THE IMPACT OF ROUTINE PNEUMOCOCCAL CONJUGATE IMMUNISATION ON BACTERIAL MENINGITIS IN SOWETAN CHILDREN

– A TIME-SERIES ANALYSIS.

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DECLARATION

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

…….

The ……..day of ……., 2013

DEDICATION

I dedicate this work to the generations who have travelled this path before me:

Dr F J Hauptfleisch MBChB (Edin) 1916

Dr P C Hauptfleisch MBChB (UCT) 1947

Dr C J Hauptfleisch MBBCh (Wits) 1987

ABSTRACT

Introduction

Invasive pneumococcal disease, including meningitis, caused by *Streptococcus pneumoniae* is a major cause of morbidity and mortality world-wide. The introduction of pneumococcal polysaccharide-protein conjugate vaccine (PCV) in the United States has resulted in a reduction in incidence of pneumococcal meningitis. PCV was introduced into the South African expanded programme on immunization (EPI) in 2009.

Objective

We evaluated the temporal association which the introduction of PCV into the South African EPI had on the incidence of pneumococcal meningitis in children.

Methods

The study was undertaken in Soweto. All children admitted to Chris Hani Baragwanath Academic Hospital (CHBAH) <14 years of age with meningitis from January 2006 to November 2011 were identified through an electronic database and their microbiological records reviewed to identify the causative bacteria. The results were time framed into two groups: prior to introduction of PCV 1 January 2006 to 31 March 2009 (pre-vaccine era) and post PCV-introduction 1 April 2009 to 30 November 2011 (post-vaccine era).

Results

783 patients were admitted with suspected meningitis during the study period, of these 243 (31.0%) met the criteria for bacterial meningitis. The incidence of pneumococcal meningitis was decreasing in the CHBAH in-patient paediatric population by 4.7% per annum prior to the introduction of the vaccine in April 2009. The decline in incidence after PCV introduction

accelerated to 18% per annum post-vaccine introduction (P=0.391). In the population most at risk for pneumococcal meningitis, children <1 year of age, the annual reduction in incidence of pneumococcal meningitis accelerated from 1.1% in the pre-vaccine era to 43.4% following PCV introduction (P= 0.011).

Conclusions

The introduction of PCV resulted in a decline in the incidence of pneumococcal meningitis in all age groups. This decline was most dramatic in the <1 year age group.

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ABBREVIATIONS

ART	Antiretroviral therapy
BHI	Brain-heart infusion broth
CHBAH	Chris Hani Baragwanath Academic Hospital
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded programme on immunisation
HIV	Human immunodeficiency virus
HREC	Human Research Ethics Committee
IPD	Invasive pneumococcal disease
MCV1	First dose of measles-containing vaccine
NHLS	National Health Laboratory Service
PCR	Polymerase chain reaction
PCV	Pneumococcal polysaccharide-protein conjugate vaccine
RMPRU	Respiratory & Meningeal Pathogens Research Unit
WHO	World Health Organization

1.0 Introduction

1.1 Background

Bacterial meningitis is an important acute infectious disease of childhood that remains a source of substantial morbidity and mortality [1]. A meta-analysis of bacterial meningitis reported an overall case fatality rate of 4.5% [2], and the World Health Organization (WHO) estimates that bacterial meningitis causes up to 263,000 deaths in low-income countries annually [3].

One of the most important causes of bacterial meningitis, in the absence of pneumococcal polysaccharide-protein conjugate vaccine (PCV), is *Streptococcus pneumoniae*, a pathogen that affects children and adults worldwide. In 2005, the WHO estimated that 0.7-1.0 million children under five years of age, mostly in developing countries, died of pneumococcal disease annually [4]. Data collected through the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) confirms that the age group at highest risk of invasive pneumococcal disease (IPD) in the South African setting are infants under one year of age [5].

A systematic literature review in 2009 estimated the global incidence of pneumococcal meningitis in children to be 17 cases per 100,000 [6]. In the United States, the estimated overall annual incidence of pneumococcal meningitis prior to PCV immunization was 1-2 cases per 100,000, with the highest incidence among children 6-24 months of age (3-30 per 100,000, with a peak of 30 per 100,000 in those 3-5 months of age) [7]. The extent of the burden of meningitis is also reflected by the high case fatality rate in the USA (19%) for pneumococcal meningitis, where it contributed to 7,600 of 40,000 annual deaths due to invasive pneumococcal disease (IPD) prior to PCV-immunization [7]. In South Africa

specifically, the case fatality rate was 19% in the period 1991-1992 [8]. This has been reflected locally in a review of all bacterial meningitis at Kalafong Hospital over the period 1990-1995 in which the morbidity and case fatality rate (9.8%) were greatest for *S. pneumoniae* meningitis [9]. A survey of meningitis throughout Africa found a case fatality rate of 45% for pneumococcal meningitis and long term sequelae in 50% of surviving cases [10]. Young children are particularly vulnerable to bacterial meningitis, possibly due to immaturity of their immune systems, and also have poorer outcomes [10]. To assess the extent of sequelae a long term retrospective study of children treated for bacterial meningitis was undertaken in the 1980's in the United States, which reported that 8.5% of children surviving bacterial meningitis had major neurological deficits (e.g. mental retardation, seizures, hydrocephalus, cerebral palsy, blindness, hearing loss), and 18.5% had learning difficulties and developmental problems which persisted up to 12 years after the initial episode [2]. The same neurological sequelae were evident in a literature review on bacterial meningitis in African children [10].

Human immunodeficiency virus (HIV) infection is a major risk factor for IPD due to impairment of both cell mediated and humoral immunity. The introduction of antiretroviral therapy (ART) in South Africa in 2004 caused a marked reduction in the incidence of IPD in HIV-infected children. In Gauteng Province, the overall estimated coverage with ART in HIV-infected children requiring treatment increased from 21.5% in 2004 to 83.4% in 2009 [11]. However, even in the era of ART management of HIV-infected children, they remain at a 20-fold greater risk for IPD compared to the general population [12]. The efficacy of PCV in preventing IPD in HIV-infected children was observed to be 53% compared to 42% in HIV-uninfected children [13].

1.2 Vaccine Review

In February 2000, a 7-valent PCV (PCV7:Prevenar®, Wyeth Lederle Vaccines) was licensed by the Food and Drug Administration for use among infants and young children in the United States [7]. In 2007, the WHO recommended that all countries include PCVs in their national immunization schedules [4]. A systematic review of pre- and post-licensure data did not identify major safety problems with PCV7 or other PCV formulations [7].

Although there are 93 different pneumococcal serotypes, the serotypes included in PCV7 are those most commonly found to cause IPD, namely serotypes 4, 6B, 9V, 14, 18C, 19F, 23. These are collectively responsible for 58-66% of all IPD worldwide [14]. 70% of IPD cases in children under the age of 5 years in South Africa are due to the serotypes contained in PCV7. PCV13 extends this cover, by including six additional serotypes (1, 3, 5, 6A, 7F, 19A). In the pre-vaccine era, pneumococcal serotypes included in PCV13 were responsible for approximately 85% of under-5 IPD episodes in South Africa [15].

In 2009, the Cochrane Collaboration undertook a systematic review of PCV randomised controlled trials to evaluate the efficacy of PCVs against IPD and pneumonia in both industrialised and developing countries. They concluded these vaccines to be efficacious in protecting against vaccine-serotype specific IPD [16].

Studies in the United States have reported marked reductions in the incidence of IPD and pneumococcal meningitis following the introduction of PCV7. This reduction is not only through the direct protection of immunised children, but also by protecting non-immunised children and adults through an indirect (or 'herd') effect, resulting from interruption of transmission of vaccine-serotype pneumococci from immunised young children to other non-immunised individuals [1]. In 2001, the rate of IPD among children under 2 years of age was 69% lower than in 1998 and 1999 in the United States [17] and the incidence of

pneumococcal meningitis in children <2years of age decreased by 64% between these periods [1].

