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Impact of the pneumococcal conjugate vaccine on culture-confirmed pulmonary tuberculosis in hospitalized children

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IMPACT OF THE PNEUMOCOCCAL CONJUGATE VACCINE ON CULTURE-CONFIRMED PULMONARY TUBERCULOSIS IN HOSPITALIZED CHILDREN

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

Johannesburg 2015

DECLARATION

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

……

The 26th day of May, 2015

DEDICATION

I dedicate this work to my parents, Marykutty and John Mammen, who have dedicated their lives for the benefit of their family. I thank them for their unconditional love and support.

ABSTRACT

INTRODUCTION

Children hospitalized with culture-confirmed pulmonary tuberculosis (PTB) frequently present with acute symptoms, possibly because underlying PTB may predispose to superimposed bacterial pneumonia. Immunization of children with pneumococcal conjugate vaccine (PCV) could protect against such superimposed bacterial pneumonia and reduce the incidence of PTB hospitalization.

OBJECTIVE

We studied the temporal association of childhood immunization with pneumococcal conjugate vaccine on the incidence of hospitalization for culture-confirmed PTB in children.

METHODS

A retrospective study on the incidence of hospitalization for culture-confirmed childhood PTB at Chris Hani Baragwanath Academic Hospital was undertaken from 2005 to 2012. This included a pre-PCV era (2005-2008) and a PCV era (2011-2012).

RESULTS

Overall, there was a 69.2% (95% CI: 62.8-74.6%) decline in the incidence of hospitalization for culture-confirmed PTB when comparing the PCV and pre-PCV-eras, with the decline in HIV-uninfected children only significant in the 3-11 month age category and the decline in HIV-infected significant across all age categories. There was

a trend for reduced pneumococcal bacteraemia in the PCV era compared to the pre-PCV era, with the odds of having a blood culture positive for pneumococcus being 1.91-fold (95% CI, 0.26-84.56) greater in the Pre-PCV era.

CONCLUSION

There has been a significant decline in culture-confirmed PTB hospitalization in children comparing the pre-PCV to the PCV-era. However, the incidence of culture-confirmed PTB prior to the introduction of PCV had been reduced to low levels due to antiretroviral therapy. This confounder together with the retrospective ecological study design and other several possible confounders limited any robust estimate as to whether there was a temporal association between PCV immunization and incidence of culture-confirmed PTB hospitalization in our study setting.

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Acronyms

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CHBAH	Chris Hani Baragwanath Academic Hospital
DST	Drug susceptibility testing
EPI	Expanded Program on Immunisation
ESBL	Extended-spectrum ß-lactamase
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
INH	Isoniazid
IQR	Interquartile range
IPD	Invasive pneumococcal disease
IPT	INH preventive therapy
LRTI	Lower respiratory tract infection
MTB	*Mycobacterium tuberculosis*
NHLS	National Health Laboratory Service
PCV	Pneumococcal polysaccharide-protein conjugate vaccine
PCV7	Heptavalent PCV
PCV13	13-valent PCV
PMTCT	Prevention of Mother to Child Transmission (of HIV)
PTB	Pulmonary Tuberculosis
RMPRU	Respiratory and Meningeal Pathogens Research Unit
SD	Standard deviation

TB	Tuberculosis
WHO	World Health Organization

1.0 Introduction

Pneumonia is a major cause of childhood morbidity and mortality worldwide, particularly in low-income countries (1). *Streptococcus pneumoniae* (the pneumococcus) and *Mycobacterium tuberculosis* (MTB) have been implicated in the aetiology of childhood pneumonia in sub-Saharan Africa (2-4). The burden of pneumococcal disease and tuberculosis (TB) has increased as a result of the human immunodeficiency virus (HIV) epidemic. The overall burden of severe invasive pneumococcal disease (IPD) and pulmonary tuberculosis (PTB) is significantly greater in HIV-infected compared to HIV-uninfected children (5, 6). Antiretroviral therapy (ART) programs have reduced the incidence of PTB and IPD in HIV-infected children (7-10).

Challenges in directly delineating the role of bacteria (including pneumococcus) and MTB as aetiological agents of pneumonia, include the lack of sensitive diagnostic tools in the absence of direct lung sampling (11-14). Gastric washing or induced sputum culture for MTB is only positive in 15-30% of PTB cases (15), whilst blood cultures positive for culture of pneumococcus occurs in only approximately 2.6% and 18.8% of HIV-uninfected and -infected children with pneumococcal pneumonia, respectively (16).

Nevertheless, *S. pneumoniae* is recognized as the leading bacterial cause of community-acquired pneumonia in children (17). At least 94 pneumococcal serotypes have been identified, distinguished based on antigenic differences in the polysaccharide cell wall, an important virulence component of the bacterium. Thirteen serotypes, however, cause the majority (70-75%) of IPD in children (18). Pneumococcal conjugate

vaccine (PCV) was introduced in the USA in 2000, and subsequently recommended by the World Health Organization (WHO) for introduction into public immunization programs in low- and middle-income countries (18).

The introduction of heptavalent PCV (PCV7) has resulted in a marked reduction in rates of IPD. The resultant impact on IPD caused by vaccine serotypes has been consistent across countries including South Africa, with a reduced incidence ranging from 79% to 100% (19, 20). In a South African study the incidence of invasive pneumococcal disease caused by vaccine serotypes in children younger than 2 years of age declined by 89% (95% CI, −92 to −86%) (20). Furthermore, PCV has resulted in a reduction of hospitalization from all-cause radiologically confirmed pneumonia as defined by WHO interpretation (21, 22).

The burden of TB in South Africa is among the highest globally (23, 24). Hospitalization for PTB in children frequently presents as an acute illness in South Africa, with 43%-77% of hospitalized children subsequently confirmed as having culture confirmed PTB presenting with cough of <7-10 days duration (6, 25). This acute presentation of PTB may be related to superimposed infections precipitating as acute illness in children with underlying primary PTB. This was alluded to in a PCV vaccine-probe study, which demonstrated a 43% (95% confidence interval [CI], 10-65%) reduction in hospitalization for culture-confirmed PTB in PCV-vaccinated compared to PCV-unvaccinated children (25).

The likely role of bacterial co-infection precipitating hospitalization in children with underlying PTB is further supported by the observation that approximately 50% of children hospitalized with culture-confirmed PTB in our setting, where >70% of such cases present with cough duration of <7 days, are discharged clinically well following empiric treatment for bacterial pneumonia and without initiation of anti-TB drugs (25). The latter is largely due to the low threshold for investigating of TB in a high burden-setting such as ours, coupled with the lag of 4-6 weeks before MTB culture results become available (25, 26). Similar acute clinical symptoms in hospitalized children subsequently diagnosed to have culture-confirmed TB have also been observed elsewhere in South Africa (6, 27).

There are a limited number of studies which have directly documented the prevalence of co-infections with bacteria or respiratory viruses in individuals with PTB. Previously in Soweto during the mid-1990s, 10% of children hospitalized with culture-confirmed PTB had concurrent bacteraemia (4). In addition, two descriptive studies among South African adults also identified *S. pneumoniae* and MTB co-infections among HIV-infected individuals presenting with community acquired pneumonia and pericarditis (28, 29).

It is postulated that several factors in TB disease contribute to the increased risk of bacterial infections. IL-4 in advanced TB cases down-regulates the protective Th1 immune responses (30). The assembly of defensins which are an important factor of the innate immune response against microorganisms have been found to be reduced with progression of TB disease (31). Gamma interferon has also been found to induce

erosion of pre-existing CD8+ T cell memory to intracellular pathogens during TB infection (31). These factors cause immune dysfunction in patients with underlying TB, which could predispose them to bacterial infections (25).

The aim of this study was to determine the temporal association of infant PCV immunization on the incidence of hospitalization for culture-confirmed PTB in HIV-infected and HIV-uninfected children.

1.1 Hypothesis and objectives

Null Hypothesis

Introduction of PCV into the South African pubic immunization program is not associated with a reduction in the incidence of hospitalization for culture-confirmed PTB in children.

Study design:

A retrospective, ecological study analyzed the incidence for hospitalization of culture-confirmed PTB in children from Soweto, South Africa, between 2005 and 2012.

Primary objective:

To determine the temporal association of infant PCV immunization on the incidence of hospitalization for culture-confirmed PTB in HIV-infected and HIV-uninfected hospitalized children in Soweto.

Secondary objective:

To determine the proportion of children with culture-confirmed PTB who had concurrent bacteraemia.

2.0 Study design and methods

2.1 Study population and setting

The study was undertaken in Soweto, which spreads over an area of 108 square kilometers. Chris Hani Baragwanath Academic Hospital (CHBAH) is a secondary/tertiary level hospital situated in this low-middle income South African township, near Johannesburg in Gauteng Province. Chris Hani Baragwanath Academic Hospital has 3,200 beds, including 408 for children. The hospital serves a population of approximately 1.25 million people including 128,000 children under-5 years of age (32, 33).

The prevalence of HIV in Gauteng has remained stable at 16-17% in adults and 2-3% in children between 2005 and 2012 (32). The roll-out of ART for the treatment of HIV infection was implemented by the South African government in April 2004 (34). Also, the vertical transmission of HIV from mother-to-child declined to <2.5% from 2010 onward with the currently used ART-regimens in pregnant women (35). In Gauteng, the percentage of children with Acquired Immunodeficiency Syndrome (AIDS) that were on ART increased from 40.6% in 2005 to 96.1% in 2012 (32) (Appendix 1, page 39). Recent studies at CHBAH showed that increased uptake of ART among HIV-infected children in Soweto was associated with decline in the incidence of hospitalization for culture-confirmed PTB (70.6%) and IPD (50.8%), which occurred prior to PCV introduction, whereas lesser changes for PTB (41.3%) and no significant changes for IPD were observed among the HIV-uninfected children (8, 10).

