Serotype-specific correlates of protection for pneumococcal carriage: an analysis of immunity in 19 countries

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Summary:

Anti-capsular pneumococcal antibody concentrations associated with protection against carriage vary between serotypes and are higher than antibody levels required for protection against disease. Higher antibody concentrations are required for protection in lower-income countries.

Abstract

Background

Pneumococcal conjugate vaccines (PCVs) provide direct protection against disease in those vaccinated, and interrupt transmission through the prevention of nasopharyngeal carriage.

Methods

We analysed immunogenicity data from 5224 infants who received PCV in prime-boost schedules. We defined any increase in antibody between the one-month post-priming visit and the booster dose as an indication of nasopharyngeal carriage ('seroincidence').

We calculated antibody concentrations using receiver-operator characteristic curves, and used generalised additive models to compute their protective efficacy against seroincidence.

To support seroincidence as a marker of carriage, we compared seroincidence in a randomised immunogenicity trial in Nepal with the serotype-specific prevalence of carriage in the same community.

Findings

In Nepalese infants, seroincidence of carriage closely correlated with serotype-specific carriage prevalence in the community.

In the larger data set, antibody concentrations associated with seroincidence were lowest for serotypes 6B and 23F (0.50 μg/mL and 0.63 μg/mL respectively), and highest for serotypes 19F and 14 (2.54 μg/mL and 2.48 μg/mL respectively). The protective efficacy of antibody at these levels was 62% and 74% for serotypes 6B and 23F, and 87% and 84% for serotypes 19F and 14.

Protective correlates were on average 2.15 times higher in low/lower middle income countries than in high/upper middle income countries (GMR 2.15, 95%CI 1.46–3.17, p=0.0024).

Interpretation

Antibody concentrations associated with protection vary between serotypes. Higher antibody concentrations are required for protection in low-income countries. These findings are important for global vaccination policy, to interrupt transmission by protecting against carriage.

Introduction

Vaccination with pneumococcal conjugate vaccines (PCV) induces IgG antibody production which peaks approximately one month after vaccination and wanes thereafter in the absence of serotype-specific exposures[1]. The concentration of circulating serum antibody that is sufficient to protect an infant against invasive pneumococcal disease (IPD) or nasopharyngeal colonisation during the post-vaccination period is not well defined but is generally higher for mucosal endpoints than IPD and varies between serotypes.[2-4]

In large efficacy trials only a small proportion of children will have antibody concentrations assessed after vaccination, precluding analyses which directly link the antibody concentration in an individual with their disease outcome. In such situations the overall trial-level estimates of the proportion with disease and the proportions with antibody below a threshold level are used to define an antibody level associated with protection which is referred to as a correlate of protection.[5-7] A pooled correlate of protection against IPD of 0.35 µg/mL was derived from a meta-analysis of three efficacy trials[68-10] Although these thresholds have provided a means to establish licensure of additional products, they cannot predict protection for the individual, as evidenced by a case report of vaccine failure in a 9-month old child, 10 days after a measured antibody concentration of 4.98 µg/mL.[11]

Current correlates of protection refer only to protection against IPD, and not to other endpoints of clinical importance such as acute otitis media, non-bacteraemic pneumonia, nor to nasopharyngeal carriage. The effector molecule for protection against mucosal colonisation is unlikely to be circulating serum IgG, but instead may be IgG or IgA that is produced by mucosally associated B-cells, or IgG which leaks from circulation to mucosa.[12,13] Nevertheless, circulating serum IgG concentrations measured 4-6 weeks post-vaccination can serve as a proxy for the level of immune response to pneumococcal conjugate vaccines which in turn provides protection from carriage. Since nasopharyngeal colonisation is a necessary precondition for development of pneumococcal disease and is the mechanism for transmission of pneumococcal infection, vaccine efficacy against this endpoint is key to preventing disease reducing transmission, inducing herd immunity, and thereby delivering maximum health benefits of PCV.[14,15]

The aim of this study was to use a large number of vaccine trials with individual participant level data to calculate the concentration of serotype-specific serum IgG which is associated with protection against nasopharyngeal colonisation in the individual.

