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Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies

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Abstract

Estimates of minimum protective antibody concentrations for vaccine preventable diseases are of critical importance in assessing whether new vaccines will be as effective as those for which clinical efficacy was shown directly.

We describe a method for correlating pneumococcal anticapsular antibody responses of infants immunized with pneumococcal conjugate (PnC) vaccine (Prevenar) with clinical protection from invasive pneumococcal disease (IPD). Data from three double blind controlled trials in Northern Californian, American Indian and South African infants were pooled in a meta-analysis to derive a protective concentration of 0.35 μg/ml for anticapsular antibodies to the 7 serotypes in Prevenar. This concentration has been recommended by a WHO Working Group as applicable on a global basis for assessing the efficacy of future pneumococcal conjugate vaccines.

The WHO Working Groups anticipated that modifications in antibody assays for pneumococcal anticapsular antibodies would occur. The principles for determining whether such assay modifications should change the protective concentration are outlined. These principles were applied to an improvement in the ELISA for anticapsular antibodies, i.e. absorption with 22F pneumococcal polysaccharide, which increases the specificity of the assay for vaccine serotype anticapsular antibodies by removing non-specific antibodies. Using sera from infants in the pivotal efficacy trial in Northern California Kaiser Permanente (NCKP), 22F absorption resulted in minimal declines in pneumococcal antibody in Prevenar immunized infants but significant declines in unimmunized controls. Recalculation of the protective concentration after 22F absorption resulted in only a small decline from 0.35 μg/ml to 0.32 μg/ml. These data support retaining the 0.35 μg/ml minimum protective concentration recommended by WHO for assessing the efficacy of pneumococcal conjugate vaccines in infants.

Keywords: Streptococcus pneumoniae; Pneumococcus; Vaccine; Conjugate vaccine; Antibody response; Protective correlate; Protective antibody level; Vaccine licensing; Vaccine regulation

1. Introduction

When antibodies are the major mechanism by which vaccines provide protection, it is theoretically possible to establish an antibody concentration which predicts protection in an immunized population [1]. In practice, protective

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antibody concentrations have been proposed and generally accepted for a number of vaccine preventable diseases, including tetanus, diphtheria, polio, Japanese encephalitis, measles, mumps, rubella, hepatitis B, varicella, influenza, meningococci and *Haemophilus influenzae type b* (Hib). [2] Recently a protective concentration has also been recommended for pneumococci [3,4], which is the subject of this report.

The most important application of protective antibody concentrations is for establishing the protective efficacy of new or improved vaccines when placebo controlled efficacy trials are no longer feasible or ethical. The protective concentration is also used as the benchmark for assessing interference between vaccines given concomitantly. In these assessments, the proportion of the test population receiving the new vaccine or new vaccine combination which achieves an antibody concentration equal or greater than the protective concentration is compared to the control population receiving the control vaccine or vaccine combination which is the current standard of practice and for which efficacy has already been established in controlled clinical trials. Achieving the protective concentration thus serves as the primary outcome in determining whether a new vaccine is not inferior to an already licensed vaccine with clinically documented efficacy and thus can be inferred also to be effective.

Certain fundamental principles must be followed in order to develop a valid estimate of the protective concentration. First, it must be established that the immune mechanism that is measured correlates with protective activity. In all cases for which protective antibody correlates have been established to date, protection is mediated by antibody [2]. It has however not been possible to establish an antibody based protective correlate for certain vaccines which are given by mucosal routes, including the intranasal cold adapted influenza vaccine [5], oral rotavirus vaccine and oral typhoid vaccine [2], perhaps because local cellular and humoral immunity may protect in the absence of measurable systemic antibody.

Second, it is important to verify that the target antigen is indeed a virulence factor of the organism or serves as a protective antigen when antibody binds to it. For many vaccines, the target antigen is the major virulence factor, including tetanus, diphtheria and pertussis toxins, capsular polysaccharides of pneumococcus, meningococcus and Hib, and critical surface exposed viral antigens such as influenza hemagglutinin.