Heptavalent PCV, which includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, was introduced into the South Africa public immunization program in April 2009. The PCV7 was replaced by 13-valent PCV (PCV13), which includes serotypes 1, 3, 5, 6A, 7F and 19A (in addition to those incorporated into PCV7), in May 2011. The PCV7 and PCV13 vaccines provided potential coverage against 67% and 88%, respectively, of serotypes associated with IPD in South African children in the absence of PCV-vaccination (18).

The PCV schedule in South Africa includes two primary doses at 6 and 14 weeks and a third (booster) dose at 9 months of age. This schedule is more immunogenic following the third dose compared to the 6, 10 and 14 week three-dose primary schedule previously studied in the same population (36). No catch-up campaign of older children was undertaken at the time of the introduction of PCV7 into the public immunization program, whilst a limited catch-up campaign targeting high-risk children 18 to 36 months of age was undertaken at the time of transitioning to PCV13 (37). Whilst the coverage of three doses of PCV in South African infants was estimated to be 10% in 2009, this steadily increased to 64% and 89% by 2010 and 2011, respectively (38). In Gauteng, coverage with the third-dose was 86% in 2010 and approximately 100% in 2011 and 2012 (39).

Children presenting with symptoms of severe pneumonia or lower respiratory tract infections (LRTI) as per WHO case definitions (40) are routinely admitted at CHBAH which, at the time of this study was the only public sector health facility with in-patient paediatric beds in Soweto. These children usually have a blood culture test undertaken

at admission and chest radiographs. Children admitted with acute respiratory tract infections are also investigated for PTB at the discretion of attending physicians, who because of the high burden of TB in the setting have a low threshold for investigating for PTB. Gastric aspirates and/or induced sputum are collected on 2-3 separate occasions and tested for MTB by microscopy and/or liquid mycobacterial culture. Gene-Xpert testing became available at CHBAH in 2012, but was not routinely used in the TB diagnostic work-up of children at the facility during the study period. Ampicillin was the first line antibiotic for the treatment of childhood community-acquired pneumonia at CHBAH at the time of this study, with the addition of gentamicin (for Gram negative cover) in immunocompromised and malnourished children.

2.2 Study procedures

Data on children hospitalized for culture-confirmed PTB from 2005 to 2009 had been previously analyzed in a study that interrogated the effect which the upscaling of the ART program in HIV-infected children had on the incidence of PTB hospitalization (8). The method used for determining the culture-confirmed PTB cases from 2005 to 2009 has been described (8). Patients aged 0-3 months were excluded from the previous analysis as they were considered to represent cases of congenital TB (41).

This study expanded on the previous study, using the same methodologies for identifying cases of culture-confirmed PTB in children less than 15 years of age hospitalized to the general paediatric wards at CHBAH from 2010 to 2012. Briefly, cases of culture-confirmed PTB were extracted from the National Health Laboratory

Service (NHLS) database, and cross-referenced against an electronic database of all admissions to the general paediatric wards at CHBAH. This electronic database is maintained by the Respiratory and Meningeal Pathogens Research Unit (RMPRU) based at CHBAH. Hospital records of these patients were retrieved and information extracted onto a standardized data collection form (Appendix 2, page 40). In cases where the hospital records were not retrievable, available clinical information was extracted from a discharge summary database. The hospital registration system (MEDICOM) and hospital admission registries were also checked for confirmation of hospitalization and outcome status.

Admission blood results (usually full blood count and blood culture) were retrieved from the NHLS database. HIV status, CD4+ lymphocyte counts, HIV viral load and duration of ART were determined by review of the NHLS and electronic paediatric databases. Anthropometry measurements were extracted from the paediatric database and weight for age z-scores in children less than 10 years were calculated as per the WHO child growth standards using WHO AnthroPlus version 1.0.4 (42).

The standard of care for diagnosis of HIV positivity at CHBAH include confirmation of a positive initial serologic result by qualitative HIV PCR in children under 18 months of age or a positive HIV ELISA test in children over 18 months of age, or a detectable HIV viral load if qualitative HIV PCR or serologic test results were unavailable. Infants with a positive HIV exposure history are tested with HIV PCR, and a positive result is confirmed with an HIV viral load test. The most recent CD4+ counts within three months

of the TB admission episode were included in the analysis, to attribute immunological status in the HIV-infected children. WHO levels of immune suppression based on CD4+ percentage/count categorization were used to stage the extent of immunosuppression (43) (Appendix 3, page 41).

Children were included in the analysis if their respiratory samples (gastric aspirates, induced sputum, expectorated sputum, tracheal aspirates or broncho-alveolar lavage secretions) were positive for MTB within one month of hospitalization for an episode of severe pneumonia, irrespective of duration of symptoms, and had not been initiated on TB treatment for more than two weeks prior to the admission. Excluded from the analysis were children 0-3 months and those older than 15 years of age.

2.3 Statistical analysis

The estimated incidence rates for culture-confirmed PTB were calculated and stratified by age group (3-11 months, 12-23 months, 24-59 months and 60-179 months) and HIV status to yield annual PTB incidence rates over an eight year period. In addition, the estimated incidence of culture-confirmed PTB was calculated in three time periods, designated the Pre-PCV era between 2005-2008 (i.e. Period 1), Transitional-PCV era between 2009-2010 (i.e. Period 2) and Established-PCV era between 2011-2012 (i.e. Period 3) based on the coverage for the third dose of PCV . As the coverage in 2009 was only 10% and 64% by 2010, these years were considered as transitional years for PCV introduction. Incidence rate ratios (IRR) were calculated to compare the burden of PTB in HIV-infected and HIV-uninfected children for Period 1 and Period 3.

The numerators for the incidence calculations were the number of culture-confirmed PTB cases hospitalized during each year/period and the denominators were based on mid-year population estimates from the Gauteng Department of Health and Social Development for region D, Soweto (Johannesburg), as per Statistics South Africa report (44). HIV prevalence in the study population was derived from the AIDS and Demographic models 2008 as per the Actuarial Society of South Africa (32) (Appendix 4, page 42).

The X^2 test (or Fisher's exact test, where appropriate) was used for the comparison of proportions for categorical variables. Student's t-test was used for the comparison of means for normally-distributed continuous variables and the Wilcoxon sign-rank test

was used to compare medians of skewed continuous variables. 95% confidence intervals (CIs) are reported, with p-values <0.05 considered as statistically significant.

The average of the previous, index and following month incidence rates were plotted against time to highlight overall trends, as was done in a previous study (8). This is also known as the 3-term moving average. It is used to smooth out short-term disparities when examining time-series data. For example, the 3-term moving average in June 2010 would be the average of the incidence rates in May 2010, June 2010 and July 2010.

Demographic data and estimates of incidence and IRR were analyzed using STATA version 12.0 (StataCorp, College Station, Texas, USA) and Epi Info version 3.5.1 (CDC, Atlanta, USA). The 3-term moving average was analyzed using R version 2.13 (Foundation for Statistical Development, Vienna, Austria).

2.4 Ethical Considerations

Permissions were obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and CHBAH Medical Advisory Committee to conduct the study (Appendix 5, page 43). Permissions were also obtained from the Paediatrics Department of the University of the Witwatersrand and from the NHLS for the use of laboratory data. Dr Ziyaad Dangor collaborated on (and co-supervised) this study and provided data for the 2005 to 2009 culture-confirmed PTB cohort. There was no need for informed consent because this was a retrospective study.

RESULTS

3.1 Identification of children hospitalized with culture-confirmed PTB (Figure 1)

In Period 1 (2005-2008), 792 children were hospitalized for culture-confirmed TB, 667 (84%) of which had PTB. Reasons for exclusion of cases between 2005 and 2008 included 93 children aged 0-3 months (potential congenital TB cases) and 32 children where culture-confirmed TB were from extrapulmonary sites (specimens other than gastric washings, sputum or broncho-alveolar lavage).

Period 2 included cases from 2009 and 2010, during which 199 children were identified with culture-confirmed TB; of whom 157 (78.9%) were hospitalized with PTB. Reasons for exclusion of cases in Period 2 included 17 children aged 0-3 months, 15 children with culture-confirmed TB from extrapulmonary sites, and 10 children who were diagnosed with PTB but not hospitalized.

Between 2011 and 2012 (Period 3), 174 children were hospitalized with culture-confirmed TB, of whom 48 were excluded, including 23 aged 0-3 months, MTB being cultured from a non-respiratory specimen in 22 children, two children never having been hospitalized and one child in whom TB treatment was initiated >2 weeks prior to admission for pneumonia. Therefore 126 children between the ages of 3 months and 15 years were hospitalized with culture-confirmed PTB in Period 3.

