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Blood stream infection is associated with altered heptavalent pneumococcal conjugate vaccine immune responses in very low birth weight infants

James L. Wynn, MD[1], **Lei Li, PhD**[2], **C. Michael Cotten, MD, MHS**[11,1], **Dale L. Phelps, MD**[3], **Seetha Shankaran, MD**[4], **Ronald N Goldberg, MD**[11], **Waldemar A. Carlo, MD**[5], **Krisa Van Meurs, MD**[6], **Abhik Das, PhD**[7], **Betty R. Vohr, MD**[8], **Rosemary D. Higgins, MD**[9], **Barbara J Stoll, MD**[10], and **Carl T D'Angio, MD**[3] **for the NICHD NRN**

[1]Department of Pediatrics, Vanderbilt University, Nashville, TN

[2]Statistics and Epidemiology Unit, RTI International, Research Triangle Park, NC

[3]University of Rochester School of Medicine and Dentistry, Rochester, NY

[4]Department of Pediatrics, Wayne State University, Detroit, MI

[5]Division of Neonatology, University of Alabama at Birmingham, Birmingham, AL

[6]Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University School of Medicine and Lucile Packard Children's Hospital, Palo Alto, CA

[7]Statistics and Epidemiology Unit, RTI International, Rockville, MD

[8]Department of Pediatrics, Women & Infants Hospital, Brown University, Providence, RI

[9]*Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, MD

[10]Emory University School of Medicine, Department of Pediatrics, Children's Healthcare of Atlanta, Atlanta, GA

Correspondence to: James L. Wynn, MD, Assistant Professor of Pediatrics, Division of Neonatal-Perinatal Medicine, Department of Pediatrics, Vanderbilt University Medical Center, Pediatrics/Neonatology, 2215 B Garland Avenue, 1125 MRB4/Light Hall, Nashville, TN 37232-0656, james.wynn@vanderbilt.edu.

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Contributors statement page
James L. Wynn, MD: Dr. Wynn conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.
Lei Li, PhD: Dr. Li performed the analyses, drafted the initial manuscript, and approved the final manuscript as submitted.
C. Michael Cotten, MD MHS: Dr. Cotten conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.
Dale L. Phelps, MD: Dr. Phelps drafted the initial manuscript and approved the final manuscript as submitted.
Seetha Shankaran, MD: Dr. Shankaran drafted the initial manuscript and approved the final manuscript as submitted.
Ronald N Goldberg, MD: Dr. Goldberg drafted the initial manuscript and approved the final manuscript as submitted.
Waldemar A. Carlo, MD: Dr. Carlo drafted the initial manuscript and approved the final manuscript as submitted.
Krisa Van Meurs, MD: Dr. Van Meurs drafted the initial manuscript and approved the final manuscript as submitted.
Abhik Das, PhD: Dr. Das performed the analyses, drafted the initial manuscript, and approved the final manuscript as submitted.
Betty R. Vohr, MD: Dr. Vohr drafted the initial manuscript and approved the final manuscript as submitted.
Rosemary D. Higgins, MD: Dr. Higgins drafted the initial manuscript and approved the final manuscript as submitted.
Barbara J Stoll, MD: Dr. Stoll conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.
Carl T D'Angio, MD: Dr. D'Angio conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

[11]Department of Pediatrics, Duke University, Durham, NC

Abstract

Objective—Sepsis in older children and adults modifies immune system function. We compared serotype-specific antibody responses to heptavalent pneumococcal conjugate vaccine (PCV7) in very low birth weight infants (<1500g,VLBW) with and without blood stream infection (BSI) during their birth hospitalization.

Patients and Methods—Retrospective analysis of prospectively collected data for the Neonatal Research Network study of PCV7 responses among VLBWs. Infants received PCV7 at 2, 4, and 6 months after birth with blood drawn 4–6 weeks after 3rd dose. Serotype antibodies were compared between infants with or without a history of BSI. Regression models were constructed with birth-weight groups and other confounding factors identified in the primary study.

Results—244 infants completed the vaccine series and had serum antibody available; 82 had BSI. After adjustment, BSI was not associated with reduced odds of serum antibody ≥0.35μg/mL.

Conclusions—BSI was not associated with reduced odds of WHO-defined protective PCV7 responses in VLBWs.

