Published in final edited form as:

Vaccine. ; 32(51): 6948–6955. doi:10.1016/j.vaccine.2014.10.060.

Maternal Immunization with Pneumococcal 9-valent Conjugate Vaccine and Early Infant Otitis Media

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Abstract

A randomized trial of an investigational 9-valent pneumococcal conjugate vaccine (PCV-9) or placebo given to pregnant women during the last trimester to prevent early infant otitis media (OM) was conducted. All infants received Prevnar® at 2, 4, 6, and 12 months. Clinic and adverse event records were reviewed to identify OM. Variables significantly related to acute OM by age 6 months ($p < 0.05$) were: vaccine group (9 valent or placebo), sibling history of tympanostomy tubes, upper respiratory infection, and number of clinic visits by 6 months. Infant OM rates were similar between 6 and 12 months (58% and 56%). Results suggested that immunizing pregnant women with PCV-9 increased infants' risk of acute OM in the first 6 months of life, and this correlated with decreased infant antibody responses to their infant *Streptococcus pneumoniae* vaccine serotypes, but did not influence antibody responses to 3 other serotypes two of which were in maternal vaccine (types 1 and 5) and one was a control (type 7F). Explanations for these results include dampening of infant antibody production by high levels of passively acquired maternal pneumococcal antibodies and/or altered B lymphocyte immune responses in infants exposed to these specific polysaccharide antigens in utero.

The trial is registered at ClinicalTrials.gov, number NCT00617682.

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Conflict of Interest statement: Dr. Ferrieri is a Principle Investigator on an NIH R01 grant studying prevention of otitis media in a chinchilla model. The other authors have no conflict of interest.

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Keywords

pneumococcus; otitis media; maternal immunization; pneumococcal antibodies

INTRODUCTION

Since the 1990s, obstetricians and pediatricians have advocated for maternal immunization during pregnancy to prevent neonatal morbidity and mortality [1–3]. The rationale for their position is that infants are immunologically immature, and typically do not produce protective antibody levels until after the primary vaccine series is given in the first 6 months of life. A cohort study conducted in the 1990s showed that infant cord blood IgG levels to *Streptococcus pneumoniae* (*S. pneumoniae*) types 14 and 19F in the lowest quartile predicted acute otitis media (AOM) onset in the first 6 months of life [4]. Others have shown that AOM onset in the first 6 months of life predicts recurrent AOM and chronic otitis media with effusion (OME) [5, 6].

Randomized trials have demonstrated that heptavalent pneumococcal conjugate vaccine (PCV-7) is moderately efficacious in preventing AOM and recurrent AOM in infants and children, especially episodes caused by *S. pneumoniae* vaccine serotypes and cross-reacting serotypes [7–9]. Reductions of –1% to 7% in AOM and 9% to 16% in recurrent AOM were demonstrated in these trials. Prior to routine infant immunization with PCV-7, *S. pneumoniae* was the most prevalent bacterium cultured from the middle ear in children with AOM and persistent AOM [10–12]; 40% of middle ear pneumococcal isolates were antibiotic resistant [13].

The Maternal Infant Vaccine Study (MIVS), a Phase I/II randomized, double-masked trial of maternal immunization with an investigational 9-valent pneumococcal conjugate vaccine, diluted in aluminum phosphate (PNCRM9), hereafter referred to as PCV-9, [Wyeth Lederle] at 30–35 weeks of pregnancy, was designed to determine safety and maternal and infant antibody response [14]. The aim of the current study was to determine whether maternal immunization during pregnancy would prevent AOM onset in early infancy. We hypothesized that since maternal antibodies cross the placenta, infants of mothers immunized in late pregnancy would have higher levels of pneumococcal antibody at birth and thus fewer AOM episodes in early life. Data to explore this aim were collected during the MIVS study and were analyzed. Secondary aims included 1) AOM and otitis media (OM) incidence from birth to 12 months and from 6 to 12 months by randomized maternal treatment group, and 2) predictors for OM in these two time periods.

MATERIALS AND METHODS

1. Patient enrollment

Research nurses recruited and enrolled pregnant women between November 2000 and March 2003 from seven HealthPartners clinics, an integrated health care system in the Minneapolis-St. Paul metropolitan area serving both urban and suburban residents. Women were recruited by letter, telephone, and personal contact at each clinic beginning at 20 weeks

of pregnancy. Eligibility of interested women was determined by interview and medical record review. They were excluded from the study if they had prior immunization with pneumococcal vaccine, were at risk for preterm delivery, or had a condition that would be compromised by immunization. Protocol and consent forms were reviewed and approved by the Institutional Review Boards of the University of Minnesota, HealthPartners and the community hospitals where study infants were delivered. An External Data Safety and Monitoring Board was appointed (see Acknowledgements for members). Consent was obtained from both parents unless the father was unavailable. To assure similar size treatment groups at each clinic, participants were block randomized within clinic to receive PCV-9 or saline placebo. The vaccine lot number was #7-5021-013A. At the 30–35 week prenatal visit, a research nurse reconfirmed eligibility of the participant and administered a single 0.5mL dose (saline placebo or PCV-9) injection into the deltoid with a 23 gauge 1 inch sterile needle. Investigators, research nurses, physicians, study staff and participants were all masked to product identity and randomization group.