Prevenar® was included in the South African Expanded Programme on Immunization (EPI) in 2009 and was available since April 2009 in Gauteng Province. Although the vaccine is registered for a three dose primary series and booster dose in the second year of life, the schedule adopted in the South African EPI was three doses: two primary doses at 6 weeks, 14 weeks and a third dose at 9 months of age. This was done to address in part the waning immunity against IPD which had been observed previously in HIV-infected children when only given a three dose primary series at 6, 10 and 14 weeks of age in the absence of a booster dose [18]. In mid-2011, PCV13 superseded PCV7 in the South African EPI.

In the United States in 2006, PCV7 coverage amongst children 19 to 35 months of age was estimated to exceed 68% for the full vaccine series of four or more doses, and to exceed 87% for three or more doses [1]. The WHO estimated the South African 3rd dose *Haemophilus influenzae* type b vaccine coverage to be around 80% in 2000 [19]. The WHO estimates 1st dose measles-containing vaccine (MCV1, given at 9 months of age) South African coverage rates in 2011 to be 95% in 2011 [20]. It is anticipated that a three dose schedule for PCV7 or PCV13 (given at 6 weeks, 14 weeks and 9 months in South Africa) will have coverage of >90%.

The effectiveness of PCV in immunization programs has exceeded expectations compared to what was observed in efficacy trials [13]. This may be partially due to the indirect effect of childhood PCV immunization. It is anticipated that the inclusion of PCV7 and PCV13 into the South African EPI will have resulted in appreciable declines in IPD, including meningitis caused by vaccine-serotype specific pneumococci, in children in South Africa.

1.3 Hypothesis and Objectives

Null Hypothesis

The introduction of PCV into the South African EPI schedule and Gauteng in April 2009 will not have changed the epidemiology of bacterial meningitis in children residing in Soweto.

Primary Objective

To analyse the impact which the introduction of PCV in the South African EPI has had on confirmed pneumococcal meningitis in children under 1 year of age in Soweto.

Secondary Objectives

- To analyse the impact which PCV has had on the incidence of confirmed pneumococcal and suspected bacterial meningitis in children less than 14 years of age in Soweto.
- To analyse the impact which PCV has had on the epidemiology of non-pneumococcal culture-confirmed bacterial meningitis in children less than 14 years of age in Soweto.

2. 0 Methods

2.1 Study Population

Chris Hani Baragwanath Academic Hospital (CHBAH) is the only public sector hospital in Soweto, and serves approximately 1.1 million, mainly black urban, South Africans. This includes 120,000 children under five years of age [21]. An estimated 90% of individuals in Soweto requiring hospitalisation are admitted to CHBAH, as less than 10% of the population has medical insurance [11]. Paediatric admissions to CHBAH over the study period (2006-2010) average around 7,000 patients per year (CHBAH Department of Paediatrics ward statistics).

2.2 Meningitis diagnosis at CHBAH

Children (< 14 years of age) with suspected meningitis who present to CHBAH have a lumbar puncture performed to aid in diagnosis as standard-of-care. Being a secondary/tertiary care academic hospital, there is a low threshold for performing lumbar punctures in children, including any child with symptoms and signs of meningism as well as a first episode of febrile convulsions.

Paper-based discharge summaries, which detail the cause and course of hospitalisation in children requiring admission to the general paediatric wards at CHBAH, are entered into an electronic Paediatric Database, with discharge diagnoses coded using the ICD-10 coding system. All discharge summaries dating back to 2006 were coded for diagnoses by two medical doctors. The electronic Paediatric Database is maintained and updated at the Respiratory & Meningeal Pathogens Research Unit (RMPRU) at CHBAH. The completeness

of the paper-based discharge summaries were cross-validated against ward registries of the general paediatric medical wards as well as the acute-care admissions ward at CHBAH, where all admitted children are registered. For subjects with missing discharge summary records, information available from the ward registries which include date of admission, age/date of birth, admission and discharge diagnosis and outcome were summarised and also coded using ICD-10.

All cerebrospinal fluid (CSF) and blood samples from patients at CHBAH are sent to the National Health Laboratory Service (NHLS) laboratory on site. CSF samples are routinely divided into aliquots for cell counts, chemistry and microbiologic testing. If the sample is insufficient, CSF samples are prioritised for visualisation of bacterial organisms with a Gram stain and inoculation of serum broth and agar plates. Second priority is cell count and biochemistry and last is bacterial antigen detection testing using latex agglutination assays.

The cell count is done manually using a Fuchs-Rosenthal counting chamber with all polymorphonuclear cells, lymphocytes and red cells recorded per cubic millimetre. A Giemsa stain is performed if the total white cell count is greater than 100 or if there is difficulty differentiating between polymorphonuclear cells and lymphocytes.

For the culture of specimens, depending on the volume of sample received, the sample is either centrifuged or, if less than 1ml received, put in a vortex mixer. The sediment (bacteria and cells) is used to inoculate the culture media. Culture media used include brain-heart infusion (BHI) broth, blood agar and chocolate agar. All plates are examined daily, for macroscopic evidence of growth up until 72 hours post-inoculation. If bacterial growth is identified, a Gram stain is performed to identify the organism. Pure cultures of organisms isolated from CSF specimens are prepared, and susceptibility to antimicrobial agents is determined using disc diffusion methods and (for pneumococcus), E-tests.

Latex agglutination tests are only done if requested by the clinician who submitted the specimen. The NHLS lab uses the Wellcogen Bacterial Antigen Kit (Wellcome Diagnostics: Product number R30859001, ZL22). The kit provides reagents for the detection of bacterial antigens of *H. influenzae* type b, *Streptococcus agalactiae*, *S. pneumoniae*, *Neisseria meningitidis* A, C, Y and W135, *N. meningitidis* B and *Escherichia coli* K1. The CSF specimen is mixed with latex reagent on a slide and if positive, agglutination will develop within 3 minutes. Positive and negative controls are performed at the same time for quality control purposes.

For requested blood cultures the NHLS lab uses Paediatric BactT/ALERT bottles (Biomerieux: Reference 259794). Isolated pathogens are identified and susceptibility patterns determined using standard laboratory techniques.

2.3 Study Method

All children <14 years of age admitted to CHBAH with confirmed or suspected meningitis from 01 January 2006 until 31 December 2010 were identified by searching against the electronic Paediatric Database using the appropriate ICD 10 codes (Appendix 1, page 33).

Patients identified as having been admitted with confirmed or suspected meningitis during the period under investigation were cross-referenced against the database of the National Health Laboratory Service (NHLS) to identify those children who had meningitis as per the study definitions.

For the purposes of this study, case definitions for bacterial meningitis were as follows:

- Confirmed bacterial meningitis: culture-confirmed bacterial meningitis, regardless of pathogen isolated;

- Probable bacterial meningitis: CSF cultures negative with presence of surrogate indicators of bacterial meningitis on baseline investigation. Surrogate indicators of bacterial meningitis included: CSF white cell count of >100 cells/$mm^3$and at least a protein of >1.0g/l or glucose of <2.2mmol/l [19].
- Cases of tuberculosis meningitis were excluded.

For those patients who met the study case definitions of bacterial meningitis (TB meningitis excluded), data as enumerated in Appendix 2 (page 34) was extracted from the NHLS database.

HIV infection status of children identified as having meningitis was attributed as follows:

- HIV enzyme-linked immunosorbent assay (ELISA) positive in a child over 18 months of age: HIV-infected;
- HIV ELISA positive in a child under 18 months of age, with positive HIV polymerase chain reaction (PCR): HIV-infected;
- HIV ELISA positive in a child under 18 months of age, with negative HIV PCR: HIV exposed, uninfected;
- HIV ELISA positive in a child under 18 months of age, with no HIV PCR: HIV-exposed but status indeterminate;
- No HIV test available, regardless of age group: HIV status undetermined.

For the evaluation of increasing access of the paediatric population to ART as a potential contributor to declining meningitis incidence in HIV-infected children, time periods were stratified according to the coverage of ART within the community as previously reported: 2006 being the intermediate ART era (51% ART coverage for HIV-infected children with AIDS) and 2007 onwards being the established ART era (>74% ART coverage in HIV-infected children eligible for treatment per national guidelines) [12].

To assess the impact that PCV has had on the population most at risk, and who would have benefitted most from the vaccine during the study time frame, the age group under 1 year of age was specifically looked at with the population stratified into <1year and >1year age groups.