Keywords

VLBW; immune response; vaccine; sepsis; blood stream infection

Introduction

Vaccination of children to prevent infectious disease represents one of the greatest contributions to pediatric health, but is dependent upon competent immune system function. Distinct adaptive immune responses occur among very preterm infants as compared to more mature neonates, infants, and children[1]. Antibody levels to routine infant vaccines are sometimes lower among very low birth weight (401–1500g, VLBW) infants[2]. The developing neonatal adaptive immune system has a very limited capacity to respond to non-protein antigens (e.g. bacterial polysaccharide capsules) until nearly 2 years of age[3]. Protein-conjugated vaccinations (e.g. heptavalent pneumococcal conjugate vaccine [PCV7]) capitalize on the largely intact T cell-dependent B cell antibody response to protein to promote production of protective immunoglobulins against polysaccharide antigens such as the capsule of Pneumococcus (*Streptococcus pneumoniae*). D'Angio et al showed when compared with larger premature infants, infants weighing ≤1000g at birth have similar antibody responses to most, but not all, PCV-7 vaccine serotypes[4].

Serious infection or sepsis in children and adults can result in significant short and long-term quantitative and qualitative alterations in adaptive immune function that alter the host's capacity to respond to infectious challenge. Specifically, sepsis results in a significant loss of dendritic cells (professional antigen presentation cells), T cells, and B cells[5, 6, 7, 8]. In addition to cellular losses, long-term functional alterations occur in T cells that may be present weeks to months after sepsis recovery[9]. Epigenetic immune system changes that occur following sepsis are the subject of intense research and have recently been associated with specific histone modifications (methylation, phosphorylation, ubiquitination and sumoylation among others) of critical DNA promoter regions[10].

It is unknown how or if sepsis affects the subsequent function of the preterm infant's developing adaptive immune system including vaccine responses. Sepsis is a clinical diagnosis that at present lacks accepted definitive criteria in preterm neonates[11]. However, the high frequency of blood stream infection (BSI) (as high as 60%) during hospitalization

in the very preterm population[12, 13, 14, 15] makes this clinically relevant. The study of whether BSI modifies subsequent vaccine responses has not been previously performed in pediatrics, specifically because we rarely have the opportunity of obtaining blood prospectively at sequential intervals following vaccination. In this follow-up study of the PCV7 vaccination trial by D'Angio et al, we identified and characterized the incidence of BSI in a cohort of VLBW infants who received PCV7 vaccine and determined whether BSI was associated with an altered vaccine response.

Materials / subjects and Methods

Patients

We performed a retrospective cohort study of patients and data prospectively collected for the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) study entitled "*Heptavalent Pneumococcal Conjugate Vaccine Immunogenicity in Very-Low-Birth-Weight, Premature Infants*" and the NRN Generic Database with respect to the occurrence of BSI during the infant's hospitalization. Infants studied were premature (<32 weeks completed gestation), had birth weight 401–1500g (VLBW), and were born between June 2004-October 2006 and cared for in one of nine participating centers in the NICHD NRN[4]. The study was reviewed and approved by each center's Institutional Review Board and monitored by the NICHD Data and Safety Monitoring Committee.

Serology

Infants received PCV7 vaccination at 2, 4, and 6 months after birth and had blood drawn 4–6 weeks after the 3rd dose[4]. As described in detail in the primary study, subjects had 3 doses of PCV-7 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 Prevention and American Academy of Pediatrics recommendations[4]. The amounts of anti-capsular polysaccharide antibody were determined for each of the 7 vaccine components (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) by a third generation enzyme-linked immunosorbent assay (ELISA)[4]. The lower detection limit of the assay was 0.01–0.03μg/mL. Percentages of infants who reached designated cutoff antibody titers of ≥0.35μg/mL were compared between infants with and without a history of BSI. This serum antibody cutoff value was chosen for analysis in the primary manuscript based on recommendations from the World Health Organization[16]. Opsonophagocytosis titers (OT) against the primary serotypes (4, 6B, 14, 23F) were defined as the serum dilution that killed 50% of the target bacteria in the presence of effector immune cells[4].

