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Serotype-Specific Immune Responses to Pneumococcal Conjugate Vaccine Among Children Are Significantly Correlated by Individual: Analysis of Randomized Controlled Trial Data

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Abstract

Background—The magnitude of an individual's serotype-specific immunoglobulin G (IgG) response to a pneumococcal conjugate vaccine (PCV) has been associated with the vaccine's protective efficacy against carriage of pneumococci of that serotype, though the relationship with other serotypes needs to be understood.

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Conflicts of interest

ML has received research support through his institution from PATH Vaccine Solutions and Pfizer. He has received honoraria or consulting fees from Merck, Pfizer, Affinivax and Antigen Discovery.

RD has received grants and consulting and speaker fees from Pfizer; grant and consulting fees from MSD and consulting fees from MeMed.

WCG, DAS, and CJ are current employees of Pfizer and may hold stock and/or stock options. SP was an employee of Pfizer Inc during the time of the study. JT is an employee of inVentiv Health Clinical, LLC, a company contracted by Pfizer Inc. All authors approved the final article.

Author contributions

Conception and design of study and post-hoc analysis: ML, RD, SP, JT, CJ, WCG, DAS. All authors were involved in the analysis and interpretation of the data, writing and revision of the manuscript, and in the decision to submit the manuscript for publication.

Previous presentations

Preliminary results were presented as a poster titled "Individual Specific Immune Responses to Pneumococcal Conjugate Vaccines (PCV13; PCV7): Do Individuals Specialize in the Same Pattern of Response Across Vaccine Serotypes?" at the 10th International Symposium on Pneumococci and Pneumococcal Diseases, 2016.

Methods—Using immunogenicity data collected during a trial comparing the 7-valent (PCV7) and 13-valent (PCV13) vaccines, we measured associations between serotype-specific IgG levels, and used multiple regressions to identify demographic predictors of response.

Results—Vaccine-induced IgG levels were moderately positively correlated with one another, with pairwise correlation coefficients of 0.40–0.70. Principal component analysis of vaccine-serotype responses yielded one principal component indicating general immune responsiveness, and a second principal component mainly describing responses to serotype 14, which was the least correlated with the other responses. Overall, demographic variables explained only 17.0 and 20.4% of the geometric mean PCV7 and PCV13 responses, respectively. In both groups, older age at enrollment and shorter time from vaccination to antibody measurement were independently associated with stronger geometric mean responses.

Discussion—Improved understanding of the nature and causes of variation in immune response may aid in optimizing vaccination schedules and identifying robust correlates of protection.

Keywords

Streptococcus pneumoniae; Pneumococcal conjugate vaccine; Antibody response; Nasopharyngeal colonization

INTRODUCTION

Pneumococcal conjugate vaccines (PCV) protect recipients against colonization with *Streptococcus pneumoniae* serotypes included in the vaccine, as well as against mucosal and invasive disease and pneumonia. The key mechanism of protection against each of these endpoints is anticapsular immunoglobulin G (IgG), which protects in a serotype-specific fashion. Correlates of protection, based on serotype-specific antibody concentrations elicited by vaccination, have been identified for invasive disease [1] and otitis media [2]. Two randomized controlled trials using 7-valent [3] or 7- and 13-valent [4] pneumococcal conjugate vaccines have shown for several serotypes that the concentration of type-specific IgG produced in response to vaccination is predictive of subsequent nasopharyngeal (NP) carriage of that serotype, with higher responses predicting a reduced risk of carriage. This finding was consistent with data from another PCV7 randomized trial, where it was seen for serotype 23F but not serotype 19F [5].

