Supplementary Methods and Definitions

Patients

We randomized 39,836 children into a double-blind placebo controlled trial to evaluate the efficacy of a 9-valent PncCV against vaccine-serotype specific invasive pneumococcal disease and World Health Organization defined radiologically confirmed pneumonia[5]. We vaccinated children at approximately 6, 10 and 14 weeks of age with either a 9-valent PncCV (19,922 children) or matching placebo (19,914 children)[1]. Children who were 28–84 days of age were eligible if they received no other vaccine except Bacille-Calmette-Gu_rin and oral polio virus vaccine at birth. All children received other childhood vaccines; including trivalent oral polio vaccine (PolioOral, Biovac), hepatitis B vaccine (Hepaccine®, Cheil Sugar Organization) and *Haemophilus influenzae* type b conjugate vaccine (HibTiter®, Wyeth Vaccines and Pediatrics, USA) , concurrently with PncCV/placebo. We gave *Haemophilus influenzae* type b conjugate vaccine as a benefit to the control children prior to the introduction of that vaccine into the routine immunization program of South Africa. We vaccinated children at one of 21 participating vaccination facilities in the community. We calculated the proportion of children randomized who were HIV infected, as the average (weighted by annual enrolment into the study) of the HIV seroprevalence of antenatal women in this community from the national seroprevalence survey of women attending public antenatal clinics in South Africa (Department of Health, Pretoria, South Africa). From 1998 – 2000 the seroprevalence for this community increased from 22.5% – 27.8% with a weighted average of 24.87%. Using a vertical transmission rate in this community at that time estimated at 26% (Gray, GE, McIntyre JA, Lyons, SF, XIth International Conference on AIDS, Vancouver, 1997, Abstract Th.C.413), the estimate of the prevalence of HIV infection in children in this community is 6.47%. No respiratory viral vaccine, including influenza vaccine, was provided during the course of the study, and there was little likelihood of access to these vaccines by children in the study as children receive their immunizations from the

Expanded Programme on Immunization in South Africa which does not provide influenza vaccine. Parents or legal guardians signed written informed consent prior to enrollment of the children and approval for the study was obtained from the Committee for the Study of Human Subjects at the University of the Witwatersrand, and the Medicines' Control Council of South Africa[1].

Enrollment into the study started in March 1998 and was completed in October 2000. We identified the outcome cases discussed in this report during follow-up until the 15th November 2001, with the median duration of follow-up of 847.3 (range 60 –1 354) days. We conducted surveillance for all-cause hospitalization at Chris Hani-Baragwanath hospital, a secondary-tertiary care public hospital that served the community from which children had been recruited. Study doctors examined all admitted children, and were responsible for determining the presence/absence of clinical signs, on the day of admission. Study-doctors were not however involved in the management of children. We performed blood cultures on all hospitalized children using an automated system (BacT – Alert, Organon, Teknika).

Investigation for respiratory viral infections

We investigated children hospitalized for lower respiratory tract infections (LRTI), i.e. those in whom a diagnosis of either bronchiolitis or pneumonia had been made, for the presence of a respiratory viral infection using respiratory tract secretions obtained by a nasopharyngeal aspirate technique. There was no difference in the proportion of children with pneumonia who were investigated for respiratory viral infections between the vaccinees and placebo groups overall (991 [94.7%] of 1 047 vs. 1 186 [95.6%] of 1 240 respectively, $P = 0.269$), as well as in HIV - uninfected (580 [96.8%] of 599 vs. 698 (96.8%) of 721, $P = 0.985$) and HIV - infected children (411 [91.7%] of 448 vs. 488 [94.0%] of 519, $P = 0.166$).

Details of the methodology for performing nasopharyngeal aspirates and screening for specific respiratory viruses; specifically RSV, influenza A/B, parainfluenza type 1–3 (PIV 1–3) and adenovirus, have been described[2]. We performed viral testing until September 1998 at the National Institute of Virology, South Africa, and thereafter at the Respiratory and Meningeal Pathogens Research Unit. Briefly stated, we performed respiratory viral screening using an indirect immunofluorescence assay (*Light Diagnostics* Respiratory Panel 1 Vital Screening and Identification Kit, Chemicon International Inc, Temecula, CA, USA) that included a pool-screen for any of the investigated viruses, which was followed by screening for influenza A, influenza B, PIV 1–3 and adenovirus. As we did not subtype PIV consistently throughout the study, the results for PIV type 1–3 include virus due any of the PIV subtypes. We screened all specimens for RSV. We performed shell-vial culture of samples testing negative for RSV, but positive on the respiratory viral panel pool screen when specimens were processed at the National Institute of Virology. We performed only indirect immunofluorescence without shell-vial culture on specimens processed at the Respiratory and Meningeal Pathogens Research Unit.

The absence of tissue culture throughout the study, as well as the lack of shell-vial culture in the latter part of the study, may have resulted in a lower sensitivity to detect the studied viruses. This risk would have been however equally distributed between children in the two arms of the study. Furthermore, the sensitivity (>90%) of immunofluorescence in diagnosing respiratory viral infections, has been reported to be similar to that of tissue-culture methods[14]. Investigators and clinicians were informed of the results of viral studies but were blinded to vaccination status of the child.

