Published in final edited form as:

Pediatr Infect Dis J. 2010 July ; 29(7): 600–606. doi:10.1097/INF.0b013e3181d264a6.

HEPTAVALENT PNEUMOCOCCAL CONJUGATE VACCINE IMMUNOGENICITY IN VERY-LOW-BIRTH-WEIGHT, PREMATURE INFANTS

Carl T. D'Angio, MD^a, Roy J. Heyne, MD^b, T. Michael O'Shea, MD, MPH^c, Robert L. Schelonka, MD^d, Seetha Shankaran, MD^e, Shahnaz Duara, MD^f, Ronald N. Goldberg, MD^g, Barbara J. Stoll, MD^h, Krisa P. Van Meurs, MDⁱ, Betty R. Vohr, MD^j, Abhik Das, PhD^k, Lei Li, PhD^k, Robert L. Burton, MS^d, Betty Hastings^k, Dale L. Phelps, MD^a, Pablo J. Sanchez, MD^b, Waldemar A. Carlo, MD^d, David K. Stevenson, MDⁱ, and Rosemary D. Higgins, MD [on behalf of on behalf of the NICHD Neonatal Research Network]^l

^aUniversity of Rochester School of Medicine and Dentistry, Rochester, NY

^bDepartment of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX

^cWake Forest University School of Medicine, Winston-Salem, NC

^dDepartments of Pediatrics and Pathology, University of Alabama at Birmingham, Birmingham, AL

^eDepartment of Pediatrics, Wayne State University, Detroit, MI

^fDepartment of Pediatrics, University of Miami, Miami, FL

^gDepartment of Pediatrics, Duke University, Durham, NC

^hDepartment of Pediatrics, Emory University, Atlanta, GA

ⁱStanford University School of Medicine, Palo Alto, CA

^jDepartment of Pediatrics, Women & Infants Hospital, Brown University, Providence, RI

^kStatistics and Epidemiology Unit, RTI International, Research Triangle Park, NC

^lEunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

Abstract

Background—The heptavalent pneumococcal-CRM₁₉₇ conjugate vaccine (PCV-7) has been incompletely studied in very-low-birth-weight (VLBW, ≤1500 grams) infants.

Corresponding Author: Carl T. D'Angio, MD, Box 651, Neonatology, Golisano Children's Hospital at Strong, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, Phone: (585) 273-4911, Fax: (585) 461-3614, carl_dangio@urmc.rochester.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All authors confirm that they made substantial contributions to the work, were involved in the drafting and/or reviewing of the article, and have approved the version to be published. No author has a relevant financial interest or other conflict of interest as regards this research.

Trial Registration: This study was registered at www.clinicaltrials.gov (NCT00273325).

Objective—To assess PCV-7 immunogenicity in VLBW, premature infants. We hypothesized that the frequency of post-vaccine antibody concentrations ≥ 0.15 $\mu\text{g/mL}$ would vary directly with birth weight.

Methods—This was a multi-center observational study. Infants 401–1500 grams birth weight and <32 0/7 weeks gestation, stratified by birth weight, were enrolled from 9 NICHD Neonatal Research Network centers. Infants received PCV-7 at 2, 4 and 6 months after birth and had blood drawn 4–6 weeks following the third dose. Antibodies against the 7 vaccine serotypes were measured by enzyme-linked immunosorbent assay.

Results—Of 369 enrolled infants, 244 completed their primary vaccine series by 8 months and had serum obtained. Subjects were 27.8 ± 2.2 (mean $\pm$ standard deviation) weeks gestation and 1008 ± 282 grams birth weight. Twenty-six percent had bronchopulmonary dysplasia and 16% had received postnatal glucocorticoids. Infants 1001–1500 grams birth weight were more likely than those 401–1000 grams to achieve antibody concentrations ≥ 0.15 $\mu\text{g/mL}$ against the least two immunogenic serotypes (6B: 96% v. 85%, $P = 0.003$ and 23F: 97% v. 88%, $P = 0.009$). In multiple logistic regression analysis, lower birth weight, postnatal glucocorticoid use, lower weight at blood draw and Caucasian race were each independently associated with antibody concentrations <0.35 $\mu\text{g/mL}$ against serotypes 6B and/or 23F.

Conclusion—When compared with larger premature infants, infants weighing ≤ 1000 grams at birth have similar antibody responses to most, but not all, PCV-7 vaccine serotypes.

Keywords

Infant, premature; infant, very low birth weight; pneumococcal vaccines; immunization; vaccines

INTRODUCTION

Streptococcus pneumoniae causes 10–25% of all pneumonias in the United States, is responsible for some 40,000 deaths each year and is a leading cause of bacteremia and meningitis in infants.^{1, 2} Epidemiologic information suggests a 2.6-fold increase in invasive pneumococcal disease among infants born at <2500 grams birth weight, when compared with normal-birth-weight infants.³

The heptavalent pneumococcal CRM₁₉₇ conjugate vaccine (PCV-7) is safe, immunogenic and effective in preventing invasive pneumococcal infections in infants and children.^{4, 5} The vaccine is also effective in premature infants, but data in very-low-birth-weight (VLBW, ≤ 1500 grams) or very premature (<32 weeks gestation) infants are sparse.³ Although preterm infants generally respond normally to vaccines given at the same postnatal ages as full-term infants, antibody concentrations to routine infant vaccines are sometimes lower among VLBW infants.^{6–11}

The primary objective of this research was to measure the immunogenicity of 3 doses of PCV-7 in VLBW (401–1500 gram) infants, when this vaccine was given as part of routine pediatric care. We hypothesized that, in this population, the frequency of antibody concentrations ≥ 0.15 $\mu\text{g/mL}$ would decrease with decreasing birth weight. Other objectives included identification of risk factors for poorer vaccine immunogenicity, description of vaccine-related adverse events and detection of pneumococcal disease in the study population.