Methodological work has emphasized the importance of accounting for heterogeneity in vaccine responses, as the distribution of responses can have important implications for the individual-level distribution of protection and for the extent of herd immunity achieved when a vaccine is used widely in a population [6–8]. Here we extend the analysis of the data from a trial of the 7- and 13-valent vaccines [4,9] to assess the degree to which variability in response to these vaccines is correlated for different serotypes: are those participants who respond strongly to one serotype (thereby achieving greater protection against that serotype [4]) also likely to respond strongly to other serotypes. Put another way, are there individuals who respond strongly to nearly all types and those who respond less strongly to nearly all types, or do responses to different types vary independently? For those trial participants who received the 7-valent vaccine, measurements of antibodies to the 6 serotypes not included in

that vaccine represent measurements of naturally acquired antibody responses. Therefore for these 6 serotypes we can compare the degree of correlation observed in response to vaccination (among those who got the 13-valent vaccine) to the degree of correlation observed in naturally acquired antibody responses (among those who got the 7-valent vaccine).

METHODS

Participants

In a randomized controlled trial in Israel for which results were previously reported [4,9], participants were randomized to receive either 13-valent pneumococcal conjugate vaccine (PCV13, N=881) or 7-valent pneumococcal conjugate vaccine (PCV7, N=873) at ages 2, 4, 6, and 12 months. At ages 7 and 13 months, blood was drawn to measure antibody concentrations. Participants were also swabbed and NP carriage of *S. pneumoniae* assessed at ages 2, 4, 6, 7, 12, 13, 18, and 24 months. Participants received concomitant vaccines on the recommended Israeli schedule throughout the study period. Detailed methods are given in reference [9].

ELISA

Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum serotype-specific IgG concentrations for each of the 13 serotypes in PCV13: the 7 that are in PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and the 6 that are in PCV13 but not PCV7 (serotypes 1, 3, 5, 6A, 7F, and 19A). For this study, the log-transformed IgG concentration measured at the 7-month visit for each serotype was defined as the response to that serotype [9]. The ELISA protocol recommended by the World Health Organization was used [10].

Statistical methods

We performed post-hoc analysis of data from the aforementioned randomized controlled trial. Scatterplots were used to explore the relationship between responses to pairs of serotypes, with each dot representing a single participant. Spearman rank correlation coefficients were estimated between responses to pairs of serotypes, and these pairwise correlations were estimated separately for those receiving PCV13 ($741 \leq n \leq 765$) and those receiving PCV7 ($715 \leq n \leq 782$). Numbers varied because a valid result could not be obtained for every serotype in every subject. As a sensitivity analysis, we repeated correlation estimates excluding a participant's response to any serotype that had been recovered from that participant's NP carriage swab at 2, 4, or 6 months ($n=284$). Principal component analysis (PCA) [11] was applied separately to responses of PCV13 and PCV7 recipients to identify groups of serotypes for which responses were correlated. Alpha of 0.05 was used for significance testing.

We performed multiple linear regressions where the explanatory variables were demographic predictors. The response variable was the mean of serotype-specific $\log_{10}$ -transformed antibody levels (hereafter mean response), the first principal component (PC1), or the $\log_{10}$ -transformed values of serotype-specific antibodies. We included the following demographic predictors in our analyses: time interval between the third dose of vaccine and blood draw,

age at enrollment, gestational age, weight, sex, ethnicity (Bedouin/Jewish), NP carriage of a vaccine serotype at the 2, 4, or 6 month visits, NP carriage of a non-vaccine serotype at the 2, 4, or 6 month visits, childcare arrangement (alone/not alone), ever breastfed before 6 months of age, and the number of siblings under the age of 5 years old at the 6 month visit. Regression coefficients (β) for mean log₁₀ antibody concentrations were transformed to percent changes in the geometric mean associated with a change in the explanatory variable by the formula (% change) = $100\% \times (10^\beta - 1)$.

