naïve**	5.87 (4.98 – 6.22)	1.38 (1.38 – 2.19)	<0.001	5.56 (4.95 – 6.34)	1.38 (1.38 – 2.96)	<0.001
	ART-experienced**	3.51 (1.38 – 4.75)	1.38 (1.38 – 1.38)	0.212	4.65 (1.38 – 5.66)	1.38 (1.38 – 3.83)	0.001

Table 36: Comparison of baseline and follow-up virological parameters in HIV-infected children treated for tuberculosis at RCWMCH

* There were 27 children with culture-confirmed tuberculosis in whom baseline and follow-up HIV viral load determinations were available: <60 months (n=14), 60-180 months (n=13); ART-naïve (n=20), ART-experienced (n=7).

[†]There were 64 children with 'other than culture-confirmed' tuberculosis in whom baseline and follow-up HIV viral load determinations were available: <60 months (n=50), 60-180 months (n=14); ART-naïve (n=39), ART-experienced (n=25).

[‡] P-values derived by comparison of median HIV viral load and percentages at baseline and follow-up determinations (using the Wilcoxon sign-rank test).

[§] Numbers in brackets are interquartile ranges.

** ART-naïve reflects children who were not on ART at tuberculosis diagnosis, ART-experienced reflects children who were on ART at time of tuberculosis diagnosis.

available; these included 42 children who were treated with PI-based regimens, and 35 who were treated with NNRTI-based regimens. Median time on ART did not differ significantly between the ART regimen groups, P=0.616. Median baseline HIV viral load was 665 000 RNA copies per millilitre (IQR 24 000 – 2 900 000), and < 25 RNA copies per millilitre (IQR 25 – 5 600) at follow-up in children treated with PI-based therapy, P<0.001. In children treated with NNRTI-based regimens, baseline median HIV viral load was 58 000 RNA copies per millilitre (IQR 1 600 – 700 000) and < 25 RNA copies per millilitre (IQR 25 – 25) at follow-up, P<0.001. Baseline (P=0.009) and follow-up (P=0.005) viral load determinations were significantly higher in children treated with PI-based ART compared to those treated with NNRTI-based ART. Similar trends were observed in the analysis of HIV viral load $\log_{10}$ reposes to ART (**Figure 11**).

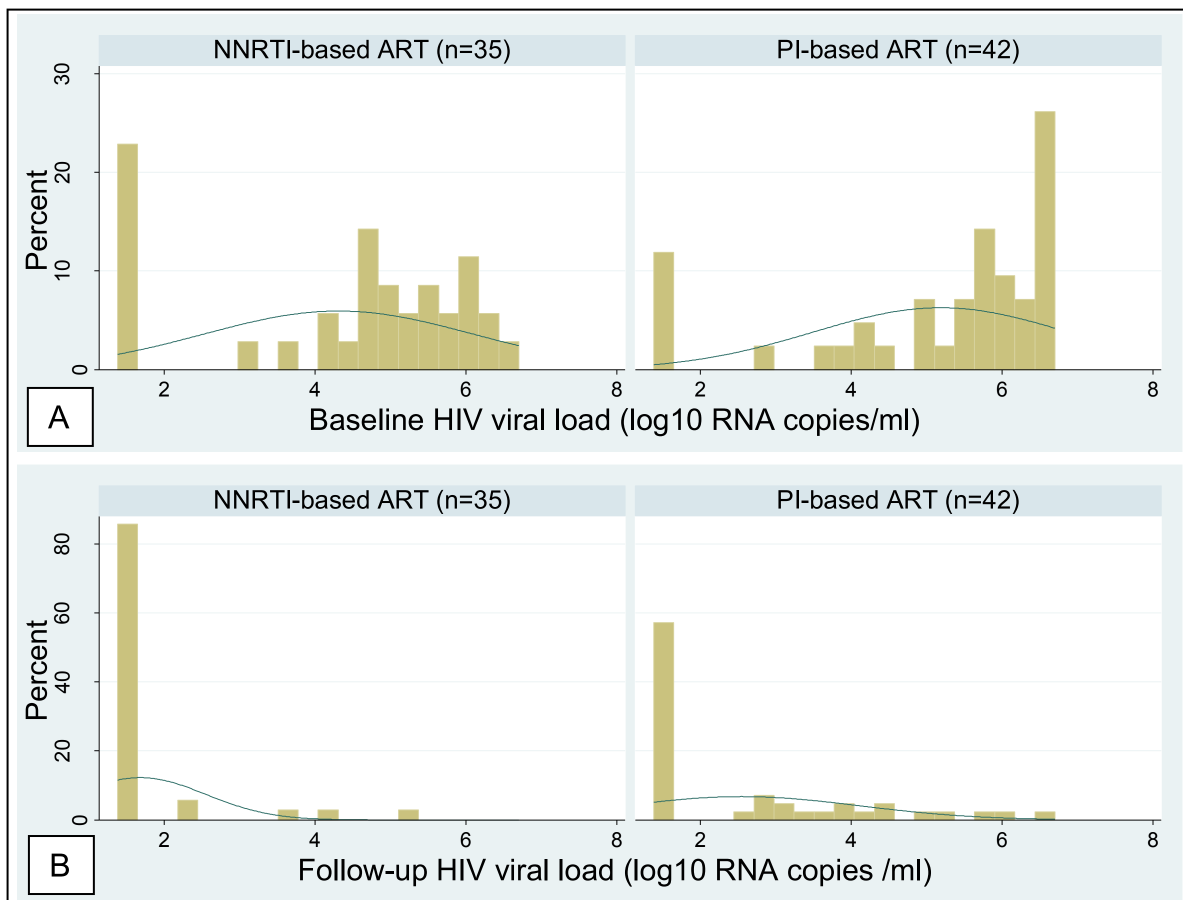

Figure 11: Baseline [A] and follow-up [B] HIV viral load ($\log_{10}$ copies/ml) stratified by ART regimen in children who had been established on ART either before or during antituberculosis treatment

Using an HIV viral load of ≥ 400 RNA copies per millilitre at follow-up viral load determination as the definition of 'virologic failure' in children who had been established on ART prior to the diagnosis of tuberculosis, (71) children who had been established on PI-based ART prior to tuberculosis diagnosis (8 [42.1%] of 19 children) tended to exhibit more episodes of virologic failure at the follow-up viral load determination than did those who were treated with NNRTI-based regimens (three [20.0%] of 15 children), OR 2.91 (95% CI, 0.50 – 20.78), P=0.271. Mean time interval between follow-up viral load determination and ART initiation in those who exhibited virologic failure was 31.9 months (SD 24.5), and did not differ between the ART regimen groups, P=0.358.

A less robust virologic response to ART according to ART regimen was also observed in children who were initiated on PI-based ART during the course of antituberculosis therapy, compared to those who were initiated on NNRTI-based ART: 10 (43.5%) of 23 children who were initiated on PI-based regimens had follow-up viral loads ≥ 400 RNA copies per millilitre, whereas there were no instances of virologic non-suppression in the 20 children who were initiated on NNRTI-containing regimens during the course of antituberculosis treatment, P=0.001. Median time interval between viral load determination and date of initiation of ART in these children was 6.5 months (IQR 6.0 – 8.0), and did not differ between ART regimen groups, P=0.884.

Combining the analyses of children who were started on ART prior to diagnosis with tuberculosis and those who started ART during the course of antituberculosis therapy, 18 (42.9%) of 42 children on PI-based ART exhibited virologic failure (defined as follow-up viral load of ≥ 400 RNA copies per millilitre), compared to three (8.6%) of 35 children treated with NNRTI-based ART (OR 8.00; 95% CI, 1.95 – 45.99), P=0.001. Median time period between ART initiation and follow-up viral load testing in the 21

children who exhibited virologic failure was 9.1 months (IQR 6.7 – 36.2), and did not differ in the comparison between ART regimen groups, P=0.315.

Using a Cox proportional hazards model to determine the risk of virologic failure amongst children who were initiated on ART either before or during antituberculosis treatment, and to control for the possible confounding effects of timing of ART initiation, age and degree of certainty of the tuberculosis diagnosis, it was possible to demonstrate that children who were initiated on ART regimens during antituberculosis treatment, and those treated with PI-based regimens tended to exhibit virologic failure (defined as being ≥ 400 HIV RNA copies per millilitre at the follow-up viral load determination) at follow-up virologic assessment more frequently than those started on ART before tuberculosis diagnosis or those treated with NNRTI-based ART (**Table 37**).

	Hazard Ratio (95% CI)	**P-values**	**Adjusted Hazard Ratio (95% CI)**	**P-values**
Start ART during antituberculosis treatment versus start ART before antituberculosis treatment	5.00 (1.65 – 15.10)	0.004	6.46 (1.95 – 21.34)	0.002
PI-based regimen versus NNRTI-based regimen	10.09 (2.32 – 43.92)	0.002	8.20 (1.12 – 59.88)	0.038
Age <60 months versus age ≥60 months*	7.63 (1.71 – 33.98)	0.008	1.20 (0.14 – 10.56)	0.867
'Other than culture-confirmed' versus culture-confirmed tuberculosis	3.01 (0.96 – 9.41)	0.058	1.84 (0.53 – 6.42)	0.341

Table 37: Cox proportional hazards model of virologic failure over time in children treated for tuberculosis[†]

* Three (14.3%) of the 21 children who exhibited virologic failure were older than 60 months of age at investigation for tuberculosis.

[†] Time to virologic failure (defined as being ≥ 400 HIV RNA copies/ml at follow-up viral load in children who were started on ART either prior to, or during antituberculosis therapy), was derived by calculating the difference between the date on which the follow-up viral load determination was conducted from the date of initiation of ART.

In the Cox proportional hazards ratio model which excluded the effect of age (as children on PI-based regimens are generally under three years of age or under 10 kilograms in weight, indicating an inherent bias for younger children to be treated with PI-based regimens), and controlling for degree of certainty of the tuberculosis diagnosis and timing of ART in relation to antituberculosis treatment, virologic failure was significantly more likely in the children on PI-based ART compared to those on NNRTI-based therapy (adjusted Hazard Ratio [HR] 9.20; 95% CI, 2.07 – 40.98), P=0.004.

Using a similar Cox proportional hazards model (with exclusion of age for the reasons stated above), and using a follow-up HIV viral load cut-off of ≥ 1 000 RNA copies per millilitre as the definition of virologic failure, children treated with PI-based ART were significantly more likely to exhibit virologic failure (14 [33.3%] of 42 exhibited virologic failure) compared to children treated with NNRTI-based regimens (three [8.6%] of 35 exhibited virologic failure), adjusted HR 7.70 (95% CI, 1.65 – 35.95), P=0.009.

ART regimens used during the course of antituberculosis treatment in children who exhibited a poor virologic response to therapy were recorded in all 17 children whose follow-up HIV viral load was ≥ 1 000 RNA copies per millilitre. Regimens used in children under five years of age (n=14) included: stavudine, lamivudine and lopinavir/ritonavir with added ritonavir (n=4); stavudine, lamivudine and lopinavir/ritonavir (n=4); zidovudine, lamivudine, lopinavir/ritonavir with added ritonavir (n=2); zidovudine, lamivudine and ritonavir (n=2), and zidovudine, didanosine and efavirenz (n=2). The three children over five years of age at clinical evaluation for tuberculosis who were failing ART (defined as a follow-up viral load of ≥ 1 000 RNA copies per millilitre) were treated with stavudine, lamivudine and efavirenz (n=1), zidovudine, didanosine and efavirenz (n=1), and stavudine, lamivudine and lopinavir/ritonavir with added ritonavir (n=1).