MATERIALS AND METHODS

Subjects

This was a prospective, observational cohort study. Subjects were enrolled between September 2004 and November 2006 at nine centers in the 16-center National Institute of Child Health and Human Development (NICHD) Neonatal Research Network. The study was reviewed and approved by each center's Institutional Review Board. All subjects' parents gave informed permission before study entry. The trial was monitored by the NICHD Data and Safety Monitoring Committee.

Subjects were eligible for enrollment if they were <32 0/7 weeks gestation at birth (as determined by best obstetrical estimate of last menstrual period with confirmation by ultrasound, or by neonatologist's estimate based on physical characteristics if an obstetrical estimate was not available), had a birth weight 401–1500 grams, their family had a telephone at home, and they were anticipated to be available for a follow-up visit at 7–9.5 months postnatal age. Children with known immunodeficiency or HIV exposure, who had not received the first dose of PCV-7 by 3 months, 0 days postnatal age or whose primary care providers chose not to participate were excluded. Subjects were enrolled with the intention of gathering at least 20 subjects in each 100-gram birth-weight increment between 501 and 1500 grams who had received a primary series of 3 PCV-7 doses, given at least 4 weeks apart, that was completed by 8 months postnatal age, and who had post-vaccine serology drawn between 4 and 6 weeks following the 3rd vaccine dose. Subjects who completed the study within the time windows were included in a pre-specified “on-time” cohort. All subjects, including those whose immunizations were given outside one or more time window, were included in a secondary “intention-to-complete” analysis.

Study Procedures

Immunization—Subjects had three doses of PCV-7 (Prevnar™, Wyeth Pharmaceuticals, Madison, NJ) purchased and administered by their clinical providers beginning before 3 months of age and spaced about 2 months apart, either in the neonatal intensive care unit or as outpatients, according to the providers' usual practices and Centers for Disease Control and Prevention¹² and American Academy of Pediatrics¹³ recommendations. There were no restrictions on the receipt or timing of other routine infant vaccines. This study did not include evaluation of the 4th, 12-month dose of PCV-7 (except in special circumstances, described below in “Antibody determination”).

Tracking—Initially, information regarding immunization status and adverse events was abstracted from the subject's hospital record. After the infant was discharged, study personnel tracked immunization status by telephone contact with primary care providers before and after each scheduled vaccine visit. Families completed a seven-day adverse event diary following each outpatient PCV-7 dose. The diaries were reviewed by telephone with study personnel 8–10 days after each vaccine visit. Possible severe adverse events, including possible cases of pneumococcal disease, were confirmed by primary record review.

Study visit—Subjects made one study visit at 4–6 weeks following the third PCV-7 dose. At that visit, interim history and immunization data were reviewed, growth indices were measured, and a 2 mL blood sample was obtained. Serum was separated and stored at –80°C.

Follow-up—We gathered information regarding adverse events, survival, hospitalization and possible invasive bacterial infection at 18–22 months of age, corrected for gestation. Data were collected on a questionnaire that included specific questions about unscheduled physician visits, emergency room visits and hospitalization, and confirmed, where necessary, by review

of primary records. The questionnaire was administered in person to parents during a Neonatal Research Network follow-up appointment routinely scheduled for children ≤ 1000 grams birth weight. The questionnaire information was collected by mail or telephone from the parents of infants >1000 grams.

Serology

Serum specimens were sent on dry ice by overnight courier for pneumococcal antibody analysis at the laboratory of Dr. Moon Nahm at the University of Alabama at Birmingham.

Antibody determination—The amounts of anti-capsular polysaccharide antibody were determined for each of the seven vaccine components (pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) by a third-generation enzyme-linked immunosorbent assay, as previously described (a detailed protocol is available at www.vaccine.uab.edu).¹⁴ The lower detection limit of the assay was 0.01–0.03 $\mu\text{g/mL}$. Any subject with antibody concentrations $<0.15 \mu\text{g/mL}$ against two or more vaccine serotypes was offered the opportunity to have repeat serologies performed following the booster dose of PCV-7 at 12–15 months' postnatal age.

Pneumococcal opsonophagocytosis—Pneumococcal-killing opsonophagocytosis assays were performed for 4 serotypes (4, 6B, 14, and 23F) as previously described (a detailed protocol is available at www.vaccine.uab.edu).¹⁵ Opsonization titers were defined as the serum dilution that killed 50% of the target bacteria.

Data Analysis

The primary outcome was the proportion of infants achieving antibody concentrations $\geq 0.15 \mu\text{g/mL}$ (an estimate of the minimum protective concentration) against serotype 6B at 4–6 weeks after the third PCV-7 dose, compared between infants 401–1000 grams birth weight (extremely [E] LBW) and those 1001–1500 grams birth weight (VLBW).⁴ Serotype 6B was chosen prospectively due to its known low immunogenicity and its high contribution to the burden of disease.^{1, 4, 16, 17} Pre-specified secondary analyses included proportions of children with antibody concentrations to the other 6 vaccine serotypes $\geq 0.15 \mu\text{g/mL}$, with antibody concentrations $\geq 1.0 \mu\text{g/mL}$ and with opsonophagocytosis titers $\geq 1:8$.^{18, 19} After the development of the study protocol, the World Health Organization recommended a reference antibody concentration standard of 0.35 $\mu\text{g/mL}$.¹⁹ As a result, additional, tertiary analyses of potential confounding variables divided antibody concentrations above and below 0.35, rather than 0.15, $\mu\text{g/mL}$.