RESULTS

Example correlations in antibody responses

Figure 1 shows the relationship (Spearman correlation) between responses to serotype 1 and 7F (Fig 1A), 23F and 9V (Fig 1B), and 1 and 14 (Fig 1C). These exemplify the general trends seen throughout the data. Serotypes 1 and 7F are both in PCV13 but not in PCV7. Responses to these two serotypes were strongly correlated in PCV13 recipients (0.62) but less so in PCV7 recipients (0.33). In contrast, 23F and 9V were included in both vaccines and the responses to these serotypes were highly correlated in both PCV13 and PCV7 recipients with correlation coefficients of 0.58 and 0.59, respectively. For the last comparison, serotype 1 is only in PCV7 whereas serotype 14 is in both. The correlation coefficient between the immune responses was higher in PCV13 recipients at 0.36 than in PCV7 recipients where there was no significant correlation ($p > 0.05$), with numerous outliers showing poor response to serotype 14 compared to their response to serotype 1.

Highly correlated vaccine responses, except for serotype 14

For the six PCV7 serotypes other than serotype 14 (Table 1, top left), responses showed significant ($p < 0.05$) pairwise Spearman correlations of 0.46–0.68 in PCV7 recipients (black numbers) and 0.43–0.67 in PCV13 recipients (blue numbers). In PCV13 recipients, pairwise correlations were high at 0.40–0.70 (Table 1, bottom right, blue numbers) for responses against PCV13-only serotypes, and also high for PCV7 types (except 14) and PCV13-only types with values ranging 0.37–0.72 (Table 1, top right, blue numbers). Serotype 14 was distinctive; its correlations with responses to other vaccine types were lower ranging from 0.25 to 0.40 in the PCV7 and PCV13 groups taken together (Table 1).

Serotype-specific vaccine responses showed weaker or no correlation with serotype-specific naturally acquired antibody responses

This comparison was possible in the PCV7 group, for whom antibody responses to the PCV7 serotypes were vaccine responses, while antibody responses to the 6 additional PCV13 serotypes were naturally acquired. An exception was the strong correlation between naturally acquired serotype 6A response and the vaccine-induced serotype 6B response, which has been noted in previous studies.

Pairwise correlations between a PCV7 serotype and a PCV13-only serotype among PCV7 recipients ranged from 0.01 to 0.48, except for the correlation between 6A and 6B responses which was higher at 0.54. While most such correlations were statistically greater than zero,

they were in all cases weaker than the same correlation in PCV13 recipients, measuring the relationship between two vaccine responses (top right of Table 1, black numbers).

The geometric mean of antibody levels against naturally acquired serotypes was lower than that of antibody levels against vaccine serotypes. The former had a median of 0.12 (interquartile range: 0.07–0.19) µg/mL whereas the latter had a median of 2.18 (interquartile range: 1.36–3.33) µg/mL.

Naturally acquired antibody responses showed intermediate levels of correlation with one another

PCV7 recipients' responses to PCV13-only serotypes showed statistically significant correlations with one another, ranging from 0.30 to 0.59, with a median value of 0.39 (bottom right of Table 1, black numbers). In comparison, vaccine responses were more highly correlated with a median Spearman correlation coefficient of 0.54 (range: 0.25–0.72). On the other hand, vaccine responses and naturally acquired responses were only weakly correlated in comparison with a median of 0.17 (range: 0.01–0.54).

Principal component analysis

Given the high frequency of positive pairwise correlations between antibody responses to different serotypes, we sought to identify independent contributors to the pattern of antibody responses, focusing on the vaccine responses to 7 serotypes by the PCV7 recipients and to 13 serotypes by the PCV13 recipients, using principal component analysis (PCA). For PCA we used $n=768$ participants for PCV7 and $n=733$ participants for PCV13 for whom all 7 or 13 vaccine-type antibody measurements were available, respectively. Table 2 shows the cumulative proportion of variance explained by the first 6 principal components. More than half the variation in either the PCV7 and PCV13 responses can be explained by a single principal component, while three can explain 79 or 71% of variation respectively. The loadings of the first two principal components are shown in Supplementary Table 1; these indicate the contribution of each serotype to the principal component. For both PCV7 and PCV13 recipients, all the vaccine serotypes had similar loadings in the first principal component, while the second principal component is dominated by serotype 14, indicating that much of the variation in individuals' responses can be explained by an overall level of responsiveness (first component), combined with a level of responsiveness to serotype 14.