2. Patient monitoring

Nurses contacted participants by phone 1–3, 4–7 and 8–14 days after immunization to gather information about local and systemic reactions and adverse events (AE), and at 34–36 and 38–40 weeks of pregnancy to determine changes in health status. Maternal AEs were monitored from immunization to delivery, infant AEs were monitored from maternal immunization until 13 months of age. Information for both mothers and infants was obtained by phone interview, maternal diary, and from medical records.

Mothers were interviewed by phone between 28 and 35 weeks gestation to gather data about demographic factors, family OM history, maternal smoking and alcohol consumption. Additional risk factor data (parental smoking, breastfeeding, daycare attendance and exposure to other children) were collected at 2 and 6 months of age by phone contact. Maternal interviews were conducted every 2 months to ascertain interim infant illnesses and visits to health care providers within and outside of HealthPartners. At the 6 month visit, mothers were asked which product they thought they had received (vaccine or placebo). Rates of infant follow-up were 99% in the first 6 months, and 80% from 6–12 months. Infants were seen an average of once a month in both groups during the first six months.

Infants received Prevnar[®], PCV-7, at 2, 4, 6, and 12 months of age.

3. Abstraction of episodes of otitis media and other respiratory illness

HealthPartners abstractors recorded data from all clinic visits entered in the HealthPartners medical record. When a mother or infant was seen outside the HealthPartners system, a medical record release form was signed for that facility, and data from those visits were obtained and abstracted by the University of Minnesota coordinator. Data on type of visit (ill, recheck, well child); symptoms (fever, ear pain, irritable/fussy, not sleeping, difficulty hearing, not eating/eating poorly), ear exam findings (abnormal tympanic membrane position, color, appearance, mobility, perforation; presence and type of middle ear effusion); and middle ear diagnosis (normal, acute [suppurative] OM, serous OM, other OM) were also

recorded through 13 months of age. An ear exam form, in use for years with other OM studies, was used in this study by the physicians.

These ear exam forms were scanned into a database. The study coordinator reviewed the database and ran queries to identify and remediate inconsistent entries (e.g. tympanic membrane recorded as not visualized, but ear exam findings present) and other data discrepancies. The coordinator also reviewed interim illness and adverse event data collected every two months by research nurse interview to identify visits outside the HealthPartners system. The medical records obtained for these visits were subsequently abstracted and added to the database.

After identifying discrepancies, the coordinator compared ear exam data against the medical record to resolve data entry, scanning and abstractor errors. Changes and corrections were recorded on Data Resolution Forms, and entered into the data base. Physician diagnosis of middle ear status was used unless it was inconsistent with middle ear findings (e.g. air-effusion level, normal diagnosis). For infants with inconsistent findings, Drs. Ferrieri and Daly reviewed ear exam findings, physicians' dictation, recorded signs and symptoms, adverse event log, and diagnosis code to determine a middle ear diagnosis code for the child using the preponderance of evidence. They were blinded to maternal vaccine vs. placebo status. Nearly all discrepancies between ICD-9 code and diagnosis were reconciled after review of the medical record. If ears had different findings (i.e. right ear serous, left ear AOM), suppurative OM rather than non-suppurative OM was used for the child's diagnosis. The term OM includes all otitis media diagnoses, while AOM referred specifically to suppurative OM diagnoses.

Upper respiratory infection (URI) was determined as follows: 1) URI was diagnosed and an ICD-9 code was recorded by a physician, or 2) the term URI (or cold) was mentioned in physician dictation or other documentation (AE log, symptom diary, illness interviews), but not coded, or 3) URI was not recorded with a code, but determined from documented signs and symptoms indicative of URI.

4. Blood draws and antibody assay

Cord blood samples were drawn at delivery, and maternal samples were drawn prior to immunization, at delivery, and at 2, 6, and 13 months post-immunization. Infant blood samples were drawn at 6, 7, 12 and 13 months. Type-specific pneumococcal antibody assays were performed on all sera.

Type-specific pneumococcal antibody titers were measured to nine vaccine serotypes (1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) and control serotype 7F. IgG antibodies against pneumococcal capsular polysaccharides (PS) were analyzed by enzyme-linked immunosorbent assay (ELISA) using Costar 96-well microtiter plates (Corning Incorporated, Corning, NY) for pneumococcal anti-PS IgG antibody against the national reference serum 89-SF (FDA/CBER, Bethesda, MD) using a modified World Health Organization protocol (described by Drs. David Goldblatt and Moon Nahm) that was validated/approved by DMID [15]. After conclusion of the assay, well optical densities (OD) were read using a Fusion Universal Microplate Analyzer (PerkinElmer, Shelton, CT)

at 405nm primary and 690nm reference filters. OD data were converted to antibody concentrations with the Fusion Data Analysis Program (Fusion Data Analysis Software, Version 1.71.0 (PerkinElmer, Shelton, CT)) which calculated the weighted average of the serial dilutions by the 4 Parameter Logistic Curve function after blank OD were subtracted from all wells. The weighted average concentrations were converted to means and coefficients of variation by an in-house program. All data inspection rules were followed to ensure the assay results were valid. Concentrations were measured against 89-SF pneumococcal serum reference standards with assigned values [16].

