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Pneumococcal antibodies in a child with type 14 pneumococcal conjugate vaccine failure☆

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ABSTRACT

We measured the concentration, opsonic activity, and avidity of serotype-specific serum antibodies in a pneumococcal conjugate vaccine (PnCRM7) efficacy trial participant who contracted serotype 14 pneumococcal bacteremia following dose 3 of PnCRM7. Controls included 18 PnCRM7- and 10 MnCC-vaccinated children without invasive pneumococcal disease (IPD). The child with vaccine failure had 4.98 mcg/mL of serotype 14 antibodies 10 days before disease onset; these antibodies had greater opsonic activity and lower avidity than those of control PnCRM7 recipients. The child had no booster response to a fourth dose of PnCRM7 for most vaccine serotypes. We conclude that antibody concentration, functional activity and avidity do not predict individual protection against IPD, and immunological correlates of protection are only useful at the population level.

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1. Introduction

Pneumococcal conjugate vaccine (PCV), first licensed in February 2000 by the Food and Drug Administration, is a routine part of the childhood immunization schedule in the United States. Immunogenicity and invasive pneumococcal disease (IPD) efficacy data from the trial that led to licensure [1] were pooled with similar data from two other PCV trials among American Indian [2,3] and South African children [4,5] to estimate the protective antibody concentration following PCV administration [6,7]. The proposed correlate of protection is 0.35 mcg/mL of serotype-specific pneumococcal antibody, when the antibody is measured without 22F absorption [6]. Antibody concentrations as low as 0.2 mcg/mL have been correlated with an opsonophagocytic titer of 8 [8]. This estimate provides a guideline for the minimal functional antibody titer required for protection at the population level. The 0.35 mcg/mL antibody correlate of protection was defined for licensure of other PCV products, but its predictive value for disease episodes among individuals is unknown.

Between April 1997 and October 2000, the Center for American Indian Health conducted a large phase III, double-blind, controlled trial of a PCV among Navajo and White Mountain Apache children less than 2 years of age [2,9]. Nested within that efficacy trial was an immunogenicity study [3]. During the trial, a PnCRM7-randomized subject developed invasive serotype 14 pneumococcal disease; that child was a participant in the nested immunogenicity study and therefore had multiple serum samples available for analysis of pneumococcal antibody status immediately prior to and following the disease episode. We assessed the magnitude and functional characteristics of the serotype-specific antibody response in this PnCRM7-immunized child with vaccine serotype IPD compared with other immunized and unimmunized children who did not develop such disease during the course of the trial.

2. Methods

2.1. Efficacy and immunogenicity trial design

The efficacy trial and the immunogenicity study have been previously described in detail [2,3]. The intervention vaccine was PnCRM7 (Prevnar, Wyeth Vaccines, serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and the control vaccine was *Neisseria meningitidis* group C protein conjugate vaccine (MnCC, Wyeth Vaccines).

Infants enrolled between 6 weeks and 6 months of age received three doses of vaccine 2 months apart (4 weeks minimum) and a booster dose at 12–15 months of age (at least 2 months after dose

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3). A subset of the participants was enrolled in an immunogenicity evaluation; blood was drawn prior to each dose, 1 month following the third and fourth doses, and at 9–12 months following the fourth dose. The vaccine failure child was an immunogenicity study participant.

2.2. Comparison groups

To interpret the antibody results from the vaccine failure child, we assayed sera from 18 children immunized with PnCRM7 and 10 children immunized with MnCC. With this sample size, we had 81.5% power to detect a statistically significant difference ($p < 0.05$) in the proportion of children achieving an OPA titer $\geq$8 in the two groups, assuming the underlying true percentages were 80% and 20%, respectively. For the avidity assay we tested samples from 9 children immunized with PnCRM7. All samples for the avidity assay had ELISA values for the serotypes of interest of at least 1 μg/mL (a minimum of 0.6 μg/mL is required). We selected all comparison subjects from the same randomization unit as the vaccine failure case when possible, to control for exposure to pneumococcus.

2.3. ELISA antibody assays

All serum samples were tested at Wyeth Vaccines (Rochester, NY) in a blinded fashion for serotype-specific anti-pneumococcal IgG antibodies to all vaccine serotypes using a standard ELISA assay without 22F absorption [10]. The pneumococcal antibody limit of quantitation was 0.01 mcg/mL; samples with a value below this limit were assigned a value of 0.005 mcg/mL.