To calculate the sample size needed, we projected that 89% of infants of 1401–1500 grams birth weight would achieve 6B concentrations $\geq 0.15 \mu\text{g/mL}$, while 55% of infants of 501–600 grams birth weight would achieve these concentrations, and that immunogenicity would vary linearly and continuously with birth weight. The seroresponse in infants born at 1401–1500 grams was estimated from limited prior data on premature infants.³ The seroresponse in infants born at 501–600 grams was estimated from *Haemophilus influenzae* type b (Hib) polyribosylribitol antibody responses in small, sick infants receiving Hib conjugate vaccines.^{9, 10} Using these assumptions, 200 infants (20 infants per 100-gram increment) were required to detect a difference in proportions of infants ≤ 1000 grams (62.6%) and >1000 grams (81.4%) with antibody concentrations $\geq 0.15 \mu\text{g/mL}$, assuming $\alpha = 0.05$ and $1-\beta = 0.80$.

Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). Weight-for-corrected-age Z-score at the time of the blood draw was estimated using the 2000 CDC growth chart (<http://www.cdc.gov/growthcharts>). The proportions of infants with antibody concentrations $\geq 0.15 \mu\text{g/mL}$, $\geq 0.35 \mu\text{g/mL}$ and $\geq 1.0 \mu\text{g/mL}$ by two birth weight categories (≤ 1000 grams and >1000 grams) were calculated for each vaccine serotype and the statistical

significance of the differences between the two birth weight groups was assessed with the chi square test. Least mean squares linear regression was used to delineate the relationship between log antibody concentration and birth weight. Multiple logistic regression models for antibody concentrations ≥ 0.35 $\mu\text{g/mL}$ were estimated using birth weight group as a predictor, with potential confounding factors (sex, race, postnatal glucocorticoids, Z-score of weight for corrected age at blood draw and age at first vaccination) chosen *a priori*.

RESULTS

Two hundred and forty four infants completed their three-dose primary PCV-7 series by 8 months, had blood drawn within 6 weeks after the third vaccine dose and had serum available (Figure 1). These infants were the subjects of the pre-specified primary analysis (the on-time cohort). All 100-gram enrollment weight strata above 600 grams birth weight achieved at least the intended 20 subjects per increment in the on-time cohort. There were 6 subjects in the 401–500 gram increment and 19 subjects in the 501–600 gram increment. Baseline and postnatal characteristics of these infants are shown in Table 1 (SDC).²⁰ Mean gestational age was 27.8 ± 2.2 (standard deviation) weeks. Twenty three of 38 infants (61%) who received postnatal glucocorticoids also had bronchopulmonary dysplasia (BPD). Although only one infant was receiving systemic glucocorticoids at the time of the first PCV-7 dose, 15 infants received at least one dose of systemic glucocorticoids after the first dose of vaccine. Of infants in the on-time cohort, 169 (69%) had their first PCV-7 immunization while still hospitalized.

In analysis of the primary outcome, infants 401–1000 grams birth weight were less likely than those 1001–1500 grams birth weight to achieve antibody concentrations ≥ 0.15 $\mu\text{g/mL}$ against serotype 6B (Table 2). Findings were similar for serotype 23F, concentrations ≥ 0.35 $\mu\text{g/mL}$, and opsonophagocytosis titers $\geq 1:8$ (Tables 2 and 3).

When antibody concentrations were plotted against birth weight (Figure 2), wide variability was noted, with no abrupt change in concentrations at any given weight. Serotypes 6B and 23F showed small, but significant, linear trends toward lower concentrations with lower birth weight and serotype 14 showed a trend of marginal significance ($P = 0.048$, not shown). No other serotype showed a significant association between concentration and birth weight.

Postnatal glucocorticoid administration was associated with diminished antibody concentrations (Table 4). The mean ($\pm$ standard deviation) birth weight of children who had received postnatal glucocorticoids was 777 ± 213 grams, while the mean birth weight of those who had not was 1050 ± 273 grams. There was no significant relationship between cumulative glucocorticoid dosage and antibody concentration among the 38 subjects who received systemic glucocorticoids (data not shown).

Since only serotypes 6B and 23F showed significant associations with birth weight on univariate analyses, only these serotypes were assessed by multiple logistic regression (Figure 3). Lower birth weight (serotypes 6B and 23F) and postnatal glucocorticoid administration (serotype 6B only) were each independently associated with antibody concentrations < 0.35 $\mu\text{g/mL}$. Caucasian race (serotype 6B) and lighter weight for corrected age (serotype 23F) also emerged as factors associated with poorer vaccine immunogenicity, even when controlled for glucocorticoid exposure. Addition of ethnicity, bronchopulmonary dysplasia and/or center to the model or using a cut-off of 0.15 $\mu\text{g/mL}$ did not materially alter the regression results (data not shown).

Fifteen children (11 in the ≤ 1000 -gram group and 4 in the > 1000 -gram group) had antibody concentrations < 0.15 $\mu\text{g/mL}$ against at least two serotypes at the time of the initial blood draw. Of these, 3 (20%) had repeat concentrations drawn 4–6 weeks following the 4th dose of PCV-7. All repeat concentrations were ≥ 0.35 $\mu\text{g/mL}$.