Methods

Data

Individual level serological data were sought from immunogenicity clinical trials in which PCV was administered to children in prime-boost schedules. Blood samples were taken for serology at one-month post-priming and at the booster dose visit. For each participant, antibody concentrations were compared at the one-month post-priming visit and the booster visit. Any increase in antibody at the latter time-point was considered evidence for natural boosting due to pneumococcal carriage acquisition at some point in the intervening period ('seroincidence').

Randomised Trial in Nepal

Additional immunogenicity data were available from an open-label randomised controlled trial of 10-valent PCV (PCV10) in Nepal (authors own data). Details of the trial have been previously published[16] (Supplementary material).

Antibody concentrations have been shown to increase after nasopharyngeal carriage.[17] To confirm that any increase in antibody between two time points is a good proxy measure for nasopharyngeal colonisation with the same serotype, antibody data from the Nepal RCT were paired with data from a cross-sectional carriage study of 600 healthy children (aged 6 weeks to 24 months) conducted during the same period and the same study centre.[18] High correlation between the proportions of children with an increase in antibody levels between the pre-boost and post-primary time points, and the proportions of community children with positive nasopharyngeal swabs was considered supportive evidence for the use of seroincidence as a marker of nasopharyngeal carriage.

Use of carriage seroincidence to define antibody thresholds of protection

Two methods were used to analyse the association between serotype-specific pneumococcal serum antibody concentrations and seroincidence.

First, serotype-specific receiver-operator characteristic (ROC) curves were created for antibody measured one-month post-priming vaccination, compared with seroincidence in the following months prior to the booster dose visit (see Supplementary material, Table S1 and Figure S1). From each ROC curve, the antibody level which maximised the sum of sensitivity + specificity was retained as the antibody level most able to discriminate between those with and without presumed carriage acquisition in the post-vaccination period.[19,20]

A second analysis used generalised additive models (GAM) to compute the protective efficacy of the antibody thresholds identified from ROC curves. A GAM model for binary data using seroincidence as the outcome was applied to each country cohort and serotype separately, with IgG at one-month post-priming fitted with a spline smooth. The models adjusted for age and sex. For each country cohort the antibody concentration at which the probability of seroincidence became negligible (probability < 0.05) was identified.

The probability of seroincidence at the level of antibody identified from ROC curves was compared to the probability of seroincidence in those with antibody below the lower limit of assay quantification (LLD) to compute the protective efficacy of each ROC threshold.

Protective thresholds from ROC curves were compared across income strata (high/upper middle income countries (H/UMIC) vs low/lower middle income countries (L/LMIC)) using mixed effects models. In addition, the proportion of infants with maternal antibody prior to vaccination was compared. Infants with antibody against pneumococcal serotypes prior to vaccination are likely indicative of mothers who had been susceptible, exposed and infected (likely with asymptomatic carriage) within a time period which enabled transplacental transfer of antibody to occur. The seroprevalence of type-specific pneumococcal antibodies in infants aged ~8 weeks is therefore

interpreted as a surrogate measure of the force of infection and its association with calculated ROC antibody thresholds was investigated.

Sensitivity analyses were undertaken as detailed in the supplementary material, which contains further statistical methods.

Results

Antibody increase and circulating serotypes in the community

There was a strong relationship (R^2=0.743) between seroincidence in the immunogenicity trial participants in Nepal, and nasopharyngeal carriage prevalence among children in the same community as the trial participants. Because antibodies to some serotypes are cross-reactive to other serotypes within the same serogroup (vaccine-related types), it was unclear which serotype might be responsible for an observed antibody increase among the immunogenicity participants. For this reason, serotype data from nasopharyngeal carriage observations were combined into serogroups. With the exception of serotypes 1 and 5, confidence intervals for nasopharyngeal carriage included the line of best fit (Figure 1).

Serotype-specific antibody thresholds of protection against seroincidence

Pneumococcal IgG serum concentrations were available from 29 studies (19 countries, and 5224 children). Studies were conducted in Europe (Denmark, The Czech Republic, Finland, France, Germany, The Netherlands, Poland, Slovakia, Spain, Sweden), Asia (India, Japan, Malaysia, Nepal, Philippines, and Singapore), Africa (Mali, Nigeria), and Chile (Table S2).