3.11.8 Possible IRIS events in HIV-infected children who commenced ART prior to TB diagnosis

Twenty-four (38.1%) of the 63* tuberculosis episodes in children who had been started on ART before tuberculosis was diagnosed occurred within six months of ART initiation, and 13 (54.2%) of these 24 episodes occurred within three months of ART initiation. Onset of tuberculosis within three months of ART initiation was considered to represent possible tuberculosis IRIS in these children. Three (23.1%) of the 13 possible IRIS episodes were culture-confirmed and 10 (76.9%) were 'other than culture-confirmed' tuberculosis events.

The only child who presented with possible unmasking tuberculosis IRIS during the course or a recurrent tuberculosis episode had discontinued antituberculosis treatment through non-adherence to therapy for a previous episode of drug-susceptible pulmonary tuberculosis. This child was readmitted 4.9 months later with dual pulmonary and extrapulmonary tuberculosis, confirmed to be caused by multidrug-resistant *M. tuberculosis*. Antiretroviral therapy was commenced during the admission episode during which the drug-resistant isolate was obtained, 36 days prior to submission of the pus swab from which *M. tuberculosis* was cultured, and 45 days prior to commencement of antituberculosis therapy.

Ten (76.9%) of the 13 possible tuberculosis IRIS events occurred in children under five years of age. From **Table 34**, ART was commenced prior to tuberculosis diagnosis in 42 episodes of tuberculosis in children under five years of age, compared to 21 episodes in children who were older than five years of age at tuberculosis diagnosis;

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* Children who started ART prior to antituberculosis therapy included 62 children who had initiated ART prior to admission for the illness during which tuberculosis was investigated for, as well as one child with culture-confirmed tuberculosis whose ART was initiated during the course of the admission episode in which tuberculosis was investigated for, but whose specimen for mycobacterial culture, and antituberculosis therapy was commenced subsequent to ART initiation (**Table 34**).

hence, 10 (23.8%) of 42 children under five years of age at tuberculosis developed possible tuberculosis IRIS compared to three (14.3%) of 21 children over five years of age (OR 1.88; 95% CI, 0.40 – 11.85), P=0.516. Mean time to tuberculosis diagnosis after ART initiation in the 13 possible tuberculosis IRIS episodes was 29 days (SD 23): 31 days (SD 24) in episodes occurring in children under five years of age (n=10) at ART initiation compared to 23 days (SD 23) in episodes occurring in children older than five (n=3) at ART initiation, P=0.499.

Table 38 explores the baseline and follow-up CD4 T-lymphocyte and virologic responses in children with (n=7) and without (n=27) possible IRIS in whom baseline and follow-up viral load and CD4 T-lymphocyte parameters were available. Children with possible tuberculosis IRIS had significantly lower baseline CD4 T-lymphocyte counts but exhibited an equally robust CD4 T-lymphocyte response to ART compared to those that did not have possible IRIS. A less robust virologic response to ART was demonstrated in children with possible IRIS (**Table 38**). There was no significant difference in interval between immunologic and virologic testing according to strength of possibility of IRIS occurrence: 7.8 months (IQR 7.4 – 10.4) in children with possible IRIS* compared to 9.4 months (IQR 6.3 – 13.9) in those that did not have IRIS, P=0.844.

Nineteen (79.2%) of the 24 children who developed tuberculosis within six months of initiation of ART had available weight-for-age z-scores; those with possible IRIS (n=3) had significantly lower mean weight-for-age z-scores (-3.46, SD 0.07) compared to those who were not considered to have IRIS (n=16; -1.37, SD 1.04), P=0.003.

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* Children considered as having possible tuberculosis IRIS had been initiated on ART before diagnosis of tuberculosis, and were diagnosed with tuberculosis within three months of initiation of ART; those who were diagnosed with tuberculosis after three months on ART were considered not to be tuberculosis IRIS cases.

	CD4 T-lymphocyte counts			CD4 T-lymphocyte percentages			HIV viral load values			HIV $\log_{10}$ viral load values		
	× 10^6/l (IQR)		P-values*	% (SD)		P-values*	RNA copies per millilitre (IQR)		P-values*	$\log_{10}$ copies per millilitre (IQR)		P-values*
	Baseline	Follow-up		Baseline	Follow-up		Baseline	Follow-up		Baseline	Follow-up	
Possible IRIS (n=7)	285 (114 – 462)	1 189 (844 – 1 258)	0.018	17.2 ± 12.1	21.6 ± 8.9	0.545	700 000 (3 600 – 3 000 000)	5 100 (25 – 70 000)	0.102	5.85 (3.56 – 6.70)	3.71 (1.38 – 4.85)	0.102
Not IRIS (n=27)	846 (508 – 1 194)	1 112 (734 – 1 539)	0.005	21.1 ± 9.8	25.0 ± 10.1	0.007	9 700 (25 – 70 000)	25 (25 – 1 600)	0.003	3.99 (1.38 – 5.04)	1.38 (1.38 – 3.20)	0.001
P-values†	0.025	0.915	-	0.375	0.423	-	0.031	0.020	-	0.024	0.020	-

Table 38: Immunologic and virologic responses to ART in children with and without possible IRIS

IQR = interquartile range; IRIS = immune reconstitution inflammatory syndrome; SD = standard deviation.
* P-values are derived using the Wilcoxon sign-rank test by comparison of the baseline and follow-up median CD4 T-lymphocyte counts, HIV viral load (RNA copies per millilitre) and HIV viral load $\log_{10}$ values within IRIS categories, in children with and without possible IRIS. The Paired *t* test was used to compare baseline and follow-up mean CD4 T-lymphocyte percentages.
† P-values are derived using the Wicoxon sign-rank test by comparison of the baseline and follow-up median CD4 T-lymphocyte counts, HIV viral load (RNA copies per millilitre) and HIV viral load $\log_{10}$ values across IRIS categories, in children with and without possible IRIS. The Student *t* test was used to compare mean CD4 percentages.

3.12 Deaths In Tuberculosis Cases And Tuberculosis-attributable Deaths

Forty-five (3.5%) of the 1 300 children ever diagnosed with tuberculosis died, of which 22 (48.9%) had culture-confirmed tuberculosis and 23 (51.1%) had 'other than culture-confirmed' tuberculosis. Nineteen (42.2%) of those that died were HIV-infected, 16 (35.6%) were HIV-uninfected and 10 (22.2%) had undefined HIV status. Case fatality rates for tuberculosis were explored only in children who were considered to be on antituberculosis treatment at the time of death (n=34), which were defined as being tuberculosis-attributable deaths; therefore, 34 (75.6%) of the 45 deaths which occurred amongst children diagnosed with tuberculosis in the cohort were tuberculosis-attributable.

Nineteen (55.9%) tuberculosis-attributable deaths occurred in children with pulmonary tuberculosis (two of these children had concomitant extrapulmonary disease), and 15 (44.1%) occurred in children with miliary tuberculosis or tuberculous meningitis; 30 (88.2%) of the 34 tuberculosis-attributable deaths occurred in children who had been notified for tuberculosis from the general medical wards at the hospital.

Median time to death in the children with pulmonary tuberculosis was 1.6 month (IQR 0.2 – 2.6) after the date of admission for the illness in which tuberculosis was identified, with no statistically significant difference in time to death according to HIV infection status, P=0.374. Median time to death in those with miliary tuberculosis or tuberculous meningitis was 0.5 month (IQR 0.3 – 2.1) after the date of admission in which these diagnoses were made; similarly, there was no significant difference in time to death in miliary tuberculosis or tuberculous meningitis patients according to HIV infection status, P=0.061.

The two children who died with extrapulmonary tuberculosis associated with pulmonary disease, died at 0.5 month after admission (a child with undefined HIV infection status) and 5.2 months after admission (an HIV-infected child).

Fourteen (41.2%) of the 34 children who died during the course of antituberculosis therapy were HIV-infected, 10 (29.4%) were HIV-uninfected and 10 (29.4%) had undefined HIV infection status. HIV-infected children had significantly higher tuberculosis-attributable case fatality rates than did those with undefined HIV infection status (**Table 39**).

	HIV-infected	**HIV-uninfected**	**HIV status undefined**	**Total**
Number of tuberculosis-attributable deaths*	14	10	10	34
Number of tuberculosis episodes	271	414	629	1 314
Tuberculosis-attributable case fatality rate (%)	5.2	2.4	1.6	2.6

	Comparison of proportions	
	Odds Ratio (95% CI)	**P-values**†
HIV-infected compared to -uninfected	2.20 (0.89 – 5.62)	0.056
HIV-infected compared to HIV status undefined	3.37 (1.37 – 8.59)	0.002
HIV-uninfected compared to HIV status undefined	1.53 (0.57 – 4.14)	0.342

Table 39: Comparison of tuberculosis-attributable case fatality rates according to HIV infection status

Median time to tuberculosis-attributable death was 1.0 month (IQR 0.3 – 2.6) after hospital admission for the illness in which tuberculosis was identified, with no statistically significant difference in time to death between children with culture-

* Tuberculosis-attributable deaths were assigned in children who died during the course of antituberculosis therapy.

† P-value compares proportions of children dying from tuberculosis, according HIV status.

confirmed tuberculosis (0.4 month [IQR 0.2 – 1.6]) and those with 'other than culture-confirmed' tuberculosis (1.9 month [IQR 0.4 – 2.7]), P=0.190. Children with undefined HIV status died significantly sooner after tuberculosis diagnosis (0.4 month [IQR 0.2 – 0.5]) compared to those with defined HIV status (1.9 month [IQR 0.3 – 2.9]), P=0.031.

3.12.1 Tuberculosis-attributable deaths in children with culture-confirmed tuberculosis

Twenty-two (5.1%) of the 433 children with culture-confirmed tuberculosis died, and 18 (81.8%: HIV-infected [n=7]; HIV-uninfected [n=6]; undefined HIV infection status [n=5]) of these deaths occurred during the course of antituberculosis therapy and were thus considered to be tuberculosis-attributable deaths.

The tuberculosis-attributable deaths in children with culture-confirmed disease occurred at a median of 0.4 month (IQR 0.2 – 1.6) after admission to RCWMCH; there was no significant difference in median time to death after hospital admission according to HIV infection status, P=0.262. Median age at death in these children was 22.8 months (IQR 13.9 – 50.8), with no significant difference in age at death between HIV infection status groups, P=0.688.

HIV infection status did not impact on tuberculosis-attributable case fatality rates in the analysis of culture-confirmed tuberculosis cases, whether drug-susceptible or -resistant isolates were isolated (data not shown).

Five (27.8%) of the tuberculosis-attributable deaths in children with culture-confirmed tuberculosis were associated with drug-resistant isolates: one had isoniazid mono-resistant tuberculosis and four had multidrug-resistant tuberculosis. The tuberculosis-attributable case fatality rate in multidrug-resistant tuberculosis cases (four deaths out

of a total of 18 identified multidrug-resistant isolates identified in the cohort, 22.2%) was significantly higher than the tuberculosis-attributable case fatality rate for children with disease caused by susceptible isolates (12 deaths in 361 tuberculosis episodes in which susceptible isolates were identified, 3.3%), RR 6.69 (95% CI, 2.39 – 18.69), P=0.005.