Definition of BSI

BSI was defined by growth of bacteria or fungi on a blood culture obtained ≤72 hours of birth (early-onset, EOBSI) or > 72 hours (late-onset, LOBSI) plus antimicrobial treatment (≥5 days)[14]. Blood cultures were performed based on clinical concern for infection and were not related to the primary study. Positive blood cultures with different pathogens (genus, species) taken ≥5 days apart or same pathogen ≥14 days apart were considered indicative of different episodes. Cultures that grew Coagulase negative *Staphylococcus* (CoNS) were included only if the infant received antimicrobial treatment for ≥5 days. Blood cultures positive with *Corynebacterium, Propionibacterium*, or *Micrococcus* were considered contaminated. Infants with cultures positive with any three organisms or 2 organisms that included a contaminant listed above were deemed uninfected and were included in the "no BSI" group. In the primary paper, 88 infants had culture-proven systemic infection prior to discharge. Among those 88, 2 had meningitis only (no BSI) and were excluded from our

analysis. An additional 3 were considered contaminants by our definition of BSI (all had cultures positive with three organisms or 2 organisms that included a contaminant). Lastly, 1 infant was recorded as having BSI but had a missing organism code. Thus, we excluded these 6 infants to focus on BSI with known etiology. BSI episodes were classified based on the pathogen recovered (gram positive, gram negative, or fungus). If more than one pathogen was recovered with a valid episode of BSI, each organism was counted. Regarding the effect of specific pathogen class on PCV7 and OT, we only considered the causative organism for the first episode of BSI for infants with more than one episode.

Primary outcome

The primary outcome was the percentage of VLBW infants with or without a history of BSI who achieved antibody titers ≥ 0.35μg/mL to PCV7 serotypes.

Statistical Considerations

Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). Weight-for-corrected-age Z score at the time of the blood draw was estimated using the 2000 Centers for Disease Control and Prevention growth chart (http://www.cdc.gov/growthcharts). Student's t-test was used to compare the mean birth weight, gestational age, 5-minute Apgar score between infants with and without BSI, and chi-square tests were used to compare the frequency of neonatal comorbidities defined in a previous NRN study[14]. The percentage of infants that achieved cutoff serum antibody titer (≥ 0.35μg/mL) following completion of the PCV-7 vaccination series, and OT >1:8 (against serotypes 4, 14, 23F) were compared between groups (with or without BSI) using Chi-square test. Logistic regression models for serum antibody cutoff, and OT (serotype 6B) were constructed with adjustment for birth weight group (≤1000g or >1000g) and other confounding factors identified in the primary study (sex, race, postnatal glucocorticoid treatment, Z-score of weight for corrected age at blood draw and age at 1st vaccination).

Results

Patient demographics

Out of 369 infants enrolled in the primary study, 244 completed their 3-dose PCV7 series by 8 months of age and had antibody levels and OT tests determined (Supplemental figure 1). Of these 244 eligible infants, 82 experienced BSI by our definition (table 1). Forty-eight percent (118/244) were ≤1000g at birth. Among the 118 infants of ≤1000g birth weight, mean BW was 826g ± 127g without BSI vs. 702g ± 159g with BSI, and mean GA 27 ± 1.6 vs. 26 ± 1.6 weeks.

BSI and associated pathogens

Because seven episodes of BSI were associated with growth of 2 valid pathogens, the number of episodes (n=122) does not equal the number of recovered pathogens (n=129, table 2). Gram positive organisms were the predominant pathogens associated with BSI episodes. CoNS was the most commonly isolated organism. Due to very low sample size, fungal infections (n=9) were not analyzed.

Timing of BSI episodes and first PCV7 vaccination

One hundred twenty-two episodes of BSI occurred in 82/244 (34%) infants. The majority (77/82) of infants who developed BSI were diagnosed only with LOBSI. Thirty-three percent (27/82) of infants with BSI experienced 2 or more episodes of BSI during their hospitalization. The median day of life for the first documented episode of BSI was 16 (25th

percentile–75th percentile: 11–27 days) and 76% (62/82) of the first BSI episode for patients in our cohort occurred <28 days after birth.