2.4. Avidity

Avidity was determined at the Centers for Disease Control and Prevention. The avidity protocol was a modification of that previously published by Antilla et al. [11]. Briefly, avidity was determined by a modified ELISA using 0.5 molar sodium thiocyanate as a chaeotropic agent to dissociate weak antigen–antibody interactions and allowing detection of higher avidity antibodies. Avidity determinations were performed on samples from the vaccine failure case and 9 PnCRM7 vaccine recipients for serotypes 14, 19F, 18C, and 23F. The protocol included the absorption of anti-CPS antibodies and other cross-reactive antibodies by Ps 22F. Avidity was estimated as the percent reduction in antibody concentration in the presence of chaeotrope (high avidity Ab) by comparison to antibody concentrations in the absence of chaeotrope (total binding Ab) and represented graphically as [100 − % reduction]. For each serum sample, both reactions were run in the same microtiter plate (Nunc-Immunoplate Maxisorp, Daigger, Vernon Hills, IL). IgG concentrations were determined by a four-parameter logistic regression analysis using the standard curve (89-SF) generated in the absence of chaeotrope or 22F absorption.

2.5. Opsonophagocytic assay

OPA determinations were performed at the CDC, Atlanta, GA. OPA to serotypes 14, 19F, 18C, and 23F were tested by a reference killing assay as previously described [12]. Lab operators were blind to the disease and vaccine status of the specimens.

2.5.1. Statistical analysis

We calculated the total antibody GMCs, the geometric mean percent reduction in IgG antibody from the avidity assay, the geometric mean OPA titer, and the proportion of subjects with OPA titer $\geq$8. We compared the vaccine failure child's results to those of other PnCRM7 recipients based on the point estimates and 95% confidence intervals (95% CI) in this group. We also compared the proportion of subjects with OPA $\geq$8 in the PnCRM7 and MnCC groups using Fisher's exact test. All analyses were conducted in STATA 9.0. We report statistical significance at the 0.05 level.

2.6. Ethical approval

All study subjects signed a written informed consent for participation in the trial and the immunogenicity sub-study. The protocol was approved by the relevant IRBs from Johns Hopkins Bloomberg School of Public Health, the Navajo Nation, the Phoenix Area Indian Health Service, the National Indian Health Service, the Centers for Disease Control and Prevention as well as the White Mountain Apache and Navajo tribes.

3. Results

3.1. Vaccine failure invasive pneumococcal disease

A PnCRM7-vaccinated child, enrolled in the nested immunogenicity study, developed occult bacteremia at 9.6 months of age, 53 days following the third dose of PnCRM7 vaccine. The child, who had a past medical history of febrile seizures, presented with a 1-week history of diarrhea, 1 day of irritability, tactile fever, and a single seizure episode. The child's physical examination was noncontributory. The child had a peripheral white blood cell count of 7700 (68% lymphocytes, 8% monocytes, 24% neutrophils), a chest X-ray with mild perihilar edema and a normal electrolyte panel. Blood culture grew serotype 14 *Streptococcus pneumonia*; the isolate was serotyped as type 14 on two separate occasions at the AIP laboratory. The illness resolved without hospital admission or antibiotic administration. On follow-up through 30 months of age the child was clinically well with no signs or symptoms of underlying

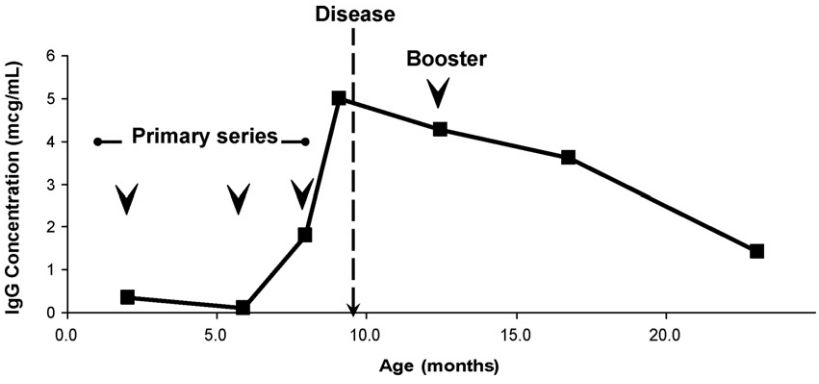

Fig. 1. Serotype 14 IgG antibody concentration (mcg/mL) and PnCRM7 vaccine doses by age from the vaccine failure child. Arrows indicate dates of vaccination at ages 2.0, 5.9, 7.9 and 12.5 months of age.