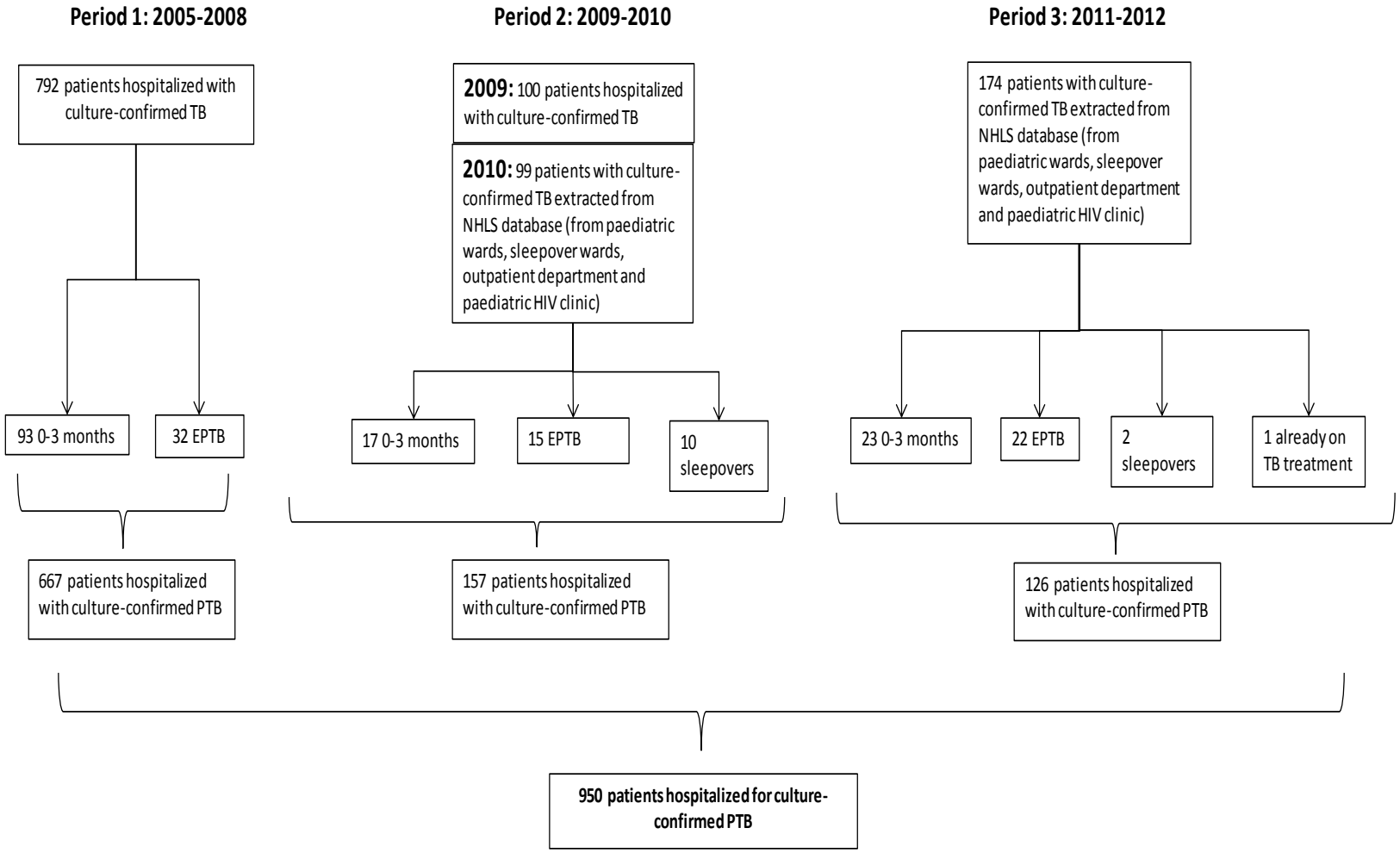


Figure 1: Flow diagram of children hospitalized with culture-confirmed PTB

3.2 Demographic information of Culture-confirmed PTB cases (Table 1)

Overall, 514 (54%) of the 950 culture-confirmed PTB cases were HIV-infected, 345 (36%) were HIV-uninfected and the HIV infection status was unknown in 91 (10%). The number of children with unknown HIV status and the number of HIV infected cases decreased over the eight year study period (Table 1).

The median age of culture-confirmed PTB cases was 25 months (Interquartile range [IQR], 9 to 85 months) across the eight year study period. The overall median age for culture-confirmed TB cases initially increased from 19 months (IQR, 8 to 72 months) in 2005 to 59 months (IQR, 15 to 123 months) in 2010 ($p<0.001$); including from 21 months (IQR, 8 to 82 months) to 92 months (IQR, 35 to 137 months) in HIV-infected children ($p<0.001$) in the respective periods. The median age of culture-confirmed PTB (all children, regardless of HIV status) subsequently decreased in 2012 to 24 months (IQR, 10 to 70 months) ($p=0.001$), but remained similar in HIV-infected children (70 months; IQR, 38 to 93 months; $p=0.933$). The median age in HIV-uninfected children increased from 11 months (IQR, 7 to 38 months) to 36 months (IQR, 14 to 109 months) ($p<0.001$) between 2005 to 2011, and then decreased to 15 months (IQR, 5 to 34 months) ($p=0.008$) in 2012.

Most (n=369; 65.2%) children were underweight for age with a median weight-for-age Z-score of -2.73 (Range, -8.89 to 5.51) for children <10 years of age.

The in-hospital case fatality rate over the eight year period was 2.6% (95% CI, 1.7-3.9%). Of the 25 children with culture-confirmed PTB who died during the eight year study period, 14 (56%) were HIV-infected and the HIV-status was not ascertained in six (24%) cases. Sixteen (64%) of the 25 deaths occurred in children under two years of age. None of these 16 children had significant blood culture isolates on admission to hospital.

3.3 Trends in the incidence of culture-confirmed PTB

There was a steady decline in annual number of culture-confirmed PTB hospitalizations, from 216 in 2005 to 62 by 2012 (Table 1). The decline was most evident in the 3-11 month and 60-179 month age-groups. This included a decrease from 88 cases in 2005 to 17 cases in 2012 in the 3-11 month age-group, and from 64 cases in 2005 to 19 cases in 2012 in the 60-179 month age-group. The number of cases decreased in all age categories among HIV-infected children, whilst the decrease was primarily evident in the 3-11 month age group in HIV-uninfected children (Table 2).

The overall incidence of culture-confirmed PTB (per 100 000) decreased by 69.2% (95% CI, 62.8-74.6%) when comparing Period 1 (47.9; 95% CI, 44.6-51.7) to Period 3 (14.8; 95% CI, 12.3-17.6) (Table 3). The percentage reduction in culture-confirmed PTB between Periods 1 and 3 was most evident in HIV-infected (80.5%; 95% CI, 73.8-85.5%) than in HIV-uninfected children (34.8%; 95% CI, 14.9-50.1%) when looking at all age categories combined (Table 3).

Table 1: Demographic data of children hospitalized with culture-confirmed PTB

Year		2005	2006	2007	2008	2009	2010	2011	2012	Total
PCV eras		**Period 1 (Pre-PCV era)**				**Period 2 (Transitional PCV era)**		**Period 3 (PCV era)**		
Total no. of cases		216	211	136	104	84	73	64	62	950
Age (months)	3-11	88 (41%)	82 (39%)	46 (34%)	31 (30%)	23 (27%)	15 (21%)	8 (13%)	17 (27%)	310 (33%)
	12-23	35 (16%)	38 (18%)	24 (18%)	16 (15%)	11 (13%)	7 (10%)	12 (19%)	14 (23%)	157 (17%)
	24-59	29 (13%)	38 (18%)	23 (17%)	26 (25%)	15 (18%)	16 (22%)	17 (27%)	12 (19%)	176 (19%)
	60-179	64 (30%)	53 (25%)	43 (32%)	31 (30%)	35 (42%)	35 (48%)	27 (42%)	19 (31%)	307 (32%)
	Median age (range): All	19 (3-176)	17 (3-173)	23 (3-170)	27 (3-167)	34 (3-163)	59 (3-178)	48 (5-167)	24 (3-165)	25 (3-178)
	HIV-infected	*21(3-158)*	*22 (3-173)*	*55 (3-170)*	*55 (3-167)*	*88 (3-163)*	*92 (3-174)*	*78 (3-167)*	*70 (3-165)*	*47 (3-174)*
	HIV-uninfected	*11 (3-170)*	*15 (3-160)*	*16 (3-159)*	*21 (3-156)*	*22 (3-160)*	*22 (3-178)*	*36 (3-159)*	*15 (3-159)*	*17 (3-178)*
Gender	Male	112 (52%)	114 (54%)	76 (56%)	52 (50%)	50 (60%)	29 (40%)	39 (61%)	33 (53%)	505 (53%)
Weight for Age (WAZ) (in children <10 years)	Median Z-score	-2.81	-2.74	-3.18	-2.89	-2.94	-2.51	-2.05	-2.59	-2.73
	Range	-7.14 – 1.87	-7.83 – 4.39	-8.89 – 1.8	-7.31 – 1.44	-6.29 – 5.51	-5.4 – 1.8	-5.21 – 1.31	-7.61 – 0.08	-8.89 – 5.51
	% WAZ <-2	54%	38%	36%	41%	58%	55%	46%	43%	45%
HIV status: 3-179 months	HIV-infected	127 (59%)	112 (53%)	80 (59%)	55 (53%)	48 (57%)	42 (58%)	29 (45%)	21 (34%)	514 (54%)
	HIV- uninfected	56 (26%)	68 (32%)	41 (30%)	42 (40%)	35 (42%)	30 (41%)	34 (53%)	39 (63%)	345 (36%)
	HIV unknown	33 (15%)	31 (15%)	15 (11%)	7 (7%)	1 (1%)	1 (1%)	1 (2%)	2 (3%)	91 (10%)
Outcomes	Discharged	169 (78%)	193 (92%)	125 (92%)	96 (92%)	81 (96%)	70 (96%)	62 (97%)	61 (98%)	857 (90%)
	Demised	5 (2%)	5 (2%)	3 (2%)	5 (5%)	2 (2%)	2 (3%)	2 (3%)	1 (2%)	25 (3%)
	Unknown	42 (19%)	13 (6%)	8 (6%)	3 (3%)	1 (1%)	1 (1%)	0 (0%)	0 (0%)	68 (7%)
	Case fatality proportion (95% CI)	2.3 (0.8 – 5.4)	2.4 (0.8 – 5.5)	2.2 (0.5 – 6.4)	4.8 (0.2 – 11.2)	2.4 (0.3 -8.6)	2.7 (0.3 – 9.9)	3.1 (0.4 – 11.3)	1.6 (0.04 – 9.0)	2.6 (1.7 – 3.9)