It was anticipated that the antibody level for each individual serotype in the vaccine group would be equivalent to or greater than the placebo group, therefore non-inferiority testing was performed. All statistical tests were performed on log concentration of antibodies, since the untransformed concentrations had skewed distributions. The null hypothesis was that the vaccine group was inferior to the saline group, i.e. the vaccine group had lower antibody concentrations. For the alternative hypothesis, noninferiority was defined as a difference in mean log concentrations (vaccine – saline) greater than $\log(0.5) = -0.693$. This criterion was equivalent to a ratio of geometric mean concentrations (vaccine/saline) greater than 0.5. The primary endpoint (*S. pneumoniae* antibody levels to the 7 serotypes in PCV-7 at seven months of age) was analyzed by a one sided t-test applied to antibody levels on a log scale. Secondary endpoints were also analyzed by a one sided t-test applied to antibody levels on a log scale at 13 months of age. Because there were 7 tests (one for each antigen in the infant vaccine) for each specimen type, a Hochberg correction for multiple tests was performed for each of the 10 specimen types [17]. The t-tests assumed that the data were normally distributed. The Wilcoxon test is a nonparametric statistical test analogous to the t-test. The Wilcoxon test assumes that the data follow a distribution that is symmetric around its median, and as applied here was a test of the difference between the two group medians. The conclusions of the t-tests and the Wilcoxon tests were the same.

5. Statistical analyses for AOM and OM

Univariate analyses included assessment of baseline comparability between the two treatment groups, Kaplan-Meier estimates of AOM-free and OM-free rates in the first 6 months of life, predictors for AOM-free and OM-free time using the log rank test, and predictors for OM between 6 and 12 months, and OM in the first 12 months using the two-sample t-test and Fisher's exact test. Multivariate analyses for predictors of AOM and OM in the first 6 months used Cox regression to estimate risk ratios, and logistic regression analyses determined odds ratios and confidence intervals for variables significantly predicting OM in the second 6 months of life. Multivariate analyses included all predictors with p values <0.10 from the univariate analyses, however, final models included only those variables with p <0.05. The statistical package used for analysis here was SAS version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

One hundred fifty-three women enrolled in the study, 152 infants were available for analysis, 74 in the vaccine group and 78 in the placebo group. Distribution of risk factors by

treatment group was balanced as seen in Table 1. Vaccine was well-tolerated by maternal and infant participants.

1. Early AOM/OM Onset

The first episode of AOM occurred at 15 days in the vaccine group and 114 days in the placebo group, whereas OM onset was similar in the two groups, 15 and 20 days respectively (Figures 1 and 2). AOM-free and OM-free rates in the first 6 months varied significantly between the two groups: 89% in the placebo group and 74% in the vaccine group were AOM-free in the first 6 months ($p = 0.03$), while 85% in the placebo group and 68% in the vaccine group were OM-free ($p = 0.02$).

Potential risk factors for AOM the first 6 months are shown in Table 2. Variables significantly related to both AOM and OM in the first 6 months of life with univariate analyses were upper respiratory infection ($p < 0.001$ for both) and maternal receipt of vaccine ($p = 0.03$ for AOM, 0.02 for OM). Sibling history of middle ear ventilation with pressure equalization tubes (PE tubes) was also significantly related to both infant AOM ($p = 0.03$) and OM ($p < 0.01$). White race and maternal lack of certainty about group assignment had borderline significant relationships with OM ($p = 0.08$ and 0.09 respectively). Infants with AOM or OM in the first 6 months had significantly more clinic visits compared to those without an AOM/OM diagnosis: mean number of visits was 7.4 (SD 2.7) for OM and 6.0 (SD 2.2) for no OM ($p = 0.01$); mean number of visits for AOM/no AOM were 7.8 and 6.1, respectively ($p = 0.01$). Cox regression analyses demonstrated significantly increased risks of AOM and OM for those in the vaccine group, and those who had an URI (Table 3A). None of the variables violated the proportional hazards assumption, and there were no significant two-way interaction terms between vaccine/placebo group and the other significant variables in the final model.

2. OM 6–12 months

Maternal receipt of PCV-9 did not affect OM incidence after 6 months of age, 58% of vaccine infants and 56% of placebo infants had OM between 6 and 12 months ($p = 0.87$). With univariate analyses, number of visits in the second 6 months of life ($p = < 0.001$) and nonparental smoker in the household at two months ($p = 0.01$) were significant predictors of OM between 6 and 12 months of age. Borderline predictors were income ($p = 0.05$), daycare ($p = 0.06$), maternal age at delivery ($p = 0.06$) and sibling history of tympanostomy tube treatment ($p = 0.08$). However, only number of clinic visits in the second 6 months of age was significantly related to OM during this period with logistic regression analyses (Table 3B).