Pneumococcal antibody thresholds of protection derived from ROC curves varied by serotype. The lowest threshold was 0.50 µg/mL and the highest was 2.54 µg/mL, for pneumococcal serotypes 6B and 19F antibodies respectively (Figure 2, Table 1).

The probability of seroincidence from GAM models declined as antibody increased, until a point where the probability became negligible and curves flattened out (Figure 3). For serotype 1 the probability of seroincidence was low for all levels of post-primary antibody thus the point at which the probability became negligible could not be defined (Figure 3). For serotypes 4, 5, 7F and 18C, the post-primary antibody thresholds from both ROC and GAM methods were essentially the same, thus seroincidence is negligible for antibody above these levels. For the remaining serotypes (6B, 9V, 14, 19F and 23F) antibody concentrations of at least 3.45 µg/mL were required to minimise seroincidence to the 5% level. However, substantial protection against seroincidence was available with antibody at levels of 0.50 to 2.54 µg/mL as defined by ROC curves, and protective efficacy estimates at these levels was between 62% and 87% (Table 1).

High and low income countries

Overall across all serotypes, thresholds derived from ROC curves were twice as high in L/LMICs, than in H/UMICs (GMR 2.15 95% CI 1.46–3.17, p=0.0024) although for individual serotypes confidence intervals overlapped (Figure 4). Seroprevalence of maternal antibodies in infants prior to vaccination was also higher in L/LMICs (p=0.0006), and there was a significant relationship between the

proportion of children with maternal antibody and the estimated correlate of protection for the same serotypes (p=0.0347) (Figure S2).

Sensitivity analyses

Sensitivity analyses showed that variations in the definition of seroincidence had little impact on calculated correlates of protection (Table S3).

Discussion

This analysis of infant immunogenicity data from 5224 infants in 29 studies in 19 countries, estimates pneumococcal serotype-specific antibody concentrations associated with protection against an increase in antibody following the primary vaccination series, which we infer as a proxy for nasopharyngeal carriage. It confirms that more antibody is needed to protect against carriage than the current level of 0.35 mg/mL associated with protection against IPD. Dagan *et al* in a study of NP carriage acquisition report more than 80% of acquisitions occurred in children with antibody higher than 0.35 µg/mL one-month post-primary vaccination.[4] All calculated antibody concentrations in our study were higher than serotype-specific correlates of protection previously derived for IPD, a finding also observed by Dagan.[2,4]

The mechanism of protection against carriage is unclear but could be mediated by either IgA or IgG. IgA mediated immunity has been demonstrated in mice and levels of the isotype are typically lower than and proportional to systemic IgG levels.[21] However, S. pneumoniae produces an IgA protease which may mean that local production of IgA at the mucosa is successfully evaded by pneumococci.[22] It may be that higher blood levels of IgG correlate with local production of IgG at the mucosa or that high levels of circulating IgG leak across to the mucosal surface from the circulation and provide local protection against colonisation.[13]

Relationship with circulating serotypes

The serogroup-specific prevalence of nasopharyngeal carriage in unvaccinated healthy children in a cross-sectional carriage study in Nepal was closely associated with the proportions of children who had antibody increases in the PCV immunogenicity trial. The two studies were conducted in the same period and location, providing compelling, indirect, evidence of the validity of using serology to infer colonisation. For serotypes 1 and 5, the frequency of seroincidence was not closely associated with nasopharyngeal carriage prevalence in the community. There were no positive swabs for serotype 1, and only one for serotype 5 from 600 children tested, yet large proportions of children in the immunogenicity trial had antibody increases by 3 years of age for these serotypes. These serotypes appear to circulate more widely than is indicated by cross-sectional carriage studies, consistent with data showing a short half-life of colonisation, and rapid transmission.[23] Serotypes 1 and 5 are associated with the majority of invasive disease among young children in Nepal,[18,24] therefore serological methods may be better at assessing colonisation for these serotypes than nasopharyngeal swabs.