Predictors of the responses

Demographic factors measured in this study are shown for PCV7 and PCV13 participants in Supplementary Figure 1. We investigated the degree to which these measured factors explained interindividual variation in immune responses to vaccines (Supplementary Figures 2–4). All results reported here were from multiple linear regressions using all 15 demographic factors listed in Methods. In total, they explained 17.0% and 20.4% of variation in the geometric mean response for vaccine serotypes in PCV7 and PCV13, respectively. Because geometric mean response to vaccine types is more readily interpretable than principal components, we report results for this quantity (Table 3), with PC1 and individual serotype results in the supplementary material (Supplementary Tables 2–4). Each one-day increase in age at enrollment was associated with a 1.4% (0.8% – 2.0%) and 1.3%

(0.8% – 1.8%) increase in the geometric mean antibody concentration for PCV7 and PCV13, respectively. On the other hand, each one-day decrease in the time interval between vaccine and blood draw was independently associated with increases of 1.3% (1.0%–1.7%) and 1.4% (1.0%–1.7%) in the geometric mean antibody concentration for vaccine serotypes in PCV7 and PCV13, respectively.

For the immune response against PCV13 serotypes only, each kilogram increase in weight at enrollment was associated with a 7.1% (0.8%–13.8%) increase. On the other hand, for the immune response against PCV7 serotypes only, breastfeeding before 6 months of age was associated with a 13.9% (4.0%–22.7%) decrease in mean antibody concentration.

There was heterogeneity between serotype-specific immune responses against different serotypes in terms of predictors and the regression coefficients (Supplementary Tables 2–3). While breastfeeding had no significant association with the mean antibody concentration against PCV13 serotypes, it was significantly associated with reduced serotype-specific antibody levels against serotypes 14, 19F, and 3. Similarly, there were no significant differences in the mean antibody concentrations between the two genders, but male compared to female participants receiving PCV7 had 12.9% (3.2%–21.6%) lower antibody response against serotype 4 and 17.5% (2.4%–30.3%) against serotype 6B.

Among PCV7 recipients, Jewish compared to Bedouin children had increases of 30.4% (7.4%–58.3%) and 22.6% (5.5%–42.6%) in serotype-specific antibody responses against serotype 5 and 19A, respectively. In PCV13 recipients, the increased serotype-specific response was only observed against serotype 5, with Jewish children having 14% (1.5%–27.9%) higher response than Bedouin children.

Among PCV13 recipients, having 2 siblings under the age of 5 as opposed to 1 was associated with 22.5% (2.4%–46.6%), 12.5% (0.8%–25.5%), and 16.6% (0.5%–35.3%) higher antibody concentrations against serotypes 6B, 9V, and 6A. These associations were not observed among PCV7 recipients. In fact, a 21.2% (5.5%–34.72%) decrease was observed in serotype-specific antibody levels against serotype 14.

DISCUSSION

We have shown in this post-hoc analysis that the magnitude of the IgG response to pneumococcal conjugate vaccines varies across individuals within a population, and that strong responders to the capsular polysaccharide of any one serotype in the vaccine tended to respond strongly to that of other serotypes, with the partial exception of serotype 14, a serotype that is epidemiologically distinctive in several other ways [12]. Vaccine-induced antibody responses were more correlated with each other than with naturally acquired antibody response.

The demographic predictors investigated in this study explained a small proportion of variation in antibody levels (less than 20%). The strongest demographic predictors of immune response to all vaccine types, and to individual serotypes, were time interval between vaccination and antibody measurement, and age at enrollment. The negative correlation with time interval between third vaccine dose and antibody measurement

suggests that peak antibody levels induced by vaccination had declined more in those participants in whom more time had passed since the most recent dose. This explanation is consistent with the finding that the PCV7 recipients did not consistently show such a relationship for antibodies to types not included in the vaccine. The two variables were negatively correlated, so infants who were enrolled at an older age on average waited a shorter period of time between vaccination and antibody measurement. The multiple regression including both age at enrollment and delay from vaccination to antibody measurement found evidence that both remained associated with the geometric mean antibody response, indicating that neither association fully explained the other.