3. Maternal/Infant Immune Response

At 28 weeks, pre-vaccination, the pregnant women had similar antibody titers. The mothers injected with PCV-9 showed mean antibody levels that were significantly higher at delivery. The mean IgG antibody levels for all serotypes in the PCV-9 pneumococcal vaccine were sustained at two months, six months and 13 months after vaccination in the mothers. Statistical analyses revealed that the titers were all non-inferior at all time points post vaccination (data not shown, but available).

In Table 4 are antibody means for each serotype of each cord and infant blood draw from infants born to vaccine versus placebo vaccinated mothers. Infant cord sera antibody levels paralleled maternal levels, and antibody responses to all seven serotypes present in both the maternal and infant vaccines were non-inferior, compared to the infant cord sera from babies born to the placebo injected mothers.

At six months of age, mean antibody concentrations for five pneumococcal serotypes were significantly lower in infants of vaccinated mothers (Table 4); however, antibody titers were non-inferior for types 6B and 23F in infants born to mothers who received the PCV-9 vaccine. At seven months of age, after their third vaccination of PCV-7, type-specific antibodies against all seven serotypes included in both the maternal and infant vaccines (4, 6B, 9V, 14, 18C, 19F, and 23F) were inferior in infants of vaccine-injected mothers.

As seen in Table 4, responses to only 3 of the 7 immunogens were non-inferior at 12 months in infants of vaccinated mothers. At 13 months, one month after the PCV-7 booster vaccine, the results were different with non-inferiority for only types 4, 9V (as seen at the 12 month bleed) and type 14. Now, the response to serotype 19F was inferior in the infants born to the vaccinated mothers.

Antibody levels to the three pneumococcal serotypes not in the infant vaccine were also measured. Serotypes 1 and 5 were in the maternal nine-valent vaccine, and maternal antibody titers in the vaccine group for these types continued to be elevated throughout the bleedings at delivery/cord, 2 months, 6 months and 13 months (Table 5). Infant cord blood results were comparable to the maternal results for these two serotypes. Antibody levels in infants at 6 and 7 months were modestly higher in infants of vaccinated mothers (see Table 5). At 12 and 13 months, the results were similar between the two infant groups. The control, serotype 7F, was not present in either vaccine; the results were comparable in the two groups (Table 5).

The immune responses to the seven antigens present in the infant PCV-7 after the third vaccination at 6 months, measured at 7 months, were inferior in the infants born to the PCV-9 vaccinated mothers compared to the infants born to mothers who were injected with placebo. It was thought that these might revert and be non-inferior over time, but responses to only three of the seven immunogens were non-inferior at 12 months before the fourth injection of PCV-7, and one month after the booster response, the antibody titers were inferior still to four of the seven serotypes in the infant vaccine. The immunological responses corresponded with the clinical study revealing respiratory illness and increased otitis media in these infants during the first six months of life. No infant developed invasive pneumococcal infection.

There was no difference in the mean number of clinic visits for infants of mothers who thought they knew which vaccine/placebo product they received versus mothers who had no prediction of which product they received.

DISCUSSION

Two well-designed clinical trials in Finland demonstrated that pneumococcal conjugate vaccine provides variable protection against infant AOM. The randomized trial by Eskola et al of PCV-7 given at 2, 4, 6 and 12 months of age showed a 6% reduction in all AOM between 6 and 24 months of age, 57% reduction in AOM caused by vaccine serotypes, and a 51% decrease in AOM caused by cross-reacting serotypes [8]. The trial with 7-valent pneumococcal polysaccharide-meningococcal protein complex vaccine by Kilpi et al showed no reduction in all AOM over the same period, a 56% reduction in AOM caused by vaccine serotypes, and a 5% increase in AOM caused by cross-reacting serotypes [9]. AOM caused by *Haemophilus influenzae* (*H. influenzae*) and *Moraxella catarrhalis* (*M. catarrhalis*) also increased in both trials [8, 9].

In the current study of maternal immunization with PCV-9, AOM occurred significantly earlier in the vaccine group than in the placebo group. Infants whose mothers received PCV-9 vaccine during pregnancy were significantly more likely to have AOM and OM diagnosed in the first 6 months of life than infants whose mothers received placebo. Placebo group infants had significantly more AOM and OME free time than infants of vaccine recipients, and had later onset of AOM compared to OM. Since OM includes both suppurative and nonsuppurative OME, fluid may remain in the middle ear for variable amounts of time after an AOM episode and viable organisms may be absent if the child was treated with antibiotics. After 6 months of age, infant AOM/OM rates were similar in the two treatment groups.