medical conditions, no other episodes of invasive bacterial disease and no evidence of recurrent respiratory tract infections. The child did not have specific testing for immunologic deficiencies conducted.

3.2. Serotype-specific antibody concentration

The serotype 14 ELISA antibody concentrations for the vaccine failure child are shown in Fig. 1. Eighteen days prior to the invasive disease episode, and 35 days after the administration of the third priming dose of PnCRM7, the child's serotype 14-specific antibody concentration was 4.98 mcg/mL.

The antibody concentration for all 7 serotypes following dose 3, prior to the booster dose and following the booster dose for the child with vaccine failure disease as well as for 387 other PnCRM7-vaccinated children are illustrated in Fig. 2. The vaccine failure child responded well to all vaccine serotypes. Post-dose 2 serotype-specific antibody levels were >1 mcg/mL for all serotypes except type 23F (data not shown). Following dose 3, antibody concentrations for types 4, 9V, 18C and 19F decreased and those for types 6B, 14 and 23F increased. The child's response to PnCRM7 did not improve after boosting, except for serotype 19F, for which the highest antibody concentration was achieved at 22 months of age. The child's response to natural infection appeared limited; serotype 14 antibody concentration was lower after the invasive disease episode (4.25 mcg/mL pre-boost) than before it (4.98 mcg/mL, post-dose 3). Compared with other PnCRM7 recipients, the vaccine failure child had antibody concentrations below the median but within the 95% CI for all serotypes at all time points, excepting serotypes 4, 9V and 18C at age 6 months, serotype 14 at age 12 months (after the disease episode) and serotype 19F at ages 13 and 24 months, for which the child's antibody concentrations were above the median value.

3.3. Opsonophagocytic assay findings

For serotypes 14, 18C and 23F, the proportion of PnCRM7 recipients with OPA titer $\geq$8 ranged from 44% to 100%, depending on the serotype and blood draw; this was significantly higher than the proportion of MnCC recipients whose OPA titer met this cutoff, which remained <20% (Fisher's exact $p < 0.02$ for all comparisons). Similarly, geometric mean OPA titers were higher in PnCRM7 vaccinees than in controls for these three serotypes (Table 1). Immunization with PnCRM7, however, did not stimulate antibodies with significant OPA to type 19F. The vaccine failure child had significantly higher OPA titers than the average PnCRM7 vaccinee for serotypes 14, 18C and 23F post-dose 3 and for serotypes 18C and 23F post-booster. The child's OPA titers were significantly lower than average pre-booster and post-booster titers for serotype 14 among the other PnCRM7-vaccinated children. The comparison of serotype 19F OPA titers is not informative given the similarity between PnCRM7 vaccinees and controls for this serotype.

3.4. Avidity of antibodies

The avidity measures of the vaccine failure child were compared with those from 9 other PnCRM7-vaccinated children (Fig. 3). We found that the vaccine failure child had a lower proportion of high avidity serotype 14 antibodies than other PnCRM7 recipients at all three time points tested. Prior to the disease episode, the vaccine failure child had 2.4 mcg/mL of high avidity type 14 antibody (3.5 mcg/mL of type 14 antibody in the 22F absorption ELISA, 68% of which was of high avidity). After the disease episode, the child's proportion of total antibody that had high avidity for the antigen decreased from 68% to 45%. For serotypes 18C, 19F and 23F, the vaccine failure child had a significantly higher proportion of antibody with high avidity than did other vaccinees post-dose 3, and a lower proportion of high avidity antibodies pre-boost and post-boost. The proportion of antibody that was of high avidity increased steadily from the post-dose 3 to the post-booster blood draw among PnCRM7 vaccine recipients. In the vaccine failure child, however, the proportion of antibody that was high avidity decreased between the post-dose 3 and the pre-booster blood draw, and then increased after the booster dose (for ST 19F the proportion remained constant).