Other demographic predictors were associated with different serotype-specific antibody responses. Breastfeeding, for instance, was negatively correlated with immune responses only against some serotypes. This negative correlation concurs with studies that have shown an inhibitory effect on vaccine-induced immunity [13].

Although the mechanisms for immunity differ between vaccine-induced and naturally acquired protection [14], weakly positive associations were found between naturally acquired and vaccine-induced antibody levels. Furthermore, the demographic factors that explained the variation in antibody levels were different for the two types of responses. The demographic factors explored in this paper could only explain around 6% of variance in the naturally acquired immunity, with prior exposure being the most important factor. Perhaps there exist host genetic factors that cause some individuals to respond strongly to both the polysaccharide-conjugate in the vaccine and the polysaccharide in a natural infection. Further research is required to determine the cause of this positive association.

Our study is limited to concentrations of binding antibodies as measured by ELISA. Measurements of functional antibodies using an opsonophagocytic assay (OPA) could be more relevant to protection as not all binding antibodies are functional [15]. However, whereas ELISA measurements are standardized across serotypes, OPAs at the time of the study were not standardized to an external reference serum. Furthermore, a *post hoc* analysis of the same clinical trial showed that antibody responses measured by OPA and by ELISA were positively correlated for serotypes targeted by the vaccines [16], which meant that binding antibody levels measured using ELISA would be similarly associated with protection against nasopharyngeal carriage of *S. pneumoniae* as functional antibody levels measured using OPA. We reported only on the analysis of IgG levels because ELISA measurements were available for 1501 subjects whereas OPA measurements were only available for only 351 subjects. Given the positive correlation between the two types of measurements, however, we do not expect our findings would change by analyzing OPA measurements instead.

Given that around 80% of variation in antibody levels remains unexplained, there must be many other factors that affect immune response. Some of these may be mechanical factors such as the quantity of antigen reaching the lymph nodes following injection (which would likely be correlated between serotypes). Pre-existing antibody to the CRM carrier protein may be associated with lower immune responses to the conjugated polysaccharides [17]. This might explain the higher vaccine-induced immune response against some serotypes in

Jewish children than in Bedouin children, as the latter tend to have higher maternal antibody levels [18]. Immunodeficiencies, either congenital or acquired, could reduce the ability to produce antibodies against the capsular polysaccharide. However, any such immunodeficiencies would have to be undiagnosed, as participants with known immunodeficiencies were ineligible for this study.

There may be host genetic predictors of the vaccine-induced immune response. For example, genes affecting vaccine-induced antibody levels have been identified for the Hepatitis B vaccine [19]. Identification of genetic markers and other immune biomarkers of immune response to PCV could improve the predictability of serotype-specific antibody response and therefore vaccine efficacy.

In addition to genetic host factors, environmental factors such as nutritional status may influence vaccine response [20]. Recently, the composition of the host gut microbiome has been associated with the magnitude of response to influenza vaccine [21]. While we found no consistent association of pneumococcal colonization at the time of vaccination with vaccine response, other components of the respiratory, gut or other host microbiomes may affect this response. The microbiome itself is a composite factor reflecting host genetics, nutrition, infection history, and antibiotic use. For example, the composition and diversity of the gut microbiome in children who are breastfed are distinct from those who were not [22]. Furthermore, there is significant seasonal variation in the composition of the microbiome, possibly due to temporal changes in pathogen exposure or in diet [23].