Of the 125 children who were enrolled in the study, but not included in the on-time cohort, 37 children had specimens available for a secondary analysis (Figure 1). Combining these infants with the on-time cohort in an intention-to-complete analysis did not change the birth-weight-based differences in proportions of children achieving 6B and 23F concentrations ≥ 0.15 $\mu\text{g/mL}$ (data not shown). Of 281 infants with any antibody concentration data available, 266 (95%) had concentrations ≥ 0.15 $\mu\text{g/mL}$ to at least 6 vaccine serotypes.

Among the 369 infants enrolled in the study, 124 (34%) had at least one adverse event at some time before 18-to-22 months. Two subjects, neither in the on-time cohort, died from sudden infant death during the 4-month course of PCV-7 immunization. Detailed analysis of adverse events was confined to the 244 subjects in the on-time cohort, since their high level of follow-up (92% through 18-to-22 months) allowed more accurate estimation of event rates. One hundred subjects (41%) experienced 201 adverse events (many of which included more than one finding, e.g. emergency department visit and hospitalization); 133 adverse events among 62 (53%) of 118 infants ≤ 1000 grams birth weight and 68 adverse events among 38 (30%) of 126 infants >1000 grams. There were 119 re-hospitalizations before 18–22 months of age among 64 infants (26%); 83 re-hospitalizations among 39 (33%) infants ≤ 1000 grams and 36 re-hospitalizations among 25 (20%) infants >1000 grams. Only 3 re-hospitalizations were within 7 days after vaccine. Six infants (2% of all infants and 4% of hospitalized infants) had “recurrent” episodes of apnea (apnea occurring with no history of apnea during the 7 days preceding the vaccine) in the 72 hours after vaccine. All recurrent apnea episodes were among infants ≤ 1000 grams, and all of these subjects were still hospitalized at the time of the episodes. One subject ($<1\%$) each experienced prolonged crying or fever $>40^{\circ}\text{C}$ within 48 hours after vaccine, or seizure without fever within 72 hours after vaccine; all were ≤ 1000 grams. Primary record review of subjects with questionnaire results suggestive of invasive bacterial disease revealed one case of culture-proven pneumococcal disease, giving a crude frequency of 0.4% (95% confidence interval [CI]: 0.1%, 2.3%) through 18–22 months corrected age. The pneumococcal serotype was not tested.

DISCUSSION

We have shown that infants ≤ 1000 grams birth weight, when compared with larger premature infants, have similar antibody responses to most, but not all, PCV-7 vaccine serotypes. Postnatal glucocorticoid exposure, lower weight at blood drawing and Caucasian race were also risk factors for lower antibody concentrations to serotypes 6B and/or 23F, which appeared to have the poorest immunogenicity overall.

The efficacy and immunogenicity of PCV-7 have been tested previously in larger premature infants. As part of an efficacy study, in which PCV-7 was administered at 2, 4 and 6 months, some 4340 premature infants (born at <38 weeks gestation) and 1756 LBW infants (<2500 grams) were evaluated.³ The vaccine had 100% efficacy in those populations. However, only 167 infants <32 weeks and 131 VLBW infants were included, and the immunogenicity of PCV-7 in these infants was incompletely addressed. In a study of 46 infants born at 32–36 weeks gestation and given PCV-7 at 3, 5 and 11 months of age, premature infants had antibody concentrations equivalent to the concentrations of full term infants following the third vaccine dose.²¹ All premature infants in that study achieved concentrations ≥ 0.15 $\mu\text{g/mL}$ against all 7 vaccine antigens after 3 doses of vaccine, but that small study did not include extremely preterm infants. In a recent study of a population more similar to ours, 69 premature infants, of whom 42 were <32 weeks gestation at birth, and 68 full-term controls received PCV-7 at 2, 3 and 4 months of age.²² Lower proportions of premature infants had concentrations ≥ 0.35 $\mu\text{g/mL}$ against serotypes 4, 6B and 9V than full-term controls following the primary vaccine series, although both premature and full-term infants responded similarly to a polysaccharide pneumococcal vaccine booster at 12 months old.

Studies of full-term infants receiving PCV-7, regardless of schedule, describe greater than 90% of infants achieving concentrations $\geq 0.15 - 0.2 \mu\text{g/mL}$ against each vaccine serotype.^{17, 22} Greater than 90% of full-term infants receiving PCV-7 on an accelerated 2, 3 and 4 month schedule achieve concentrations $\geq 0.35 \mu\text{g/mL}$ against all serotypes except 6B (to which 79% achieve these concentrations).²² Full-term infants receiving PCV-7 on a 2, 4, and 6 month schedule achieve concentrations $\geq 1.0 \mu\text{g/mL}$ in proportions ranging from 51% (serotype 9V) to 89% (serotype 14), in a pattern similar to that seen among even the most immature infants in the current study.¹⁷

The current study complements previous studies in suggesting that, under circumstances predisposing to reduced vaccine immunogenicity (e.g. less immunogenic serotypes or accelerated vaccine schedules), PCV-7 may be less immunogenic in ELBW (<1000 grams birth weight) infants. As antibody concentrations decay over time, lower concentrations may become clinically significant prior to a booster dose of vaccine.^{21, 22}

The finding in our population of an independent effect of postnatal glucocorticoid administration on the immunogenicity of serotype 6B in premature infants agrees with findings for several other vaccines.^{23–25} Contrary to the findings of others regarding Hib vaccine,^{9, 10} glucocorticoid administration, rather than the presence of BPD for which glucocorticoids were administered, appeared in our study to be more strongly associated with poor vaccine immunogenicity. We recognize the possibility that receipt of postnatal glucocorticoids could be a marker for severe BPD; our analysis did not consider severity of BPD.