Table 2: Number of cases hospitalized for culture-confirmed PTB stratified by age and HIV status

Year		2005	2006	2007	2008	2009	2010	2011	2012	Total
PCV eras		**Period 1 (Pre-PCV era)**				**Period 2 (Transitional PCV era)**		**Period 3 (PCV era)**		**Combined**
3-11 months	HIV-infected	50 (57%)	42 (51%)	22 (48%)	14 (45%)	9 (39%)	5 (33%)	4 (50%)	2 (12%)	148 (48%)
	HIV-uninfected	29 (33%)	26 (32%)	17 (37%)	14 (45%)	13 (57%)	10 (67%)	4 (50%)	13 (77%)	126 (41%)
	HIV unknown	9 (10%)	14 (17%)	7 (15%)	3 (10%)	1 (4%)	0 (0%)	0 (0%)	2 (12%)	36 (12%)
	Total: 3-11 months	88	82	46	31	23	15	8	17	310
12-23 months	HIV-infected	17 (49%)	16 (42%)	8 (33%)	5 (31%)	5 (46%)	1 (14%)	3 (25%)	1 (7%)	56 (36%)
	HIV-uninfected	10 (29%)	18 (47%)	14 (58%)	11 (69%)	6 (55%)	5 (71%)	9 (75%)	13 (93%)	86 (55%)
	HIV unknown	8 (23%)	4 (11%)	2 (8%)	0 (0%)	0 (0%)	1 (14%)	0 (0%)	0 (0%)	15 (10%)
	Total: 12-23 months	35	38	24	16	11	7	12	14	157
24-59 months	HIV-infected	17 (59%)	17 (45%)	15 (65%)	11 (42%)	6 (40%)	10 (63%)	6 (35%)	6 (50%)	88 (50%)
	HIV-uninfected	7 (24%)	14 (37%)	5 (22%)	11 (42%)	9 (60%)	6 (38%)	11 (65%)	6 (50%)	69 (39%)
	HIV unknown	5 (17%)	7 (18%)	3 (13%)	4 (15%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	19 (11%)
	Total: 24-59 months	29	38	23	26	15	16	17	12	176
60-179 months	HIV-infected	43 (67%)	37 (70%)	35 (81%)	25 (81%)	28 (80%)	26 (74%)	16 (59%)	12 (63%)	222 (72%)
	HIV-uninfected	10 (16%)	10 (19%)	5 (12%)	6 (19%)	7 (20%)	9 (26%)	10 (37%)	7 (37%)	64 (21%)
	HIV unknown	11 (17%)	6 (11%)	3 (7%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	21 (7%)
	Total: 60-179 months	64	53	43	31	35	35	27	19	307

Table 3: Incidence of culture-confirmed PTB for Period 1 to Period 3 in children 3-179 months of age

Incidence of PTB[1] (95% CI) [N of PTB cases]				IRR comparing the Pre-PCV and Transitional PCV eras[2] (95% CI)	IRR comparing the Transitional PCV and PCV eras[3] (95% CI)	IRR comparing the Pre-PCV and PCV eras[4] (95% CI)	% reduction and P-value comparing Pre-PCV and PCV eras (95% CI)
	Period 1 (Pre-PCV era) (2005-2008)	Period 2 (Transitional-PCV era) (2009-2010)	Period 3 (PCV era) (2011-2012)				
Overall	47.9 (44.4-51.7) [667]	21.3 (18.1-25.0) [157]	14.8 (12.3-17.6) [126]	0.45 (0.37-0.53)	0.69 (0.55-0.87)	0.31 (0.25-0.37)	69.2 (62.8-74.6); p<0.001
HIV-infected	1,144.9 (1,032.4-1,266.2) [374]	441.2 (355.0-542.1) [90]	223.2 (165.7-294.2) [50]	0.39 (0.31-0.49)	0.51 (0.36-0.71)	0.20 (0.15-0.26)	80.5 (73.8-85.5); p<0.001
HIV-uninfected	15.2 (13.2-17.5) [207]	9.1 (7.0-11.6) [65]	9.9 (7.8-12.5) [73]	0.60 (0.45-0.79)	1.09 (0.78-1.52)	0.65 (0.50-0.85)	34.8 (14.9-50.1); p=0.002
HIV-infected EX[5]	1,313.3 (1,192.6-1,442.7) [429]	446.1 (359.4-547.5) [91]	218.8 (161.9-289.1) [49]	0.34 (0.27-0.43)	0.49 (0.35-0.69)	0.17 (0.12-0.22)	83.3 (77.6-87.6); p<0.001
HIV-uninfected EX[6]	17.5 (15.4-19.9) [238]	9.2 (7.1-11.7) [66]	10.2 (8.0-12.8) [75]	0.53 (0.40-0.69)	1.10 (0.79-1.54)	0.58 (0.45-0.76)	41.8 (24.5-55.1); p<0.001
HIV-uninfected EX[7]	21.6 (19.2-24.2) [293]	9.4 (7.3-11.9) [67]	10.3 (8.1-12.9) [76]	0.43 (0.33-0.57)	1.10 (0.79-1.53)	0.48 (0.37-0.62)	52.1 (38.3-62.8); p<0.001

[1] Number of cases per 100 000

[2] IRR = Incidence risk ratios IRR: Period 1 vs. Period 2

[3] IRR = Incidence risk ratios IRR: Period 2 vs. Period 3

[4] IRR = Incidence risk ratios IRR: Period 1 vs. Period 3

[5] Incidence of PTB in HIV-infected extrapolated proportionally to include HIV-unknown as HIV-infected and HIV-uninfected

[6] Incidence of PTB in HIV-uninfected extrapolated proportionally to include HIV-unknown as HIV-infected and HIV-uninfected

[7] Incidence of PTB in HIV-uninfected extrapolated to include all cases with HIV-unknown as HIV-uninfected

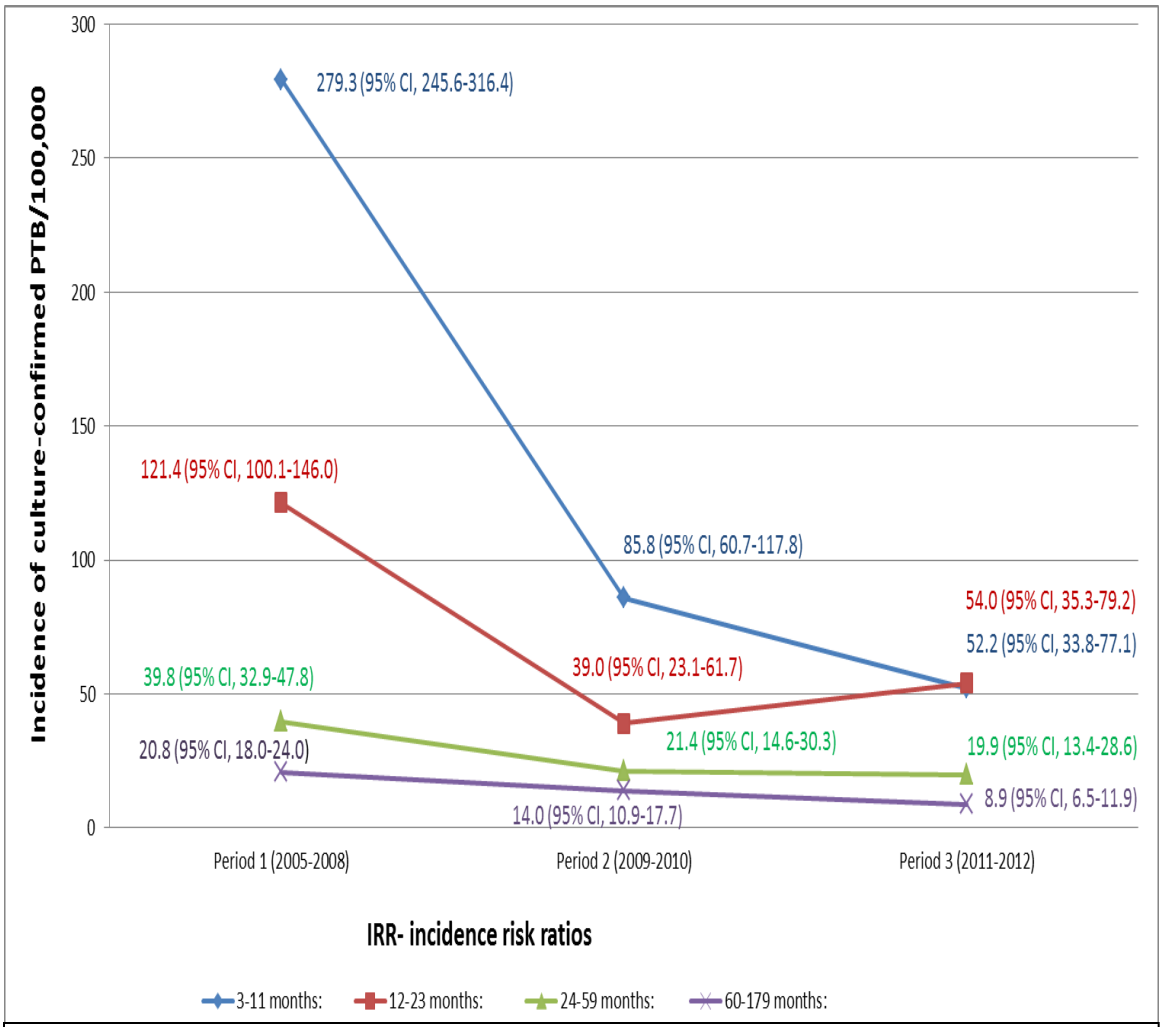
Despite the decline in incidence (per 100 000) of culture-confirmed PTB in HIV-infected children, the incidence remained 22-fold higher in Period 3 (223.2; 95% CI, 165.7-294.2) compared to that observed in HIV-uninfected children (9.9; 95% CI, 7.8-12.5).