Understanding the reasons for heterogeneity in vaccine response, particularly for heterogeneities like those documented here that affect responses to multiple antigens in the same way, could have implications for the optimal timing of vaccine administration or potentially for the design of other interventions to enhance vaccine response and thereby protection against pneumococcal acquisition [4].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IgG	Immunoglobulin G
PCV	pneumococcal conjugate vaccine
ELISA	enzyme-linked immunosorbent assay
PCA	principal component analysis

NP	nasopharyngeal
SNP	single nucleotide polymorphism

References

1. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine*. 2007; 25:3816–26. DOI: 10.1016/j.vaccine.2007.01.119 [PubMed: 17368878]
2. Jokinen JT, Ahman H, Kilpi TM, Mäkelä PH, Käyhty MH. Concentration of antipneumococcal antibodies as a serological correlate of protection: an application to acute otitis media. *J Infect Dis*. 2004; 190:545–50. DOI: 10.1086/422531 [PubMed: 15243930]
3. Dagan R, Givon-Lavi N, Fraser D, Lipsitch M, Siber GR, Kohberger R. Serum serotype-specific pneumococcal anticapsular immunoglobulin g concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *J Infect Dis*. 2005; 192:367–76. DOI: 10.1086/431679 [PubMed: 15995949]
4. Dagan R, Juergens C, Trammel J, Patterson S, Greenberg D, Givon-Lavi N, et al. Modeling pneumococcal nasopharyngeal acquisition as a function of anticapsular serum antibody concentrations after pneumococcal conjugate vaccine administration. *Vaccine*. 2016; 34:4313–20. DOI: 10.1016/j.vaccine.2016.06.075 [PubMed: 27422342]
5. Millar EV, O'Brien KL, Bronsdon MA, Madore D, Hackell JG, Reid R, et al. Anticapsular Serum Antibody Concentration and Protection against Pneumococcal Colonization among Children Vaccinated with 7-Valent Pneumococcal Conjugate Vaccine. *Clin Infect Dis*. 2007; 44:1173–9. DOI: 10.1086/513199 [PubMed: 17407035]
6. Gomes MGM, Lipsitch M, Wargo AR, Kurath G, Rebelo C, Medley GF, et al. A Missing Dimension in Measures of Vaccination Impacts. *PLoS Pathog*. 2014; 10:10–2. DOI: 10.1371/journal.ppat.1003849
7. Gomes MGM, Gordon SB, Laloo DG. Clinical trials: The mathematics of falling vaccine efficacy with rising disease incidence. *Vaccine*. 2016; 34:3007–9. DOI: 10.1016/j.vaccine.2016.04.065 [PubMed: 27177948]
8. Poland GA, Ovsyannikova IG, Jacobson RM, Smith DI. Heterogeneity in Vaccine Immune Response: The Role of Immunogenetics and the Emerging Field of Vaccinomics. *Clin Pharmacol Ther*. 2007; 82:653–64. DOI: 10.1038/sj.clpt.6100415 [PubMed: 17971814]
9. Dagan R, Patterson S, Juergens C, Greenberg D, Givon-Lavi N, Porat N, et al. Comparative immunogenicity and efficacy of 13-valent and 7-valent pneumococcal conjugate vaccines in reducing nasopharyngeal colonization: A randomized double-blind trial. *Clin Infect Dis*. 2013; 57:952–62. DOI: 10.1093/cid/cit428 [PubMed: 23804191]
10. World Health Organization. Recommendations for the production and control of pneumococcal conjugate vaccines. WHO Tech Rep Ser. 2005; 927:64–98.
11. Johnson, DE. Applied Multivariate Methods for Data Analysts. Duxbury Resource Center; 1998.
12. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis*. 2005; 5:83–93. DOI: 10.1016/S1473-3099(05)01280-6 [PubMed: 15680778]
13. Niewiesk S. Maternal antibodies: Clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front Immunol*. 2014; 5doi: 10.3389/fimmu.2014.00446
14. Wilson R, Cohen JM, Reglinski M, Jose RJ, Vogel D, Gordon S, et al. Naturally Acquired Human Immunity to *Pneumococcus* Is Dependent on Antibody to Protein Antigens. 2017; :1–26. DOI: 10.1371/journal.ppat.1006137
15. Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D, Nahm MH. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. *Clin Vaccine Immunol*. 2006; 13:165–9. DOI: 10.1128/CVI.13.2.165-169.2006 [PubMed: 16467321]
16. Juergens C, Patterson S, Trammel J, Greenberg D, Givon-Lavi N, Cooper D, et al. Post hoc analysis of a randomized double-blind trial of the correlation of functional and binding antibody

responses elicited by 13-valent and 7-valent pneumococcal conjugate vaccines and association with nasopharyngeal colonization. *Clin Vaccine Immunol.* 2014; 21:1277–81. DOI: 10.1128/CVI.00172-14 [PubMed: 24990907]