Other authors have reported an elevated risk of local vaccine reactions in premature or low-birth-weight infants receiving PCV-7.³ We did not analyze local reactions, but found a low incidence (<1%) of fever $>40^{\circ}\text{C}$ in our vaccine recipients. Others have reported a 2.3% incidence of temperature $>39^{\circ}\text{C}$ following PCV-7 in premature infants.³ Similarly, apnea or other respiratory compromise has been reported following several types of vaccines in hospitalized premature infants.^{6, 26–31} In a preliminary report, Sumner described a 17% incidence of recurrent apnea within 72 hours after PCV-7 in hospitalized VLBW infants who had had no apnea in the 3 days preceding immunization.³² Among our hospitalized infants, a lower proportion (4%) suffered recurrent episodes of apnea following PCV-7, although our study used a more restrictive definition of recurrent apnea. We detected no incidents of post-vaccine apnea in non-hospitalized subjects, although our surveillance for this event was less comprehensive than for in-hospital apnea. Our data support apnea monitoring of hospitalized infants receiving PCV-7 and provide some limited reassurance about apnea in non-hospitalized infants.

Our study was designed to measure PCV-7 immunogenicity over a range of birth weights in VLBW, premature infants only, without the use of a full-term control group. This limits comparisons to historic data on full-term infants from other studies, which may have used differing methodologies. When the current study was designed, agreement was lacking on a meaningful antibody concentration associated with protection from pneumococcal disease, and a concentration of $0.15 \mu\text{g/mL}$ was used to generate hypotheses. Since that time, a reference antibody concentration of $0.35 \mu\text{g/mL}$, based on meta-analysis of major trials, has been established by the World Health Organization.¹⁹ The findings of the current study remained consistent using antibody cutoffs ranging from $0.15 \mu\text{g/mL}$ to $1.0 \mu\text{g/mL}$, including $0.35 \mu\text{g/mL}$. With the exception of a few infants with low antibody concentrations whose parents desired repeat testing at 12–15 months, we did not measure antibody concentrations following the booster dose of vaccine. This limited our ability to assess responses to the 4th dose of vaccine, which may be important for long-term protection.²²

In summary, in this study of PCV-7 immunogenicity among VLBW infants, even the smallest and least mature infants usually achieved antibody concentrations similar to those described in full-term infants. The favorable immunogenicity of PCV-7 among VLBW infants supports current recommendations to immunize premature infants at the same postnatal ages as full-term infants. In keeping with our hypothesis, however, lower birth weight appeared to be an independent risk factor for poorer vaccine immunogenicity, especially for less immunogenic serotypes. It may be particularly important to offer a timely booster dose of PCV-7 to infants who have multiple risk factors (extremely low birth weight, postnatal glucocorticoid exposure, poor weight gain and/or Caucasian race) for poorer vaccine immunogenicity.

Acknowledgments

The Neonatal Research Network's PCV-7 Study (Recruitment 2004–2006) was supported by grants from the National Institutes of Health and from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The funding agency provided overall oversight for study conduct. PCV-7 vaccine was purchased directly by infants' clinical care providers.

Data collected at participating sites of the NICHD Neonatal Research Network (NRN) were transmitted to RTI International, the Data Coordinating Center (DCC) for the NRN, which stored, managed and analyzed the data for this study. On behalf of the NRN, Drs. Abhik Das (DCC PI) and Lei Li (DCC Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

We are indebted to our medical and nursing colleagues and the infants and their parents who agreed to take part in this study. The following investigators, in addition to those listed as authors, participated in this study:

NRN Chairs: Alan Jobe, MD PhD, University of Cincinnati (2001–2006); Michael S. Caplan, MD, Northwestern University (2006–2011).

Duke University University Hospital, Alamance Regional Medical Center, and Durham Regional Hospital (GCRC M01 RR30, U10 HD40492) – C. Michael Cotten, MD; Ricki F. Goldstein, MD; Kathy J. Auten, MSHS; Melody B. Lohmeyer, RN.

Emory University Children's Healthcare of Atlanta, Grady Memorial Hospital, and Emory Crawford Long Hospital (GCRC M01 RR39, U10 HD27851) – Ira Adams-Chapman, MD; Ellen C. Hale, RN BS.

RTI International (U01 HD36790) – W. Kenneth Poole, PhD; Elizabeth McClure, MEd; Rebecca L. Perritt, MS; Steve Emrich, MS; Kristin Zaterka-Baxter, RN; Carolyn Petrie Huitema, MS; Jamie E. Newman, MPH; Scott E. Schaefer, BS MS; Jeanette O'Donnell Auman, BS.

Stanford University Lucile Packard Children's Hospital (GCRC M01 RR70, U10 HD27880) – Susan R. Hintz, MD MS; Maria Elena DeAnda, PhD; M. Bethany Ball, BS CCRC.

University of Alabama at Birmingham Health System and Children's Hospital of Alabama (GCRC M01 RR32, U10 HD34216, AI-30021) – Myriam Peralta-Carcelen, MD MPH; Monica V. Collins, RN BSN; Shirley S. Cosby, RN BSN; Vivien A. Phillips, RN BSN; Moon H. Nahm, MD.