Third, the antibody assay chosen ideally should directly measure the functional activity mediating protection. Examples of such functional assays include antitoxin assays for tetanus and diphtheria, bactericidal assays for Hib and meningococcus, opsonophagocytic assays for pneumococcus and direct virus neutralization assays for measles and other viruses. Because of technical difficulties or variability associated with complex bioassays, it is often desirable to use a binding or binding inhibition assay which correlates highly with functional activity as surrogate assays. Examples are influenza hemagglutination inhibition (HAI) as a surrogate for viral neutralization and ELISA assays for IgG class anticapsular polysaccharide antibodies to Hib and pneumococcal capsular polysaccharides as surrogates for bactericidal and opsonic activity respectively.

Recently a WHO working group has proposed a protective concentration for pneumococcal conjugate (PnC) vaccine in infants [3,4]. A concentration of IgG anticapsular polysaccharide antibodies measured by ELISA ≥ 0.35 μg/ml measured one month after primary immunization was recommended as the protective threshold based on three double-blind controlled efficacy trials for invasive pneumococcal disease (IPD) performed in Northern California Kaiser Permanente (NCKP) [6], American Indians [7] and South Africa [8].

This report describes the serologic data and statistical methods used to derive this estimate. In addition, we present information on the effect on pneumococcal antibody concentrations and on the protective correlate of performing an additional absorption of the test sera with pneumococcal type 22F polysaccharide. This modification has been introduced to remove antibodies to non-capsular pneumococcal antigens and thereby make the ELISA more specific for antibodies to vaccine-type capsular polysaccharides which confer protection [9].

2. Materials and methods

2.1. Patient populations

Three double blind controlled efficacy trials of pneumococcal conjugate vaccine were utilized in a meta-analysis to estimate the concentration of anticapsular polysaccharide antibodies associated with protection against invasive pneumococal disease (IPD). Two trials were conducted using 7 valent PnC vaccine, (Prevenar®, Wyeth Vaccines) given on a US schedule (2, 4, 6 and 12 months) using individual randomization in 37,868 infants at Northern California Kaiser Permanente [6] and group randomization in 8292 American Indian infants in the South Western US [7]. The third study was conducted with 9 valent PnC vaccine given on the EPI schedule (6, 10 and 14 weeks) using individual randomization in 19,922 infants in Soweto, South Africa [8]. The 9 valent PnC vaccine contained the seven serotypes in Prevenar plus types 1 and 5 which are common in developing countries [10]. Only efficacy estimates in the non-HIV infected infants were used to calculate protective concentrations.

Table 1 summarizes the number of patients by treatment group which were immunized per protocol in the three trials, the numbers of IPD cases observed per protocol caused by the 7 vaccine serotypes in Prevnar and, the numbers of sera assayed for pneumococcal antibodies to generate the protective correlate.

Table 1
Three controlled double blind efficacy trials of pneumococcal conjugate vaccine used in meta-analysis of protective pneumococcal antibody concentration

Study/Author	Evaluable patients (per protocol)		IPD Cases (7vPnC types, per protocol)		Efficacy (95% CI)	No. of sera assayed by single absorption ELISA with C-Ps	
	Control	PnC	Control	PnC		Control	PnC
NCKP (2000) Black et al. [6]	10,995 (MnCC)	10,940 (7vPnC)	39	1	**97.4%** (82.7, 99.9)	189 (180)[a]	190 (188)[a]
American Indian (2003) O'Brien et al. [7]	2818 (MnCC)	2974 (7vPnC)	8	2	**76.8%** (−9.4, 95.1)	481	445
South Africa (2003) Klugman et al. [8]	18,550 (Placebo)	18,557 (9vPnC)	10	1	**90%** (29.7, 99.8)[b]	302	256
Pooled studies	33,363	32,471	57	4	**93.0%** (81, 98.2)	972	891

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).
[a] No. of sera re-assayed by double absorption ELISA with both C-Ps and type 22F Ps.
[b] Efficacy for the 7 serotypes in Prevnar in non-HIV infected children [8].

2.2. Pneumococcal antibody assays

IgG antibodies for type-specific pneumococcal capsular polysaccharides were measured by validated ELISA using only C-Ps absorption of both unknown sera and the 89SF standard serum as described [11]. This assay was performed as described in the WHO ELISA protocol [12] except that : (1) the WHO protocol uses double absorption of unknown sera with both C-Ps and 22F Ps; and (2) the Wyeth assay used a C-Ps preparation made by Wyeth rather than C-Ps from the Staten Serum Institute, Denmark. The performance of the two C-Ps absorbents was shown to be equivalent in assay validation studies (data not shown). To further reduce binding by non-specific pneumococcal antibodies, double absorption of sera with both C-Ps and type 22F pneumococcal polysaccharide was introduced [9] but was applied only to unknown sera and not to the standard 89SF serum according to the WHO protocol. The 89SF standard is not absorbed in order to retain the original assignments of anti-capsular polysaccharide antibody concentrations which were done without 22F absorption [11].