3.12.2 Tuberculosis-attributable deaths in HIV-infected children

Timing of ART in relation to the diagnosis of tuberculosis, culture-confirmation of tuberculosis, and possible tuberculosis IRIS generally did not impact significantly on tuberculosis-attributable case fatality rates amongst HIV-infected children; however, culture-confirmed possible tuberculosis IRIS (tuberculosis occurring within three months of ART initiation) was associated with appreciably higher tuberculosis-attributable mortality than did culture-confirmed disease in children who developed tuberculosis beyond three months after ART initiation, P=0.018 (**Table 40**).

3.12.3 Multivariate analysis of factors that were associated with death in children with tuberculosis

Multiple logistic regression techniques were applied in order to explore the factors which were associated with death in children investigated and treated for tuberculosis at RCWMCH; low weight-for-age z-score and drug resistant tuberculosis were significantly associated with an increased adjusted odds of death (**Table 41**).

3.12.4 Deaths in children notified for conditions other than tuberculosis

Twelve (2.4%) of the 435 children who were notified for diseases other than tuberculosis, died; there was no significant difference in time to death after admission to RCWMCH in the comparison between children who died from notifiable conditions

Patient Category		Number of deaths	Total number of tuberculosis episodes (n=271)	Tuberculosis-attributable case fatality rate (%)[*]	Odds Ratio (95% CI)	P-values[†]
All HIV-infected children with tuberculosis	Ever started ART	10	167	6.0	1.59 (0.44 – 7.13)	0.577[‡]
	Never started ART	4	104	3.8		
HIV-infected children with culture-confirmed tuberculosis	Ever started ART	5	60	8.3	1.45 (0.22 – 16.05)	1.000[‡]
	Never started ART	2	34	5.9		
HIV-infected children on ART	ART started before antituberculosis therapy	5	63	7.9	1.41 (0.31 – 6.43)	0.743[‡]
	ART started during antituberculosis therapy[§]	5	87	5.7		
	ART started before or during antituberculosis therapy[§]	10	150	6.7	-	1.000[‡]
	ART started after completion of antituberculosis therapy	0	14	0.0		
HIV-infected children with culture-confirmed tuberculosis and on ART	ART started before antituberculosis therapy	2	19	10.5	1.14 (0.09 – 10.97)	1.000[‡]
	ART started during antituberculosis therapy[§]	3	32	9.4		
	ART started before or during antituberculosis therapy[§]	5	51	9.8	-	1.000[‡]
	ART started after completion of antituberculosis therapy	0	6	0.0		
HIV-infected children who started ART before antituberculosis therapy	Possible tuberculosis IRIS[**]	3	13	23.1	7.20 (0.70 – 92.51)	0.055[‡]
	Not tuberculosis IRIS	2	50	4.0		
HIV-infected children with culture-confirmed tuberculosis that started ART before antituberculosis therapy	Possible tuberculosis IRIS	2	3	66.7	-	0.018[‡]
	Not tuberculosis IRIS	0	16	0.0		

Table 40: Tuberculosis-attributable case fatality rates in HIV-infected children, according to antiretroviral therapy (ART) experience and timing of initiation of ART in relation to tuberculosis episode

* Tuberculosis-attributable deaths were assigned in children who died during the course of antituberculosis treatment.
[†] P-values are derived by comparison of case fatality rates according to each patient category.
[‡] Fisher's exact test.
[§] Three children with culture-confirmed tuberculosis who commenced ART at down-referral facilities, in whom timing of ART initiation (during or after antituberculosis therapy) was uncertain, are excluded from this analysis.
** IRIS = immune reconstitution inflammatory syndrome.

		Univariate Analysis		Multivariate Analysis*	
		Odds Ratio (95% CI)	P-values	Adjusted Odds Ratio (95% CI)	P-values
Year of study	2007 versus 2006	5.62 (1.28 – 24.74)	0.022	4.46 (0.52 – 38.18)	0.173
	2008 versus 2006	6.43 (1.47 – 28.00)	0.013	4.46 (0.52 – 38.41)	0.173
Age (months)		1.00 (0.99 – 1.01)	0.608	-	-
Age group < 60 months versus 60–180 months		1.12 (0.52 – 2.42)	0.778	-	-
Weight-for-age z-score		0.58 (0.41 – 0.83)	0.002	0.54 (0.37 – 0.79)†	0.001
Duration of illness		0.99 (0.95 – 1.02)	0.402	-	-
Surgical versus medical care		0.49 (0.07 – 3.65)	0.488	-	-
History of TB contact		2.46 (0.80 – 7.62)	0.118	2.39 (0.73 – 7.78)	0.148
HIV result known versus HIV status undefined		2.29 (1.08 – 4.82)	0.030	0.89 (0.28 – 2.79)	0.836
Positive score chart		0.68 (0.26 – 1.74)	0.420	-	-
ART naïve versus ART experienced at tuberculosis diagnosis		0.50 (0.16 – 1.56)	0.234	-	-
ART started during antituberculosis treatment versus ART started before antituberculosis treatment		0.90 (0.25 – 3.24)	0.872	-	-
Ever commenced ART versus never commenced ART		1.64 (0.50 – 5.39)	0.234	-	-
Drug-resistant tuberculosis		2.99 (1.01 – 8.87)	0.048	4.25 (1.31 – 13.87)	0.016

Table 41: Multiple logistic regression analysis of factors which influenced mortality in children investigated for tuberculosis at RCWHCH

* The multivariate analysis utilised only the parameters which had achieved P-values of ≤ 0.20 in the univariate analyses.

† The coefficient attributable to each 'unit decrease' in weight-for-age z-score was 0.62 (95% CI, 0.24 – 0.99) in the adjusted analysis.

other than tuberculosis (0.3 month [IQR 0.0 – 1.4]) and children who had tuberculosis-attributable death (1.0 month [IQR 0.3 – 2.6]), P=0.118.

Only six of the 12 children who died of notifiable disease processes other than tuberculosis had defined HIV infection status: these included two HIV-infected children and four HIV-uninfected children. The HIV-infected children died of *M. bovis* BCG disease and varicella pneumonitis, respectively, and the HIV-uninfected children each died of disseminated *M. bovis* BCG disease (n=1: a child with presumed primary immunodeficiency), congenital syphilis (n=1) and unspecified notifiable conditions (n=2). The six children who died of notifiable conditions other than tuberculosis and who had no retrievable HIV result died of diphtheria (n=1), hepatitis A (n=1), measles (n=1), and unspecified notifiable conditions (n=3).

Case fatality rates (2.4%) for notifiable diseases other than tuberculosis did not differ significantly from the tuberculosis-attributable case fatality rate (2.6%, **Table 39**) observed in children with tuberculosis (OR 1.07; 95% CI, 0.50 – 2.14), P=0.847.

3.13 Length Of Hospitalisation In Children With Culture-confirmed Tuberculosis

Information regarding the length of hospitalisation was obtainable in 412 (96.3%) of the 428 culture-confirmed tuberculosis episodes for which folder reviews were conducted. HIV-infected children (n=91) and those who were HIV-uninfected (n=149) had significantly longer durations of hospitalisation (6 days [IQR 1 – 19] and 7 days [IQR 2 – 14], respectively) compared to children with undefined HIV infection status (n=172: 2 days [IQR 1 – 4]), P<0.001.

Children who were managed in the outpatient setting (n=222) had significantly shorter median duration of admission (2 days [IQR 1 – 3]) compared to those who were managed as inpatients (n=168: 10 days [IQR 5 – 23]), P<0.001.

3.14 Proportion Of Cape Town Child Tuberculosis Identified Through RCWMCH

The proportions of Cape Town's child tuberculosis cases managed at RCWMCH in each year during the study period are tabulated below (**Table 42**).

Year of Study	Age < 60 months		Age 60 – 180 months		All Children	
	City of Cape Town (N)*	RCWHCH (n [%]; 95% CI)	City of Cape Town (N)*	RCWHCH (n [%]; 95% CI)	City of Cape Town (N)*	RCWHCH (n [%]; 95% CI)
2006	2 444	241 (9.9; 8.7 – 11.2)	1 186	111 (9.4; 7.7 – 11.3)	3 630	352 (9.7; 8.7 – 10.8)
2007	2 486	349 (14.0; 12.6 – 15.6)	1 080	132 (12.2; 10.2 – 14.5)	3 566	481 (13.5; 12.3 – 14.7)
2008	2 829	350 (12.4; 11.1 – 13.7)	1 120	131 (11.7; 9.8 – 13.9)	3 949	481 (12.2; 11.1 – 13.3)
2006 to 2008	7 759	940 (12.1; 11.4 – 12.9)	3 386	374 (11.0; 10.0 – 12.2)	11 145	1 314 (11.8; 11.2 – 12.4)

Table 42: Proportion of Cape Town child tuberculosis managed at RCWMCH during the study period

* Denominators (i.e. total child tuberculosis cases registered in the City of Cape Town Health Department tuberculosis register [15]) against which RCWMCH tuberculosis cases are compared are derived from **Table 2**.

4.0 DISCUSSION

4.1 General Comments

This observational study, designed to explore aspects of tuberculosis case management at an academic children's hospital in Cape Town, South Africa, highlights a substantial case load of tuberculosis amongst children managed by clinicians working at the facility. One thousand, three hundred and fourteen episodes of tuberculosis were identified in 1 300 children treated at RCWMCH between 01 January 2006 and 31 December 2008, and clinicians at the hospital diagnosed and managed 12% of the Cape Town Metropole's child tuberculosis case load over the study period (**Table 42**). Tuberculosis was also demonstrated to contribute to the majority (74.0%) of notified illness at the facility (**Table 3**).

That three-quarters of the child tuberculosis episodes in which place of residence data were available arose from high-density, lower socioeconomic status residential areas in the Cape Town Metropole reinforces the fact that tuberculosis is a disease of social inequity, (74, 75) and that measures to improve the quality of life of South Africans are crucial to the alleviation of disease burden in our communities.

4.2 Untreated Culture-confirmed Tuberculosis Episodes

Barriers to effective case reporting appear to exist even in this setting with a well-established laboratory-clinical communication framework which attempts to ensure that children with culture-confirmed tuberculosis access appropriate treatment. It is concerning that 120 (27.7%) of the 433 culture-confirmed tuberculosis episodes were not registered in

the hospital's paper-based Notifications Register (**Figure 1**); 30 (25.0%) of these children were not started on antituberculosis therapy.

In this cohort, the 30 children with culture-confirmed tuberculosis who were not notified, and who appear through folder review not to have been initiated on appropriate antituberculosis therapy, represent the minority (7.1%) of the 421 episodes in which data relating to type of antituberculosis treatment used was available by folder review. This rate of non-initiation of specific therapy in children with culture-confirmed tuberculosis compares favourably with those (17 – 49%) observed at other public hospitals in South Africa, (27, 53, 60, 63, 76) and probably relates to the fact that an established laboratory-clinical feedback mechanism has been established and utilised at RCWMCH since 2007; however, any omission of appropriate therapy in children with culture-confirmed tuberculosis represents a severe breakdown in case management, and strategies to further minimise untreated cases need urgently to be explored.