University of Miami Holtz Children's Hospital (GCRC M01 RR16587, U10 HD21397) – Charles R. Bauer, MD; Ruth Everett-Thomas, RN BSN.

University of Rochester Golisano Children's Hospital at the University of Rochester Medical Center (GCRC M01 RR44, U10 HD40521) – Gary J. Myers, MD; Cassandra A. Horihan, MS; Rosemary L. Jensen; Diane L. Hust, RN PNP.

University of Texas Southwestern Medical Center at Dallas Parkland Health & Hospital System and Children's Medical Center Dallas (GCRC M01 RR633, U10 HD40689) – Charles R. Rosenfeld, MD; Walid A. Salhab, MD; Janet S. Morgan, RN; Jackie F. Hickman, RN; Alicia Guzman; Nancy A. Miller, RN; Gaynelle Hensley, RN.

Wake Forest University Baptist Medical Center, Brenner Children's Hospital, and Forsyth Medical Center (GCRC M01 RR7122, U10 HD40498) – Robert G. Dillard, MD; Nancy J. Peters, RN CCRP; Debbie C. Hiatt, BSN; Dia D. Roberts, BSN, PNP.

Wayne State University Hutzel Women's Hospital and Children's Hospital of Michigan (U10 HD21385) – Yvette Johnson, MD MPH; Athina Pappas, MD; Rebecca Bara, RN BSN; Geraldine Muran, RN BSN; Deborah Kennedy, RN BSN.

Funding: The Neonatal Research Network's PCV-7 Study was supported by grants from the National Institutes of Health and from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

REFERENCES

1. Zangwill KM, Vadheim CM, Vannier AM, Hemenway LS, Greenberg DP, Ward JI. Epidemiology of invasive pneumococcal disease in southern California: implications for the design and conduct of a pneumococcal conjugate vaccine efficacy trial. *Journal of Infectious Diseases* 1996;174:752–759. [PubMed: 8843213]
2. Centers for Disease Control and Prevention. Preventing pneumococcal disease among infants and young children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2000;29(No. RR-9):1–35.
3. Shinefield H, Black S, Ray P, Fireman B, Schwalbe J, Lewis E. Efficacy, immunogenicity and safety of heptavalent pneumococcal conjugate vaccine in low birth weight and preterm infants. *Pediatr Infect Dis J* 2002;21:182–186. [PubMed: 12005078]
4. Rennels MB, Edwards KM, Keyserling HL, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. *Pediatrics* 1998;101:604–611. [PubMed: 9521941]
5. Black S, Shinefield H, Fireman B, et al. Northern California Kaiser Permanente Vaccine Study Center Group. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 2000;19:187–195. [PubMed: 10749457]
6. D'Angio CT, Maniscalco WM, Pichichero ME. Immunologic response of extremely premature infants to tetanus, Haemophilus influenzae, and polio immunizations. *Pediatrics* 1995;96:18–22. [PubMed: 7596716]
7. Khalak R, Pichichero ME, D'Angio CT. Three-year follow-up of vaccine response in extremely preterm infants. *Pediatrics* 1998;101:597–603. [PubMed: 9521940]
8. Smolen P, Bland R, Heiligenstein E, Lawless MR, Dillard R, Abramson J. Antibody response to oral polio vaccine in premature infants. *J Pediatr* 1983;103:917–919. [PubMed: 6644426]
9. Munoz A, Salvador A, Brodsky NL, Arbeter AM, Porat R. Antibody response of low birth weight infants to Haemophilus influenzae type b polyribosylribitol phosphate-outer membrane protein conjugate vaccine. *Pediatrics* 1995;96:216–219. [PubMed: 7630672]
10. Washburn LK, O'Shea TM, Gillis DC, Block SM, Abramson JS. Response to Haemophilus influenzae type b conjugate vaccine in chronically ill premature infants. *J Pediatr* 1993;123:791–794. [PubMed: 8229493]
11. Lau YL, Tam AY, Ng KW, et al. Response of preterm infants to hepatitis B vaccine. *J Pediatr* 1992;121:962–965. [PubMed: 1447667]
12. Anonymous. Recommended Childhood and Adolescent Immunization Schedule --- United States, January--June 2004. *MMWR* 2004;53:Q1–Q4. [PubMed: 14733209]
13. American Academy of Pediatrics. Immunization in special clinical circumstances. In: Pickering, LK., editor. *Red Book: 2003 Report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2003. p. 66–68.
14. Wernette CM, Frasci CE, Madore D, et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clin Diagn Lab Immunol* 2003;10:514–519. [PubMed: 12853378]
15. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clin Vaccine Immunol* 2006;13:1004–1009. [PubMed: 16960111]
16. Shapiro ED, Austrian R. Serotypes responsible for invasive Streptococcus pneumoniae infections among children in Connecticut. *J Infect Dis* 1994;169:212–214. [PubMed: 8277185]