Protective thresholds

Substantial variation exists in the level of antibody associated with protection against carriage, as potentially expressed by seroincidence. Thresholds defined using ROC curves represent the antibody level which best distinguishes those who have greater levels of protection.

Using ROC curves, the lowest correlates of protection (0.50 μg/mL and 0.63 μg/mL) were observed for serotypes 6B and 23F respectively, and the highest were 2.54 μg/mL and 2.48 μg/mL for serotypes 19F and 14. Andrews *et al.* calculated correlates of protection using IPD cases in UK children and found similar serotype-specific variation, with higher levels of antibody required for 19F and lower levels for 6B, and 23F.[2] Similarly, Dagan *et al* report the antibody level above which 10% of carriage acquisitions occurred was high for serotypes 14 and 19F, and low for 23F, however 6B was also high in that study.[4] These findings are consistent with in vitro evidence that more IgG is required to kill serotype 19F than 6B.[25]

GAM thresholds can be interpreted as the level of antibody at which the probability of seroincidence becomes negligible (<5%). For serotypes 4, 5, 7F and 18C, the ROC and GAM thresholds were similar. For serotypes 6B, 9V, 14, 19F and 23F, much higher levels of antibody were consistent with a probability of seroincidence of 5% than those obtained from ROC curves, thus the ROC correlates for these serotypes indicate less than complete protection for the individual. Such difference may relate to a longer duration of carriage.[26,27]

These antibody concentrations show similarities with thresholds derived for other mucosal endpoints. Our estimate of 0.50 μg/mL for serotype 6B derived using ROC methods, had a protective efficacy of 62%. This is very similar to the predicted vaccine efficacy (VE) of ≥ 65% against acute otitis media (AOM) at 0.5 μg/mL reported by Jokinen *et al*.[28] Thus an antibody concentration of 0.5 μg/mL more than halves the probability of 6B carriage and the majority of protection occurs with induction of this small amount of antibody. In Dagan's model of carriage acquisition rates which used similar regression model methodology, 0.5 μg/mL of antibody against serotype 6B equated to approximately 6.5% probability of acquisition in the Jewish cohort, and 13.5% acquisition in the Bedouin community. 10% of carriage acquisitions occurred in those with post-vaccination antibody of at least 7.4 or 10.8 μg/mL in the Jewish and Bedouin cohorts respectively.[4]

In contrast, VE against serotype 23F AOM was negligible in Jokinen *et al* at 0.65 μg/mL with wide confidence intervals[28] whereas in our analysis protective efficacy against colonisation was 74% at this antibody level. Millar *et al* investigated 17 cases of serotype 23F carriage acquisition after vaccination with PCV7 and report no carriers achieving serotype 23F antibody concentrations greater than 4 mg/mL before acquisition. This threshold value for total protection is slightly lower than the estimate from GAM analysis of 5.81 mg/mL (95%CI 4.64–7.28).[29] In Dagan *et al* 10% of acquisitions occurred at antibody levels above 2.97 mg/mL.

The probability of seroincidence predicted from GAM models for serotypes 9V, 14, 19F and 23F was between 10% and 22% for antibody at the level of the ROC thresholds. Thus these levels of antibody provide substantial protection even though not representing levels which provide absolute protection for an individual.

High and low income countries

Protective antibody concentrations were on average twice as high in L/LMICs than in more wealthy countries. Carriage of pneumococci increases with factors such as household size, exposure to smoke (including from cooking fires), and underlying illnesses.[30-32] Such factors are also related to socioeconomic status. Higher rates of nasopharyngeal colonisation in low income settings have been observed from carriage studies in young children[32] and in rural settings compared with urban.[31] Dagan *et al* showed that the relationship between carriage acquisition rates and antibody levels can be quite different for high and low socioeconomic groups in a study of Jewish and Bedouin communities.[4] Higher rates of colonisation in the community (i.e. higher force of infection) would increase the probability that such colonisation occurs during pregnancy, resulting in higher maternal antibody in early infancy. Seroprevalence of maternal antibodies is therefore a useful proxy for exposure. Our analysis shows the seroprevalence of maternal antibodies in infants (a surrogate for the force of infection) is higher in L/LMICs, and is significantly related to the antibody concentration required for protection against seroincidence for the same serotypes. The higher level of antibody needed for protection in L/LMICs may relate to a higher infectious load delivered at each nasopharyngeal exposure event, requiring higher levels of antibody for individual protection. In addition, the variation across serotypes in both force of infection and antibody levels required for protection indicates biological variation in characteristics of the bacteria resulting in varying abilities of serotypes of pneumococci to colonise the nasopharynx.