These findings were contrary to our hypothesis that transfer of pneumococcal antibodies from mother to fetus during pregnancy would result in lower AOM and OM incidence in infants in the PCV-9 vaccine group. All infants in the current study received PCV-7 at 2, 4, 6, and 12 months of age. However, the basis for the above observations includes the possibility of altered B lymphocyte responses in infants exposed to these specific polysaccharide antigens in utero, altering immune responses to their conventional pneumococcal vaccine of infancy. Invoking the concept of “immune tolerance” is appealing, but cannot be proved [18, 19]. After studying infant 7 month blood draw antibody results, our interpretation was that, conceivably, maternal immunization and/or high maternal antibody levels may have modulated the pneumococcal immune responses of their infants. In contrast to studies showing that infant pneumococcal conjugate vaccine conferred relatively moderate rates of protection against AOM [20], maternal PCV-9 pneumococcal vaccine did not demonstrate this, and, in fact, was associated with higher rates of AOM in their infants in our study.

High levels of passive maternal pneumococcal antibody may have suppressed infants’ ability to produce an adequate immune response to middle ear pathogens. There is precedent from studies of immunological responses to other bacterial pathogens, that elevated titers at the onset of a challenge may dampen a new immunological response [21]. Infants in the vaccine group may have been less able to mount an immune response against vaccine and non-vaccine serotypes of *S. pneumoniae*, as well as against *H. influenzae* and *M. catarrhalis*,

common causes of otitis media. However, it was not possible for us to measure antibodies to Hib.

More than half of the children in this study experienced upper respiratory infection, which predisposes infants and children to AOM [22–27]. In a study of URI and OM, 61% of URI episodes were complicated by OM, 37% had AOM and 24% had OME [22]. The daycare setting enhances transmission of viruses responsible for URI, and these viruses predispose infants to Eustachian tube dysfunction, bacterial colonization and AOM [22]. Although nearly half of the infants in each randomized group in this study were in daycare, this factor did not significantly increase the risk of AOM/OM, which is contrary to previous studies of early OM onset [4–6, 28, 29]. In an earlier study conducted in HealthPartners, risk of AOM in the first 6 months of life was considerably higher among infants with URI than for those in daycare (RR 7.1 vs.1.7) [30].

Our study demonstrated that infants born to mothers who received vaccine during the last trimester were more likely to have AOM/OM in the first 6 months of life than those whose mothers received placebo. This difference in OM rates did not persist past 6 months of age, possibly due to maturation of other components of their immune systems. High levels of maternal pneumococcal antibody at birth may have suppressed early infant response to their PCV-7 immunization, resulting in AOM and OM. This study does not allow determination of the causative agents of AOM and OM, e.g. vaccine serotypes, cross-reacting serotypes, non-vaccine serotypes, *Haemophilus influenzae* or *Moraxella catarrhalis*. Exposure to pneumococcal antigens *in utero* may also have inhibited a ntibody response when infants were infected with *S. pneumoniae*. Although it is possible that high levels of maternal pneumococcal antibody could inhibit early infant antibody response resulting in AOM and OM, the effect appeared to be limited to the first 6 months of life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support was provided by the National Institute of Deafness and Communication Disorders: P50 DC03093, P50 DC03093-05S1, and R01 DC005974, the National Institute of Allergy and Infectious Diseases was the Investigational New Drug (IND) holder. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Deafness and Other Communication Disorders or the National Institutes of Health. Wyeth Lederle Vaccine and Pediatrics provided the 9-valent pneumococcal conjugate vaccine and placebo for maternal use, and the 7-valent pneumococcal conjugate vaccine (Pneumovax®) for study infants. We would like to thank the mothers and their infants whose dedication made this study possible, research nurses Elaine Stier, Debi Frerichs and Mary Meester of the Health Partners Research Foundation (HPRF), coordinators Julie Toth and Pam Kaufman, HealthPartners physician advisors Joan Madden, Mary Meland, Lawrence Condon and nurse practitioner Georgeanne Croft, HealthPartners physicians and nurse practitioners who performed infant examinations, HPRF medical record abstractor Linda Loes, Data Safety and Monitoring Board members Drs. Kathryn Edwards, Maurizio Maccato, Shrikant Bangdiwala, Kenneth Trofatter, Eugene Shapiro, and Timothy Landers, NIAID/DMID sponsor Dr. George Curlin, NIDCD sponsor Dr. Julianna Gulya, and regulatory affairs specialists Wendy Fanaroff-Ravick and Janie Russel (NIAID). This trial would not have been possible without the expertise and contributions of all these individuals and entities.

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Highlights

- AOM/OM occurred earlier in the first 6 months of life in the vaccine group.
- Maternal receipt of vaccine did not affect OM incidence after 6 months of age.
- Mothers' perception of receiving vaccine may have influenced infant clinic visits.
- Treatment groups were balanced for examined risk and demographic variables.
- Infant pneumococcal abs of vaccinated mothers vs. placebo mothers were inferior.