We specifically examined the timing of BSI episodes and first PCV7 vaccination in our cohort of 244 infants to determine the degree of overlap of these two events (Supplemental figure 2). We first chose the day of life where <10% of infants had received their first dose of PCV7 (57 days). We then determined the number of infants that had experienced at least one episode of BSI (79/82, 96%) as well as the percent of all episodes of BSI that had occurred by that time point (102/122, 84%). Thus very little overlap occurred between the timing of BSI and the timing of the first vaccination.

BSI and the percentage of infants that reached protective PCV7 antibody cutoff

Overall, there was a difference between groups (BSI versus No BSI) in achieving the protective cutoff of ≥ 0.35μg/mL for serotypes 4, 6B, and 23F. After adjustment for other covariates the difference was no longer significant (table 3). When the analysis was restricted to infants with BW≤1000g (n=118) or to only infants <28 weeks no changes in odds of serum antibody ≥ 0.35μg/mL occurred.

Association of BSI and PCV7 opsonophagocytosis titers

In unadjusted analyses, infants with BSI had a reduced percentage of OT >1:8 against serotype 6B compared to infants without BSI (83 vs. 97%, p<0.01). The association of BSI with reduced 6B OT persisted when we restricted the analyses to BSI caused by only gram positive pathogens, only gram positive pathogens with specific exclusion of BSI due to CoNS, and only gram negative pathogens (table 4). OT against serotypes 4, 14, and 23F were not different between infants with or without a history of BSI or by specific pathogen class. Due to the high percentage (nearly 100%) in both groups that reached OT >1:8 for serotypes 4, 14, and 23F; it was only possible to perform an adjusted analysis on results for 6B. In the adjusted analysis, a reduced response against 6B was found following BSI (OR=0.26 [0.72, 0.92], p=0.04) and followed a similar trend for gram positive BSI (OR=0.28 [0.07, 1.03], p=0.05).

Discussion

BSI was not associated with a reduced protective response to PCV7 as defined by the WHO (≥0.35μg/mL[16]) in preterm VLBW infants when measured 4–6 weeks after vaccination at 2, 4, and 6 months of life. However, BSI was associated with altered responses including a reduced OT >1:8 against serotype 6B in this cohort of preterm neonates. To our knowledge, this is the first study to report the association of BSI and the subsequent vaccine response in the VLBW infant.

Because the majority of vaccines are given in early life, evaluations of the effect of pediatric and adult sepsis on subsequent vaccine responses are scarce. We are not aware of any previous study in neonates that has examined the impact of BSI on subsequent adaptive immune function in general or specifically through the production of antibodies following vaccination. Immunologic functional studies of adaptive immune responses following sepsis are available from experiments performed in adult animals but these may not accurately reflect the situation in preterm infants[9, 10, 17]. However, in those studies, altered adaptive immune cellular responses (altered T helper cell function) persisted *for months* in fully recovered, previously septic adult mice. Specifically, *Scumpia et al* showed T-cell dependent B cell responses (IgM and IgG2a) are reduced after polymicrobial peritoneal sepsis (induced by cecal ligation and puncture [CLP]) and *Delano et al* demonstrated these deficiencies persist for up to 3 months[9, 17]. These changes represent a shift from a T-helper

1 (Th1) to a Th2 immunophenotype that is associated with an altered immunoglobulin production profile particularly for IgG2a. Neonates manifest a Th2 phenotype at baseline and produce less IgG2[18] that is important for protection against encapsulated organisms such as pneumococcus[19].

Our findings are potentially relevant for several reasons. First, vaccine response (either achievement of protective cutoff concentration and/or adequate OT) for serotypes 23F and 6B were low in the primary study, and this finding has been recently confirmed in another cohort of preterm infants[20]. In addition, these serotypes are associated with invasive disease in infants and 6B is the most common serotype for breakthrough infection following vaccination[21, 22]. Second, the frequency of BSI in the preterm infant can be as high as 60% in the most immature infants[14]. Our findings that BSI alters the host response to PCV7 vaccination may also be true for other vaccine responses and may partially explain why preterm infants may exhibit reduced vaccine responses[2]. Of note is the relationship between attaining the prescribed "protective" cutoff of antibody concentrations and *effective* antibody-mediated opsonophagocytosis of the live pathogen. Effective opsonophagocytosis was determined using He-La cells in the presence of additional rabbit serum as a source for complement in the primary study. While this assay was used to assess all samples obtained from preterm infants, the *in vivo* function of preterm phagocytes and serum level of complement components may not mirror the effectiveness demonstrated using this method of *ex vivo* immune function modeling[23].