Limitations and strengths

Deriving robust thresholds of protection from any single study is problematic due to the large degree of variation between studies in both VE and estimated correlates of protection.[2,6,8,10] The main strength of this analysis is therefore the uniquely large dataset of paired immunogenicity data in infants from a large number of studies. All results were assayed in one laboratory. This has the benefit of reducing the influence of inter-laboratory variation, however also means caution must be applied when extrapolating to results from other laboratories and other vaccines.

The use of seroincidence as a proxy for carriage improves the detection of acquisition events as the data are not limited by the timing and frequency of nasopharyngeal swabs but instead create a historical record of the acquisition events experienced during the period between the post primary age and the booster age. This improves the differential misclassification that affects cross-sectional carriage studies, however cannot remove it entirely. Misclassification of true carriage events in our data can be due to many reasons including (1) rises and then falls in antibody between blood draws resulting in an overall observed decline, or (2) rises in antibody from transient exposure but not a true carriage event. These data were not from studies designed to compute antibody thresholds and are speculative in nature. The lengths of time between priming and booster doses varied across studies which may affect misclassification rates and the thresholds calculated from the data.

Conclusion

The variation between serotypes in antibody levels which are associated with an inferred protection against carriage in this investigation support previous observations that combined correlates of protection for all serotypes are inaccurate for some serotypes.[17]

We recommend considering the use of serotype-specific thresholds of protection in clinical trials to demonstrate induction of antibody levels associated with high protection against colonisation to support vaccine development, dosing schedule optimization, and the assessment of vaccine impact.

In settings with high force of infection, often low-income settings, higher levels of antibody are required to interrupt transmission. These findings are important for global vaccination policy especially with regard to assessment of alternative schedules aimed at reducing transmission to gain indirect effects (herd immunity) of the vaccine and maximizing the impact of the vaccine.

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MV obtained and analysed the data and wrote the paper. AJP and TRF supervised the work. All authors provided advice, reviewed the manuscript, and approved the final version for submission. MV had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Disclaimer

The views expressed in this manuscript are those of the authors and do not necessarily reflect the views of the JCVI, the DH, the NIHR, the NHS, or the WHO. All data included in these analyses were provided by GlaxoSmithKline through an independent third party (http://clinicalstudydatarequrest.com). The company were not involved in the decision to submit the manuscript and did not review the manuscript or any analyses prior to submission.

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Declarations of interest statement

AJP has previously conducted studies on behalf of Oxford University funded by vaccine manufacturers, but currently does not undertake industry funded clinical trials. Trials of vaccines or observational studies previously funded by Okairos, Novartis, Pfizer were completed within the past 3 years. His department received unrestricted educational grants from Pfizer/GSK/Astra Zeneca in July 2016 for a course on Infection and Immunity in Children. AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunisation (JCVI) and is a member of the World Health Organization's (WHO) Strategic Advisory Group of Experts.

KOB is a member of the WHO Strategic Advisory Group of Experts. KOB has research grants from Pfizer and GSK in the past 3 years and has served on a voluntary capacity on expert advisory committees for GSK, and Merck.

DFK has previously received support from vaccine manufacturers to attend scientific meetings.

JH has received research funding from GSK, SPMSD, PATH and Pfizer in the past 3 years and is co-founder of BUGS Bioscience, a not-for-profit spin-out company.

All other authors declare no conflicts of interest.