17. Shinefield HR, Black S, Ray P, et al. Safety and immunogenicity of heptavalent pneumococcal CRM197 conjugate vaccine in infants and toddlers. *Pediatr Infect Dis J* 1999;18:757–763. [PubMed: 10493334]
18. Jodar L, Butler J, Carlone G, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine* 2003;21:3265–3272. [PubMed: 12804857]
19. World Health Organization. Pneumococcal conjugate vaccines. Recommendations for the production and control of pneumococcal conjugate vaccines. WHO Technical Report Series 2005;927:64–98. (Annex 2).
20. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol* 1996;87:163–168. [PubMed: 8559516]
21. Esposito S, Pugni L, Bosis S, et al. Immunogenicity, safety and tolerability of heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 11 months post-natally to pre- and full-term infants. *Vaccine* 2005;23:1703–1708. [PubMed: 15705475]
22. Ruggeberg JU, Collins C, Clarke P, et al. Immunogenicity and induction of immunological memory of the heptavalent pneumococcal conjugate vaccine in preterm UK infants. *Vaccine* 2007;25:264–271. [PubMed: 17070968]
23. Robinson MJ, Campbell F, Powell P, Sims D, Thornton C. Antibody response to accelerated Hib immunisation in preterm infants receiving dexamethasone for chronic lung disease. *Archives of Disease in Childhood Fetal & Neonatal Edition* 1999;80:F69–F71. [PubMed: 10325817]
24. Robinson MJ, Heal C, Gardener E, Powell P, Sims DG. Antibody response to diphtheria-tetanus-pertussis immunization in preterm infants who receive dexamethasone for chronic lung disease. *Pediatrics* 2004;113:733–737. [PubMed: 15060220]
25. Losonsky GA, Wasserman SS, Stephens I, et al. Hepatitis B vaccination of premature infants: a reassessment of current recommendations for delayed immunization. *Pediatrics* 1999;103:e14. [PubMed: 9925860]
26. Botham SJ, Isaacs D. Incidence of apnoea and bradycardia in preterm infants following triple antigen immunization. *J Paediatr Child Health* 1994;30:533–535. [PubMed: 7865269]
27. Topsis J, Kandall S, Weinstein J, Wilets I. Tolerance of initial diphtheria-tetanus-pertussis immunization in preterm infants. *J Perinatol* 1996;16:98–102. [PubMed: 8732555]
28. Botham SJ, Isaacs D, Henderson-Smart DJ. Incidence of apnoea and bradycardia in preterm infants following DTPw and Hib immunization: a prospective study. *J Paediatr Child Health* 1997;33:418–421. [PubMed: 9401886]
29. Sanchez PJ, Laptook AR, Fisher L, Sumner J, Risser RC, Perlman JM. Apnea after immunization of preterm infants. *J Pediatr* 1997;130:746–751. [PubMed: 9152284]
30. Pourcyrous, M.; Korones, SB.; Crouse, D.; Bada, HS. Interleukin-6, C-reactive protein, and abnormal cardiorespiratory responses to immunization in premature infants. *Pediatrics*. 1998. URL: <http://www.pediatrics.org/cgi/content/full/101/103/e103>
31. Slack MH, Schapira D. Severe apnoeas following immunisation in premature infants. *Archives of Disease in Childhood Fetal & Neonatal Edition* 1999;81:F67–F68. [PubMed: 10375367]
32. Sumner JL, Goodman B, Bush MA, Badhwar P, Sanchez PJ. Immunization of very-low-birth-weight (VLBW) infants in the neonatal intensive care unit (NICU) with Prevnar™: is it OK? [abstract]. *Pediatr Res* 2003;53:311A.

Figure 1. Flow of study participants

Of a total of 1310 infants assessed for eligibility, 30% did not qualify (most would have been unable to return for follow-up), 42% were eligible but did not enroll (most had parents decline to give permission), and 28% were enrolled. The birth weights, gestational ages and demographic characteristics of the non-enrolled subjects were similar to those of the enrolled subjects. Overall, 66% of enrollees completed study procedures within specified windows and had serum from 4–6 weeks after the third PCV-7 dose available for the primary analysis (“on-time” cohort). Of the 125 children who were enrolled in the study, but were not included in the primary analysis cohort, 4 died between enrollment and the blood draw (2 of these died during the vaccine series and 2 before vaccines were begun), 7 withdrew, 14 were lost to follow-up, 54 did not complete the course of vaccines and blood draws, and 9 had inadequate blood samples (5 had had blood drawn within the prescribed window and 4 outside it). This left 37 children with specimens for a secondary analysis.

Figure 2. Pneumococcal antibody concentrations at 4–6 weeks after the third PCV-7 dose by birth weight

Serotypes 6B (A) and 23F (B) are shown. Each plot includes the 244 subjects in the primary analysis cohort. Diamonds represent individual values and lines show least mean squares regression and 95% confidence intervals. The dashed lines represent the WHO reference antibody concentration (0.35 µg/mL). In both cases, although the association between antibody concentration and birth weight was significant (6B $P=0.006$, 23F $P=0.02$), $\leq 3\%$ of variability in antibody concentration was attributable to birth weight ($R^2 \leq 0.03$). Similar results were found if antibody concentrations were plotted against gestational age (not shown).

A **Serotype 6B****B** **Serotype 23F**

Figure 3. Multiple logistic regression for pneumococcal antibody concentration ≥ 0.35 $\mu\text{g/mL}$ at 4–6 weeks after the third PCV-7 dose

Multiple logistic regression was performed on 243 available subjects in the primary analysis cohort using the independent variables shown on the plots (one subject had missing weight data). The dependent variable was an antibody concentration ≥ 0.35 $\mu\text{g/mL}$. Results are depicted for serotypes 6B (A) and 23F (B). The X axis shows the odds ratio for the effect of each variable, adjusted for all of the other independent variables. Diamonds depict odds ratios and whiskers represent 95% confidence intervals.

Table 1

(on-line only). Baseline and postnatal characteristics of subjects completing study within time window and having serum available (N = 244).