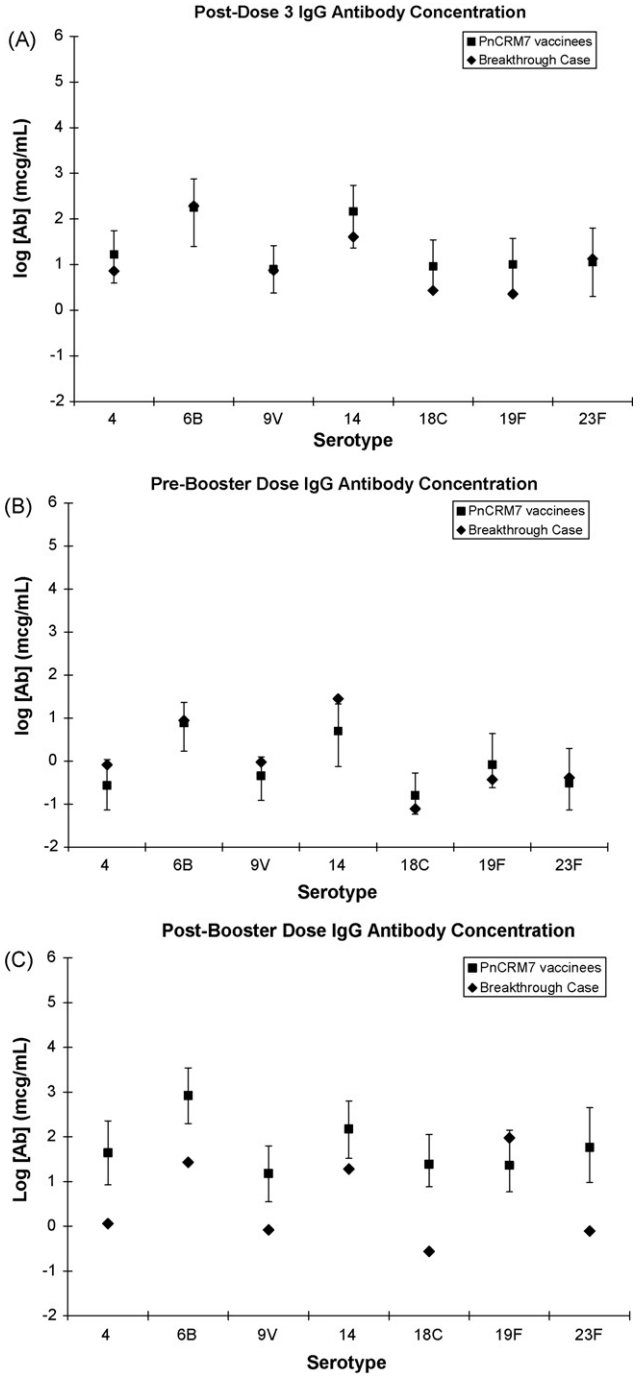

Fig. 2. Serotype-specific IgG antibody concentrations (mcg/mL) among PnCRM7-vaccinated children who did not develop vaccine serotype disease and among the child with serotype 14 disease. Vaccine failure child shown in diamonds and median value of comparison children without vaccine serotype disease shown in squares. Errors bars represent inter-quartile range. Panel A: post-dose 3; Panel B: pre-booster dose; Panel C: post-booster dose.

Table 1
Geometric mean OPA (95% CI) among PnCRM7 vaccinees, MnCC vaccinees and vaccine failure child.