3.4 Trends in the incidence of culture-confirmed PTB stratified by age

The decline in culture-confirmed PTB incidence when comparing Period 1 and Period 3 was most marked in the two youngest age categories; i.e. 81.3% (95% CI, 71.8-87.6; p<0.001) in 3-11 months of age and 55.5% (95% CI, 31.8-70.9%; p<0.001) in 12-23 months of age (Figure 2).

The incidence of culture-confirmed PTB in HIV-infected children decreased in all age categories when comparing Periods 1 and 3 (p<0.001) (Figure 3), whereas the decline in HIV-uninfected children was only significant in the 3-11 month age-category; IRR 0.36 (95% CI, 0.21-0.61; p<0.001) (Figure 4).

The incidence of culture-confirmed PTB among HIV-uninfected children in the 12-23 month age category comparing Periods 2 and 3 was similar.



3-11 months: IRR Period 1 vs 2: 0.31 (95% CI, 0.22-0.43; p<0.001); 55.5% (95%CI; 47.0-62.6%)*

IRR Period 2 vs 3: 0.61 (95% CI, 0.37-1.01; p=0.051); 39.1% (95%CI, -0.10-63.2%)*

IRR Period 1 vs 3: 0.19 (95% CI, 0.12-0.28; p<0.001); 81.3% (95% CI, 71.8-87.6%)*

12-23 months: IRR Period 1 vs 2: 0.32 (95% CI, 0.20-0.53; p<0.001); 67.9% (95% CI, 47.2-80.5%)*

IRR Period 2 vs 3: 1.39 (95% CI, 0.76-2.53; p=0.286); 27.8% (95% CI, -31.7-60.4%)*

IRR Period 1 vs 3: 0.45 (95% CI, 0.29-0.68; p<0.001); 55.5% (95% CI, 31.8-70.9%)*

24-59 months: IRR Period 1 vs 2: 0.54 (95% CI, 0.36-0.80; p=0.002); 46.2% (95% CI, 20.1-63.8%)*

IRR Period 2 vs 3: 0.93 (95% CI, 0.56-1.55; p=0.783); 6.9% (95% CI, -54.5-43.9%)*

IRR Period 1 vs 3: 0.48 (95% CI, 0.32-0.73; p<0.001); 51.7% (95% CI, 27.0-68.0%)*

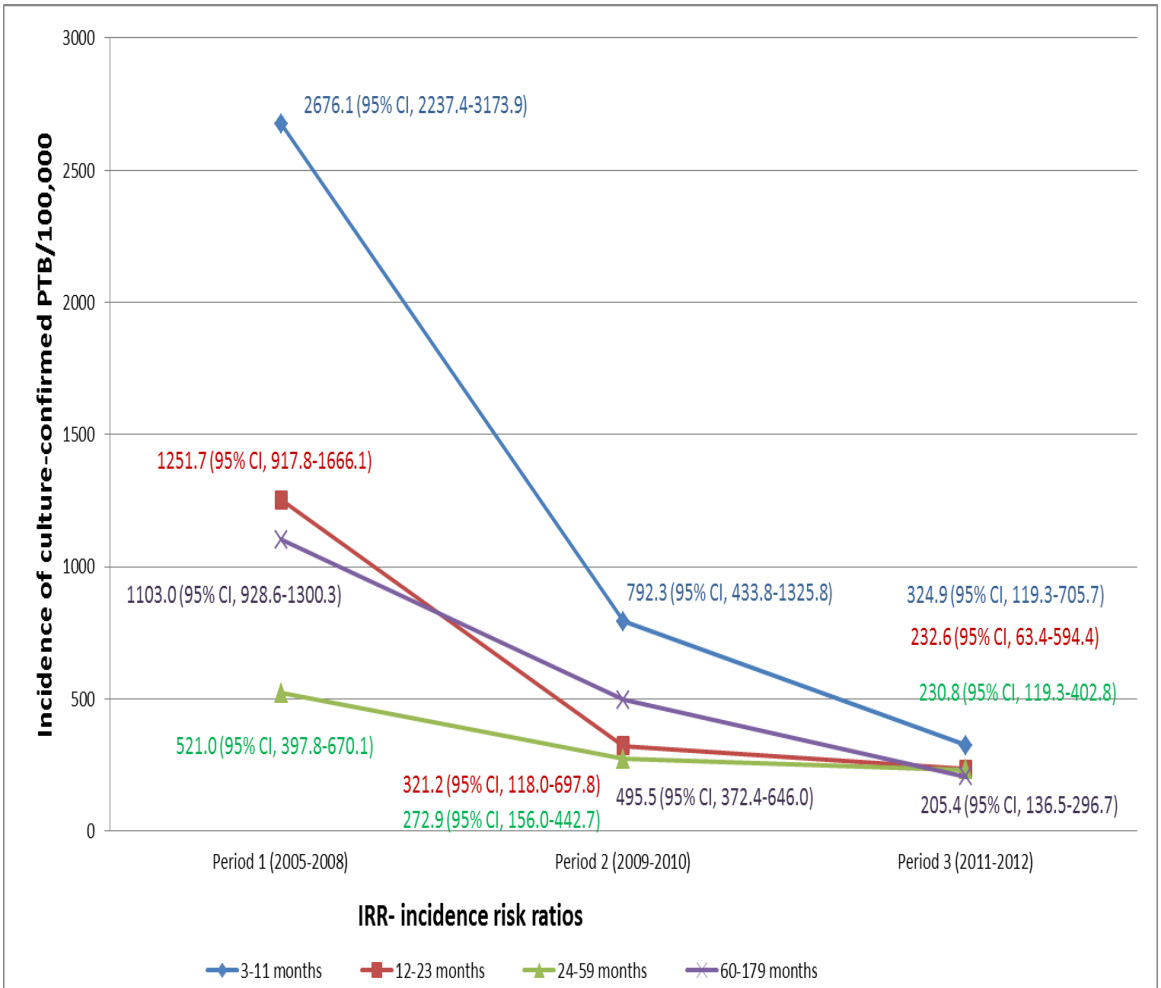
60-179 months: IRR Period 1 vs 2: 0.67 (95% CI, 0.51-0.88; p=0.004); 32.8% (95% CI, 11.6-48.9%)*

IRR Period 2 vs 3: 0.64 (95% CI, 0.44-0.92; p=0.017); 36.3% (95% CI, 7.6-56.1%)*

IRR Period 1 vs 3: 0.50 (95% CI, 0.33-0.75; p<0.001); 49.9% (95% CI, 24.8-66.7%)*

*Percentage reduction

Figure 2: Incidence trends in culture-conformed PTB by PCV-Period: All children



3-11 months: IRR Period 1 vs 2: 0.30 (95% CI, 0.17-0.51; p<0.001); 70.4% (95% CI; 48.7-82.9%)*

IRR Period 2 vs 3: 0.41 (95% CI, 0.16-1.07; p=0.058); 60.0% (95% CI, 64.6-84.2%)*

IRR Period 1 Vs 3: 0.12 (95% CI, 0.05-0.28; p<0.001); 88.0% (95% CI, 73.0-94.6%)*

12-23 months: IRR Period 1 vs 2: 0.26 (95% CI, 0.11-0.60; p<0.001); 74.3% (95% CI, 40.0-89.0%)*

IRR Period 2 vs 3: 0.72 (95% CI, 0.21-2.56; p=0.615); 27.6% (95% CI, -156.1-79.5%)*

IRR Period 1 vs 3: 0.19 (95% CI, 0.07-0.52; p<0.001); 81.4% (95% CI, 48.5-93.3%)*

24-59 months: IRR Period 1 vs 2: 0.52 (95% CI, 0.30-0.91; p=0.019); 47.6% (95% CI, 9.2-69.8%)*

IRR Period 2 vs 3: 0.85 (95% CI, 0.40-1.79; p=0.660); 15.4% (95% CI, -78.6-60.0%)*

IRR Period 1 vs 3: 0.44 (95% CI, 0.24-0.82; p=0.008); 55.7% (95% CI, 17.8-76.2%)*

60-179 months: IRR Period 1 vs 2: 0.45 (95% CI, 0.33-0.61; p<0.001); 55.1% (95% CI, 38.6-67.2%)*

IRR Period 2 vs 3: 0.42 (95% CI, 0.26-0.65; p<0.001); 58.6% (95% CI, 34.6-73.7%)*

IRR Period 1 vs 3: 0.19 (95% CI, 0.12-0.28; p<0.001); 81.4% (95% CI, 72.1-87.6%)*

*Percentage reduction

Figure 3: Incidence trends in culture-conformed PTB by PCV-Period: HIV-infected children



3-11 months: IRR Period 1 vs 2: 0.53 (95% CI, 0.33-0.83; p=0.005); 47.4% (95% CI; 16.7-66.8%)*

IRR Period 2 vs 3: 0.68 (95% CI, 0.37-1.28; p=0.230); 31.7% (95% CI, -28.8-63.5%)*

IRR Period 1 Vs 3: 0.36 (95% CI, 0.21-0.61; p<0.001); 64.1% (95% CI, 39.6-78.6%)*

12-23 months: IRR Period 1 vs 2: 0.42 (95% CI, 0.22-0.80; p=0.007); 58.1% (95% CI, 19.8-78.1%)*

IRR Period 2 vs 3: 1.91 (95% CI, 0.93-3.93; p=0.075); 47.6% (95% CI, (-8.1-74.6%)*