4.3 Comparison Of Numbers Of Children With Culture-confirmed Tuberculosis At RCWMCH (2006 – 2008) To The Culture-confirmed Cohort Described In Cape Town (2003 – 2005)

Through the approach of describing all child tuberculosis diagnosed at RCWMCH from 01 January 2006 to 31 December 2008, our analysis builds on that of an extensive prospective study which describes the characteristics of children with culture-confirmed tuberculosis who were managed at RCWMCH and Tygerberg Children's Hospital (TCH) in Cape Town between 01 March 2003 and 28 February 2005. (58) It is, therefore, interesting to draw comparisons between the 433 culture-confirmed child tuberculosis episodes

identified in this analysis with the 596 culture-confirmed cases described in the former study.

In the study conducted by Schaaf et al. (58) 596 children with culture-confirmed tuberculosis were identified, 277 (46.5%) of whom were managed at RCWMCH. These numbers reflect approximately 139 children with culture-confirmed disease per year (in 2003/4 and 2004/5) who were managed at RCWMCH, compared to 95, 167 and 163 children with culture-confirmed tuberculosis who were treated at RCWMCH in 2006, 2007 and 2008, respectively in the cohort of children described in our analysis.

The substantially depleted case-load of culture-confirmed tuberculosis identified at RCWMCH in 2006 (n=95), as described in our study, likely represents a spurious under-identification of cases which arose because of the different case-finding strategies adopted for 2006 and 2007/8 in this study: the method by which tuberculosis cases were identified for 2006 was by review of the paper-based Notifications Register only, whereas the 2007 and 2008 cohorts were derived by review of the paper-based Notifications Register in addition to the Culture-confirmed Tuberculosis Database which relied on active and regular feedback from the GSH microbiology laboratory. When considering that 28% of all episodes of culture-confirmed tuberculosis were not recorded in the paper-based Notifications Register, as mentioned above, the paper-based Notifications Register-derived case-load of 95 culture-confirmed tuberculosis cases in 2006 may have been underestimated by 28%; hence, a derived expected number of culture-confirmed cases managed at RCWMCH in 2006 could have been 131 culture-confirmed tuberculosis cases, similar to the case load of culture-confirmed cases observed at RCWMCH in 2003/4 and 2004/5.

Schaaf et al. (58) describe that 31% of children identified with culture-confirmed tuberculosis at RCWMCH and TCH had undefined HIV infection status in 2003 – 2005, compared to 41% (176 of 425 children who ever presented with culture-confirmed tuberculosis, **Figure 1**) in the cohort of culture-confirmed tuberculosis cases described in this dissertation.

As the decision to test suspected cases of child tuberculosis for HIV infection depended on clinicians' discretion in both studies, it is difficult to explain the reduction in completeness of HIV work-up in the latter cohort; this omission may possibly reflect a lack of awareness amongst clinicians of the impact which HIV infection has on child tuberculosis, as previous studies conducted in the Western Cape have observed that 20-30% of children with a hospital-based diagnosis of culture-confirmed tuberculosis are HIV co-infected, (26, 58, 63) which may appear to be less of a burden compared to the 45-70% HIV-infection prevalence in hospitalised culture-confirmed tuberculosis paediatric cohorts described from Gauteng and KwaZulu-Natal. (27, 53)

Whereas 133 (32.1%) of all children whose HIV infection status was determined in the former cohort (n=414) were confirmed to be HIV-infected; (58) 90 (36.1%) of those with culture-confirmed tuberculosis whose HIV infection status was determined (n=249) were HIV-infected at RCWMCH from 01 January 2006 to 31 December 2008, P=0.289 (or 264 [39.3%] of the 671 children in the cohort who were tested for their HIV infection status, regardless of the strength of tuberculosis diagnosis, P=0.016 in the comparison of the proportion of HIV co-infected cases reported by Schaaf et al. (58)).

Increasing antenatal HIV seroprevalence in Cape Town over the past decade (from 8.7% in 2000 to 15.1% in 2006, with a peak prevalence of 16.1% in 2008), (77, 78) despite

improved uptake of prevention of mother-to-child transmission (PMTCT) of HIV strategies in the Western Cape Province from 2003 – 2008 (79) with the utilisation of more effective PMTCT regimens, (26, 80-82) may explain the higher proportion of HIV-infected children amongst those whose HIV infection status had been determined in our cohort.

It is anticipated that, as the HIV epidemic in the Western Cape reaches maturity over the next few years and the antenatal seroprevalence rates stabilise, coupled with improved uptake of PMTCT and better PMTCT regimens, (83) fewer HIV-infected babies will be born to HIV-infected mothers. Additionally, with the current thrust to implement ART in early infancy, as soon as HIV infection has been confirmed, (84, 85) it is anticipated that few HIV-infected children will develop profound HIV-associated immunodeficiency. Both of these effects – a diminishing population of HIV-infected children, and better immunological outcomes in those who are HIV-infected – would be anticipated to impact favourably on child tuberculosis incidence rates in the future.

4.4 HIV Test Coverage In Children With Tuberculosis At RCWMCH

As HIV infection has been demonstrated to be the most potent risk factor for the progression to active tuberculosis in individuals latently infected with *M. tuberculosis*, (24, 25) the WHO recommends that children undergoing evaluation for possible tuberculosis who reside in highly HIV and tuberculosis endemic regions should be screened for co-infection with HIV. (86) The lower proportion of children with defined HIV infection status in the RCWMCH cohort compared to the cohort described by Schaaf et al. (58) is concerning, as children with undiagnosed HIV infection represent missed opportunities for treatment using co-trimoxazole preventive therapy and ART.

This study has demonstrated a significant improvement in the proportions of children with tuberculosis who were screened for concomitant HIV infection over the time period 2006 – 2008 (**Figure 4**), possibly in response to a greater awareness of the importance of testing as a result of the publication, in 2006 and 2007, of the WHO Child Tuberculosis Guidance document. (65, 68, 86, 87)

In a multivariate analysis conducted on our cohort, management of children with tuberculosis in the outpatient setting or by the surgical disciplines was associated with appreciably lower adjusted odds of being tested for HIV; conversely, those who were found to have culture-confirmed tuberculosis, and who were therefore likely to be more ill at clinical evaluation, were 1.4-fold (95% CI, 1.08 – 1.83) more likely to have been tested for HIV infection (**Table 6**).

Missed opportunities for HIV testing in children managed in the outpatient and surgical settings at RCWMCH highlight the need for up-scaling of HIV-tuberculosis integration strategies at the facility.

As the majority of children identified with tuberculosis in this cohort were managed in the outpatient setting (**Figure 3**), strategies to expedite the acquisition of HIV results in children who are transiently evaluated in outpatient settings and whose HIV exposure status is unknown should be explored; this could be achieved by the use of rapid test kits, whereby evidence for HIV seropositivity can be obtained in a short time period (by definition, within 20 minutes), (88) minimising the lag time between testing and result retrieval when using ELISA techniques to identify seropositivity. Such an intervention would require considerable additional deployment of experienced counsellors and laboratory technologists, in order to ensure appropriate pre-and post-test counselling of

families (particularly in the sensitive scenario where parents are confronted with a positive screening result on their child), and to provide quality-assured performance of rapid tests and recording of results, respectively. Children found to be HIV seropositive through rapid testing could be streamlined to access same-day confirmatory testing according to age group, parental counselling and specific therapeutic interventions if confirmed to be HIV-infected, as appropriate. A streamlined testing algorithm using rapid tests has been operational at RCWMCH since October 2008.

Evidence obtained through this analysis suggests that children with tuberculosis who remained untested for their HIV infection status presented with less severe disease compared to those with defined HIV infection status. Children with undefined HIV infection status were less likely to have culture-confirmed tuberculosis (**Table 8**), presented with a shorter median duration of cough (**Table 11**), were significantly more likely to present with a history of acute cough (defined as being less than 10 days' duration, **Section 3.7.1**), presented with better nutritional status (**Section 3.7.3**), and had lower positive microscopy yields (possibly indicating a greater likelihood of paucibacilliary disease, **Section 3.8.1**), compared to children with defined HIV infection status. Additionally, children with undefined HIV infection status who had culture-confirmed tuberculosis had positive tuberculosis algorithm scores significantly less frequently compared to those with defined HIV infection status (**Table 24**).

Clinical and laboratory features indicating less severe disease may confound the diagnosis of tuberculosis, leading clinicians who are well-versed in the dogma that tuberculosis is a subacute or chronic disease which frequently presents with features of chronicity, not to consider the diagnosis in children presenting with more acute symptoms. If the diagnosis

of tuberculosis is not considered, there may be a perception that it is not necessary to test a child for HIV infection.

4.5 Pulmonary Tuberculosis Presenting With Acute Clinical Manifestations

An important finding of this study was that 86 (44.1%) of the 209 children with culture-confirmed pulmonary tuberculosis whose cough duration was recorded, presented with cough duration of less than 10 days. This proportion of culture-confirmed pulmonary tuberculosis cases who presented with acute symptoms is equivalent to those (42.3% and 43.0%, respectively) observed by Schaaf et al. (58) in Cape Town and Jeena et al. (53) in Durban, and similar to that (55.6%) observed in children who presented with illness that was subsequently identified as being due to culture-confirmed pulmonary tuberculosis during the course of a pneumococcal polysaccharide-protein conjugate vaccine trail in Soweto from 1998 – 2006. (D.P. Moore, unpublished data)

There is a growing appreciation that previous or concurrent infection with certain micro-organisms may influence the immunologic response to infections caused by other micro-organisms, through a process which is termed ‘heterologous immunity’. (89) Latent infection with *M. tuberculosis* may predispose children to infection with bacterial pathogens such as *S. pneumoniae* through such heterologous immune effects, which may explain a proportion of cases of culture-confirmed pulmonary tuberculosis that presented with short cough duration in these cohorts. (27)

Forty (54.8%) of 73 children with culture-confirmed pulmonary tuberculosis whose HIV infection status remained undefined presented with cough of less than 10 days’ duration,

compared to 16 (35.6%) of 45 HIV-infected children with culture-confirmed pulmonary tuberculosis (OR 2.20; 95% CI, 0.96 – 5.09), P=0.042 (**Table 12**).

The proportion of HIV-infected children with culture-confirmed pulmonary tuberculosis presenting with acute cough duration observed in our analysis corresponds with the proportions of HIV-infected children with culture-confirmed pulmonary tuberculosis presenting with short cough duration (7-37%) described at other sites. (51, 54, 57, 59) It is noteworthy that the study which observed the most similar proportion (36.6%) of HIV-infected children with culture-confirmed pulmonary tuberculosis who presented with short cough duration to that observed in our cohort (35.6%) was conducted in Cape Town, (54) which reinforces the impression that regional differences in access to health care, patterns of health care seeking and referral, and vulnerability to infection with co-pathogens may play a role in the clinical presentation of child tuberculosis. (90)

4.6 Tuberculosis Disease Classification Within The Cohort

A third (n=433) of all child tuberculosis cases treated at RCWMCH from 01 January 2006 to 31 December 2008 were culture-confirmed, and those between the ages of five and 15 years at investigation for tuberculosis were significantly more likely to have culture-confirmed disease than those who were under five years of age at presentation (**Table 7**). In the analysis only of cases in which specimens were submitted for microscopy and mycobacterial culture (n=1 053), 12% were microscopy-positive overall (**Table 25**) and 41% were culture-positive (**Table 27**). Crude microscopy yields improved (25%) when analysed amongst culture-confirmed episodes (n=433: **Table 26**), and time to culture-positivity was significantly shorter in children who had smear-positive specimens. These

microscopy and culture yields are commensurate with findings from other researchers with child tuberculosis experience. (22, 23)

Until recently, clinicians have been made aware of the prolonged time to mycobacterial culture positivity (up to eight weeks) after specimen submission in children with suspected tuberculosis. (63, 64) These delays reflect the expected time to positive culture of paucibacilliary specimens submitted from children, using solid-phase selective media such as Löwenstein-Jensen medium. (91) In our cohort, 70% of cases investigated with submission of specimens for mycobacterial culture, and which were subsequently classified as having culture-confirmed tuberculosis, were positive within three weeks of specimen submission. Clinicians working in settings where laboratories conduct mycobacterial cultures using liquid-phase methodologies should be made aware of the considerably shorter time to culture positivity of specimens, so that follow-up appointments can be scheduled sooner.