2.4 Statistical Analysis

The numerators for bacterial meningitis incidence calculation were the number of meningitis cases identified in each year, and the population denominators were based on mid-year population estimates from the Gauteng Department of Health and Social Development for region D (Soweto) in Johannesburg as per Statistics South Africa report [21], shown in Appendix 3 (page 35).

The HIV prevalence in the study population was estimated from the projections of the AIDS and Demographic models developed by the Actuarial Society of South Africa [21] and tabulated in Appendix 4 (page 36).

Unadjusted data were quoted in the results. However, to account for those cases with an unknown HIV result, we extrapolated our data to include these cases. In this sensitivity analysis, children with unknown HIV status were assigned as being either HIV-infected or HIV-uninfected based on the proportional percentage of known results in the HIV tested group.

X^2 Test (or Fisher's exact test) were used to compare the distribution of categorical variables, and 95% confidence intervals are reported with p-values <0.05 considered as being statistically significant. Demographic data and estimates of incidence and risk ratio were analysed using STATA version 11.0 (StataCorp, College Station, Texas).

A Poisson regression model with terms for quarter and cyclical terms were fitted to the data. To then compare the pre-PCV and post-PCV time periods an additional indicator variable for the period post-vaccine was included. This allowed comparison of the incidence in the two groups and rate ratio calculation.

2.5 Study approval

Permission to conduct the study at CHBAH was obtained from the Medical Advisory Committee at the hospital and also from the NHLS for access to electronic data and laboratory records.

Ethics approval was granted by the Human Research Ethics Committee (HREC), University of Witwatersrand (HREC number: M111176), who granted a waiver for patient consent due to this being a retrospective review of medical records.

3.0 Results

3.1 Overall meningitis

Seven hundred and eighty three children were admitted with suspected meningitis from January 2006 to December 2010, with a male-to-female ratio of 1.55:1. Of the 783 cases, 243 (31%) met the study criteria for bacterial meningitis, among whom the male-to-female ratio was 1.36:1. The median age of the children who met the study criteria was 8.8 months (Interquartile Range [IQR], 0 – 159.4 months).

Of the 243 with bacterial meningitis, 116 (48%) were HIV-uninfected, 82 (34%) were HIV-infected, and 45 (18%) had undetermined HIV status.

Table 1: Demographic data of children with meningitis

	Suspected meningitis	**Bacterial meningitis**
Total	783	243
Age		
Mean (months)	36.3 (SD 45.8 months)	32.0 (SD 43.6 months)
Median (months)	13.6 (IQR 3.0 – 58.8 months)	8.8. (IQR 0 – 159.4 months)
Gender		
Male	476 (61%)	140 (58%)
Female	307 (39%)	103 (42%)
HIV Status		
HIV-uninfected	508 (65%)	116 (48%)
HIV-infected	159 (20%)	82 (34%)
HIV status undetermined	116 (15%)	45 (18%)

Culture-positive CSF findings are tabulated in Table 2. This identified that *S. pneumoniae* contributed towards 55% of all culture-confirmed meningitis cases in the 5-year period.

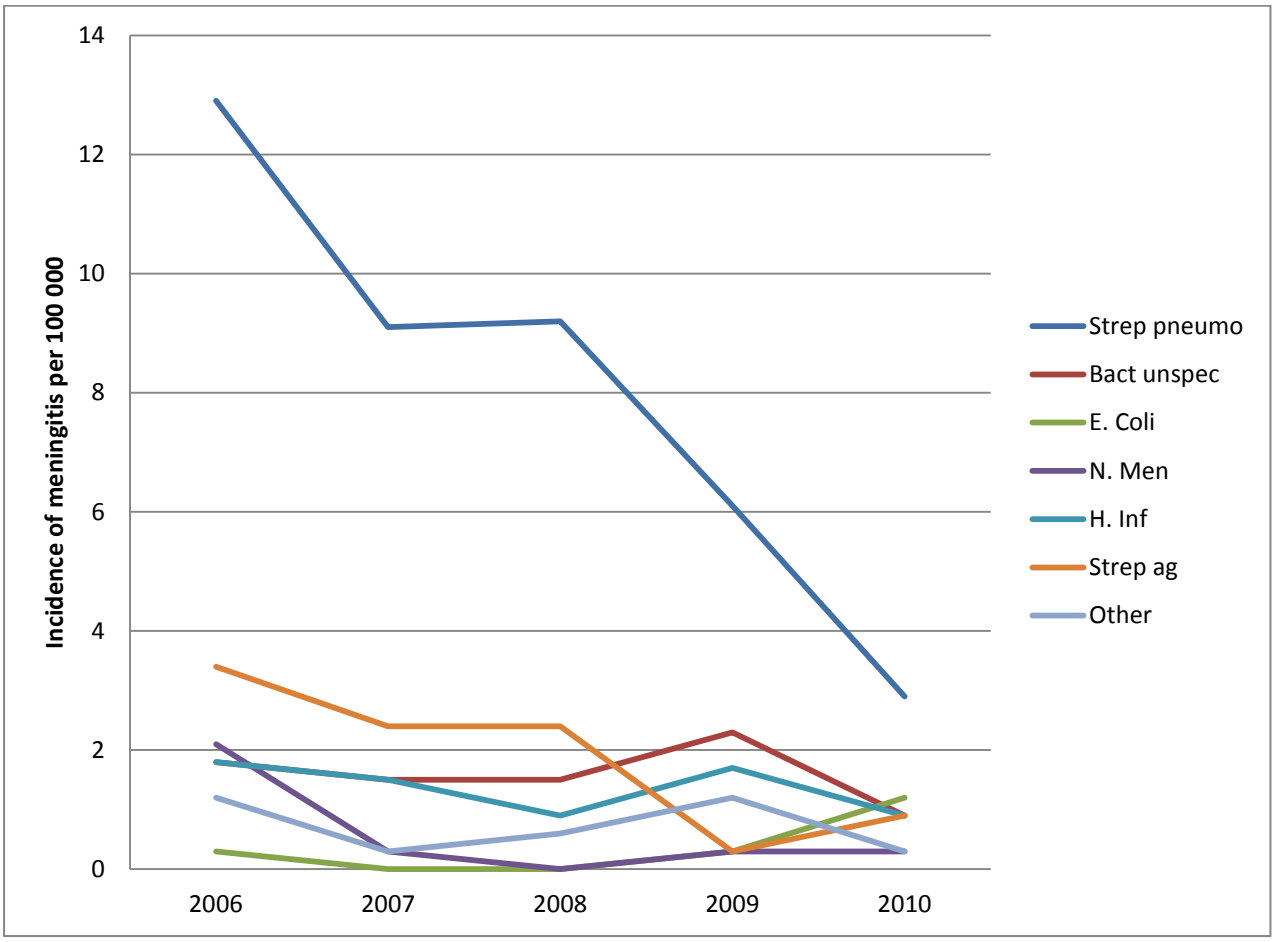
The observed change in incidence for the different bacterial meningitis pathogens is plotted in Figure 1. The graph shows a rapid decline in pneumococcal meningitis from 2009 and does not show any marked variation in the incidence of the other meningitis pathogens over the years.

Table 2: The number of cases of all bacterial meningitis pathogens during the study period.

	2006 N (%)	2007 N (%)	2008 N (%)	2009 N (%)	2010 N (%)	Total N (%)
Streptococcus pneumonia	42 (55)	30 (60)	31 (63.3)	21(50)	10 (40)	134(55.1)
Bacteria unspecified	6 (8)	5 (10)	5 (10.2)	8 (19)	3 (12)	27 (11.1)
Escherichia coli	1 (1)	0 (0)	0 (0)	1 (2.4)	4 (16)	6 (2.5)
Neisseria meningitides	7 (9)	1 (2)	0 (0)	1 (2.4)	1 (4)	10 (4.1)
Haemophilus influenza	6 (8)	5 (10)	3 (6.1)	6 (14.3)	3 (12)	23 (9.5)
Streptococcus agalactiae	11 (14)	8 (16)	8 (16.3)	1 (2.4)	3 (12)	31 (12.7)
Other*	4 (5)	1 (2)	2 (4.1)	4 (9.5)	1 (4)	12 (5)
TOTAL CASES	77 (100)	50 (100)	49 (100)	42 (100)	25 (100)	243 (100)

* Of other: *Acinetobacter baumannii* (one case in 2006), *Klebsiella* spp (two cases in 2009), *Salmonella* spp (one case each in 2006, 2007 and 2009), *Staphylococcus aureus* (one case in 2010), *Streptococcus* spp (two cases in 2006, two cases in 2008).