IRR Period 1 vs 3: 0.80 (95% CI, 0.49-1.32; p=0.377); 20.0% (95% CI, -31.5-51.4%)*

24-59 months: IRR Period 1 vs 2: 0.93 (95% CI, 0.52-1.64; p=0.790); 7.5% (95% CI, -64.4-47.6%)*

IRR Period 2 vs 3: 0.99 (95% CI, 0.51-1.94; p=0.978); 0.9% (95% CI, -94.0-49.4%)*

IRR Period 1 vs 3: 0.92 (95% CI, 0.52-1.63; p=0.770); 8.3% (95% CI, -62.8-48.4%)*

60-179 months: IRR Period 1 vs 2: 0.96 (95% CI, 0.52-1.75; p=0.880); 4.5% (95% CI, -74.6-47.8%)*

IRR Period 2 vs 3: 1.04 (95% CI, 0.52-2.05; p=0.921); 3.4% (95% CI, -91.2-51.2%)*

IRR Period 1 vs 3: 0.99 (95% CI, 0.55-1.79; p=0.978); 1.2% (95% CI, -78.6-45.3%)*

*Percentage reduction

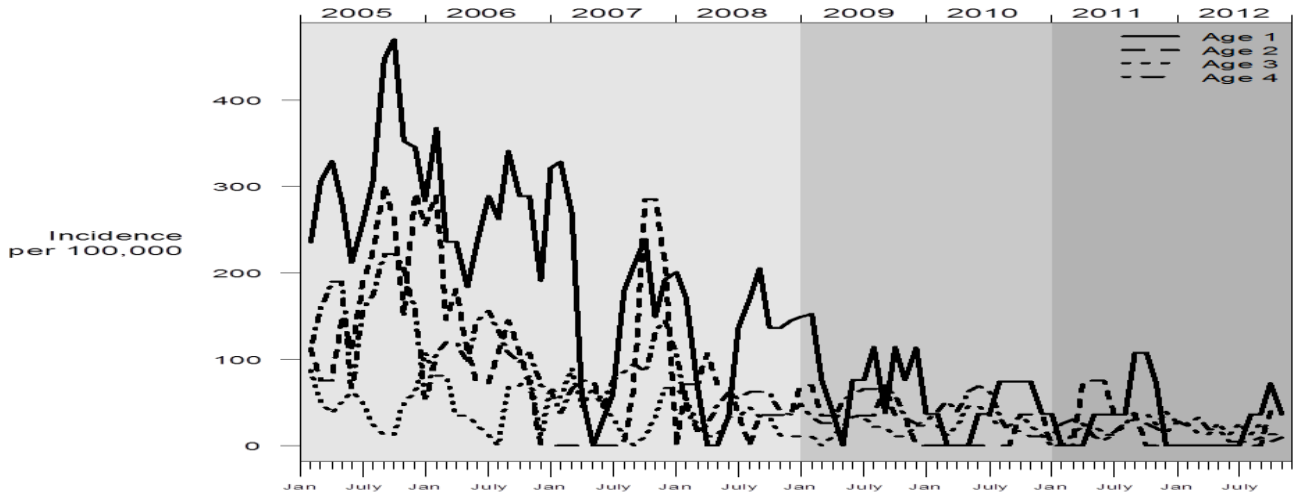
Figure 4: Incidence trends in culture-conformed PTB by PCV-Period: All children: HIV-uninfected children

3.5. The 3-term moving average and regression analysis of incidence of culture-confirmed TB in HIV-infected and -uninfected children stratified by age (Figure 5)

The 3- term moving average showed a decreasing trend, particularly in the 2 youngest age-categories, in the incidence of culture-confirmed PTB in hospitalized children in HIV-infected and –uninfected children.

Although the regression analysis showed a significant decline in the incidence of culture-confirmed TB from 2005 to 2009 in age-groups 3 months to <2 years and 2 years to <5 years as previously reported (8), the low incidence of culture-confirmed PTB at the time of PCV introduction precluded any further analysis being undertaken with regard to the association of PCV introduction on the year-on-year decline in hospitalization for culture-confirmed PTB in HIV-infected (Figure 5) and HIV-uninfected children of all age-groups.

(A)



(B)

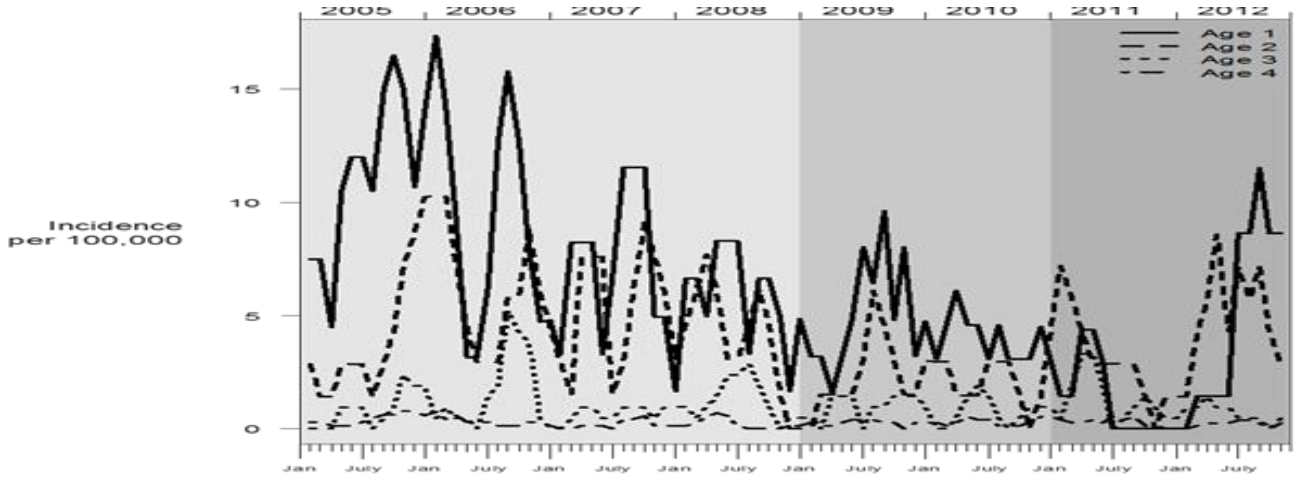


Figure 5: Three-term moving average showing the incidence of culture-confirmed TB in HIV-infected (A) and -uninfected (B) children stratified by age

Legend: Age 1: 3-11 months

Age 2: 12-23 months

Age 3: 24-59 months

Age 4: 60-179 months

3.6 Immunological classification in HIV-infected children hospitalized with PTB (Table 4)

Three hundred and eighty nine (76.0%) of the 514 HIV-infected cases had CD4+ lymphocyte counts done within three months of PTB diagnosis. Two hundred and sixty eight (68.9%) of these patients were categorized as severely immunocompromised. The number of HIV-infected children with available CD4+ counts increased significantly during the study time-period (test-for-trend, p=0.039), however, the proportion of severely immunocompromised children was similar across the eight year period. The mean CD4 percentage was 14% (Standard Deviation [SD], 10%).

Table 4: WHO immunological classification* for established HIV infection in children hospitalized for culture-confirmed PTB

Year	PTB cases	HIV-infected (% of PTB cases)	HIV-infected with available CD4 results (% of HIV-infected PTB cases)	Degree of Immunosuppression*			
				Not significant	Mild	Advanced	Severe
2005	216	127 (58.8%)	90 (70.9%)	9 (10.0%)	6 (6.7%)	10 (11.1%)	65 (72.2%)
2006	211	112 (53.1%)	77 (68.8%)	9 (11.7%)	3 (3.9%)	10 (13.0%)	55 (71.4%)
2007	136	80 (58.8%)	55 (68.8%)	3 (5.5%)	3 (5.5%)	9 (16.4%)	40 (72.7%)
2008	104	55 (52.9%)	43 (78.2%)	3 (7.0%)	2 (4.7%)	10 (23.3%)	28 (65.1%)
2009	84	48 (57.1%)	46 (95.8%)	4 (8.7%)	3 (6.5%)	4 (8.7%)	35 (76.1%)
2010	73	42 (57.5%)	33 (78.6%)	3 (9.1%)	3 (9.1%)	9 (27.3%)	18 (54.5%)
2011	64	29 (45.3%)	25 (96.2%)	6 (24.0%)	2 (8.0%)	3 (12.0%)	14 (56.0%)
2012	62	21 (33.9%)	20 (95.2%)	4 (20.0%)	0 (0.0%)	3 (15.0%)	13 (65.0%)
Total	950	514 (54.1%)	389 (75.7%)	41 (10.5%)	22 (5.7%)	58 (14.9%)	268 (68.9%)

- WHO immunological classification for established HIV infection (CD4% or absolute number per mm3) (43) (Appendix 3, page 41)

3.7 Culture-confirmed PTB with concurrent bacterial infections (Table 5)

Eight hundred and sixty two (90.7%) of the 950 children with culture-confirmed PTB had blood cultures done at the time of their admission to hospital (Table 5). Concurrent bacteraemia was identified among 37 (6.2%) of 594 cases, five (3.4%) of 146 cases and three (2.5%) of 122 PTB cases in Periods 1, 2 and 3, respectively. The proportion of non-contaminated blood cultures with significant isolates decreased during the study time-period (test-for-trend, p=0.012). In period 1, 28 (80%) of the 37 cases were HIV-infected compared to 4 (80%) out of 5 cases in period 2 and 3 (100%) of 3 cases in period 3.