Figure 1.
Kaplan-Meier estimates for time to first AOM in 6 months

Figure 2.
Kaplan-Meier estimates for time to first OM in 6 months

Table 1

Comparison of demographic variable vaccine and treatment groups

Variable	Vaccine n=74 n (%)	Placebo n=78 n (%)	P-value ^a
Maternal smoking	2/74 (2.7)	7/78 (9.0)	0.17
Paternal smoking	15/74 (20.3)	13/78 (16.7)	0.68
Maternal OM history	13/73 (17.8)	10/76 (13.2)	0.50
Paternal OM history	9/70 (12.9)	10/69 (14.5)	0.81
Sibling OM history	14/69 (20.3)	14/71 (19.7)	1.00
Maternal education			
High school or less	7/74 (9.5)	10/78 (12.8)	0.73
Up to college graduation	51/74 (68.9)	49/78 (62.8)	
Graduate school	16/74 (21.6)	19/78 (24.4)	
Paternal education			
High school or less	11/74 (14.9)	14/78 (18.0)	0.70
Up to college graduation	49/74 (66.2)	46/78 (59.0)	
Graduate school	14/74 (18.9)	18/78 (23.1)	
Parents married or living together	71/74 (96.0)	70/78 (89.7)	0.21
Income			
\$50,000	19/74(25.7)	24/77(31.2)	0.63
\$50,001 to \$80,000	28/74(37.8)	24/77(31.2)	
>\$80,000	27/74(36.5)	29/77(37.7)	
Premature birth (<37 wks)	5/74 (6.8)	1/78 (1.3)	0.11
White race	58/73 (79.5)	63/77 (81.8)	0.84
1 sibling in the household	34/74 (46.0)	41/78 (52.6)	0.42
Maternal smoking at 2 mo	4/74 (5.4)	9/78 (11.5)	0.25
Paternal smoking at 2 mo	13/74 (17.6)	13/78 (16.7)	1.00
Others smoking at 2 mo	3/74 (4.1)	2/78 (2.6)	0.68
Mother sure she received vaccine	22/69 (31.9)	20/77 (26.0)	0.47
Breast feeding 2 mo	47/70 (67.1)	56/77 (72.7)	0.48
^c Day-care	36/74 (48.7)	32/78 (41.0)	0.42
^d URI	42/74 (56.8)	40/78 (51.3)	0.52

Variable	Vaccine n=74 n (%)	Placebo n=78 n (%)	P-value ^a
	Mean (SD)	Mean (SD)	P-value ^b
Maternal age at delivery	30.3(5.3)	30.1(5.3)	0.84
No. visits within 6 mo	6.3(2.4)	6.5(2.4)	0.58

^a Fisher's exact test

^b two sample t-test

^c preceding AOM

^d preceding or concurrent with AOM

Table 2

Univariate analysis for Kaplan-Meier estimates of the AOM-free and OM-free rates in the first 6 months of life (N = 152)

Risk factor	N	AOM-free rate by 6 months	P-value ^a	OM-free rate by 6 months	P-value ^a
Maternal smoking					
Yes	9	0.89	0.60	0.89	0.40
No	143	0.81		0.76	
Paternal smoking					
Yes	28	0.75	0.26	0.75	0.78
No	124	0.83		0.77	
Maternal OM history					
Yes	23	0.83	0.85	0.74	0.88
No	126	0.81		0.76	
Paternal OM history					
Yes	19	0.90	0.33	0.84	0.35
No	120	0.80		0.74	
Sibling OM history					
Yes	28	0.71	0.13	0.64	0.10
No	112	0.84		0.79	
Sibling history of ear tubes					
Yes	9	0.56	0.03	0.44	<0.01
No	131	0.84		0.78	
Maternal education					
High school or less	17	0.77	0.76	0.77	0.99
Up to college graduation	100	0.82		0.76	
Graduate school	35	0.83		0.77	
Paternal education					
High school or less	25	0.84	0.97	0.84	0.19

Risk factor	N	AOM-free rate by 6 months	P-value ^a	OM-free rate by 6 months	P-value ^a
Up to college graduation	95	0.81		0.78	
Graduate school	32	0.81		0.66	
Marital status					
Married or living together	141	0.82	0.39	0.77	0.75
Not married or living together	11	0.73		0.73	
Income					
<=\$50,000	43	0.88	0.41	0.84	0.38
\$50,001 to \$80,000	52	0.79		0.75	
>\$80,000	56	0.79		0.71	
Premature birth					
37 wks	146	0.82	0.92	0.76	0.69
<37 wks	6	0.83		0.83	
Race					
White	121	0.80	0.11	0.74	0.08
Non-white	29	0.93		0.90	
Siblings living with baby					
1	75	0.80	0.63	0.75	0.63
0	77	0.83		0.78	
Maternal smoking at 2 mo					
Yes	13	0.85	0.79	0.85	0.48
No	139	0.81		0.76	
Paternal smoking at 2 mo					
Yes	26	0.73	0.17	0.73	0.60
No	126	0.83		0.77	
Others smoking at 2 mo					
Yes	5	0.80	0.84	0.80	0.94
No	147	0.82		0.76	