Figure 1: Annual incidence of meningitis per 100,000 stratified by meningitis type.



Trends in incidence of all bacterial meningitis

To analyse the impact that the introduction of PCV had on all bacterial meningitis the number of cases of meningitis according to the study definition were divided into annual quarters for all age groups (Table 3) and for children under 1 year of age (Table 4). The highlighted column in the tables indicates the time of introduction of the PCV. Using the Soweto region population figures as the denominator, the incidence was calculated and a regression model with a term for pre-vaccine (before the second quarter of 2009) or post-vaccine (from the second quarter of 2009, onwards) was generated.

Table 3: Bacterial meningitis by annual quarters in all ages:

Year	2006				2007				2008				2009				2010			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
All bacterial meningitis	17	21	22	17	12	17	13	8	9	14	17	9	15	7	15	5	10	4	3	8
Pneumococcal meningitis	8	14	11	9	4	12	9	5	6	10	9	6	5	6	6	4	4	1	2	3
Other	9	7	11	8	8	5	4	3	3	4	8	3	10	1	9	1	6	3	1	5

Table 4: Bacterial meningitis by annual quarters in children <1 year of age:

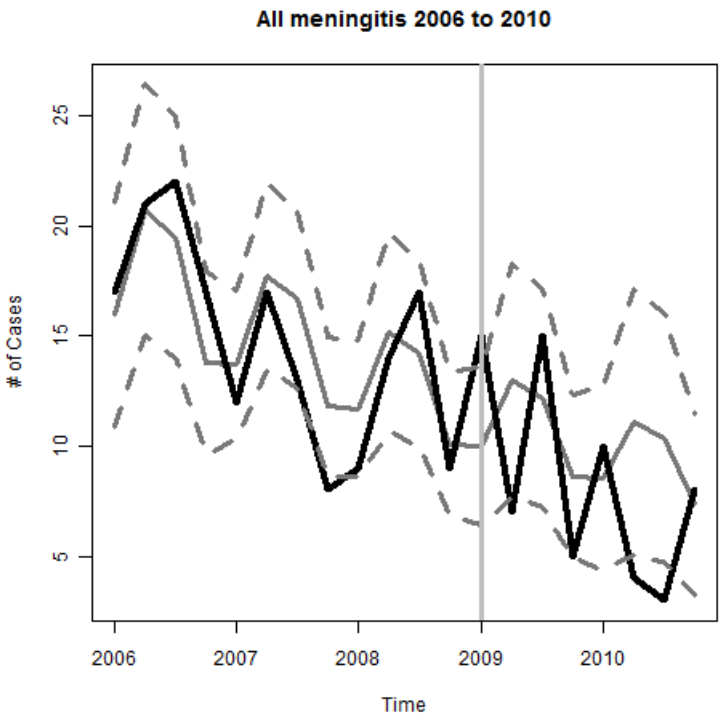
Year	2006				2007				2008				2009				2010			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
All bacterial meningitis	7	10	14	9	9	7	9	3	5	11	10	6	10	3	11	0	7	4	1	3
Pneumococcal meningitis	3	5	8	5	2	5	6		2	7	7	4	2	3	5	0	1	1	0	0
Other	4	5	6	4	7	2	3	2	3	4	3	2	8	0	6	0	6	3	1	3

The rate ratio of all meningitis between consecutive months was 0.953 for all children before 2009 (Table 5) indicating that, even prior to inclusion of PCV into the South African EPI there was a decline of 4.7% per annum in culture-confirmed bacterial meningitis amongst children being admitted to CHBAH. After 2009 the rate ratio declined further to 0.895 (10.5% per annum), however, this rate of decline between the pre- and post-PCV periods was not statistically significant; P=0.391. This change in reduction is graphically represented in Figure 2.

Table 5: Regression Model for all meningitis pre and post vaccine introduction in all age groups

	Estimate Std	Error	z value	Pr (>|z|)
Intercept	1.81	0.144	12.567	0.000
Time	-0.048	0.019	-2.449	0.014
Pre	0.811	1.183	0.685	0.493
Time:pre	-0.063	0.073	-0.859	**0.391**

Figure 2: Regression model for all bacterial meningitis in all age groups



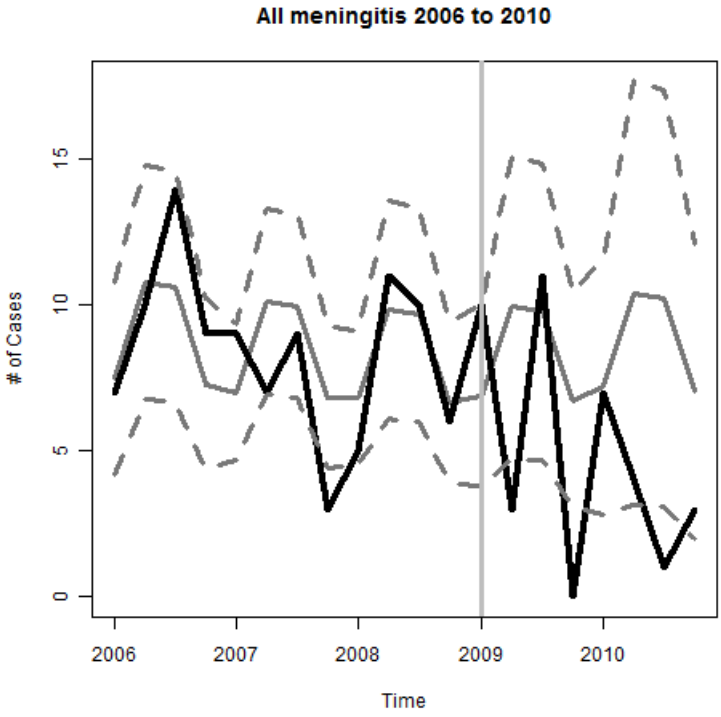
The thick black line is the number of observed culture-confirmed bacterial meningitis cases.
The solid grey line is the predicted number of cases when fitting the model to just the data from 2006-2009 (i.e., removing the effect of PCV on meningitis incidence trends).
The dashed grey lines are the prediction intervals for the predicted values.

The same regression model was used to analyse the effect of PCV immunization in children less than one year of age, the results of which were tabulated (Table 6). In this age-group, the rate ratio of all meningitis between consecutive months was 0.99 before 2009. After 2009 this changed to 0.857. This indicates that the incidence of meningitis was decreasing by 1% year-on-year in the pre-PCV era, while the decline accelerated to 14.3% year-on-year in the post-PCV era; P=0.145. This change is graphically represented in Figure 3.

Table 6: Regression Model for all meningitis pre and post vaccine introduction in children <1 year of age.

	Estimate Std	Error	z value	Pr (>|z|)
Intercept	3.738	0.197	18.994	0.000
Time	-0.010	0.025	-0.403	0.687
Pre	1.757	1.590	1.105	0.269
Time:pre	-0.144	0.099	-1.458	**0.145**

Figure 3: Regression model for all bacterial meningitis in children <1 year of age



The thick black line is the number of observed culture-confirmed bacterial meningitis cases.
The solid grey line is the predicted number of cases when fitting the model to just the data from 2006-2009 (i.e., removing the effect of PCV on meningitis incidence trends).
The dashed grey lines are the prediction intervals for the predicted values.