Of the 45 patients with significant bacteremia, 34 (75.6%) were HIV-infected and the isolates (Table 6) were *S. pneumoniae* (n=8), Methicillin-sensitive *Staphylococcus aureus* (n=6), Methicillin-resistant *S. aureus* (n=1), *Enterococcus faecalis* (n=2), *Streptococcus bovis* (n=1), *Escherichia coli* (n=5), *Salmonella spp* (n=5), *Haemophilus influenzae* (n=3), extended-spectrum ß-lactamase (ESBL) producing *Klebsiella pneumoniae* (n=1), *Shigella flexneri* (n=1) and dual *Klebsiella pneumoniae* and *Proteus mirabilis* infection (n=1). Eight (17.7%) patients with significant bacteremia were HIV-uninfected and 3 (6.7%) were HIV-unknown.

The odds of having a blood culture positive for pneumococcus was 1.91-fold (95% CI, 0.26-84.56) greater in the Pre-PCV era, p=1.000 (exact). The numbers were too small to show statistical significance, although the trend was for reduced pneumococcal bacteraemia in the PCV era.

Table 5: Admission blood culture results of cases hospitalized with culture-confirmed PTB

Year		**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**Total**
PCV eras		**Period 1 (Pre-PCV era)**				**Period 2 (Transitional PCV era)**		**Period 3 (PCV era)**		**Combined**
TOTAL		216	211	136	104	84	73	64	62	950
Negative		154 (71.3%)	135 (64.0%)	92 (67.6%)	76 (73.1)	56 (66.7%)	52 (71.2%)	52 (81.3%)	49 (79.0%)	666 (70.1%)
Blood culture not done		18 (8.3%)	28 (13.3%)	15 (11%)	12 (11.5%)	5 (6.0%)	6 (8.2%)	1 (1.6%)	3 (4.8%)	88 (9.3%)
Significant blood culture isolates		14 (6.5%)	14 (6.6%)	6 (4.4%)	3 (2.9%)	3 (3.6%)	2 (2.7%)	2 (3.1%)	1 (1.6%)	45 (4.7%)
Gram-positive organisms	*Streptococcus pneumoniae*	4	3	2	0	1	0	1	0	11
	Staphylococcus aureus	2	1	1	0	1	0	0	1	6
	Methicillin-resistant *Staphylococcus aureus*	1	0	0	0	0	0	0	0	1
	Enterococcus faecalis	0	0	1	1	1	0	0	0	3
	Streptococcus bovis	0	0	0	1	0	0	0	0	1
	Staphylococcus aureus + *Enterococcus faecalis*	1	0	0	0	0	0	0	0	1
Gram-negative organisms	*Escherichia coli*	2	5	1	0	0	0	0	0	8
	Salmonella spp	3	2	1	0	0	0	0	0	6
	Haemophilus influenzae	1	1	0	1	0	0	0	0	3
	Klebsiella pneumoniae ESBL*	0	0	0	0	0	0	1	0	1
	Moraxella catarrhalis	0	0	0	0	0	1	0	0	1
	Shigella flexneri	0	0	0	0	0	1	0	0	1
	Neisseria meningitidis	0	1	0	0	0	0	0	0	1
	Klebsiella pneumoniae + *Proteus mirabilis*	0	1	0	0	0	0	0	0	1

Presumed Contaminants	30 (13.9%)	34 (16.1%)	23 (16.9%)	13 (12.5%)	20 (23.8%)	13 (17.8%)	9 (14.1%)	9 (14.5%)	151 (15.9%)
Coagulase negative staphylococcus	19	23	14	5	14	7	6	3	91
Bacillus spp	3	4	1	2	1	2	1	1	15
Corynebacterium spp	1	2	3	2	3	1	2	0	14
Micrococcus spp	3	3	1	2	0	0	0	1	10
Viridans streptococci	2	1	2	2	0	1	0	1	9
Streptococcus salivarius	0	0	0	0	0	1	0	0	1
Lactobacillus spp	0	0	0	0	0	0	0	1	1
Viridans streptococci + Coagulase negative staphylococcus	1	0	1	0	0	0	0	1	3
Coagulase negative staphylococcus + *Corynebacterium* spp	0	1	0	0	1	0	0	0	2
Enterococcus faecium + Coagulase negative staphylococcus	0	0	0	0	0	1	0	0	1
Acinetobacter baumannii + Coagulase negative staphylococcus	0	0	0	0	0	0	0	1	1
Staphylococcus aureus + *Bacillus* spp	0	0	0	0	1	0	0	0	1
Methicillin-resistant *Staphylococcus areus* + Viridans streptococci	0	0	1	0	0	0	0	0	1
Viridans streptococci + *Bacillus spp*	1	0	0	0	0	0	0	0	1

* ESBL = extended-spectrum ß-lactamase

4.0 DISCUSSION

This study demonstrates a significant reduction in the incidence of hospitalization for culture-confirmed PTB in Sowetan children. This included a 69.2% (95% CI, 62.8-74.6) reduction in hospitalization for culture-confirmed PTB in children 3-179 months of age in Period-1 compared to Period-3 (Table 3). When analysing the data stratified by HIV status, the decline was greatest in HIV-infected children across all age groups, particularly the 3-11 month age category (Figure 3). Although there was a decline in the incidence of hospitalization with culture-confirmed PTB in all age categories in HIV-uninfected children, this was only statistically significant in the 3-11 month age category (Figure 4). The improved access to ART in HIV-infected children could be the major factor that contributed to the reduction of culture-confirmed PTB these children (8). Although we had hypothesized that the implementation of PCV could have affected the incidence of PTB hospitalization, the incidence of culture confirmed PTB prior to the introduction of PCV had been reduced to low rates, which limited any robust measure as to whether there was a temporal association between PCV immunization and incidence of culture-confirmed PTB hospitalization in our study setting. We did, however, observe a trend toward lower prevalence of concurrent bacteremia over the study period, the most common pathogen of which was *S. pneumoniae* in the pre-PCV era and which was uncommon in the PCV era.

The only comparative study that has looked at the incidence of hospitalization of culture-confirmed PTB associated with PCV (9-valent formulation) vaccination was a vaccine probe study conducted in Soweto from 1997-2003 (25). This vaccine probe

study indicated that hospitalization for culture-confirmed PTB was 43% (95% CI, 9.7-65.1%) less likely among children vaccinated with PCV compared to placebo recipients. Furthermore, the decline in culture-confirmed PTB hospitalization was significant only in HIV-infected children (25). Reductions in hospitalization for culture-confirmed PTB in the context of access to PCV suggest that pneumococcal pneumonia is a precipitating illness which prompts admission to hospital in children with PTB residing in high-burdened TB settings. Immunologic perturbations associated with TB may predispose affected children to developing bacterial community acquired pneumonia (25, 30, 31).

In comparison to the vaccine-probe study, our study also showed a significant reduction in culture-confirmed PTB in HIV-uninfected children in the 3-11 month age group. The 3-11 month age category was the category with the highest burden of hospitalization for culture-confirmed PTB in Period 1 but also had the largest reduction in the incidence of hospitalization for culture-confirmed PTB when comparing Period 1 and Period 3 (Figures 2, 3 and 4). Children in the 3-11 month age category were programmatically targeted to have received three doses of PCV (at ages 6 and 14 weeks, and 9 months of age) according to the EPI, and introduction of this vaccine was a major public health intervention between Period 1 and Period 3. The youngest child vaccinated with the third dose of PCV in South Africa would have been 3 years and 8 months old on the 31st December 2012 (the cut-off date for inclusion in the analysis of the present study), as PCV was introduced in the EPI in April 2009. The national coverage of the third dose of PCV in South Africa was reported by WHO to be 10% in 2009, increasing to 89% in 2011 (38). This suggests that by 2011 and 2012, only in the 3-11 month age category

would the majority of children have been fully vaccinated. Of note, there was no catch-up campaign of PCV immunisation at the time of introduction of PCV7 into the South African EPI in 2009. Therefore, the true impact of PCV on hospitalizations for culture-confirmed PTB would not be seen in the older age categories using the time scale cut-offs of the present analysis.

It is well documented that ART reduces the incidence of TB in HIV-infected children (7-10). In 2010 the South African Department of Health implemented further improvements in HIV prevention and care programs in the country. These included changes to the PMTCT program as well as starting all HIV-infected infants on ART regardless of immunologic staging of disease across the country, including our study population (45). A study conducted at CHBAH showed that the up-scaling of the ART program in South Africa has been associated with a significant decline in the incidence of culture-confirmed TB between 2005 and 2009, more so in HIV-infected (70.6%; $p < 0.0001$) than HIV-uninfected (41.3%; $p = 0.0003$) children in the era before PCV was introduced into the South African EPI (8), which suggests that access to ART was the main driver of reductions in culture-confirmed PTB hospitalizations. By 2010, a high proportion (94.8%) of children with AIDS in Gauteng were already on ART (32). This would mean that children would have been more immune reconstituted.

Another potential contributing factor for declining culture-confirmed PTB in children would be a reduction in transmission of MTB from adult PTB cases to children in households and the community at large; however, this scenario is unlikely to be

occurring in our setting as the South African TB incidence has continued to escalate over the past number of years (from 940 per 100 000 population in 2006 to 1003 per 100 000 population in 2012) (46, 47).

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The median age at hospitalization in ‘all’ (HIV-infected and HIV-uninfected) children with culture-confirmed PTB increased over time from 2005 to 2010/11, and then decreased. This is an expected finding, as the decline in culture-confirmed PTB was greatest in infants, most probably due to ART initially from 2005, accelerated by PCV from 2009 onwards. The subsequent decrease in median age at hospitalisation with culture-confirmed PTB in 2011 and 2012 may be due to improved management of older HIV-infected children with ART.

