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Original article

# Correlation of higher antibody levels to pneumococcal proteins with protection from pneumococcal acute otitis media but not protection from nasopharyngeal colonization in young children

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ABSTRACT

*Objectives:* We previously found that nasopharyngeal (NP) colonization by *Streptococcus pneumoniae* elicits mucosal antibody responses to three protein vaccine candidates: pneumococcal histidine triad protein D (PhtD), pneumococcal choline-binding protein A (PcpA), and detoxified pneumolysin (PlyD1). Here we sought to determine if mucosal antibody levels to the proteins correlated with protection from acute otitis media (AOM) and NP colonization.
*Methods:* A total of 228 NP samples were prospectively collected from 100 healthy infants at 6–24 months of age. Whenever children were diagnosed with AOM, middle ear fluids were collected to confirm the diagnosis by microbiological culture. NP mucosal IgG and IgA were quantified by ELISA.
*Results:* Higher NP mucosal antibody levels to *S. pneumoniae* proteins correlated with significantly decreased likelihood of developing AOM caused by *S. pneumoniae* during 3 to 12 months of subsequent prospective monitoring. Specifically, children who did not experience AOM ($n = 111$ samples) caused by *S. pneumoniae* had two- to five-fold higher mucosal IgG levels to PcpA (all p values <0.01), six- to eight-fold higher IgA to PhtD (all p values <0.05); two- to three-folder higher IgA to PcpA (all p values <0.05), and two- to three-fold higher IgA to PlyD1 (p 0.08, p 0.03 and p 0.08) compared with children who did experience AOM ($n = 18$ samples). No association between mucosal antibody levels to the three proteins and NP colonization with *S. pneumoniae* was found.
*Conclusion:* Higher NP mucosal IgG levels to PcpA, and IgA to PhtD, PcpA and PlyD1 correlate with reduced risk of development of *S. pneumoniae* AOM infection but not with reduced risk of NP colonization in young children. **Q. Xu, Clin Microbiol Infect 2017;23:487.e1–487.e6**


## Introduction

Current licensed pneumococcal vaccines, including the 23-valent pneumococcal polysaccharide vaccine, and 7-, 10- and 13-valent pneumococcal conjugate vaccines (PCV-7, -10, -13) are serotype (polysaccharide)-based vaccines that protect against *Streptococcus pneumoniae* infections caused by strains expressing the included serotypes. To date, there are 97 distinct serotypes according to capsular polysaccharide composition [1]. Within a few years of introduction of each of the PCVs, emergence of non-vaccine replacement serotypes was noted in numerous studies [1–3]. Therefore, we and others have been evaluating next-generation purified pneumococcal protein vaccines that will be composed of highly conserved proteins expressed by virtually all *S. pneumoniae* [2,3]. We have studied three pneumococcal proteins: histidine triad protein D (PhtD), pneumococcal choline binding protein A (PcpA), and pneumolysin (Ply) and have shown that natural exposure to *S. pneumoniae* following nasopharyngeal (NP) colonization elicits both serum and mucosal antibody responses in young children [4,5]. We have also shown that vaccination with monovalent and trivalent vaccines containing PhtD, PcpA or detoxified Ply (PlyD1)

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and a combination of these three proteins confers protection against pneumonia and sepsis in a mouse model [6]. Our work and that of others has provided sufficient promise for the potential of a trivalent PhtD, PcpA and PlyD1 vaccine that has entered human clinical trials [7,8].

Pneumococcal protein vaccines have the potential to prevent *S. pneumoniae* infections by all strains irrespective of the capsular serotype expressed. However, if pneumococcal protein vaccines, like PCVs, completely eliminate *S. pneumoniae* NP colonization then a concern arises regarding the potential for providing a vacant niche that might be filled by other invasive bacteria such as *Staphylococcus aureus* [9]. We recently reported that higher mucosal antibody levels to PhtD, PcpA and PlyD1 in the NP was associated with reduced acute otitis media (AOM) caused by *S. pneumoniae* in young children at onset of AOM [5]. Here we sought to determine if mucosal antibody levels to these three proteins correlated with protection from future risk of episodes of *S. pneumoniae* AOM and/or *S. pneumoniae* NP colonization over defined time spans.