	Serotype 14			Serotype 18C		
	Post-dose 3	Pre-dose 4	Post-dose 4	Post-dose 3	Pre-dose 4	Post-dose 4
Vaccine failure child	1024*	4*	64*	2048*	32	2048*
PnCRM7 vaccinees	362.0 (146.8; 892.6)	105.6 (42.6; 261.4)	762.5 (506.4; 1118.2)	149.3 (53.8; 414.4)	48.9 (18.7; 127.8)	812.7 (346.5; 1906.4)
MnCC vaccinees	6.5 (2.2; 19.5)	8 (2.7; 23.3)	8.6 (2.6; 28.5)	4 (4; 4)	4 (4; 4)	4 (4; 4)

	Serotype 19F			Serotype 23F		
	Post-dose 3	Pre-dose 4	Post-dose 4	Post-dose 3	Pre-dose 4	Post-dose 4
Vaccine failure child	8	8*	8	2048*	32	2048*
PnCRM7 vaccinees	5.4 (3.6; 8.2)	4.2 (3.8; 4.5)	5.9 (3.5; 9.7)	149.3 (53.8; 414.4)	48.9 (18.7; 127.8)	812.7 (346.5; 1906.4)
MnCC vaccinees	4 (4; 4)	4.3 (3.7; 5.0)	4 (4; 4)	4 (4; 4)	4 (4; 4)	4 (4; 4)

* $p \leq 0.05$ compared with PnCRM7 vaccinees.