Significant blood culture yields were observed in 4.7% of children hospitalized with culture-confirmed PTB (Table 5). This was lower than in a previous South African study on severe pneumonia, which reported bacteremia in 7% of HIV-uninfected children with severe pneumonia and 15% of HIV-infected children in the pre-ART, pre-PCV era (4). This could be due to pneumococcal infections that were not being prevented in that study prior to PCV immunization, as the pneumococcus constituted the majority (10.1%) of blood culture isolates (4). Although the trend was for reduced pneumococcal bacteraemia in the PCV era, the numbers were too small to show statistical significance. There was also a decline in all significant blood culture isolates from 2005 (6.5%) to 2012 (1.6%) (Table 5). Therefore, other factors could have also influenced this decline. Studies in developed countries have reported bacteremia in 2-4% of childhood

acute pneumonia episodes (51, 52). Although blood culture yield is low, a significant blood culture result is indicative of the bacterial pathogen contributing to infection and could guide appropriate antibiotic therapy. The high blood culture contamination rates observed in our study (15.9% overall) may have impaired the identification of significant pathogens.

HIV-infected children with low CD4 counts are at increased risk of hospitalization with culture-confirmed PTB. The mean CD4 percentage in this study was 14% (SD, 10%) and 69% of all HIV-infected children hospitalized for culture-confirmed PTB in our study were severely immunocompromised (Table 4). Other studies have also reported low CD4 counts in HIV-infected children with culture-confirmed TB, with mean CD4 percentages of 21% (SD, 11%) (48).

The case fatality rate in our study (2.6%; 95% CI, 1.7-3.9%) was similar to case fatality rates (2.3%; 95% CI, 1·6–3·4) for severe acute lower respiratory tract infections in children under-5 years of age treated in developing countries, and in children with TB in another African study (3.9%) (49, 50).

4.1 Study Limitations

An important limitation in this study is that the incidence of culture confirmed PTB prior to the introduction of PCV had been reduced to low rates, which hampered any robust measure as to whether there was a reduction of culture-confirmed PTB prior to the introduction of PCV into the South African EPI due to improved access to ART.

Another limitation in this study is the possible disadvantages of the retrospective ecological study design that makes it difficult to detect complicated exposure-outcome relationships and may show association at the population level which may not exist at the individual level.

A further limitation of this study is that we have not taken into account whether access to INH preventive treatment (IPT) as prophylaxis against TB in children at risk who have a close contact with PTB, has increased over the study time frame. If access to and utilization of IPT has increased in the recent past in the study setting, this would be expected to have decreased the burden of paediatric culture-confirmed PTB in the study setting. A Cape Town study showed that IPT may offer additional protection against TB in immuncompromised HIV-infected children on ART (53), however, another study failed to show any benefit of primary IPT in improving TB disease-free survival among HIV-infected children or TB infection-free survival among HIV-uninfected children immunized with BCG vaccine (54). The uptake of IPT as part of the integrated preventative approach amongst TB-exposed household contacts, including children of newly diagnosed infectious PTB cases, is suboptimal at best in high-burdened settings in South Africa (55).

Another parameter which may cloud the intended outcomes of the study would have been altered clinician-initiated investigation of children with suspected PTB at the hospital. We cannot with confidence exclude a reduced detection of culture-confirmed

PTB cases in the latter stage of the study due to changes in clinical practice which may have resulted in fewer children with suspected PTB being sampled by submission of gastric washings. This is because we did not match number of gastric washings submitted per individual case of suspected PTB to the numbers of gastric washings submitted per year. However, there were 61 to 86 gastric washings submitted per 100 paediatric admissions between 2005 and 2012; p=0.614 (test-for-trend) and 1.6 to 2.7 gastric washings submitted per LRTI admitted between 2005 and 2012; p=0.488 (test-for-trend). As there were no significant trends towards a reduction in the number of gastric washings submitted per 100 paediatric admissions to the hospital, or the numbers of gastric washings submitted per paediatric LRTI case in the study time period, it is reasonable to assume that significant changes in clinical practice did not occur at the institution between 2005 and 2012.

5.0 CONCLUSION

There has been a significant decline in culture-confirmed PTB hospitalization in children comparing the pre-PCV to the PCV-era. However, the incidence of culture-confirmed PTB prior to the introduction of PCV had been reduced to low levels due to antiretroviral therapy. This confounder together with the retrospective ecological study design and other several possible confounders limited any robust estimate as to whether there was a temporal association between PCV immunization and incidence of culture-confirmed PTB hospitalization in our study setting. We did, however, observe a trend toward lower prevalence of concurrent bacteremia over the study period, the most common pathogen of which *was S. pneumoniae* in the pre-PCV era and which was uncommon in the PCV era.

6.0 RECOMMENDATIONS

This study might not have shown the full impact of PCV on culture-confirmed PTB, as we focused on the initial years after PCV was introduced in this analysis. It will be useful to reappraise the impact of PCV on hospitalization for culture-confirmed PTB over a more extended period which incorporates an experience since the roll out of PCV13.

7.0 Appendix

Appendix 1: Percentage of children with Acquired Immunodeficiency Syndrome that were on Antiretroviral Therapy in Gauteng (32)

	2005	2006	2007	2008	2009	2010	2011	2012
Total number of children with AIDS	**6709**	**8911**	**11884**	**14176**	**17867**	**21599**	**24519**	**26812**
Number of children with AIDS, on ART	2721	5782	8133	11142	16859	20469	23435	25772
Number of children with AIDS, not on ART	3988	3129	3751	3034	1008	1130	1084	1040
Percentage (%) of children with AIDS, on ART	**40.6**	**64.9**	**68.4**	**78.6**	**94.4**	**94.8**	**95.6**	**96.1**

Footnotes

AIDS: Acquired Immunodeficiency Syndrome

ART: Antiretroviral Therapy

Appendix 2: Data extraction form

a. Date of admission
b. Patient identification number
c. Age
d. Gender
e. HIV status
f. Date of HIV result
g. CD4+ count (absolute count and percentage)
    i. Date of CD4+ count
h. HIV viral load (RNA copies per milliliter and log values)
i. Date of viral load
j. TB result
    i. Smear microscopy
    ii. Culture
    iii. Susceptibility
    iv. Date of TB result
k. TB specimen type: gastric aspirate, expectorated sputum, induced sputum or broncho-alveolar lavage
l. Nutritional assessment
    i. Weight on admission (kg)
    ii. Length on admission (cm)
    iii. Oedema (Yes/No)
m. Discharge Diagnosis
n. ART status (for HIV-infected children)
    i. On ART: Yes/No
    ii. ART Regimen used
    iii. Date of initiation of ART
o. Blood culture result
    i. Date of blood culture
p. Nasopharyngeal swab result
    i. Date of nasopharyngeal swab
q. TB Classification: Confirmed/Probable/Possible
r. Patient outcome: discharged/died
    i. Date of patient outcome
s. Started on TB treatment before discharge from hospital? Yes/No

Appendix 3: WHO immunological classification for established HIV infection (CD4% or absolute number per mm3) (43)

	Not significant	Mild	Advanced	Severe
<11 months	>35%	30-35%	25-29%	<25%
12-35 months	>30%	25-30%	20-24%	<20%
36-59 months	>25%	20-25%	15-19%	<15%
>5yrs	>500	350-499	200-349	<200/<15%

Appendix 4: Population denominators and HIV prevalence by age-group in Soweto, South Africa for years 2005 to 2012

PCV Vaccination Era	Year	Age	Total population[1]	HIV prevalence[2]
Pre-PCV	2005	<12 months	23640	6.00%
		12-23 months	24154	3.67%
		24-59 months	72490	3.67%
		60-119 months	115649	1.66%
		120-167 months	101335	0.18%
Pre-PCV	2006	<12 months	22382	5.68%
		12-23 months	23526	3.91%
		24-59 months	73271	3.91%
		60-119 months	120787	2.04%
		120-167 months	104776	0.30%
Pre-PCV	2007	<12 months	21326	5.24%
		12-23 months	22830	4.09%
		24-59 months	72977	4.09%
		60-119 months	124359	2.42%
		120-167 months	109994	0.47%
Pre-PCV	2008	<12 months	21077	4.63%
		12-23 months	22578	4.14%
		24-59 months	72620	4.14%
		60-119 months	126511	2.75%
		120-167 months	115290	0.70%
Transitional PCV	2009	<12 months	21627	4,04%
		12-23 months	22794	4,15%
		24-59 months	72358	4,15%
		60-119 months	127464	3,06%
		120-167 months	120454	0,97%
Transitional PCV	2010	<12 months	22660	3,94%
		12-23 months	23342	3,95%
		24-59 months	72431	3,95%
		60-119 months	127835	3,30%
		120-167 months	124897	1,29%
Established PCV	2011	<12 months	23777	3.89%
		12-23 months	24009	3.68%
		24-59 months	72878	3.68%
		60-119 months	128204	3.48%
		120-167 months	128423	1.62%
Established PCV	2012	<12 months	24083	3.83%
		12-23 months	24113	3.47%
		24-59 months	72568	3.47%
		60-119 months	128196	3.53%
		120-167 months	131551	1.95%

[1]The population estimates for region D, Soweto (Johannesburg) as per Statistics South Africa

[2]HIV prevalence in the study population from the AIDS and Demographic models 2008 as per the Actuarial Society of South Africa

Appendix 5: Ethics Clearance Certificate

R14/49 Dr Vijay Mammen

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130109

NAME: (Principal Investigator)	Dr Vijay Mammen
DEPARTMENT:	Department of Paediatrics CH Baragwanath Academic Hospital
PROJECT TITLE:	Impact of Pneumococcal Conjugate on Culture-Confirmed Pulmonary Tuberculosis in Hospitalized Children
DATE CONSIDERED:	25/01/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shabir Madhi
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	25/01/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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