3.2 Pneumococcal Meningitis

Trends in incidence of Pneumococcal meningitis in all age groups

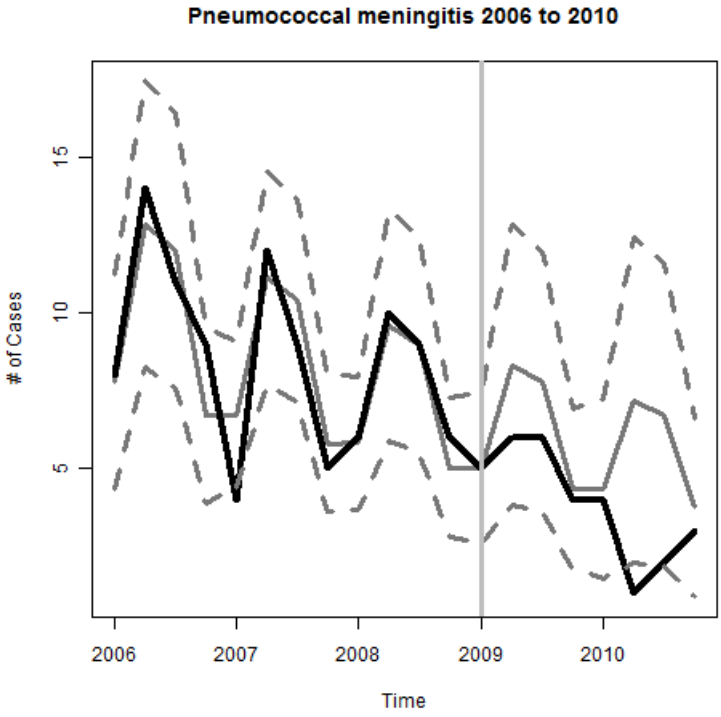
Data from (Table 3) was used to infer the impact which the introduction of PCV has had on the incidence of pneumococcal meningitis, by applying a regression model with a term for pre-vaccine or post-vaccine.

The results of the regression model are represented in Table 7 and show the rate ratio of all meningitis between consecutive months was 0.953 for all children before 2009. After 2009 this changed to 0.817. This means that the incidence was decreasing by 4.7% prior to the vaccine being introduced and post introduction this decrease accelerated to 18%, albeit also not statistically significant, P=0.146. This change is graphically represented in Figure 4.

Table 7: Regression Model for pneumococcal meningitis pre and post vaccine introduction in all age groups

	Estimate Std	Error	z value	Pr (>|z|)
Intercept	1.242	0.191	6.485	0.000
Time	-0.048	0.026	-1.852	0.064
Pre	2.192	1.692	1.295	0.195
Time:pre	-0.154	0.106	-1.453	**0.146**

Fig 4: Regression model for pneumococcal meningitis in all age groups



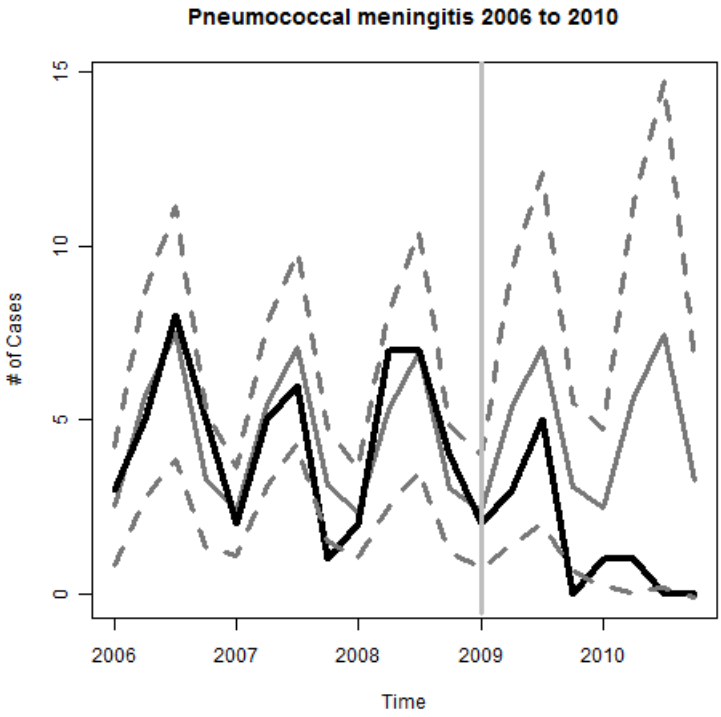
The thick black line is the number of observed pneumococcal meningitis cases.
The solid grey line is the predicted number of pneumococcal meningitis cases when fitting the model to just the data from 2006-2009 (i.e., removing the effect of PCV on pneumococcal meningitis incidence trends).
The dashed grey lines are the prediction intervals for the predicted values.

The temporal association of PCV immunization on the incidence of pneumococcal meningitis in children less the one year of age is shown in Table 4, which was applied to a regression model graph with a term for pre-vaccine or post-vaccine. This age group was specifically looked at, as this is the population most at risk of IPD and would have received the maximum benefit of the PCV immunization program, in the absence of a catch-up campaign of vaccination of older children. The results of the regression model are depicted in Table 8 and show the rate ratio of all meningitis between consecutive months was 0.989 for all children before 2009. After 2009 this changed to 0.566. This indicates that the incidence was decreasing by 1.1% in the pre-vaccine era, which accelerated to a 43.4% year-on-year decline post PCV introduction, P=0.011. Pneumococcal meningitis incidence trends in children less than one year of age are graphically represented in Figure 5.

Table 8: Regression Model for pneumococcal meningitis pre and post vaccine introduction children <1 year of age

	Estimate Std	Error	z value	Pr (>|z|)
Intercept	3.086	0.273	11.297	0.000
Time	-0.011	0.035	-0.310	0.757
Pre	7.886	3.326	2.371	0.018
Time:pre	-0.558	0.220	-2.537	**0.011**

Fig 5: Regression model for pneumococcal meningitis in children <1 year of age



The thick black line is the number of observed pneumococcal meningitis cases.
The solid grey line is the predicted number of pneumococcal meningitis cases when fitting the model to just the data from 2006-2009 (i.e., removing the effect of PCV on pneumococcal meningitis incidence trends).
The dashed grey lines are the prediction intervals for the predicted values.

3.3 Trends in the HIV-infected population

Previous studies have shown a reduction in IPD in HIV-infected children following increasing usage of ART. The periods 2006 and 2007-2010 were defined as intermediate and established ART access eras, respectively. By comparing the incidence of pneumococcal meningitis in the two periods we were able to assess the impact improved ART access had on pneumococcal meningitis. To assess the impact that PCV contributed to the decline in the established era, we split the established ART era into two time periods 2007-2008 and 2009-2010 (without and with PCV availability respectively).

To calculate the incidence of pneumococcal meningitis in the study population the study results were stratified according to age and HIV status (Table 9).

To assess the impact that the up-scaling of the ART program had on the incidence of pneumococcal meningitis in HIV-infected children, the two eras (excluding the years in which the vaccine was available) were compared using Chi squared trends (Table 10). The incidence declined from 219 per 100,000 in the intermediate era to 152 per 100,000 in the established era, a 30% reduction in incidence (95%CI: -22.1-60.3%; P=0.20).

The combined effect that PCV and up-scaled ART access had on pneumococcal meningitis in HIV-infected children is depicted in Table 11. The incidence of pneumococcal meningitis in the HIV-infected population declined from 219 per 100,000 to 108 per 100,000, i.e. a 49.4% reduction (95%CI: 29.2-83.3%; P=0.02) and was greater than the reduction caused by increased ART access alone. Although there was an overlap in the confidence intervals of the two groups, the reduction was only significant in the combined ART and PCV group.

Sensitivity Analysis: All Age Groups

The sensitivity analysis (Tables 10 and 11) showed pneumococcal meningitis incidence amongst HIV-infected children in the years excluding vaccine availability was 296 per 100,000 in the intermediate era and 167 per 100,000 in the established era, a 43% reduction in incidence (95%CI: 6.2-65.8%; P=0.02). When including the years that PCV was available in the established era, the incidence dropped further from 167 per 100,000 to 119 per 100,000, a 40% reduction (95%CI: 25.5-64.4%; P=0.0001) which was significant, but not different to the impact seen from up-scaling of ART alone.