2.3. Derivation of protective concentration of pneumococcal antibodies

The theoretical relationship between risk of IPD and anticapsular antibody concentration can be modeled as a continuous logistic function shown in Fig. 1. In the absence of anticapsular antibody, IPD rates are high, shown by the higher plateau. This disease rate is presumably determined by host factors such as innate immune defenses and specific immunity to non-capsular antigens, by the virulence of the pathogen and by the inoculum size of the exposure. In the presence of high anticapsular antibody, IPD rates would be low, shown by the lower plateau. If antibody provides sterilizing immunity this rate would be zero. Given the low incidence of IPD, it is not feasible to define this curve precisely for pneumococcus or for most human pathogens.

In order to estimate a specific level of antibody associated with protection, several simplifying assumptions must be made. The first is to assume that the relationship of the immune response and the probability of IPD is a step function as shown by the dotted line in Fig. 1, rather than a logistic function. It is not necessary to define the rates of IPD at the upper and lower plateau in order to utilize the method. This step function can then be linked to vaccine efficacy (VE) as follows:

Let,

p_v = % subjects with antibody levels less than $\times$ $[C]_{prot}$ in the vaccinated group,

where $[C]_{prot}$ is the protective concentration

p_c = % subjects with antibody levels less than $\times$ $[C]_{prot}$ in the control group

a = probability of IPD when serum antibody is $< [C]_{prot}$

b = probability of IPD when serum antibody is $\geq [C]_{prot}$

Then,

Prob (IPD event in vaccines) $= ap_v + b \times (1 - p_v)$

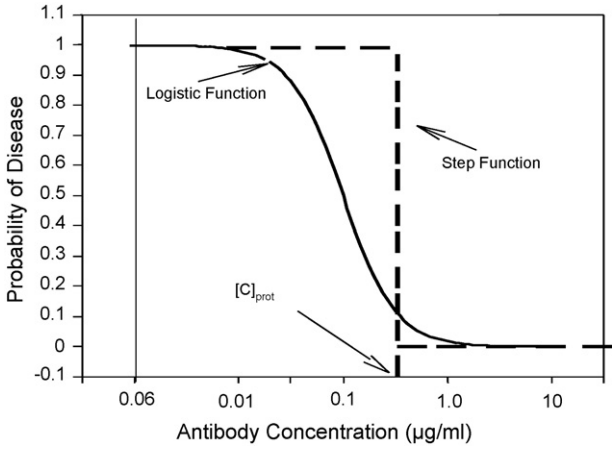

Fig. 1. Theoretical relationship between risk of disease and concentration of protective antibodies. The step function represents the simplifying assumption required to calculate a protective concentration, $[C]_{prot}$.

Prob (IPD event in controls) $= ap_c + b \times (1 - p_c)$

Since

$$\mathrm{VE} = 1 - \left[\frac{\text{prob(IPD event in vaccines)}}{\text{prob(IPD event in controls)}}\right]$$

$$\mathrm{VE} = \frac{[(a-b)\times(p_c - p_v)]}{[b + p_c(a-b)]}$$

If b, is close to zero, then this relationship is simplified to: $\mathrm{VE} \approx 1 - [p_v/p_c]$. In other words, the relative risk of IPD is the same as the relative risk of having antibody concentration less than $[C]_{prot}$.

When VE is known, $[C]_{prot}$ may be determined directly from the reverse cumulative distribution curves (RCDC) of the antibody concentrations of the vaccinated group and the control group. The variability in $[C]_{prot}$ is a function of the variability of the vaccine efficacy estimate and the serology data. Because the sample size of the serology data is so large compared to the cases in the efficacy estimate, the variability in $[C]_{prot}$ is dominated by the variability in the efficacy estimate. Confidence limits on $[C]_{prot}$ were therefore estimated by calculating the protective level at the lower and upper confidence limits of vaccine efficacy.

2.4. Simplifying assumptions to estimate protective concentration of pneumococcal capsular antibodies

As described by Jodar et al. [3], in addition to the "step function" model described above, additional simplifying assumptions were made to estimate the protective antibody concentration.