The high positive yields of tissue specimens (70.5%) submitted for mycobacterial culture (**Table 27**) and histology specimens (82.5%) amongst cases who were subsequently found to be culture-confirmed indicate that children presenting with extrapulmonary manifestations which are amenable to biopsy sampling should have specimens submitted for mycobacterial culture in addition to histology, where feasible. Fine-needle aspiration biopsy for mycobacterial culture is a valuable technique which has been shown to give high yields in well-selected patients, (92-94) and obviates the need for lymph node excision biopsy under general anaesthesia in critically ill patients. At present, fine-needle aspiration biopsy is underutilized at RCWMCH.

As has been demonstrated in other child tuberculosis studies, (23, 27, 35) HIV infection status did not impact on bacteriologic yield overall (**Tables 25, 26** and **27**); however, 38% of HIV-uninfected children less than five years of age were observed to have culture-confirmed disease compared to 26% of HIV-infected children (P=0.006) and 24% of children with undefined HIV infection status (P<0.001), **Table 8**.

HIV-uninfected children in this cohort had a younger median age at culture-confirmed tuberculosis diagnosis compared to those who were HIV-infected (**Figure 2**), which reflects the persistently elevated risk of tuberculosis disease in HIV-infected children beyond the vulnerable early years of life during which immunocompetent children are most at risk for disease progression. Data obtained in the pre-chemotherapeutic era established that 50% of immunocompetent children newly infected with *M. tuberculosis* under the age of one year developed disease compared to only 5% of those who were infected between the ages of two and five years; (21) diminishing risk of disease progression in older children is attributed to maturation of the cell-mediated immune response. (18) The 24- to 29-fold increased risk for active tuberculosis which HIV confers through disrupted cell-mediated immune surveillance, (26, 27) coupled with possible increased and on-going exposure to *M. tuberculosis* through contact with caregivers who may themselves have undiagnosed pulmonary tuberculosis, (95) may explain the older median age at tuberculosis diagnosis in HIV-infected children in this cohort.

The majority (78%) of tuberculosis episodes in our cohort were classified as being pulmonary in nature, 12% were extrapulmonary and 9% were miliary or tuberculous meningitis episodes. The proportion of extrapulmonary tuberculosis cases observed in our study corresponds to that reported in the Western Cape in 2006 and 2007, (14) although amongst culture-confirmed cases, extrapulmonary tuberculosis contributed to a greater

proportion (17.3%) of cases (**Table 28**). Older children had a 3-fold (95% CI, 1.80 – 5.34) greater likelihood of having culture-confirmed extrapulmonary tuberculosis compared to those under the age of five years, although there was no age predilection for miliary tuberculosis or tuberculous meningitis within the cohort.

Whereas Schaaf et al. (58) describe a lower prevalence of culture-confirmed miliary tuberculosis in HIV-infected children compared to HIV-uninfected children, in our study HIV-infected children were as likely as HIV-uninfected children or those with undefined HIV infection status to have been diagnosed with culture-confirmed miliary tuberculosis or tuberculous meningitis (**Table 28**), although in the analysis of all cases, regardless of the strength of evidence for tuberculosis, HIV-infected children were significantly less likely to be diagnosed with these forms of disseminated disease. These observations differ from descriptions of two child tuberculosis cohorts conducted in the pre-ART (35) and early access to ART (96) eras, respectively, which indicate that HIV-infected children are burdened with more miliary tuberculosis than are HIV-uninfected children.

These discrepancies may be explained by the fact that dedicated descriptions of chest radiographic changes in all children with tuberculosis were conducted by radiologists and/or child tuberculosis experts in the previous studies, (35, 58, 96) whereas the method of data collection in our analysis relied on disease classification as entered in the hospital paper-based Notifications Register, or clinicians' descriptions of chest radiographic changes obtained by folder review (for children with culture-confirmed disease), and may have missed some instances of miliary tuberculosis in the HIV-infected children. Alternatively, the observation made in our study that HIV-infected children did not have an increased risk of disseminated tuberculosis compared to HIV-uninfected children may represent a changing spectrum of HIV-related childhood tuberculosis in the ART era, in

which HIV-infected South African children who have more widespread and earlier access to ART may be at less risk for miliary tuberculosis and tuberculous meningitis than they were in the past. The beneficial impact which ART has in terms of alleviating the burden and incidence of tuberculosis in HIV-infected children is now well recognised, (97-100) and it is therefore feasible that the spectrum of severity of tuberculosis amongst HIV-infected children may have changed in comparison to the disease spectrum observed in HIV-infected children in the pre-ART era.

4.7 Mantoux Testing And Tuberculosis Score Chart Evaluations In The Cohort

A robust cell-mediated immune response is crucial in maintaining latency of tuberculosis infection by elaboration of the cytokine milieu which enables granuloma formation. (101) An indirect marker of cell-mediated immune functionality is the Mantoux test, which relies on a delayed-type hypersensitivity response of sensitised T-lymphocytes to a standard dose of inoculated tuberculin. (102) Although numbers of HIV-infected children in whom Mantoux tests were performed in this cohort were small, those with positive Mantoux responses had significantly higher CD4 T-lymphocyte counts than did those with negative Mantoux responses (**Figure 7A**); this correlation between immunologic and clinical factors has been well-recognised for more than a decade. (31)

Tuberculin skin test anergy was significantly more frequent in HIV-infected children compared to those with undefined HIV infection status; however, it is noteworthy that, in HIV-infected children whose Mantoux responses were reactive, 13 out of 14 reactions were greater than 10 millimetres in diameter (**Figure 6**). This ‘all-or-nothing’ response to tuberculin in HIV-infected individuals has been observed in other cohorts. (27, 103)

Van Rheenen (41) describes a score chart sensitivity of 91% in 23 HIV-infected children with confirmed, probable or possible tuberculosis, and a score chart specificity of only 25% by evaluating the chart in a cohort of 147 HIV-infected and -uninfected children in Zambia. It is noteworthy that, in our cohort, evaluation of score chart sensitivity in the context of culture-confirmed tuberculosis was similar regardless of whether the SANTCP chart or Van Rheenen's chart was used (**Table 18** and **Table 21**).

There was no significant disparity in the proportion of malnourished HIV-infected children in the Cape Town cohort (42/93 HIV-infected children: 45.2%, **Table 14**) compared to Van Rheenen's Zambian cohort (14/23 HIV-infected children: 61.0%), P=0.177. Malnutrition was noted by Van Rheenen to be an important contributor to the over-diagnosis of tuberculosis through the use the score chart, as malnutrition is commonly encountered in HIV-infected children in sub-Saharan Africa and children who may not have tuberculosis could be scored as having the disease on the basis of malnutrition. (41) The score chart did not 'over-score' HIV-infected children in comparison to the HIV-uninfected children in our multivariate analysis (**Table 24**), indicating that discrepant anthropometric parameters between these groups did not contribute significantly towards score positivity in the HIV-infected children in our cohort.

Through the evaluation of the tuberculosis score chart in 341 children with culture-confirmed tuberculosis, our analysis indicates that the tuberculosis score chart as formulated in the SANTCP Guidelines (2004) (42) may be a worthwhile tool for augmenting the sensitivity of the Mantoux response in hospitalised HIV-infected children who are screened for active tuberculosis in urban settings in South Africa (**Table 22**).

The sensitivity of the Mantoux response in children with culture-confirmed tuberculosis was equivalent to that of the score chart in HIV-uninfected children, but considerably more sensitive than the score chart in children with undefined HIV infection status (**Table 22**); therefore, it may be argued that the routine use of the score chart to aid the diagnosis of tuberculosis in HIV-uninfected children and those whose HIV infection status is unknown, is unwarranted. It is noteworthy, however, that the kappa-statistic for the degree of correlation between the Mantoux test and the tuberculosis score chart indicated at best, only fair agreement between these diagnostic modalities in the RCWMCH cohort, regardless of HIV infection status (**Table 23**); therefore, it may be useful for clinicians to adopt the score chart as a means of tailoring the clinical evaluation of children managed at RCWMCH, so as to highlight the possibility of tuberculosis in the differential diagnosis.

Hatherill et al. (104) report on a recent prospective comparison between nine different structured diagnostic approaches developed to assist in the diagnosis of tuberculosis in children enrolled in a BCG vaccine trial in the Western Cape. Nutritional status was > 80% of expected weight-for-age in 76% of the children (n=1 445) screened for tuberculosis (on the basis of a positive family contact or symptoms suggestive of the disease), and only 54 (3.7%) were HIV-infected. Six-hundred an eleven (42.3%) children were commenced on antituberculosis treatment, and 172 (11.9%) had culture-confirmed disease. Tuberculosis, when it occurred within that community-based cohort, likely included a large proportion of 'mild' cases which may account for the poor performance (145 [10.0%] of cases screened were classified as having tuberculosis) of the modified Edwards scoring system advocated for use by the SANTCP Guidelines (2004) (42) in that cohort. (104) The approach utilised by Hatherill et al. (104) did not assess the sensitivity of the different scoring systems in the context of culture-confirmed tuberculosis cases. The context – community- or hospital-based, in settings with high or low HIV endemicity – in which such scoring systems are

used, will impact on the performance of different structured diagnostic approaches to child tuberculosis. (104)

4.8 Tuberculosis In HIV-infected Children

The majority (70%) of the HIV-infected children with tuberculosis in our cohort appear to have been diagnosed with HIV infection in response to, or in conjunction with, the diagnosis of tuberculosis; tuberculosis may therefore have functioned as the sentinel illness of HIV infection in these children. The latter point may be supported by the fact that in 208 (76.8%) of all 271 tuberculosis episodes in HIV-infected children, the children were ART-naïve at clinical evaluation for tuberculosis (**Table 34**), which is similar to the proportion (77.6%) of HIV-infected children who commenced antituberculosis treatment before they commenced ART at two paediatric HIV treatment facilities in Johannesburg. (105)

Access to ART in HIV and tuberculosis co-infected children (n=264, **Figure 1**) at RCWMCH (n=162; 61.4%) was considerably better than access to ART (n=563: 49.6%) observed in a cohort of co-infected children (n=1 136) managed at CHBH, Soweto, (48) and reflects the importance of active participation of the IDC clinicians in in-patient management of HIV-infected children at RCWMCH.