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Tables and Figures

Table 1 Serotype-specific pneumococcal antibody correlates of protection against seroincidence in infancy

Serotype	*Protective antibody concentration (ROC) (µg/mL)*	*LCL (µg/mL)*	*UCL (µg/mL)*	*Antibody concentration equivalent to 5% seroincidence probability (GAM)* (µg/mL)*	*LCL (µg/mL)*	*UCL (µg/mL)*	*Probability† of seroincidence at antibody level from ROC curves (column 2)*	*PE of ROC antibody threshold compared to antibody at the LLD*
1	0.81	0.54	1.22	N/A	N/A	N/A	N/A	N/A
4	1.16	0.90	1.49	0.87	0.81	0.95	5%	>90%
5	0.73	0.42	1.26	0.62	0.42	0.90	5%	>90%
6B	0.50	0.40	0.61	5.35	4.01	7.14	33.1%	62%
7F	1.60	1.25	2.05	1.73	1.53	1.95	5%	>90%
9V	1.31	1.08	1.58	3.45	2.90	4.11	12.4%	86%
14	2.48	2.02	3.04	8.90	7.49	10.58	15.0%	84%
18C	1.32	0.95	1.82	1.58	1.37	1.84	5%	>90%
19F	2.54	1.83	3.53	9.48	7.39	12.15	9.9%	87%
23F	0.63	0.47	0.83	5.81	4.64	7.28	21.7%	74%

Data are displayed in Figure 2. PE: protective efficacy, ROC: receiver operator characteristic curve method for derivation of correlates of protection, GAM: generalised linear model method for derivation of thresholds of protection, LLD: lower limit of assay detection, LCL: lower 95% confidence limit, UCL: upper 95% confidence limit

Protective efficacy is calculated as 1-(pr(ROC)/pr(LLD))x 100%.

pr(ROC) is the probability of seroincidence predicted from GAM model at a level of antibody equivalent to the ROC correlate of protection.

pr(LLD) is the probability of seroincidence predicted from GAM model at the lower limit of detection of the assay (0.05 mg/ml)

*Antibody level at which the probability of seroincidence is less than 5%

†Predicted probability from GAM model

For serotypes 4, 5, 7F and 18C ROC and GAM thresholds had substantially overlapping confidence intervals. The probability of seroincidence is considered to be the same (5%) for both methods.

Figure 1 The percentage of participants in a randomised PCV immunogenicity trial in Nepal with an increase in vaccine serotype IgG between 10 months and 3 years of age, and serogroup-specific nasopharyngeal carriage prevalence in a cohort of healthy unvaccinated children from the same community.

Dotted line represents the line of best fit from linear regression with intercept set at 0. There was no carriage of serotype 1 detected. 95% confidence intervals use the binomial exact method.

Figure 2 Serotype-specific pneumococcal antibody correlates of protection against seroincidence in infancy

Blue circles represent serotype-specific thresholds of protection against carriage derived from ROC curves and 95% confidence intervals. Red squares represent thresholds of protection and 95% confidence intervals from GAM models. For reference, grey bars are antibody correlates of protection for IPD from Andrews et al (reference 2) (not available for serotype 5). Data are also presented in Table 1. ROC: Receiver operator characteristic; GAM: generalised additive models; IPD invasive pneumococcal disease

Figure 3 The relationship between the serotype-specific probability of seroincidence estimated from generalised additive models, and the antibody concentration measured one-month after administration of 2 or 3 priming doses of pneumococcal conjugate vaccine

Each dot represents one participant. Horizontal dashed lines at a probability of 0.05.

Figure 4 Serotype-specific pneumococcal antibody correlates of protection in infancy stratified by income status of country setting.

Serotype specific threshold correlates of protection and their 95% confidence intervals. Closed squares: Low or lower middle income countries (L/LMIC): India, Mali, Nepal, Nigeria, The Philippines; Open Squares: High or upper middle income countries (H/UMIC): Chile, Czech Republic, Denmark, Finland, France, Germany, Japan, The Netherlands, Norway, Poland, Singapore, Slovakia, Spain, Sweden. L/LMIC vs H/UMIC: GMR 2.15, 95%CI (1.46–3.17), p=0.0024