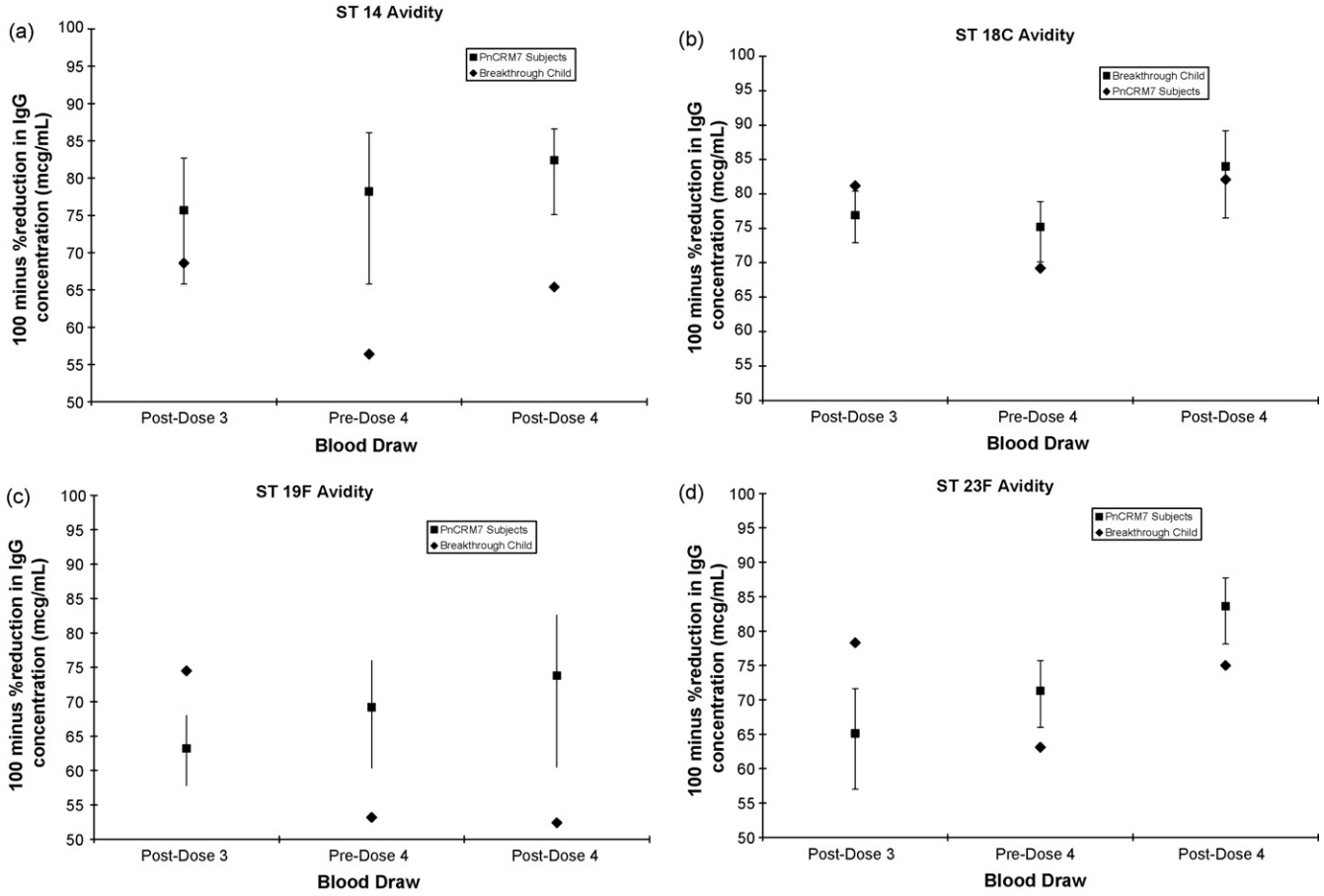

Fig. 3. Avidity (100% reduction in IgG concentration (mcg/mL)) post-dose 3, pre-boost and post-boost for PnCRM7 comparison children and vaccine failure child (shown in diamond). Median of PnCRM7 comparison children shown in squares. Panel A: serotype 14; Panel B: serotype 18C; Panel C: serotype 19F; Panel D: serotype 23F.

4. Discussion

We explored the immune response to PnCRM7 of a child with serotype 14 pneumococcal disease occurring 53 days after receipt of his third priming dose of PnCRM7. Prior to the disease episode, the child had a high concentration of ST 14 antibodies (4.98 mcg/mL) which were functional (based on the OPA assay) and avid. After disease, however, the child's serotype 14 antibody concentration had decreased; the antibodies remaining had low functional activity, with an OPA titer below the assay's detection limit, and a low proportion of them were of high avidity. This suggests that the existing functional antibodies at the time of disease were consumed through binding with the antigen. Following the booster dose, the child's serotype 14 antibody OPA and avidity increased slightly, indicating that new antibodies were formed in response to stimulation by vaccine. The fact that the child's serotype 14 antibody concentration met the proposed correlate of protection of 0.35 mcg/mL after dose 3 and prior to the disease episode illustrates that the proposed ELISA cutoff for population vaccine efficacy does not predict protection against disease for a specific individual. A confluence of factors determine whether a given exposure in an individual ultimately results in clinical invasive disease. These include intensity of exposure, co-infections, immunologic status, and environmental conditions such as smoke and allergen exposures in addition to antibody concentration. It is possible that the ELISA assay used to measure antibody concentration detected some non-specific or low-avidity antibodies and therefore does not reflect the concentration of serotype 14 IgG antibodies available to combat infection.

However, this is unlikely given that the antibody concentrations measured by the ELISA assay without 22F do not differ significantly from those measured with 22F absorption among American Indian children in this trial [7].

The child's response to other serotypes included in the vaccine was not consistent. For serotype 18C, the child's antibody concentration post-primary series was low, but these antibodies were highly functional. The 18C antibody concentration increased minimally following the booster dose but the antibodies increased in both avidity and opsonic activity. This indicates that an increase in avidity can result in higher OPA even if the total binding IgG decreases. For ST 23F, the child had high levels of antibody following the 3-dose primary series; like serotype 18C, the booster dose did not lead to a large increase in antibody concentration yet resulted in increased opsonic activity; unlike 18C, however, there was no concomitant increase in the antibody avidity. Serotype 19F had similar post-primary series antibody concentrations to ST 18C and is the only serotype for which there was a significant antibody concentration booster response. However, the rise in antibody concentration did not translate into greater opsonic activity or avidity.

The vaccine failure child's antibody responses were within the 95% confidence interval for the median for all serotypes at each time point, although they were lower than the median in most cases and seemed particularly low following the booster dose. Surprisingly, the vaccine failure child's antibodies had higher functional activity than those of other PnCRM7 recipients for serotypes 14, 18C and 23F post-primary series (prior to the disease episode) and for serotypes 18C and 23F following the booster dose. Thus, neither antibody concentration nor functional activity predicted protection against invasive disease in this case.