HIV-infected children with culture-confirmed tuberculosis had significantly lower CD4 T-lymphocyte counts compared to those with ‘other than culture confirmed’ tuberculosis. Additionally, children under five years of age at work-up for tuberculosis were 2.4-fold (95% CI, 1.23 – 4.75), P=0.005, more likely to be classified as being immunodeficient compared to older children (**Table 33**), which was also demonstrated in the analysis of

ART-naïve children stratified according to age group (OR 2.68; 95% CI, 1.13 – 6.22), P=0.011. The degree of immunologic severity noted in the cohort, as stratified according to age group, echoes the findings of Newell et al. (106) and Violari et al. (84) who report the rapidity of progression of HIV infection in infants and young children. The relatively spared immunologic parameters in HIV-infected children older than five years of age at tuberculosis diagnosis, even if ART-naïve at evaluation for tuberculosis, reflect the survivor effect of slow HIV disease progression in these children as noted by Davies et al. (107)

Excellent immunologic (**Table 35**) and virologic (**Table 36**) responses to ART were noted in HIV-infected children who accessed ART before or during antituberculosis treatment, and it is striking (as well as being intuitive) that those who did not access ART over the surveillance period exhibited a 2-fold (95% CI, 1.21 – 4.41) increase in risk of worsening immunologic status compared to those who were treated with ART.

Recent attention has been drawn to poorer virologic outcomes in HIV-infected children with tuberculosis co-treated with rifampicin and PI-based ART, as a result of the adverse pharmacokinetic interaction brought about by rifampicin-induced enhanced clearance of protease inhibitors via the cytochrome P450 enzyme system. (71, 105) This interaction leads to severely depleted serum concentrations of most of the protease inhibitor drugs. (108, 109) Our data support poorer virologic outcomes in HIV-infected children co-treated with rifampicin and PI-based ART, with an adjusted hazard ratio of 8.20 (95% CI, 1.12 – 59.88), P=0.038, for virologic non-suppression at a virologic failure cut-off of 400 RNA copies per millilitre in those treated with PI-based ART compared to those treated with NNRTI-based ART (**Table 37**); however, small sample size and the retrospective nature of our analysis necessarily impacts on the strength of this observation.

Poorer virologic response to ART was also demonstrated amongst children classified as having possible unmasking tuberculosis IRIS in this cohort (**Table 38**), as has been demonstrated in another South African child cohort in which HIV-infected children who developed suspected IRIS were nearly three times (95% CI, 1.14 – 7.29) more likely to exhibit virologic non-suppression after six months of ART in an analysis which controlled for baseline factors. (110)

Thirteen (20.6%) of the 63 child tuberculosis episodes in which ART had been initiated prior to commencement of antituberculosis therapy in our cohort, occurred within three months of ART initiation at a mean of 29 days (SD 23) after commencement of ART. Time to onset of possible unmasking tuberculosis IRIS in our cohort is similar to that observed by Zampoli et al. (70) in their description of 11 presumed tuberculosis IRIS cases which arose in children treated at RCWMCH between January 2004 and March 2006.

Although the number of patients (n=13) who were deemed to have possible unmasking tuberculosis IRIS in our study were too small to permit multivariate analysis in order to assess risk factors associated with the development of unmasking IRIS, univariate analyses give some indication as to possible risk factors. Age at tuberculosis diagnosis did not affect the likelihood of developing possible unmasking IRIS; however, children who developed possible unmasking IRIS had significantly lower baseline CD4 T-lymphocyte counts and weight-for-age z-scores compared to those who did not have possible IRIS. These data corroborate earlier observations that immunologically and clinically advanced HIV disease at ART initiation are risk factors for the development of IRIS. (37)
Careful pre-treatment screening to exclude occult tuberculosis, (70) and enhanced efforts to ensure earlier age at initiation of ART (84) are two strategies that require urgent

attention in order to minimise the occurrence of IRIS in HIV-infected children in settings such as ours.

4.9 Antituberculosis Treatment Used In The RCWMCH Cohort

As folder reviews were conducted only in children with culture-confirmed tuberculosis in our cohort, information regarding type of antituberculosis treatment used was inferred by extrapolation from the type of tuberculosis in each child (**Table 29**). This approach necessarily assumed that all tuberculosis in the cohort was drug susceptible, and therefore the 35% estimate of children who would require treatment regimens other than Regimen III, has been underestimated in our analysis. Forty percent of children with culture-confirmed tuberculosis were treated with regimens that are not routinely available at down-referral clinics (**Table 30**). These proportions of children who required complicated treatment regimens reflect the severity of the spectrum of paediatric tuberculosis encountered in hospital-based cohorts in high-burdened settings.

It is hoped that, with the adoption of the revised South African Department of Health National Tuberculosis Management Guidelines (2009), (111) which endorses the use of ethambutol in paediatric antituberculosis treatment regimens, optimised management of children with complicated forms of tuberculosis who have been down-referred from hospital settings for treatment at Primary Health Care facilities, will become a reality in the near future.

That one third of children over eight years of age were treated using Regimen III, despite the SANTCP recommendation that Regimen I be used in case management of this age group, may indicate that clinicians are unfamiliar with management guidelines for child

tuberculosis. Alternatively, treatment dilemmas do arise when older children are underweight for age, and use of adult fixed-dose combination formulations of Regimen I intensive phase drugs according to weight-based dosing tables would lead to supratherapeutic doses of first-line antituberculosis drugs. Additionally, with the WHO currently endorsing the use of ethambutol even in young infants with severe forms of tuberculosis, (65, 112) current formulations of ethambutol (400 mg tablets) have proven to be unwieldy in clinical practice. The updated SANTCP Guidelines (2009) (111) make provision for the availability of ethambutol 100mg tablets, and this should simplify ethambutol dosing in young infants.

Concerns relating to optimised case management through the use of currently recommended regimens highlight the need for enhanced advocacy so that pharmaceutical companies may be guided to formulate more appropriate fixed-dose combination tablets for children. (113)

4.10 IPT And Drug Susceptibility Profiles Within The RCWMCH Cohort

IPT has been identified as being an important secondary preventive measure to prevent progression to active tuberculosis in high-risk close contacts of pulmonary tuberculosis patients; (87, 111, 114) however, concerns relating to the incorporation of this preventive strategy into over-burdened national tuberculosis programmes, potential toxicities of isoniazid, and the concern that single-agent treatment given to children with undiagnosed active tuberculosis may drive the development of isoniazid resistance have limited wide-spread use of this intervention.

As has recently been demonstrated in another South African cohort, (115) exposure to IPT did not appear to drive the development of drug-resistance in children who had been treated with IPT prior to the development of culture-confirmed tuberculosis; in our cohort, only one of the 13 children who developed culture-confirmed tuberculosis after having received a course of IPT, had drug-resistant tuberculosis which was likely not selected out for by isoniazid monotherapy, as the isolate was multidrug-resistant (**Section 3.7.2**). The low priority given by clinicians to administering IPT to high-risk children exposed to pulmonary tuberculosis contacts in this high-burdened setting is illustrated by the fact that a history of IPT use had been enquired about in only 12% of the culture-confirmed tuberculosis episodes in which a positive tuberculosis contact was recorded, and that 71% of those whose IPT-exposure history was enquired about had not received preventive therapy.

Efforts to improve access to IPT, once active tuberculosis has been excluded, need to be strengthened in high-burdened settings, (116) although the prevalence of isoniazid resistance in our cohort, and the cohort of children described by Schaaf et al. (58) (over one in every 10 children with culture-confirmed tuberculosis had isoniazid resistance, **Table 43**) raises concerns about the effectiveness of IPT in our setting. (117)

The prevalence and profile of drug resistance within our cohort (**Table 31**) mirrors that observed by Schaaf et al. (58) (**Table 43**), although rifampicin mono-resistance was more frequently encountered in our cohort.

Drug resistance profiles	Schaaf et al. 01 March 2003 – 28 February 2005 (n=592)	RCWMCH 01 December 2006 – 31 December 2008 (n=406)	P-values*
Drug susceptible isolates	525 (88.7)	356 (87.7)†	0.630
Isoniazid mono-resistant	43 (7.3)	25 (6.2)	0.496
Rifampicin mono-resistant	2 (0.3)	8 (2.0)	0.019‡
Multidrug-resistant	22 (3.7)	17 (4.2)	0.706
All isoniazid resistance	65 (11.0)	42 (10.3)	0.750
All rifampicin resistance	24 (4.1)	25 (6.2)	0.131

Table 43: Comparison of drug resistance patterns in *M. tuberculosis* isolates between RCWMCH cohort (first-episode cases only) and the cohort described by Schaaf et al. (58)

As six of the eight rifampicin mono-resistant isolates detected in our cohort were isolated in 2006 (n=2) and 2007 (n=4) (**Table 31**), the higher proportion of rifampicin mono-resistant isolates detected in our cohort cannot be explained by the change in laboratory methodologies used to detect *M. tuberculosis* drug susceptibility, as a switch from phenotypic drug susceptibility testing to widespread use of genotypic drug susceptibility testing only occurred in 2008. (118) Rapid genotyping methods for detection of drug resistance in *M. tuberculosis* may not detect a proportion of isoniazid resistant isolates, (119) and may therefore underestimate the prevalence of multidrug-resistant tuberculosis. A trend towards increasing prevalence of rifampicin mono-resistant tuberculosis over the time-period March 2003 to February 2009, particularly in HIV-infected and HIV-exposed uninfected children, has recently been observed in children with culture-confirmed tuberculosis in Cape Town. (120)

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* P-values are derived by the comparison of proportions according to drug susceptibility profiles between the cohorts described in the two studies.

† Numbers in brackets reflect column percentages.

‡ Fisher's exact test.

Our study has highlighted a disproportionate burden of multidrug-resistant tuberculosis in HIV-infected children, who had an adjusted odds of 4.03 (95% CI, 1.01 – 16.13), P=0.049, over HIV-uninfected children of being diagnosed with multidrug-resistant tuberculosis (**Table 32**). Previous studies have failed to demonstrate an increased burden of multidrug-resistant tuberculosis in HIV-infected children, (58, 121, 122) and although a recent meta-analysis, which focused on data obtained from studies conducted in adult patients, (123) failed to demonstrate an increased risk of multidrug-resistant tuberculosis in HIV-infected adults, it is likely that in settings where a high HIV prevalence drives tuberculosis epidemics, increased rates of drug-resistant tuberculosis will arise. (124)

It is noteworthy that drug resistant tuberculosis was associated with a 4-fold (95% CI, 1.31 – 13.87), P=0.016, adjusted likelihood of death in our cohort (**Table 41**).

4.11 Death In Children With Tuberculosis

No difference in likelihood of tuberculosis-attributable death was demonstrated HIV-infected children according to ART experience in the RCWMCH cohort (**Table 41**), although in a cohort of children diagnosed with HIV and tuberculosis co-infection in Soweto, non-commencement of ART was associated with a 2.6-fold (95% CI, 1.07 – 6.13) increased risk of death. (48) This probably reflects the significantly higher ART coverage in the RCWMCH cohort, indicating that a lower proportion of HIV-infected tuberculosis patients were never exposed to ART at that facility, and therefore that a lower proportion of HIV-infected children were at risk of dying.

5.0 STUDY LIMITATIONS

Although this study has sought to examine numerous aspects of child tuberculosis case management at a children's hospital in Cape Town, South Africa, and the methods used have attempted to examine case management as thoroughly as possible, information is limited by numerous factors, chiefly in that it is a retrospective study.