Risk factor	N	AOM-free rate by 6 months	P-value ^a	OM-free rate by 6 months	P-value ^a
Maternal assessment of group assignment					
Vaccine	42	0.88	0.21	0.86	0.09
Don't know or not certain	104	0.79		0.72	
Breast feeding					
<2 mo	44	0.75	0.20	0.71	0.38
2 mo	103	0.84		0.78	
<i>b</i> Day-care					
Yes	65	0.80	0.73	0.75	0.82
No	87	0.83		0.78	
Group					
Vaccine	74	0.74	0.03	0.68	0.02
Placebo	78	0.89		0.84	
<i>c</i> URI					
Yes	82	0.71	<0.001	0.65	<0.001
No	70	0.94		0.90	

^a p values with log rank test

^b preceding AOM

^c preceding or concurrent with AOM

Table 3A

Cox regression analyses for predictors for AOM, OM by age 6 months

Risk factor	AOM Risk Ratio N = 152	OM Risk Ratio N = 140
Vaccine group (infants born to maternal vaccine group)	3.9 (95% CI 1.7, 9.1) p <0.01	3.4 (95% CI 1.6, 7.4) p <0.01
URI	9.2 (95% CI 2.8, 30.5) p <0.001	6.6 (95% CI 2.5, 17.0) p <0.001
Clinic visits in 6 months	1.3/visit (95% CI 1.1, 1.5) p < 0.01	1.2/visit (95% CI 1.0, 1.4) p = 0.02
Sibling history of tubes	Not significant in the final model (p >0.05)	2.8 (1.0, 7.5) p = 0.04

Table 3B

Logistic regression analysis for OM predictors between 6 and 12 months

Risk Factors	Odds ratio N = 144	P-value
Clinic visits in second 6 months	2.0/visit (95% CI 1.6, 2.6)	<0.001

Table 4

Infant pneumococcal antibody concentrations at five time periods

Sample Type	Antigen	Mean (µg/mL)		Mean Log		T Statistic	p-value	Test Result	Conclusion
		Vaccine	Placebo	Vaccine	Placebo				
Infant Cord	4	5.155	0.315	0.979	-1.770	17.816	4.06E-39		Totally Non-inferior
Infant Cord	6B	24.219	1.384	2.148	-0.168	14.140	3.44E-28		
Infant Cord	9V	6.925	1.007	1.259	-0.671	13.813	9.25E-29		
Infant Cord	14	30.626	4.129	2.659	0.448	12.295	9.17E-25		
Infant Cord	18C	15.067	0.661	2.010	-0.908	18.273	8.20E-39		
Infant Cord	19F	10.771	3.594	1.956	0.870	10.628	3.35E-20		Non-Inferior for 6B, 23F
Infant Cord	23F	23.216	1.034	2.227	-0.457	15.784	3.34E-32		
Infant 6-Mo	4	0.675	1.410	-0.773	0.021	-0.664	7.46E-01	Inferior	
Infant 6-Mo	6B	0.977	0.487	-0.657	-1.085	6.900	1.25E-10		
Infant 6-Mo	9V	0.744	1.381	-0.662	-0.051	0.520	3.02E-01	Inferior	
Infant 6-Mo	14	3.043	6.971	0.694	1.340	0.246	4.03E-01	Inferior	Totally Inferior
Infant 6-Mo	18C	1.023	1.440	-0.666	0.022	0.028	4.89E-01	Inferior	
Infant 6-Mo	19F	1.243	3.564	-0.170	0.865	-2.314	9.89E-01	Inferior	
Infant 6-Mo	23F	1.539	0.696	-0.716	-1.061	4.940	1.16E-06		
Infant 7-Mo	4	1.751	2.079	0.120	0.470	2.475	7.34E-03	Inferior	
Infant 7-Mo	6B	2.684	4.949	0.016	0.975	-1.241	8.92E-01	Inferior	Non-Inferior for 4, 9V, 19F
Infant 7-Mo	9V	1.631	2.324	0.066	0.518	1.613	5.46E-02	Inferior	
Infant 7-Mo	14	7.301	10.874	1.439	1.995	0.729	2.34E-01	Inferior	
Infant 7-Mo	18C	1.451	3.183	-0.158	0.915	-2.397	9.91E-01	Inferior	
Infant 7-Mo	19F	1.941	3.471	0.356	1.011	0.295	3.84E-01	Inferior	
Infant 7-Mo	23F	1.051	2.583	-0.469	0.551	-1.893	9.70E-01	Inferior	Non-Inferior for 4, 9V, 19F
Infant 12-Mo	4	0.351	0.429	-1.300	-1.088	3.928	7.01E-05		
Infant 12-Mo	6B	0.877	1.474	-0.690	-0.043	0.286	3.88E-01	Inferior	
Infant 12-Mo	9V	0.598	0.611	-0.983	-0.784	3.576	2.52E-04		
Infant 12-Mo	14	2.101	3.086	0.268	0.762	1.081	1.41E-01	Inferior	
Infant 12-Mo	18C	0.441	0.644	-1.570	-0.716	-0.999	8.40E-01	Inferior	