The serotype-specific correlates of protection developed for PCV predict population-level protection against invasive pneumococcal disease [6,8] but are inadequate to predict individual-level protection. Total binding antibody measures are influenced by the presence of non-specific and low-avidity antibodies. The low OPA titers found for serotype 19F in this study are consistent with the lower vaccine efficacy for this serotype [1], and support the notion that functional assays such as opsonophagocytosis may constitute a better correlate of protection. However, the child with vaccine failure pneumococcal disease had both a serotype 14 antibody concentration well above 0.35 mcg/mL and an OPA titer $\geq$8 only 18 days prior to developing disease. This child may have developed disease due to an inability to elicit a rapid antibody response to the disease-causing serotype, indicating impaired immunological memory. This explanation is consistent with the child's minimal booster responses to the fourth dose of PnCRM7 vaccine for a number of serotypes. A recent report indicates that even in children who fail to respond to pneumococcal polysaccharide vaccine, antibody responses to PnCRM7 vaccine rapidly increase by day 7 after a booster dose [13]. Another possible explanation is that the disease-causing strain was highly encapsulated, causing both functional and non-functional antibodies to be rapidly consumed. Opsonophagocytic titers are markedly reduced when highly encapsulated strains are used [14]. Finally, it is notable that this child had the tendency to produce antibodies of lower avidity (except for serotype 18C) than other PnCRM7-vaccinated children.

This publication is the first report to systematically evaluate the pneumococcal antibody status of a child with breakthrough disease prior to and following the disease episode. Children immunized with PnCRM7 who developed vaccine serotype invasive disease were identified in each of the vaccine efficacy studies but immunologic assessments were not conducted. A case report of breakthrough disease in the routine vaccine use PnCRM7 era which evaluated the post-disease immunologic status of a partially vaccinated child is published, however, no pre-disease blood was available for testing and no comparison children were assessed hence causal inferences about the post-disease antibodies cannot be drawn [15]. Systematic evaluations of vaccine failures following *Haemophilus influenza* type b conjugate vaccine [16], and meningococcal group C conjugate vaccine [17] with comparison group evaluations revealed that reduced avidity and antibody persistence, respectively were risk factor for development of disease following immunization with these products. The assessment of the child with breakthrough type 14 disease does not implicate either of these mechanisms as the a priori reason for the disease episode.

Although this child had serotype 14 bacteremia, the clinical episode was mild and self-resolving, likely attributable to the high concentration of type-specific antibody. The child must have been colonized with serotype 14 pneumococcus prior to the bacteremic episode, and the existing antibodies failed to prevent the carriage acquisition or to limit the organism to the nasopharyngeal mucosal surface. We and others have estimated the protective correlate for nasopharyngeal carriage as 4–5 mcg/mL of type-specific antibody, a concentration which was attained by this child [18,19]. In some respects the failure of vaccine for this child was not so much failure to prevent an invasive disease episode, which was mild and transient, but failure to prevent the colonization state which then led to the invasive episode. The child did not have a systematic immunologic evaluation so it is possible that there is some underlying deficiency, although the subsequent health history of the child does not support this possibility.

Although speculative, a further hypothesis about the occurrence of invasive type 14 disease in the face of high concentrations of functional and avid antibody is that the polysaccharide capsule of the strain may have been altered. Recently, epitope differences in isolates of serotype 6A have been described wherein the position of a single hydroxyl group on the galactose sugar of the capsule was sufficient to alter the polysaccharide structure to a double glucose instead of a galactose–glucose [20]. This seemingly small change altered the antigenicity of the modified polysaccharide, resulting in reduced cross-reactivity with type 6B. The change was stable and sufficient to warrant a new serotype designation, 6C. We cannot rule out that antigenic differences in this disease-causing strain of serotype 14 may have played a role in the failure of the PnCRM7-induced type 14 antibodies to protect against invasive disease.

The analysis of this case and relevant control subjects illustrates that antibody concentration alone is not a sufficient measure of the response to pneumococcal conjugate vaccines among individuals. Comprehensive immunological evaluations of vaccine failures illustrate the complex relationships between antibody concentration, functional activity, and avidity maturation in response to vaccine doses and exposure to natural infection. A more detailed understanding of the correlates of protection for both invasive disease and mucosal colonization or disease at the individual level will help to elucidate the likely protective characteristics of pneumococcal conjugate vaccines for both individuals and the community. Studies of PCV effect on acquisition of pneumococcus in the nasopharynx with appropriate serologic or mucosal fluid specimen characterization of antibody responses could address the relationship with organism acquisition, clearance and protection from progression to disease.

Conflict of interest

K.O.B. and M.S. have received research funding and honoraria from Wyeth Vaccines. None of the other authors have any conflicts of interest to report.

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