17. Findlow H, Borrow R. Interactions of conjugate vaccines and co-administered vaccines. *Hum Vaccines Immunother.* 2016; 12:226–30. DOI: 10.1080/21645515.2015.1091908
18. Hagerman A, Posfay-Barbe KM, Grillet S, Ochs MM, Brookes RH, Greenberg D, et al. Failure to elicit seroresponses to pneumococcal surface proteins (pneumococcal histidine triad D, pneumococcal choline-binding protein A, and serine proteinase precursor A) in children with pneumococcal bacteraemia. *Clin Microbiol Infect.* 2012; 18:756–62. DOI: 10.1111/j.1469-0691.2011.03629.x [PubMed: 21851490]
19. Ryckman KK, Fielding K, Hill AV, Mendy M, Rayco-Solon P, Sirugo G, et al. Host genetic factors and vaccine-induced immunity to HBV infection: Haplotype analysis. *PLoS One.* 2010; 5doi: 10.1371/journal.pone.0012273
20. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. *Am J Clin Nutr.* 2011; 474:327–36. DOI: 10.1038/nature10213
21. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci U S A.* 2011; 108:5354–9. DOI: 10.1073/pnas.1019378108 [PubMed: 21402903]
22. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, et al. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr.* 2017; doi: 10.1001/jamapediatrics.2017.0378
23. Davenport ER, Mizrahi-Man O, Michelini K, Barreiro LB, Ober C, Gilad Y. Seasonal variation in human gut microbiome composition. *PLoS One.* 2014; 9doi: 10.1371/journal.pone.0090731

Highlights

- Some individuals have stronger immune responses against all serotypes in PCV
- Increasing time since vaccination was associated decreasing antibody levels
- Older participants tended to have higher antibody levels than younger participants

Figure 1.

Serotype-specific IgG concentrations ($\mu\text{g/mL}$) in PCV13 and PCV7 recipients one month after the 3-dose infant series. Regression lines are shown for IgG concentrations in PCV13 (dashed) and PCV7 (solid) recipients. Comparisons are made between immune responses against (A) serotypes 7F and 1 which are both in PCV13 only, (B) serotypes 9V and 23F which are in both PCV13 and PCV7, and (C) serotypes 14 and 1, where the former is in both vaccines whereas the latter is only in PCV13.

Spearman correlation coefficients of immunoglobulin G (IgG) responses 1 month after the infant series for 13-valent pneumococcal conjugate vaccine (PCV13) and PCV7 recipients. All values are significant ($P<0.05$) unless indicated by an asterisk #.

Table 1

PCV13 Serotypes:	4	6B	9V	14	18C	19F	23F	1	3	5	6A	7F	19A
PCV7 Serotypes:	4	6B	9V	14	18C	19F	23F						
4		0.43 0.50	0.62 0.68	0.39 0.40	0.67 0.65	0.60 0.51	0.58 0.59	0.72 0.13	0.54 0.15	0.61 0.18	0.57 0.35	0.60 0.18	0.51 0.37
6B			0.52 0.50	0.25 0.28	0.46 0.46	0.49 0.52	0.54 0.62	0.47 0.13	0.37 0.16	0.52 0.22	0.62 0.54	0.42 0.10	0.52 0.41
9V				0.33 0.38	0.61 0.60	0.60 0.53	0.58 0.59	0.64 0.16	0.48 0.21	0.65 0.27	0.59 0.43	0.61 0.23	0.59 0.48
14					0.36 0.38	0.38 0.36	0.30 0.36	0.36 0.01#	0.40 0.04#	0.29 0.11	0.33 0.15	0.37 0.09	0.27 0.21
18C						0.60 0.49	0.57 0.55	0.65 0.04#	0.54 0.07#	0.58 0.18	0.57 0.31	0.58 0.07	0.52 0.32
19F							0.62 0.53	0.60 0.01#	0.48 0.07#	0.58 0.16	0.57 0.34	0.53 0.09	0.59 0.32
23F								0.57 0.10	0.46 0.15	0.61 0.19	0.61 0.41	0.53 0.14	0.57 0.44
1									0.53 0.42	0.70 0.53	0.58 0.35	0.62 0.33	0.54 0.43
3										0.48 0.51	0.44 0.31	0.55 0.29	0.40 0.38
5											0.63 0.52	0.54 0.39	0.64 0.59
6A												0.51 0.31	0.55 0.59
7F													0.50 0.30
19A													