Folder reviews were only conducted for children with culture-confirmed tuberculosis, and therefore more meaningful conclusions relating to the differential clinical presentations of children with less convincing evidence for the diagnosis of tuberculosis (those designated as having 'other than culture-confirmed' tuberculosis) could not be made. Retrospective folder reviews are challenging to perform and interpret, because study-specific information would not have been systematically recorded at the time of initial interaction with study participants; the description of culture-confirmed tuberculosis cases in the cohort has necessarily been eroded because of this limitation. Although clinical data pertaining to children with culture-confirmed tuberculosis was incomplete in this study, record reviews were conducted in the overwhelming majority (428: 98.8%) of the 433 culture-confirmed tuberculosis episodes.

Our data represent a minimum estimate of the numbers of tuberculosis cases treated at RCWMCH during the period 01 January 2006 to 31 December 2008. Culture-negative tuberculosis cases may have been under-recorded in the paper-based Notifications Database. Furthermore, as the study time periods over which the Notifications Database (01 January 2006 to 31 December 2008) and Culture-confirmed Tuberculosis Database (14 November 2006 and 17 December 2008) were complied did not overlap, it is likely that not all culture-confirmed tuberculosis cases occurring amongst children treated at

RCWMCH during the study period were identified. This implies that the description of the spectrum of disease in culture-confirmed cases is incomplete.

HIV infection status definitions were based upon a single positive, age-specific assay (serologic in children ≥ 18 months of age, and nucleic-acid amplification in younger children) in this study; although unlikely, because of the highly specific nature of these assays, this approach may potentially have over-estimated the HIV infection prevalence in the cohort if false-positive results occurred. The fact that such a large proportion (629 [48.4%] of 1 300 children ever diagnosed with tuberculosis) of the cohort remained untested for HIV infection is an important confounder in this study, and limits the interpretation of study findings as they relate to the HIV infection status of children with tuberculosis. A benefit of this observation is that it will hopefully improve the coverage of HIV testing in child tuberculosis suspects in the study setting, and other health care facilities, in future.

Important information, such as the length or height of infants and children at clinical examination (which would have completed the description of nutritional status in children with culture-confirmed disease according to current WHO recommendations) (72) was not obtained. Use of the Wellcome Classification (73) in our cohort was justified, however, as that method of categorisation of child nutritional status was used in the tuberculosis score chart recommended for use by the SANTCP Guidelines (2004). (42)

Although this study assisted in estimating the sensitivity of the tuberculosis score chart recommended for use by the SANTCP Guidelines (2004) (42) it was unable to quantify the tuberculosis score chart specificity in this cohort, as folder reviews were not conducted in all children. Children diagnosed with notifiable conditions other than tuberculosis could

have served as a possible control group against which to estimate score chart specificity, even though *M. tuberculosis* was cultured from specimens submitted from 13 (3.1%) of the 421 children who had been notified for conditions other than tuberculosis.

Further limitations relating to the assessment of the tuberculosis score chart included the fact that no chest radiographic interpretation of tuberculosis cases by specialist radiologists, paediatric pulmonologists or experts in child tuberculosis was undertaken in this study, which weakened the description of the true spectrum of intrathoracic tuberculosis (both culture-confirmed and 'other than culture-confirmed') within the cohort. The use of routine clinical notes to obtain information about chest radiographic changes, as adopted in this study, can be justified however, as these descriptions of radiographic changes reflect day-to-day paediatric practice and clinical interpretation of radiographic findings from which clinicians based their decision to commence antituberculosis treatment.

Finally, in sub-analyses of the available data, some of the sample sizes were small, necessitating the use of exact methods to determine statistical significance; it is expedient to comment on the fact that the interpretation of such results is problematic, as the possibility of a Type I error – attributing statistical significance to the difference in findings between groups when, in fact, no such difference exists – is substantial. Well-designed, prospective studies to discern whether HIV-infection is indeed associated with an increased prevalence of childhood MDR tuberculosis, or whether PI-based ART in the context of antituberculosis therapy is significantly more frequently associated with poor virologic outcomes compared to co-treatment with NNRTI-based ART, as demonstrated in our analysis, are warranted and eagerly anticipated.

6.0 CONCLUSIONS

Tuberculosis is frequently encountered in children managed at RCWMCH, and the spectrum of disease observed mirrors that described in other child tuberculosis cohorts. Barriers to optimal case management, including omission of HIV testing, under-reporting of culture-confirmed cases in the paper-based Notifications Register, lack of awareness of the importance of tuberculosis preventive therapy, and utilisation of age-inappropriate treatment regimens in older children, occur at this institution which is situated in one of the highest-resourced settings in South Africa. It is likely that similar deficiencies exist in child tuberculosis case management at other centres in South Africa (as has been demonstrated through comparison of aspects of case management at CHBH).

Although childhood tuberculosis usually has a subacute or chronic course, and most children with pulmonary tuberculosis present with a history of chronic, unremitting cough, (125) our study highlights the fact that culture-confirmed pulmonary tuberculosis often presents acutely with cough duration of less than 10 days. Clinicians treating children with pneumonia in this high-burdened tuberculosis setting should have a high index of suspicion for tuberculosis, and investigate children appropriately.

Tuberculosis appears to have been the sentinel illness in 70% of the HIV-infected children with HIV-tuberculosis co-infection at RCWMCH. Access to ART in HIV-infected children co-infected with tuberculosis (61.4%) is considerably better than access to ART observed in a cohort of co-infected children in Soweto (9.3%), (48) reflecting the beneficial effects of active involvement of the IDC clinicians in case management of all HIV-infected children admitted to RCWMCH; however, as pulmonary tuberculosis and extrapulmonary tuberculosis are classified as WHO HIV Clinical Stage III and IV disease states, (69) all

HIV-infected children diagnosed with tuberculosis qualify for commencement of ART, regardless of CD4 T-lymphocyte counts. (85) Ways of up-scaling the capacity to diagnose HIV infection in all tuberculosis suspects, and to provide ART to all HIV-infected children diagnosed with tuberculosis at RCWMCH and other centres that care for children in South Africa, need to be explored.

Gains in combating tuberculosis-related morbidity and mortality in sub-Saharan Africa will only be achievable in the setting of the dual tuberculosis and HIV epidemics through a concerted and co-ordinated effort to pool resources in such a way as to integrate HIV and tuberculosis case management in effective and sustainable ways. (126-129)

Sub-Saharan Africa is currently far off target in terms of achieving the Millennium Development Goal of halving the 1990 tuberculosis prevalence and mortality rates by 2015. (1, 2) What is also of grave concern is that tuberculosis poses not only a risk to the present, as a result of the enhanced morbidity and mortality of children in high-burdened settings, but also a risk to the future as a large population of individuals infected in childhood pose potential risks for reactivation disease and perpetuation of tuberculosis transmission in years to come. (22, 130)

The capacity for tuberculosis control in sub-Saharan Africa may be eroded well into the next century at current disease incidence rates, unless concerted efforts are urgently adopted in order to improve the health of children under our care.

7.0 RECOMMENDATIONS

The following recommendations may assist in optimising tuberculosis case management in children with suspected tuberculosis at RCWMCH, and other child-centred health facilities in South Africa:

1. Integration of tuberculosis and HIV management services at Primary, Secondary and Tertiary Health Care facilities need to be up-scaled according to WHO recommendations. (126-129) HIV-tuberculosis integration strategies include:
    a. Enhancement of tuberculosis contact tracing in families in which a tuberculosis source case has been identified;
    b. HIV testing of all tuberculosis suspects;
    c. Utilisation of tuberculosis preventive therapy in vulnerable close contacts in whom active tuberculosis has been excluded;
    d. Administration of appropriate antituberculosis treatment regimens in those diagnosed as having active disease, according to current guidelines; (65, 111)
    e. Streamlining of HIV-infected patients for access to co-trimoxazole preventive therapy and ART;
    f. Emphasis on infection control in health care settings so that the risk for tuberculosis transmission in congregate settings (such as busy health care clinics, hospital outpatient departments and wards) is minimised.

2. Regular education of junior staff regarding tuberculosis in children may be warranted, and would be especially useful if incorporated into the orientation programme of junior doctors at the start of their medical and surgical paediatric

rotations. Such seminars should be conducted at least three times per year (to coincide with the four-month clinical rotations scheduled in the two-year internship programme), and attendance at such seminars should ideally be included as one of the compulsory requirements to be achieved before completion of the clinical rotation. Such seminars should focus on highlighting:

a. Clinicians' familiarity with current tuberculosis case management guidelines, including the cornerstones of the integrated HIV-tuberculosis approach as mentioned above (which could possibly be assessed by means of a written examination);
b. The burden of tuberculosis in the communities being served by the facility;
c. Risk factors for tuberculosis – including the important association between HIV and tuberculosis;
d. The need for a high index of suspicion in considering tuberculosis, even in children presenting with acute respiratory illness in our setting;
e. The possible utility of the tuberculosis score chart to assist as a 'screening tool' in suspected tuberculosis cases, as this may help in taking a goal-directed history and examination in such cases;
f. Correct notification and referral procedures to complete when children are diagnosed with tuberculosis;
g. The need for systematised and timeous follow-up of children who have had specimens submitted for tuberculosis culture.

3. Up-scaling of HIV testing in children who are being investigated for tuberculosis may be achieved through the introduction of rapid test kits for the testing of children whose HIV exposure status is unknown, and should be incorporated into outpatient (medical and surgical) treatment services. Such a strategy has been in operation at

RCWMCH since October 2008. The logistics of incorporating this strategy are not insignificant, and require buy-in from local government, hospital management, medical and nursing staff, HIV counsellors, the NHLS, laboratory staff, and the community; however, the benefits of such a strategy are potentially far-reaching through permitting HIV-infected children to be identified and stream-lined for ART in a timeous manner.

4. Clearly, optimised tuberculosis case co-ordination in any high-burden setting would benefit from the creation of a full-time tuberculosis co-ordinator post which could be filled by a nursing sister, principal medical officer, or paediatrician with particular interest in child tuberculosis. Such an individual would assist in tracing culture-confirmed tuberculosis cases that may not have been initiated on anti-tuberculosis therapy during interaction with the clinical staff at the facility. Important administrative functions, particularly liaison with local health directorates, regarding child tuberculosis burden, planning and conduct of internal audits and ongoing research, and quality control relating to tuberculosis case investigation, preventive strategies, and treatment at the facility may be fulfilled by such an individual. Such an individual could also assist in tuberculosis out-break investigations in communities served by the hospital.

5. Advocacy for child tuberculosis cases who had been diagnosed in hospital, and who often have severe forms of disease which necessitate the use of complicated treatment regimens which may not be routinely available at down-referral settings, needs to be implemented. This will require discussion between paediatric infectious disease specialists, local health directorates and the pharmaceutical industry.

APPENDICES

<u>Appendix 1:</u> Childhood Tuberculosis Diagnostic Score Chart Recommended For Use By The South African National Tuberculosis Control Programme (2004) (42)

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: Ethics Clearance Certificate For Notifications Database

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925

Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: lamees.emjedi@uct.ac.za

06 June 2008

REC REF: 234/2008

Dr DP Moore
Paediatrics
Red Cross

Dear Dr Moore

PROJECT TITLE: COMPARATIVE STUDY TO ASSESS MANAGEMENT OF TUBERCULOSIS IN CHILDREN AT TWO TERTIARY HOSPITALS IN SOUTH AFRICA

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 15th June 2009.