Pneumococcal Meningitis Incidence Trends in HIV–infected Infants

As was observed in the analysis of all meningitis and pneumococcal meningitis incidence trends regardless of HIV infection status, the maximum benefit of PCV immunization amongst HIV-infected children was in infants. We therefore analysed this specific age group (less than one year of age) to determine the impact of increased ART compared to increased ART with PCV coverage (Tables 12 and 13). In the intermediate ART era the incidence of pneumococcal meningitis was 531 per 100,000. In the established ART era, when the vaccine was not available, pneumococcal meningitis incidence in HIV-infected children less than one year of age declined to 371 per 100,000. This showed a 30% reduction due to increased ART access (95%CI: -10.1-75.6%; P=0.51). When the years that PCV was available were included, the incidence in the established ART era decreased further to 220 per 100,000, showing a 58% reduction (95%CI: -14.5-84.8; P=0.079).

Sensitivity Analysis: Children Less Than One Year of Age

To again account for the cases with an unknown HIV result in the less than one year age group, we conducted a sensitivity analysis as described previously. Prior to the introduction of the vaccine (Tables 12 and 13) the incidence of pneumococcal meningitis was 796 per 100,000 in the intermediate ART era, and 418 per 100,000 in the established ART era. This represents a 47% reduction (95%CI: -32.4-79%; P=0.52). In the comparison including the years in which PCV was available, the incidence in the established ART era reduced further to 264 per 100,000, i.e. a reduction of 67% (95%CI: 20.9-85.9%; P=0.0089) which was greater than the reduction obtained from simply up-scaling ART.

Table 9: Number of cases of pneumococcal meningitis stratified by age and HIV status

	2006				**2007**				**2008**				**2009**				**2010**			
	+	**-**	**?**	**Total**	**+**	**-**	**?**	**Total**	**+**	**-**	**?**	**Total**	**+**	**-**	**?**	**Total**	**+**	**-**	**?**	**Total**
<1	6	8	7	**21**	4	7	3	**14**	4	14	2	**20**	2	5	3	**10**		2		**2**
1-2	8			**3**	4	1		**5**	1	2	1	**4**		1		**1**	3			**3**
2-5	2	2	1	**5**	4			**4**				**0**	2	1		**3**	1	1		**2**
5-14	9	1	3	**13**	7			**7**	7			**7**	6	1		**7**	2	1		**3**
Total	20	11	11	**42**	19	8	3	**30**	12	16	3	**31**	10	8	3	**21**	6	4		**10**

HIV Status
+ = HIV-infected
- = HIV-uninfected
? = HIV status undetermined

Table 10: Incidence of pneumococcal meningitis in children <14yrs in the Intermediate and Established ART eras prior to vaccine introduction

	Intermediate-ART 2006 Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**Established-ART 2007-2008** Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**IRR[2]**	**P-value[3]**	**% reduction[4]**
Total	12.2 [8.8-16.5] (42)	8.6 [6.6-11] (61)	0.7 [0.48-1.05]	0.08	29.4 [-4.5-52.4]
HIV-infected	219.1 [133.9-338.2] (20)	152.5 [103.6-216.4] (31)	0.69 [0.39-1.22]	0.2	30.3 [-22.1-60.3]
HIV-uninfected	3.3 [1.6-5.9] (11)	3.5 [2.2-5.2] (24)	1.1 [0.52-2.17]	0.87	Not applicable
HIV-infected EX[5]	295.8 [195-430] (27)	167.3 [115.9-233.7] (34)	0.57 [0.34-0.94]	0.02	43.4 [6.2-65.8]
HIV-uninfected EX[6]	4.5 [2.5-7.4] (15)	3.9 [2.6-5.7] (27)	0.88 [0.47-1.65]	0.68	12.3 [-64.8-53.4]

[1]Number of cases per 100,000
[2]Incidence risk ratios – Intermediate vs. Early ART era
[3] X^2 test or Fisher exact test
[4] % reduction between Intermediate and Established ART era
[5] Incidence of pneumococcal meningitis in HIV-infected children extrapolated proportionally to include HIV unknown
[6]Incidence of pneumococcal meningitis in HIV-uninfected children extrapolated proportionally to include HIV unknown

Table 11: Incidence of pneumococcal meningitis in children <14yrs in the Intermediate and Established ART eras including years after vaccine introduction

	Intermediate-ART 2006 Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**Established-ART 2007-2010** Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**IRR[2]**	**P-value[3]**	**% reduction[4]**
Total	12.2 [8.8-16.5] (42)	6.4 [5.1-7.8] (92)	0.5 [0.36-0.75]	0.0004	47.7 [24.7-63.7]
HIV-infected	219.1 [133.9-338.2] (20)	108 [79.4-143.6] (47)	0.49 [0.29-0.83]	0.0069	49.4 [29.2-83.3]
HIV-uninfected	3.3 [1.6-5.9] (11)	2.6 [1.8-3.6] (36)	0.78 [0.4-1.5]	0.478	21.7 [-53.9-60.1]
HIV-infected EX[5]	295.8 [195-430] (27)	119.5 [89.3-156.7] (52)	0.4 [0.25-0.64]	0.0001	40.5 [25.5-64.4]
HIV-uninfected EX[6]	4.5 [2.5-7.4] (15)	2.9 [2-3.9] (40)	0.64 [0.35-1.16]	0.135	36.1 [-15.6-64.7]

[1]Number of cases per 100,000
[2]Incidence risk ratios – Intermediate vs. Early ART era
[3] X^2 test or Fisher exact test
[4] % reduction between Intermediate and Established ART era
[5] Incidence of pneumococcal meningitis in HIV-infected children extrapolated proportionally to include HIV unknown
[6]Incidence of pneumococcal meningitis in HIV-uninfected children extrapolated proportionally to include HIV unknown

Table 12: Incidence of pneumococcal meningitis in children <1yr in the Intermediate and Established ART eras including years prior to vaccine introduction

	Intermediate-ART 2006 Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**Established-ART 2007-2008** Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**IRR[2]**	**P-value[3]**	**% reduction[4]**
Total	93.8 [58-143.4] (21)	80 [55.5-112] (34)	0.85 [0.49-1.47]	0.57	14.5 [-47.2-50.3]
HIV-infected	531 [195-1152] (6)	371 [160-731] (8)	0.7 [0.24-2]	0.51	29.9 [-10.1-75.6]
HIV-uninfected	37.6 [16.2-74.2] (8)	52.2 [32-79.7] (21)	1.4 [0.6-3.2]	0.41	
HIV-infected EX[5]	796 [364.8-1506.5] (9)	418.2 [191.4-792.4] (9)	0.52 [0.21-1.12]	0.169	47.2 [-32.4-79]
HIV-uninfected EX[6]	56.4 [29.2-98.6] (12)	62.1 [40.2-91.7] (25)	1.1 [0.55-2.19]	0.78	

[1]Number of cases per 100,000
[2]Incidence risk ratios – Intermediate vs. Early ART era
[3] X^2 test or Fisher test
[4] % reduction between Intermediate and Established ART era
[5] Incidence of pneumococcal meningitis in HIV-infected children extrapolated proportionally to include HIV unknown
[6]Incidence of pneumococcal meningitis in HIV-uninfected children extrapolated proportionally to include HIV unknown

Table 13: Incidence of pneumococcal meningitis in children <1yr in the Intermediate and Established ART eras including years after vaccine introduction

	Intermediate-ART 2006 Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**Established-ART 2007-2010** Incidence of pneumococcal meningitis [1] [95%CI] (N of pneumococcal cases)	**IRR[2]**	**P-value[3]**	**% reduction[4]**
Total	93.8 [58-149.4] (21)	52.8 [38.7-70.5] (46)	0.56 [0.34-0.94]	0.027	43.6 [5.6-66]
HIV-infected	531 [195-1152] (6)	220.7 [105.9-405.5] (47)	0.42 [0.15-1.14]	0.079	58.3 [-14.5-84.8]
HIV-uninfected	37.6 [16.2-74.2] (8)	33.9 [22.5-49] (28)	0.9 [0.41-1.98]	0.79	9.8 [-97.8-58.9]
HIV-infected EX[5]	796 [364.8-1506.5] (9)	264.8 [136.9-462.2] (12)	0.33 [0.14-0.79]	0.0089	66.6 [20.9-85.9]
HIV-uninfected EX[6]	56.4 [29.2-98.6] (12)	41.2 [28.5-57.6] (34)	0.73 [0.38-1.41]	0.346	27 [-40.9-62.2]

[1]Number of cases per 100,000
[2]Incidence risk ratios – Intermediate vs. Early ART era
[3] X^2 test or Fisher test
[4] % reduction between Intermediate and Established ART era
[5] Incidence of pneumococcal meningitis in HIV-infected children extrapolated proportionally to include HIV unknown
[6]Incidence of pneumococcal meningitis in HIV-uninfected children extrapolated proportionally to include HIV unknown

4.0 Discussion

The introduction of PCV into the South African immunization program was temporally associated with a decrease in incidence of pneumococcal meningitis in both HIV-infected and HIV-uninfected children. This reduction was greatest in children less than one year of age who have been specifically targeted for immunization since May 2009.