Characteristic	
Birth weight (in grams) (mean $\pm$ SD)	1008 $\pm$ 282
Gestation (in weeks) (mean $\pm$ SD) [range]	27.8 $\pm$ 2.2 [23.0, 32.0]
Gender, male	134 (55%)
Race, Caucasian	153 (63%)
Ethnicity, Latino	73 (30%)
Medicaid insurance	114 (47%)
Small for gestational age [*]	32 (13%)
Antenatal glucocorticoids	168 (69%)
Any mechanical ventilation prior to 28 days old	194 (80%)
Bronchopulmonary dysplasia ^{**}	63 (26%)
Postnatal glucocorticoids prior to hospital discharge	38 (16%)
Culture-proven systemic infection prior to hospital discharge	88 (36%)
Systemic glucocorticoid therapy at time of 1 st dose PCV-7	1 (0.4%)
Median age (months) at 1 st dose PCV-7 [range]	2.1 [1.4, 3.0]
Median age (months) at 3 rd dose PCV-7 [range]	6.2 [5.0, 7.9]
Median age (months) at blood draw [range]	7.4 [6.1, 9.0]
Weight (kg) at 1 st dose PCV-7 (mean $\pm$ SD)	2.2 $\pm$ 0.7
Weight (kg) at blood draw (mean $\pm$ SD)	6.5 $\pm$ 1.1
Weight-for-corrected-age Z-score at blood draw [†] (mean $\pm$ SD)	-0.5 $\pm$ 1.3

Values expressed as N (%), unless otherwise specified.

SD = standard deviation. PCV-7 = heptavalent pneumococcal conjugate vaccine.

^{*} Birth weight <10th percentile on Alexander²⁰ reference for fetal growth.

^{**} Supplemental oxygen administration at 36 weeks postmenstrual age. Data missing on 12 subjects.

[†] Z-score based on the 2000 CDC growth chart (<http://www.cdc.gov/growthcharts/>).

Table 2

Pneumococcal antibody concentrations at 4–6 weeks after the third PCV-7 dose.

Serotype	Concentration	Percent of infants achieving concentration		P value (Chi-square test)
		401–1000 grams (N = 118)	1001–1500 grams (N = 126)	
4	≥0.15 µg/mL	117 (99.2%)	125 (99.2%)	1.0
	≥0.35 µg/mL	113 (95.8%)	123 (97.6%)	0.49
	≥1.0 µg/mL	87 (73.7%)	97 (77.0%)	0.56
6B	≥0.15 µg/mL	100 (84.8%)	121 (96.0%)	0.003
	≥0.35 µg/mL	90 (76.3%)	115 (91.3%)	0.001
	≥1.0 µg/mL	74 (62.7%)	96 (76.2%)	0.02
9V	≥0.15 µg/mL	117 (99.2%)	125 (99.2%)	1.0
	≥0.35 µg/mL	111 (94.1%)	122 (96.8%)	0.30
	≥1.0 µg/mL	76 (64.4%)	96 (76.2%)	0.04
14	≥0.15 µg/mL	116 (98.3%)	124 (98.4%)	1.0
	≥0.35 µg/mL	115 (97.5%)	122 (96.8%)	1.0
	≥1.0 µg/mL	110 (93.2%)	120 (95.2%)	0.50
18C	≥0.15 µg/mL	117 (99.2%)	126 (100%)	0.48
	≥0.35 µg/mL	115 (97.5%)	125 (99.2%)	0.36
	≥1.0 µg/mL	92 (78.0%)	111 (88.1%)	0.03
19F	≥0.15 µg/mL	117 (99.2%)	126 (100%)	0.48
	≥0.35 µg/mL	117 (99.2%)	125 (99.2%)	1.0
	≥1.0 µg/mL	102 (86.4%)	118 (93.7%)	0.06
23F	≥0.15 µg/mL	104 (88.1%)	122 (96.8%)	0.009
	≥0.35 µg/mL	94 (79.7%)	119 (94.4%)	0.0005
	≥1.0 µg/mL	69 (58.5%)	88 (69.8%)	0.06

Values expressed as N (%).

Table 3

Pneumococcal opsonophagocytosis titers at 4–6 weeks after the third PCV-7 dose.

Serotype	Percentage of infants with titer $\geq 1:8$ [*]		P value (Chi-square test)
	401–1000 grams (N = 113)	1001–1500 grams (N = 122)	
4	111 (98.2%)	122 (100%)	0.23
6B	97 (86.6%)	119 (97.5%)	<0.01
14	112 (99.1%)	120 (98.4%)	1.0
23F	107 (95.5%)	121 (99.2%)	0.11

^{*}Titers $\geq 1:8$ associated with protection against pneumococcal disease.^{18, 19}

Table 4

Percentage of children with concentrations ≥ 0.35 $\mu\text{g/mL}$ at 4–6 weeks after the third PCV-7 dose by postnatal glucocorticoid use.

Serotype	Postnatal glucocorticoid exposure		P value (Chi-square test)
	Yes (N = 38)	No (N = 206)	
4	35 (92.1%)	201 (97.6%)	0.11
6B	23 (60.5%)	182 (88.4%)	<0.01
9V	34 (89.5%)	199 (96.6%)	0.07
14	35 (92.1%)	202 (98.1%)	0.08
18C	36 (94.7%)	204 (99.0%)	0.12
19F	37 (97.4%)	205 (99.5%)	0.29
23F	26 (68.4%)	187 (90.8%)	<0.01