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Table 2
Cumulative percentage of variance explained by principal components for PCV7 and PCV13.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Cumulative % of variance explained by PCV 13 PC	53	64	71	76	80	84	87	90	93	95	97	99	100
Cumulative % of variance explained by PCV 7 PC	54	70	79	86	93	97	100						

Table 3

Predictors of the geometric mean of serotype-specific immune responses against vaccine serotypes in 7-valent pneumococcal conjugate vaccine 7 (PCV7) and PCV13.

	PCV7	R^2	PCV13	R^2
	change		change	
Time interval (days) between third dose and blood draw	-1.3% (-1.7%, -1%) ***	0.113	-1.4% (-1.7%, -1%) ***	0.135
Age at enrollment (days)	1.4% (0.8%, 2%) ***	0.022	1.3% (0.8%, 1.8%) ***	0.027
Gestational age (weeks)	-1.2% (-3.6%, 1.3%)	0.003	0.7% (-1.6%, 3.1%)	0
Weight (kg)	6% (-0.8%, 13.3%)	0.027	7.1% (0.8%, 13.8%) *	0.028
Ethnicity (Jewish vs Bedouin)	4.1% (-5.5%, 14.7%)	0	3.3% (-5.4%, 12.8%)	0
Sex (male vs female)	-7.3% (-15.4%, 1.7%)	0.003	3.4% (-4.8%, 12.4%)	0
NP carriage of a VT at 2-month visit	13.5% (-7%, 38.4%)	0	-4.8% (-19.6%, 12.6%)	0
NP carriage of a NVT at 2-month visit	2.3% (-10.2%, 16.7%)	0.003	8.9% (-5.1%, 25%)	0.001
NP carriage of a VT at 4-month visit	-12.4% (-26.6%, 4.5%)	0.004	13.9% (-2.5%, 32.9%)	0
NP carriage of a NVT at 4-month visit	10.7% (-2.4%, 25.5%)	0.002	-11.6% (-22.7%, 1.1%)	0
NP carriage of a VT at 6-month visit	-6.9% (-21%, 9.6%)	0.003	-14.6% (-25.7%, -1.9%) *	0.005
NP carriage of a NVT at 6-month visit	-3% (-13.4%, 8.7%)	0.001	5.8% (-6.1%, 19.3%)	0.001
Childcare arrangement (group childcare vs alone)	-0.6% (-14.2%, 15.1%)	0	-5% (-16.5%, 8.1%)	0
Breastfed before 6 months	-13.9% (-22.7%, -4%) **	0.011	-6.2% (-14.7%, 3.1%)	0.007
Number of siblings under 5 (2 vs. 1)	0.4% (-10%, 12%)	0	7% (-2.3%, 17.3%)	0.003

NP = nasopharyngeal, VT = vaccine type, and NVT = non-vaccine type. 'Change' refers to the percentage change calculated based on regression coefficients, with the 95% confidence interval bounds given in brackets. Significant changes are in bold. Asterisks denote significant results:

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$. R^2 is the proportion of variation in antibody levels that can be explained by a demographic predictor.