## Materials and methods

### *Study cohort and sample collection*

This study derives from a cohort of children prospectively enrolled during a 10-year time span (2006–2015) to evaluate immunity to *S. pneumoniae* and non-typeable *Haemophilus influenzae* NP colonization and AOM in young children. The subject enrolments, sample collections and AOM diagnosis criteria have been described previously [4,5]. Briefly, healthy infants without previous episodes of AOM were enrolled at 6 months of age in a private paediatric practice in Rochester, NY. NP swabs and nasal wash samples were collected at seven prospective visits of children at 6, 9, 12, 15, 18, 24 and 30–36 months of age. Whenever the children were diagnosed with AOM, tympanocentesis was performed and middle ear fluid (MEF) samples were collected to confirm the diagnosis with microbiological culture for otopathogens. Identification of the major bacterial AOM pathogens was determined by standard culture methodology. All of the children received routine vaccinations according to the US schedule including PCV-7- or -13 (Prevnar, Wyeth Pharmaceuticals, Collegeville, PA) at the appropriate age. The study was approved by the Institutional Review Board of Rochester General Hospital, and written informed consent was obtained from parents or guardians of all children.

From 589 eligible children we randomly selected 100 children to assess the correlation of NP mucosal IgA and IgG antibody titres to PhtD, PcpA and PlyD1 with AOM incidence; the sample size was based on statistical power calculations based on our previous work [5]. The average age of the 100 children was 14.5 ± 5.9 months and 37, 32, 42, 36, 40 and 39 samples were tested when children were age at 6, 9, 12, 15, 18 and 24 months of age, respectively. The characteristics of the children are summarized in Table 1.

Nasal wash samples from the prospective visits were analysed for IgG and IgA antibody to *S. pneumoniae* proteins as described below. Then we determined if an episode of AOM or NP colonization caused by *S. pneumoniae* occurred during the subsequent 3, 6 or 12 months of follow up. In this way a measurement of NP mucosal antibody could be correlated with future risk of AOM or NP colonization by *S. pneumoniae* over defined time spans.

### *ELISA*

Pneumococcal protein-specific IgG and IgA antibody, and total IgG and IgA, were determined in the nasal wash by quantitative ELISA, and results were expressed as geometric means (GM) with 95% CI of ratios of specific IgG to total IgG or specific IgA to total IgA (ng/μg) as previously described [5]. Samples with a total IgG or IgA <0.05 μg/mL were excluded because in preliminary studies we determined that such samples were from children with a difficult or failed sampling process, and had undetectable antigen-specific antibodies.

**Table 1**
Characteristics of study cohorts

| | All children enrolled *n* (%) | Children for ELISA *n* (%) |
|---|---|---|
| Total | 589 (100%) | 100 (100%) |
| Female | 282 (48%) | 47 (47%) |
| Male | 307 (52%) | 53 (53%) |
| White | 401 (68%) | 68 (68%) |
| Non-white | 188 (32%) | 32 (32%) |
| Breastfeeding >6 months | 241 (41%) | 40 (40%) |
| Formula or breast feeding <6 months | 348 (59%) | 60 (60%) |
| Daycare | 164 (28%) | 29 (29%) |
| Non-daycare | 425 (72%) | 71 (71%) |
| Otitis-prone children[a] | 47 (8%) | 7 (7%) |
| Non-otitis-prone children | 542 (92%) | 93 (93%) |

[a] Children experiencing three episodes within 6 months or four episodes in 12 months.

### *Statistics*

Statistical analysis was performed with R Project version 2.13.2 and graphs were made with GraphPad Prism 6.0. For the purpose of statistical analysis, samples with undetectable specific antibody were arbitrarily assigned a value equivalent to half the lower limit of detection of corresponding specific antibodies. Antibody levels between groups were compared using two-tailed Mann–Whitney *U* test. Because antibodies were found to correlate significantly with age, antibody levels were adjusted for age using a generalized estimating equation model, assuming a within-subject autoregressive correlation. Association of NP antibody levels with future *S. pneumoniae* colonization and *S. pneumoniae* AOM was confirmed using the Regression Estimating Wald Test. A value of $p < 0.05$ was considered statistically significant.

## Results

### *Comparison of NP mucosal antibody levels to pneumococcal proteins between children who did and did not develop AOM infection caused by S. pneumoniae*

Among 228 samples, 116 were collected from children who had *S. pneumoniae* colonization detected and 112 were collected from children who did not have *S. pneumoniae* colonization detected. Overall, the children with detected colonization had fewer AOM infections in the following 12 months of prospective monitoring (21%; 24/116) than children who had not had colonization detected (35%; 39/112) (p 0.02), indicating that previous colonization led to fewer AOM infections as expected since colonization is an immunizing event [4].

As we have previously shown that *S. pneumoniae* colonization elicits significant mucosal antibody responses to pneumococcal proteins [5], we used the samples that had *S. pneumoniae* colonization detected to further analyse the correlation of mucosal antibody levels and incidence of AOM. We compared nasal wash IgG and IgA levels to PhtD, PcpA and PlyD1 of children that did and did not have at least one episode of AOM in the following 3–12 months of prospective monitoring. We found that children with no AOM infections during prospective monitoring had significantly higher