Definitions

Per protocol (PP) analysis: Children were included in the PP analysis 14 days after receipt of three doses of the same study vaccine, with an interval of at least 21 days between doses of the

study-vaccine and with the third dose of study-vaccine given by 270 days of age. Intent-to-treat (ITT) analysis: All children randomized and who received at least a single dose of study vaccine. Children were included in the ITT analysis from the date of receipt of the first dose of vaccine. Clinical LRTI: All hospitalized children in whom a diagnosis of LRTI was made by a study doctor. The criteria used to diagnose clinical LRTI included: the presence of the WHO case - definition of severe or very severe LRTI[15], and/or the presence of other adventitious sounds on chest auscultation and/or in the presence of WHO-AC on chest radiography (CXR)[5]. This clinical diagnosis of LRTI included children with pneumonia and bronchiolitis, but excluded children that were diagnosed as only having laryngo-tracheobronchitis.

Pneumonia: Children were diagnosed as having "pneumonia" in the presence of WHO-AC, or if the child fulfilled the clinical diagnosis of LRTI without wheeze on chest auscultation, but with rales and/or bronchial breathing.

Bronchiolitis: Children were diagnosed as having bronchiolitis in the presence of wheezing on chest auscultation performed by one of the study doctors in the absence of documented alveolar consolidation on chest radiograph.

WHO-AC[5]: The presence of a dense opacity that may be a fluffy consolidation of a portion or whole of a lobe or of the entire lung, often containing air bronchograms and sometimes associated with pleural effusion, or a pleural effusion in the lateral pleural space associated with a pulmonary infiltrate / or an effusion large enough to obscure such an opacity.

Statistical methods

We performed analyses using STATA version 8.0 (StataCorp LP, College Station, TX, USA) and Epi Info version 6.04d (Centers for Disease Control, Atlanta, GA, USA). We calculated

vaccine efficacy (E) calculations and relative risks (RR) for first episodes of illness from Epi Info version 6.04d as E = 1 – RR, using the Taylor series expansion to calculate the confidence limits of the relative risk. We compared proportions using the Pearson Chi square test or the 2-tailed Fischer exact test if the expected cell value for any observation was ≤5. We designed the trial to end follow-up with the occurrence in the per protocol analysis of invasive disease cases in the non-HIV+ population sufficient to obtain 80% power to detect 70% vaccine efficacy. We determined that simultaneously with this endpoint approximately 380 cases of WHO-AC pneumonia cases would be obtained in the non-HIV+ve population. This provided 80% power to detect 26% vaccine efficacy. WE thus designed the trial with sample sizes large enough to detect quite low VE against WHO-AC pneumonia. The power available with 95% confidence to make the observations found on vaccine efficacy against total clinical pneumonia, and pneumonia with any identified virus in fully immunized HIV uninfected children are 95.8% and 91.5% respectively. The lack of significant differences between the point estimates and 95% confidence intervals of vaccine efficacy against individual viral associated pneumonias suggests that the vaccine has similar activity against each viral associated pneumonia.

We analyzed data for hospitalizations that occurred until 15 November 2001, the date of closing of the database for analysis of the primary objectives of the study. We analyzed vaccine efficacy calculations for the first episode of pneumonia and bronchiolitis independently of each other, i.e. the same child would have been included for the 1st episode of either event if eligible. Similarly vaccine efficacy calculations against the 1st episode of individual respiratory viral associated illness were analyzed independently of other viruses, viz. each child was considered as an independent individual for each of the analyses and was not censored from inclusion if included in an alternate analysis, but no child was included twice in the same analysis. If multiple episodes of the same endpoint occurred, we included only the first episode in that analysis.

Appendix (Supplementary Data)

The Vaccine Trialists Group includes the International Advisory Board chaired by N. Pierce and the Safety Board chaired by S. Abdul Karim, University of Natal, South Africa. The other members of these Boards include C. Broome, Centers for Disease Control, Atlanta, Georgia, U.S.A; N.E. Khomo, Department of Health, Germiston, Gauteng, South Africa; K. Mulholland, University of Melbourne, Australia; P. Cooper, Department of Pediatrics, University of the Witwatersrand, Johannesburg, South Africa; R. Breiman, ICDDRB, Bangladesh; B. Blackwelder, formerly of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.; I. Kleinschmidt, South African Medical Research Council. The remaining members of the Vaccine Trialists Group are T. Cherian, Vaccines and Biologicals, and H. Ostensen, Diagnostic Imaging and Laboratory Technology, World Health Organization, Geneva, Switzerland; H. Konradsen, Streptococcus Unit, Statens Serum Institut, Copenhagen, Denmark; R. Kohberger, I. Chang, B. Forrest, J. Hackel, W. Hausdorff, A. Kimura, D. Madore, F. Malinoski, G. Siber, Wyeth Lederle Vaccines and Pediatrics, Pearl River, New York, U.S.A.; H. Crewe-Brown, M. Khoosal, H. Koornhof, Department of Clinical Microbiology and Infectious Diseases, University of the Witwatersrand, Johannesburg, South Africa; G. Mistry, A. Musan-Craayenstein, Department of Radiology, University of the Witwatersrand, Johannesburg, South Africa; R. Huebner, N. Mbelle, L. Kuwanda, S. Alekar, C. Cutland, V. Davis, L. DeGouveia, B.C. Dhladla, H. Gani, K. Ismail, A. Madhi, L. Maseko, L. Nzutha, C. O'Reilly, R. Peruman, K. Petersen, D. Pillay, V. Quan, P. Ramekgoa, N. Ramasamy, K. Shantilal, A. Singh, A. Wasas, N. Zulu, Respiratory and Meningeal Pathogens Research Unit, Johannesburg, South Africa.

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