The incidence of pneumococcal meningitis was decreasing in the paediatric population of children admitted to CHBAH by 4.7% per annum prior to the introduction of PCV, and this decline was accelerated to 18% per annum following PCV introduction. The decreasing pneumococcal incidence rates observed in the era of access to PCV did not reach statistical significance in our analysis.

The population at highest risk of invasive pneumococcal disease in our setting are infants under one year of age [5], which was also the age-group specifically targeted for PCV immunization (three doses, given at six and fourteen weeks, and nine months of age) and who stand to benefit from the direct effect of the vaccine, as there had not been any catch-up campaign for older children until 2011. In the under-one year age-group there was a statistically significant decline in the incidence of pneumococcal meningitis (from 1.1% per annum prior to PCV introduction, to 43.4% after the introduction of PCV). This PCV-related decline in the incidence of hospitalisation for confirmed pneumococcal meningitis occurred in the context, and accelerated, a secular decline in meningitis incidence which was occurring prior to vaccine introduction (Figure 1).

A study in the United States showed a decline in pneumococcal meningitis of 64% in children under two years of age, four years after the introduction of pneumococcal vaccine [1]. However this time period included a catch-up campaign and the predicted serotype

coverage (of PCV7) in the United States is 85% compared to 55-60% in South Africa [14]. Our study only looked at the impact of PCV in the immediate 21 months period following its introduction, and yet demonstrated a statistically significant vaccine effect in children less than one year of age.

There is a theoretical concern that with the reduction of one meningitis type (e.g. pneumococcal meningitis) there will be an increase in other types of meningitis attributed to bacterial species that were not as prevalent in the pre-vaccine era. When looking at the incidence of all meningitis, there was a persistent decline in the total incidence of meningitis pre- and post-introduction of the vaccine. This was to be expected as pneumococcal meningitis contributed over 50% of all confirmed bacterial meningitis cases in the period prior to PCV introduction. The numbers of non-pneumococcal meningitis where unfortunately too small to do a statistical time series analysis for each pathogen, however, a graphical representation showed the incidence to be declining in most cases when comparing 2006 to 2010 (Figure 1).

In light of the high burden of HIV in the South African population, we assessed the impact that up-scaled delivery of ART had on pneumococcal meningitis incidence and whether this impact was a greater contribution to the decline in incidence of pneumococcal meningitis in the HIV population than that of PCV.

The first statistical analysis interrogated the incidence of pneumococcal meningitis in the HIV-infected population in the intermediate ART era (2006) compared to disease incidence in the established ART era (2007-2010).

Analysis of the two ART-eras when PCV was not available showed a non-statistical significant decline (30%: 95%CI -22.1-60.3%; P=0.20). However the analysis of the two ART-eras, including the years that PCV was available, showed a statistically significant

reduction (49.4%: 95%CI 29.2-83.3; P=0.02) in the incidence of pneumococcal meningitis. This showed that although the ART programs had a significant role in reducing the incidence of pneumococcal meningitis in HIV-infected children, this decline accelerated following the further introduction of PCV into the EPI.

Sensitivity analysis conducted by extrapolating the number of unknown HIV results in all children proportionally into both HIV-infected and HIV-uninfected groups (based on the proportion that was HIV-infected amongst those who were tested for HIV), demonstrated a less substantial (43%: 95%CI 6.2-65.8%; P=0.02) but statistically significant difference from up-scaling ART alone and a similar reduction in the era with ART combined with PCV (40%: 95%CI 25.5-64.4%; P=0.0001).

As children less than one year of age derived maximum benefit from the vaccine, the effect of ART and ART combined with the vaccine was looked at specifically in this population. HIV-infected children less than one year showed a reduction of 29.9% (95%CI: -10.1-75.6; P=0.51) due to the up-scaled delivery of ART alone. Those children who gained the benefit of both up-scaled ART and PCV showed a reduction of 58.3% (95%CI: -14.5-84.8; P=0.079) in pneumococcal meningitis. These results indicate that PCV is effective in reducing the incidence of pneumococcal meningitis in the HIV-infected population, although the small sample size limited our ability to demonstrate statistical significance in these analyses.

Sensitivity analysis conducted by extrapolating the number of unknown HIV results in those children under one year of age proportionally into both HIV-infected and HIV-uninfected groups, increased the reduction in the combined ART and PCV group and reached statistical significance and the reduction in the combined group was greater, although the confidence intervals of the two groups did overlap: 47.2% reduction in the group with only up-scaled

ART (95%CI: -32.4-79) compared to a 66.6% (95%CI: 20.9-85.9) reduction in the group who received ART and PCV.

We were able to describe statistically significant outcomes in our analyses, despite the necessity to rely on observational and clinical databases from which raw data were obtained. Our study has a few limitations. The first limitation of this study is that we used the population base as that of Soweto. Although there is only one public sector hospital (CHBAH) serving this region, some children may have been admitted elsewhere. Furthermore, children not resident in Soweto may have accessed care at CHBAH. However, the same population (with likely population flux affecting results throughout the study time frame) was looked at pre- and post-PCV implementation and it was the incidence in this group that was compared, so this should negate any discrepancy.

A second limitation is that we were using the paediatric database which has only recently been finalised (2011) and may have not completely documented all cases. The possible omission of additional confirmed bacterial meningitis cases in our analysis would have enhanced our sample size, and may have increased the power of our study to demonstrate statistical significance in certain of our analyses. There is no reason to believe that deficiencies in data capturing would have affected one time period of the paediatric database more than another, so time-trends of an overall reduction of confirmed bacterial meningitis cases likely represents the true disease incidence trends.

The third limitation is that at the time of starting the study the data base was only up to date till December 2010. This means that the study interrogated the impact which PCV had in the first 21 months after it was introduced into the EPI, and this is not necessarily a true reflection of the impact the vaccine will have. In comparison the study done in the United States compared the incidence of pneumococcal meningitis prior to the introduction of PCV

to the incidence 4 years after its introduction, therefore allowing proper uptake of the vaccine into the EPI and the benefit of indirect effect. It is noteworthy that, even in the context of a newly implemented preventive strategy (PCV vaccination) where initial coverage may have been adversely affected by stock-outs, we were able to demonstrate statistically significant vaccine effects on pneumococcal meningitis disease incidence.

The fourth limitation is the assumption that vaccine coverage would have been similar to that of *H. influenzae* type b conjugate vaccine and that there were no problems encountered in the rollout of a new vaccine into the EPI. If the coverage of PCV was low, this would have reduced the efficacy of the vaccine and therefore not reflect the full potential of PCV immunization. With improved access, delivery and catch-up campaigns there may be a greater decline in the incidence of pneumococcal meningitis.

The fifth limitation of the study is that there were small numbers of cases as we looked at a specific hospital population, which limited the power to demonstrate statistical significance.

A further limitation of the study is that we did not interrogate the pneumococcal serotype data for the cases, hence we were unable to demonstrate that the vaccine did not increase the incidence of those pneumococcal serotypes not covered in the 7-valent PCV. However this was not one of the aims of the study.

5.0 Conclusion

The introduction of PCV was temporally associated with a decline in the incidence of pneumococcal meningitis in children. This decline was most pronounced in infants (i.e. children <1 year of age), among whom there was a 1.1% per annum decline prior to PCV-introduction, which increased to a 43.4% decline post-PCV introduction.

Improved access to ART was shown to contribute to the decline in the incidence of pneumococcal meningitis, however, when combined with PCV the decline was far greater than that of up-scaled ART alone.