Limitations

One specific weakness of this retrospective study is the non-random distribution of patients. It is possible that infants develop BSI as a result of altered immune function that may also be associated with reduced vaccine responses. However, the role of the adaptive immune system in the risk of developing BSI and the host response to sepsis is inadequately characterized in neonates. Furthermore, unlike in adults and children, where a large part of the post sepsis-associated immune alterations occur in the adaptive immune system, data are lacking that describe a similar dependence of the preterm infant on the adaptive immune system for protection against infection [and point more to the critical importance of innate immune system function[24]]. Nasopharyngeal colonization with pneumococcus may reduce the immune response to vaccination[25]. However, PCV7 vaccine serotypes were not identified in a recent examination of nasopharyngeal colonization of infants in this cohort[26]. In our study, BSI was detected and treated in infants evaluated for sepsis based on clinical suspicion of infection. Specific clinical parameters to discriminate between cases of sepsis and septic shock versus BSI are not well established in preterm infants[11]. Thus, it is possible that further alterations in the PCV7 immune response were associated with neonatal sepsis or septic shock that we were not able to detect. Lastly, multiple subgroup analyses can potentially overstate the significance of findings. We did not adjust the P-value for multiple comparisons and thus the significance of the estimated effects from multiple subgroup analysis could be inflated. However, we intended to show if there was a significant difference between the no BSI group and *one pre-defined BSI subgroup* (not any BSI subgroup). These subgroups are not complementary (jointly forming the whole BSI group) and there was only one subgroup analysis done for each BSI definition.

Conclusions

VLBW infants with a history of BSI achieve protective antibody cutoffs after PCV7 vaccination. However, BSI was associated with an altered vaccine response for selected serotypes often associated with infection after vaccination; an association not explained by

GA or BW. This finding may represent the presence of sepsis-induced immune system modifications with clinical significance in this fragile population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

VLBW	Very low birth weight
PCV7	heptavalent pneumococcal conjugate vaccine
WHO	World Health Organization
DNA	deoxyribonucleic acid
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NRN	Neonatal Research Network
ELISA	enzyme-linked immunosorbent assay
OT	opsonophagocytosis titers
EOBSI	early-onset blood stream infection
LOBSI	late-onset blood stream infection
CoNS	Coagulase negative *Staphylococcus*
BW	birth weight
GA	gestational age
IgM	immunoglobulin M
IgG2a	immunoglobulin G2a
CLP	cecal ligation and puncture
Th1	T-helper 1
Th2	T-helper 2

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Appendix

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NRN Steering Committee Chairs: Alan H. Jobe, MD PhD, University of Cincinnati (2003–2006); Michael S. Caplan, MD, University of Chicago, Pritzker School of Medicine (2006–2011).

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

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Table 1

Demographics

	No BSI (n=162)	BSI (n=82)
Neonatal variables		
Mean ± std or N (%)		
BW (g) *	1096 ± 245	835 ± 271
GA (weeks) *	28.5 ± 1.9	26.5 ± 2.1
Race		
Non-Hispanic Black	56 (35)	29 (35)
Non-Hispanic White	50 (31)	31 (38)
Hispanic or Latino	53 (33)	20 (24)
Other	3 (2)	2 (2)
Sex (male)	87 (54)	47 (57)
5-minute Apgar score <5	8 (4.9)	10 (12.2)
DR resuscitation		
CPR	3 (2)	5 (6)
Intubation *	76 (47)	67 (82)
Length of hospital stay (days) median (min-max)	63 (9–147)	102 (26–244)
Postnatal glucocorticoids	26 (68)	12 (32)
Comorbidities		
NEC *	6 (4)	12 (15)
IVH *	8 (5)	11 (13)
BPD *	23 (14)	42 (51)
ROP *	51 (32)	57 (70)
Maternal variables		
Age (years) mean ± std	27.6 ± 7.1	27.2 ± 6.0
Education (<High school) **	53 (33)	25 (30)
Insurance (private)	33 (20)	14 (17)
Antenatal steroids	112 (70)	56 (69)
Antenatal antibiotics	119 (74)	59 (72)
Delivery mode (cesarean)	103 (64)	49 (60)
PROM	31 (20)	10 (12)