Sample Type	Antigen	Mean (µg/mL)		Mean Log		T Statistic	p-value	Test Result	Conclusion
		Vaccine	Placebo	Vaccine	Placebo				
Infant 12-Mo	19F	1.288	1.643	-0.207	0.108	2.436	8.15E-03		
Infant 12-Mo	23F	0.345	0.599	-1.530	-0.783	-0.357	6.39E-01	Inferior	
Infant 13-Mo	4	4.075	3.404	0.950	0.860	4.651	4.27E-06		Non-Inferior for 4, 9V, 14
Infant 13-Mo	6B	13.727	20.071	1.643	2.519	-0.778	7.81E-01	Inferior	
Infant 13-Mo	9V	4.805	4.547	1.169	1.139	4.437	1.02E-05		
Infant 13-Mo	14	13.357	12.702	2.138	2.206	3.460	3.75E-04		
Infant 13-Mo	18C	3.416	6.034	0.743	1.453	-0.101	5.40E-01	Inferior	
Infant 13-Mo	19F	4.256	6.551	1.148	1.550	2.050	2.13E-02	Inferior	
Infant 13-Mo	23F	4.036	10.360	0.794	1.821	-1.604	9.44E-01	Inferior	

Table 5

Maternal and Infant Pneumococcal Antibody Concentrations for Two Antigens Only in Maternal Vaccine and 7F Control

Sample Type	Antigen	Mean (µg/mL)		Mean Log		T Statistic	p-value
		Vaccine	Placebo	Vaccine	Placebo		
Maternal 28 (23–35) Week	1	0.553	0.576	−0.859	−0.830	6.145	3.53E-09
Maternal 28 (23–35) Week	5	0.744	0.879	−0.627	−0.590	4.701	2.94E-06
Maternal 28 (23–35) Week	7F	0.604	0.629	−0.814	−0.851	5.143	4.25E-07
Maternal Delivery	1	5.061	0.501	0.935	−0.958	17.237	6.76E-34
Maternal Delivery	5	11.423	0.796	1.366	−0.757	14.118	8.03E-28
Maternal Delivery	7F	0.534	0.518	−0.911	−1.014	5.928	1.01E-08
Maternal 2-Month	1	5.917	0.816	0.964	−0.542	11.296	1.10E-18
Maternal 2-Month	5	19.913	1.321	1.468	−0.177	9.883	7.00E-16
Maternal 2-Month	7F	1.162	1.082	−0.137	−0.199	4.905	1.76E-06
Maternal 6-Month	1	3.938	0.824	0.750	−0.502	12.860	2.49E-24
Maternal 6-Month	5	7.965	1.442	1.220	−0.080	11.081	2.51E-20
Maternal 6-Month	7F	1.122	1.114	−0.162	−0.233	5.588	5.58E-08
Maternal 13-Month	1	2.821	0.859	0.567	−0.467	11.308	1.57E-20
Maternal 13-Month	5	5.862	1.496	0.988	−0.084	9.604	1.07E-16
Maternal 13-Month	7F	1.204	1.024	−0.074	−0.308	6.477	8.99E-10

Sample Type	Antigen	Mean (µg/mL)		Mean Log		T Statistic	p-value
		Vaccine	Placebo	Vaccine	Placebo		
Infant Cord	1	3.881	0.546	0.894	−0.839	17.816	1.83E-35
Infant Cord	5	7.861	0.843	1.145	−0.767	13.615	3.07E-27
Infant Cord	7F	0.511	0.538	−0.982	−1.059	5.354	1.60E-07
Infant 6-Month	1	0.153	0.086	−2.230	−2.680	9.079	9.57E-16
Infant 6-Month	5	0.328	0.115	−1.839	−2.546	7.189	4.13E-11
Infant 6-Month	7F	0.047	0.053	−3.436	−3.322	3.719	1.47E-04
Infant 7-Month	1	0.090	0.082	−2.651	−2.715	6.618	4.40E-10
Infant 7-Month	5	0.204	0.149	−2.025	−2.268	5.994	9.58E-09

Sample Type	Antigen	Mean (µg/mL)		Mean Log		T Statistic	p-value
		Vaccine	Placebo	Vaccine	Placebo		
Infant 7-Month	7F	0.044	0.054	-3.557	-3.296	2.437	8.06E-03
Infant 12-Month	1	0.086	0.103	-2.785	-2.569	3.309	6.13E-04
Infant 12-Month	5	0.246	0.281	-1.809	-1.638	3.350	5.38E-04
Infant 12-Month	7F	0.075	0.087	-3.073	-2.773	2.491	7.08E-03
Infant 13-Month	1	0.174	0.135	-2.353	-2.300	4.151	3.17E-05
Infant 13-Month	5	0.365	0.342	-1.443	-1.376	4.053	4.56E-05
Infant 13-Month	7F	0.137	0.126	-2.609	-2.419	3.099	1.23E-03