Please submit a progress report if the study continues beyond the expiry date or a closure report if completed within the period of approval.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

[signature]

pp

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

lemjedi

<u>Appendix 2, continued:</u> Extension Of Ethics Approval For Completion Of Research

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Annual Progress Report

	12 May 2010
	234/2008
	Comparative study to assess management of tuberculosis in children at two tertiary hospitals in South Africa
	David Paul Moore
	School of Child & Adolescent Health, Red Cross Children's Hospital and Department of Paediatrics Chris Hani Baragwanath Hospital

List of documentation

Please find revised research proposal, which has combined the original studies (REC REF 234/2008 & REC REF 233/2008) so that they can be incorporated into a single dissertation entitled: "Aspects of Tuberculosis Case Management at Red Cross Children's Hospital", and draft version of the final study write-up attached herewith.

This dissertation is being submitted in partial fulfilment of the requirements for the degree of Master in Philosophy in the branch of Paediatrics of the University of Cape Town.

The study has been finalised, and is due for submission this month; an expedited ethics approval would be greatly appreciated, if possible.

The principal investigator takes full responsibility for late application for extension of ethics approval for this study. He relocated to Johannesburg in March 2009, and although he submitted a revised proposal to the School of Child & Adolescent Health Investigator Review Board in June 2009, was unaware that application for extension of ethics approval needed to be applied for in June 2009.

☑ Approved		
☐ Not approved		
	☑ Expedited	☐ Full committee
	28 MAY 2011	
	[signature]	14/5/2010

11 May 2010 Page 5 of 5 FHS016

<u>Appendix 3:</u> Ethics Clearance Certificate For Culture-confirmed Tuberculosis Database

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: lamees.emjedi@uct.ac.za

06 June 2008

REC REF: 233/2008

Dr DP Moore
Paediatrics
Red Cross

Dear Dr Moore

PROJECT TITLE: ANALYSIS OF CHILDREN WITH POSITIVE TUBERCULOSIS CULTURES AT RED CROSS CHILDREN'S HOSPITAL.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 15th June 2009.

Please submit a progress report if the study continues beyond the expiry date or a closure report if completed within the period of approval.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

pp **<u>PROFESSOR M BLOCKMAN</u>**
<u>CHAIRPERSON, HSF HUMAN ETHICS</u>

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

lemjedi

Appendix 3, continued: Extension Of Ethics Approval For Completion Of Research

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Annual Progress Report

[illegible]	12 May 2010
[illegible]	233/2008
[illegible]	Analysis of children with positive tuberculosis cultures at Red Cross Children's Hospital
[illegible]	David Paul Moore
[illegible]	School of Child & Adolescent Health, Red Cross Children's Hospital and Department of Paediatrics Chris Hani Baragwanath Hospital

List of documentation

Please find revised research proposal, which has combined the original studies (REC REF 234/2008 & REC REF 233/2008) so that they can be incorporated into a single dissertation entitled: "Aspects of Tuberculosis Case Management at Red Cross Children's Hospital", and draft version of the final study write-up attached herewith.

This dissertation is being submitted in partial fulfilment of the requirements for the degree of Master in Philosophy in the branch of Paediatrics of the University of Cape Town.

The study has been finalised, and is due for submission this month; an expedited ethics approval would be greatly appreciated, if possible.

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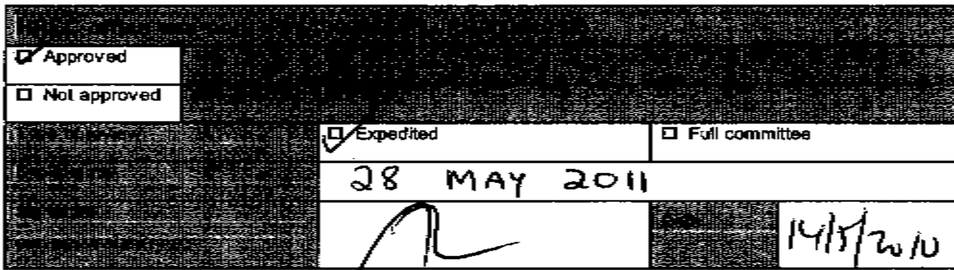

☑ Approved
☐ Not approved

[illegible]	☑ Expedited	☐ Full committee
[illegible]	28 MAY 2011	
[illegible]	[signature]	14/5/2010

11 May 2010 Page 5 of 5 FHS016

Appendix 4: Parameters Entered Into The Electronic Notifications Database

Data extracted from the paper-based Notifications Register and CliniCom™ System		
Name	Surname	Gender
Date of birth	RCWMCH Folder Number	
Date of admission	Ward to which admitted	Type of disease for which notified
Address	Outcome of case – discharged/referred/died	Date of discharge/referral/death

Data extracted from the National Health Laboratory System	
Tuberculosis investigation results:	**Dates:**
Type of specimen	Date specimen obtained
Smear positive or negative for acid-alcohol fast bacilli	
Culture positive or negative for mycobacteria*	Date specimen culture positive
Susceptibility of mycobacterial isolate	
Tuberculosis histology results:	**Dates:**
Tissue type	Date specimen submitted
Histology suggestive of tuberculosis or not	
HIV results:	**Dates:**
ELISA/PCR result	Date of HIV test
CD4 T-lymphocyte count and percentage prior to or at TB diagnosis admission episode HIV viral load determination (count and $\log_{10}$ values) prior to or at TB diagnosis admission episode	Date of CD4 T-lymphocyte and HIV viral load determinations at time of TB diagnosis
CD4 T-lymphocyte count and percentage after TB diagnosis admission episode HIV viral load determination (count and $\log_{10}$ values) after TB diagnosis admission episode	Date of CD4 T-lymphocyte and HIV viral load determination after TB diagnosis
Acute phase reactants:	**Dates:**
C-reactive protein (CRP) at TB diagnosis	Date of CRP determination
Erythrocyte sedimentation rate (ESR) at TB diagnosis	Date of ESR determination
White cell counts and monocyte counts at TB diagnosis admission episode	Date of full blood count determination
Adenosine deaminase (ADA) determination at TB diagnosis	Date of ADA determination
Type of fluid from which ADA was assayed	

Continued on next page

* Children with culture-confirmed disease are further evaluated by using the data collection form in **Appendix 5**. TB = tuberculosis.

Data extracted from the Infectious Diseases Unit Database	
On antiretroviral treatment (ART)?	**Dates:**
ART regimen used	Date ART started
First-line or second-line ART?	Date of commencement of alternate line ART regimen

Appendix 5: Parameters Entered Into The Positive TB Culture Database

Data extracted from the Groote Schuur Hospital (GSH) Microbiology Laboratory List of children with culture-confirmed tuberculosis and CliniCom™ System		
Name	Surname	Gender
Date of birth	RCWMCH Folder Number	
Date of admission	Ward to which admitted	Type of tuberculosis*
Address	Outcome of case – discharged/referred/died	Date of discharge/referral/death

Data extracted from Patient Clinical Records

Patient history:

Presenting complaint	Duration of presenting complaint
Family history of tuberculosis	Which family member had tuberculosis
	If family tuberculosis history positive: contact smear positive or smear negative?

Clinical examination:

Weight on admission

Weight trends (assessed by review of growth charts)

Presence of clinical features for scoring using the tuberculosis score sheet:

- Adenopathy (site)
- Ascites
- Bone or joint involvement
- Hepato- or splenomegaly
- Meningism or suggestive cerebrospinal fluid findings

Other features of extrapulmonary tuberculosis:

- Pericardial effusion
- Pleural effusion

Radiographic investigations:

Chest radiographic changes as recorded in clinical notes

Abdominal sonar reports as recorded in clinical notes

Tuberculin skin test:

Tuberculin skin test done?

Tuberculin skin test read?	If read – diameter of induration as recorded in clinical notes (if not measured, record whether Mantoux reaction was 'positive' or 'negative')

Antituberculosis treatment initiated:

Regimen used	Date of initiation of antituberculosis therapy

Diagnosis at hospital discharge:

Tuberculosis Score Sheet using Parameters assessed in Patient Clinical Records

Number of parameters used to derive score	Total score

Continued on next page

* Pulmonary or extrapulmonary tuberculosis. TB = tuberculosis.

Data extracted from the National Health Laboratory System		
Mycobacterial investigation:		**Dates:**
Type of specimen from which *M. tuberculosis* was cultured	Specimen smear positive for acid-alcohol fast bacilli or not? Drug susceptibility profile of isolate	Date of specimen submission Date specimen culture positive
HIV results:		**Dates:**
ELISA/PCR result		Date of HIV test
CD4 T-lymphocyte count and percentage prior to or at TB diagnosis admission episode HIV viral load determination (count and log_{10} values) prior to or at TB diagnosis admission episode		Date of CD4 T-lymphocyte and HIV viral load determinations at time of TB diagnosis
CD4 T-lymphocyte count and percentage after TB diagnosis admission episode HIV viral load determination (count and log_{10} values) after TB diagnosis admission episode		Date of CD4 T-lymphocyte and HIV viral load determination after TB diagnosis
Data extracted from the Infectious Diseases Unit Database		
On antiretroviral treatment (ART)?		**Dates:**
ART regimen used		Date ART started
First-line or second-line ART?		Date of commencement of alternate regimen

Appendix 6: Categorisation Of Childhood Tuberculosis

Culture-confirmed tuberculosis	**Other than culture-confirmed tuberculosis** (66)	
	Probable tuberculosis	**Possible Tuberculosis** (67)
Mycobacterium tuberculosis cultured from clinical specimens in a patient with compatible clinical features of active tuberculosis	Criteria for the diagnosis of possible tuberculosis, with additional evidence as listed below: Acid alcohol fast bacilli visualised in clinical specimens, without culture confirmation of disease caused by *M. tuberculosis* and/or Positive tuberculin skin test* and/or Suggestive chest radiograph and/or Suggestive histology and/or Favourable response to anti-tuberculosis therapy	Any ill child with: Family history of smear-confirmed pulmonary tuberculosis and/or Not regaining health after measles of pertussis and/or Weight loss, cough or wheeze not responding to antibiotic therapy for bacterial lower respiratory tract infection and/or Painless swelling in a group of superficial lymph nodes

* Tuberculin skin test positivity cut-offs vary by HIV- and nutritional status, and are defined as being ≥ 5mm in HIV-infected or severely malnourished children, and ≥ 10mm in HIV-uninfected children.

<u>Appendix 7:</u> World Health Organization Immunologic Classification For Established HIV Infection (69)

HIV-associated immunodeficiency	**Age-related CD4 T-lymphocyte values**			
	<12 months	**≥ 12 – 36 months**	**≥ 36 – 60 months**	**≥ 60 months**
	CD4 T-lymphocyte percentages (%)			**CD4 T-lymphocyte count (× 10^6 cells/l) or percentage (%)**
None or not significant	> 35	> 30	> 25	> 500
Mild	30 – 35	25 – 30	20 – 25	350 – 499
Advanced	25 – 29	20 – 24	15 – 19	200 – 349
Severe	< 25	< 20	< 15	< 200 or < 15%

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