* **p<0.05**

** 61 infants had missing maternal education levels

BW-Birth weight

GA-gestational age

DR-delivery room

CPR-cardiopulmonary resuscitation

NEC-proven necrotizing enterocolitis, modified Bell's stage 2 or greater, ref# [27]

IVH-intraventricular (grade 3) or intraparenchymal hemorrhage (grade 4)

BPD (need for supplemental oxygen at 36 weeks postmenstrual age)

ROP-Retinopathy of prematurity (any stage)

PROM-premature rupture of membranes > 24 hours prior to delivery

Table 2

Causative organisms of BSI episodes

Gram positive	**97 (75%)**
Coagulase negative Staphylococcus	73
Staphylococcus aureus	7
Streptococcus sp includes Enterococcus	6
Group B Streptococcus	5
Streptococcus viridans	2
Enterococcus sp	2
Group D Streptococcus	1
Listeria sp	1
Staphylococcus epidermidis	0
Gram negative	**23 (18%)**
Klebsiella sp	7
Escherichia coli	6
Enterobacter cloacae	5
Pseudomonas sp	3
Enterobacter sp	1
Serratia marcescens	1
Serratia sp	0
Candida	**9 (7%)**
Candida albicans	4
Candida parapsilosis	3
Candida sp	2
Candida glabratta	0

Because 7 episodes of BSI were associated with growth of 2 valid pathogens, the number of episodes (n=122) does not equal the number of recovered pathogens (n=129).

Table 3

Achievement of geometric mean titer cutoff by group

	Unadjusted					Adjusted		
	No BSI		BSI					
Serotype	**n**	**%**	**n**	**%**	**χ^2 test p value**	**OR**	**95% CI**	
4								
≥0.35μg/mL	160	98.8	76	92.7	**0.02** *	0.31	0.04	2.57
6B								
≥0.35μg/mL	145	89.5	60	73.2	**<0.01** *	0.49	0.21	1.14
9V								
≥0.35μg/mL	157	96.9	76	92.7	0.19	0.69	0.16	3.02
14								
≥0.35μg/mL	159	98.2	78	95.1	0.23	0.46	0.06	3.63
18C								
≥0.35μg/mL	160	98.8	80	97.6	0.60	0.75	0.08	7.07
19F								
≥0.35μg/mL	161	99.4	81	98.8	1.0		n/a	
23F								
≥0.35μg/mL	147	90.7	66	80.5	**0.02** *	0.88	0.34	2.27

* p<0.01

Table 4

Association of BSI with PCV7 opsonophagocytosis titers

	Reached >1:8 N (%)	χ^2 test p Value
PN4		
No BSI (n=159)	158 (99.4)	reference
BSI (n=76)	75 (98.7)	1.0
gram positive organism (n=57)	56 (98.3)	1.0
gram positive no CoNS (n=9)	8 (88.9)	.10
gram negative organism (n=16)	16 (100)	1.0
PN6B		
No BSI (n=159)	154 (96.9)	reference
BSI (n=76)	**62 (82.7)**	**<.01** *
gram positive organism (n=56)	**46 (82.1)**	**<.01** **
gram positive no CoNS (n=8)	**6 (75)**	**.04**
gram negative organism (n=16)	**13 (81.3)**	**.03**
PN14		
No BSI (n=159)	157 (98.7)	reference
BSI (n=76)	75 (98.7)	1.0
gram positive organism (n=57)	56 (98.3)	1.0
gram positive no CoNS (n=9)	9 (100)	1.0
gram negative organism (n=16)	16 (100)	1.0
PN23F		
No BSI (n=159)	157 (98.7)	reference
BSI (n=76)	71 (94.7)	.09
gram positive organism (n=56)	53 (94.6)	.11
gram positive no CoNS (n=8)	8 (100)	1.0
gram negative organism (n=16)	15 (93.8)	.25

* -Adjusted odds ratio = 0.26 (95% CI 0.07, 0.92), p=0.04

** -Adjusted odds ratio = 0.28 (95% CI 0.07, 1.03), p=0.05