6.0 Appendices

Appendix 1: ICD 10 Codes for Meningitis

ICD-10 Code	Description
A39.0	Meningococcal meningitis
A87	Viral meningitis
G02	Meningitis in other infectious and parasitic diseases classified elsewhere
G03.9	Meningitis unspecified
G00.0	Haemophilus meningitis
G00.1	Pneumococcal meningitis
G00.2	Streptococcal meningitis
G00.3	Staphylococcal meningitis
G00.8	Other bacterial meningitis
G00.9	Bacterial meningitis unspecified

Appendix 2: Data extracted from the NHLS System

- Date of specimen submission;
- CSF findings on admission: white cell count (cells/mm^3), protein (g/l), glucose (mmol/l), bacterial latex test (if done), Gram stain, bacterial culture, bacterial susceptibility test results (including penicillin and cefotaxime minimum inhibitory concentrations [MICs]);
- Blood culture result on admission;
- Full blood count results on admission: white cell count (cells x 10^9/l), haemoglobin (g/dl), mean cell volume (fl), platelets (x 10^9/l), and differential count (%);
- C-reactive protein (CRP) on admission (mg/l);
- HIV status;
    - HIV infection status was attributed as follows:
    1. HIV enzyme linked immunosorbent assay (ELISA) positive in a child over 18 months of age: HIV-infected;
    2. HIV ELISA positive in a child under 18 months of age, with positive HIV polymerase chain reaction (PCR): HIV-infected;
    3. HIV ELISA positive in a child under 18 months of age, with negative HIV PCR: HIV exposed, uninfected;
    4. HIV ELISA positive in a child under 18 months of age, with no HIV PCR: HIV-exposed but status indeterminate;
    5. No HIV test available, regardless of age group: HIV status undetermined.
    - For HIV-infected children, CD4+ lymphocyte count (absolute [cells x 10^6/l], and CD4+ per cent) and HIV viral load (RNA copies/ml) results conducted within 2 months of the meningitis episode were recorded.

Appendix 3: Population denominators by age group in Soweto

Year	Age	Total population
2006	< 1	22,382
	0-14yrs	344,742
2007	< 1	21,326
	0-14yrs	351,486
2008	< 1	21,077
	0-14yrs	358,076
2009	< 1	21,627
	0-14yrs	364,697
2010	< 1	23,008
	0-14yrs	371,165

Appendix 4: HIV prevalence by age-group in Soweto, South Africa

	2006	2007	2008	2009	2010
0-4 Prevalence	**5.05%**	**5.05%**	**5.12%**	**5.25%**	**5.39%**
Population	119,179	117,133	116,275	116,779	118,433
HIV-infected	6,014	5,914	5,956	6,386	6,386
HIV-uninfected	11,3165	111,219	110,319	110,644	112,047
5-9 Prevalence	**2.47%**	**2.94%**	**3.28%**	**3.49%**	**3.6%**
Population	120,787	124,359	126,511	127,464	127,835
HIV-infected	2,981	3,653	4,147	4,453	4,603
HIV-uninfected	117,806	120,706	122,364	123,011	123,232
10-14 Prevalence	**0.13%**	**0.22%**	**0.13%**	**0.54%**	**0.76%**
Population	104,776	109,994	104,776	120,454	124,897
HIV-infected	134	244	133	651	944
HIV-uninfected	104,642	109,750	104,642	119,803	123,953
Total Population	344,742	351,486	358,076	364,697	371,165
HIV-infected	9,129	9,811	10,518	11,239	11,933
HIV-uninfected	335,613	341,675	347,558	353,458	359,232

7.0 References

1. Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, Farley MM, Jorgensen JH, Lexau CA, Petit S *et al*: **Effect of pneumococcal conjugate vaccine on pneumococcal meningitis**. *N Engl J Med* 2009, **360**(3):244-256.
2. Grimwood K, Anderson P, Anderson V, Tan L, Nolan T: **Twelve year outcomes following bacterial meningitis: further evidence for persisting effects**. *Arch Dis Child* 2000, **83**(2):111-116.
3. World Health Organization. The Global Burden of Disease. 2004 Update. Available at: **http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf** (Accessed 23 December 2013)
4. World Health Organization. Pneumococcal conjugate vaccine for childhood immunization - WHO position paper. **Wkly Epidemiol Rec** 2007 Mar 23; 82(12):93-104. Available at: http://www.who.int/wer/2007/wer8212.pdf (Accessed 13 January 2014)
5. **GERMS-SA Annual Report 2008**. In. Edited by Group for Enteric RaMDSiSA. Johannesburg; 2008.
6. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, Lee E, Mulholland K, Levine OS, Cherian T: **Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates**. *Lancet* 2009, **374**(9693):893-902.
7. **Updated recommendations for prevention of invasive pneumococcal disease among adults using the 23-valent pneumococcal polysaccharide vaccine (PPSV23)**. *MMWR Morbidity and mortality weekly report* 2010, **59**(34):1102-1106.
8. Peltola H: **Burden of meningitis and other severe bacterial infections of children in Africa: implications for prevention**. *Clin Infect Dis* 2001, **32**(1):64-75.
9. Grobler AC, Hay IT: **Bacterial meningitis in children at Kalafong Hospital, 1990-1995**. *S Afr Med J* 1997, **87**(8 Suppl):1052-1054.
10. Ramakrishnan M, Ulland AJ, Steinhardt LC, Moisi JC, Were F, Levine OS: **Sequelae due to bacterial meningitis among African children: a systematic literature review**. *BMC Med* 2009, **7**:47.
11. Dangor Z, Izu A, Hillier K, Solomon F, Beylis N, Moore DP, Nunes MC, Madhi SA: **Impact of the Antiretroviral Treatment Program on the Burden of Hospitalization for Culture-Confirmed Tuberculosis in South African Children: A Time-Series Analysis**. *Pediatr Infect Dis J* 2013.
12. Nunes MC, von Gottberg A, de Gouveia L, Cohen C, Moore DP, Klugman KP, Madhi SA: **The impact of antiretroviral treatment on the burden of invasive pneumococcal disease in South African children: a time series analysis**. *AIDS* 2011, **25**(4):453-462.
13. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N: **A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection**. *N Engl J Med* 2003, **349**(14):1341-1348.
14. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Freimanis Hance L, Reithinger R, Muenz LR, O'Brien KL: **Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project**. *PLoS Med* 2010, **7**(10).
15. von Gottberg A: **Prevention of pneumococcal disease in children: The age of the polysaccharide-protein conjugate pneumococcal vaccine.** *Communicable Diseases Surveillance Bulletin* 2007, **5**(2):2.
16. Lucero MG, Dulalia VE, Nillos LT, Williams G, Parreno RA, Nohynek H, Riley ID, Makela H: **Pneumococcal conjugate vaccines for preventing vaccine-type invasive pneumococcal disease and X-ray defined pneumonia in children less than two years of age**. *Cochrane database of systematic reviews* 2009(4):CD004977.

17. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D *et al*: **Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine**. *N Engl J Med* 2003, **348**(18):1737-1746.
18. Madhi SA, Adrian P, Kuwanda L, Jassat W, Jones S, Little T, Soininen A, Cutland C, Klugman KP: **Long-term immunogenicity and efficacy of a 9-valent conjugate pneumococcal vaccine in human immunodeficient virus infected and non-infected children in the absence of a booster dose of vaccine**. *Vaccine* 2007, **25**(13):2451-2457.
19. Martin M, Casellas JM, Madhi SA, Urquhart TJ, Delport SD, Ferrero F, Chamany S, Dayan GH, Rose CE, Levine OS *et al*: **Impact of Haemophilus influenzae type b conjugate vaccine in South Africa and Argentina**. *Pediatr Infect Dis J* 2004, **23**(9):842-847.
20. World Health Organization. Vaccine-preventable diseases: monitoring system. 2013 global summary. Available at: **http://apps.who.int/immunization_monitoring/globalsummary/countries?countrycriteria%5Bcountry%5D%5B%5D=ZAF** (Accessed 08 January 2014)
21. **ASSA2003 AIDS and Demographic model**. In., ASSA 2003 full. edn: Actuarial Society of South Africa