First, the antibody concentration measured ~ 4 weeks after the primary immunization of infants was assumed to predict long-term protection. Second, the protective antibody concentrations were assumed to be similar for all pneumococcal serotypes and therefore a single estimate was used for all types. In order to estimate type specific protective antibody concentrations, we would require precise type-specific efficacy estimates. However, even the largest controlled trial at NCKP showed statistically significant type specific efficacy for only 3 of 7 types with wide confidence intervals due to the small numbers of IPD cases. When all three controlled trials are pooled together, significant efficacy was found for 6 of 7 types but the confidence intervals around the point estimates remain too wide to be useful in estimating protective concentrations for each serotype (Table 2). Licensure of Prevenar was, in fact, based on aggregate efficacy for all 7 serotypes, not type-specific efficacy for individual serotypes.

Therefore the WHO committee elected to accept a single protective concentration applicable to all seven serotypes in the seven valent PnC vaccine using pooled efficacy and pooled serology results from the three controlled studies.

Table 2
Pooled[a] Efficacy Estimates for Individual Serotypes in 3 controlled trials of PnC Vaccine and a post-marketing case-control study

Serotype	Pooled per protocol				Pooled intent-to-treat				Post-marketing Case-Control Study (17)	
	Number of cases		VE (95% CI)	*p*-value	Number of cases		VE (95%CI)	*p*-value	Discordant pairs	VE (95%CI)
	Control	PnC CRM			Control	PnC CRM				
4	2	1	**50%** (−100, 99.2)	1.000	2	1	**50%** (−100, 99.2)	1.000	19	**93%** (65, 99)
6B	9	0	**100%** (49.3, 100)	0.004	14	2	**85.7%** (37.8, 98.4)	0.004	32	**94%** (77, 98)
9V	6	1	**83.3%** (−37.4, 99.6)	0.125	7	1	**85.7%** (−11.2, 99.7)	0.070	20	**100%** (88, 100)
14	12	1	**91.7%** (43.7, 99.8)	0.003	16	1	**93.8%** (59.8, 99.9)	<0.001	47	**94%** (81, 98)
18C	9	0	**100%** (49.3, 100)	0.004	11	0	**100%** (60.2, 100)	0.001	30	**97%** (85, 99)
19F	13	1	**92.3%** (48.8, 99.8)	0.002	15	2	**86.7%** (42.7, 98.5)	0.002	34	**87%** (65, 95)
23F	6	0	**100%** (15.1, 100)	0.031	8	0	**100%** (41.4, 100)	0.008	18	**98%** (80, 100)
All types	57	4	**93.0%** (81, 98.2)		73	7	**90.4%** (79.2, 96.3)		200	**94%** (90, 96)

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).
[a] Studies by Black et al. [6], O'Brien et al. [7] and Klugman et al. [8].

2.5. Statistical methods for estimating protective antibody concentrations from the pooled efficacy trials

Because of the differences in the efficacy trials, pooling of the correlates will obtain a more widely applicable level. Such pooling is valid since there was not a statistically different protective concentration across the trials. Three pooling methods were used:

(1) Simple unweighted pooling. This method merely combines all observations: pneumococcal invasive disease cases and antibody concentrations. It will give greater weight to trials with more observations for both IPD cases and immunogenicity results.
(2) Weighted pooling. This method weights the immunogenicity data for each trial according to the number of subjects in the trial.
(3) Weighted average of $[C]_{prot}$. This method weights each trial's $[C]_{prot}$ by the variability of the estimate. The actual variability is not known but a good approximation is the number of disease cases in the trial. Because the actual variability is not known, it should be viewed as a confirmation of the other pooling methods.

3. Results

3.1. Antibody concentrations in immunized and control infants by single absorption ELISA

Table 3 summarizes the geometric mean antibody concentrations (GMC) for 7 vaccine types and all types combined in the three controlled trials.

The antibody responses in each of the trial populations have been reported separately [13–15] but samples utilized in this study and the published studies differed, based on availability of specimens [13–15] and differing timing of post-immunization samples [15].

The GMCs after immunization differed significantly among the three populations for 6 of 7 serotypes. Higher antibody responses in the South African infants were responsible for most of the differences. The American Indian and NCKP infants had overlapping confidence intervals for 4 types but higher responses were observed to types 4 and 19F in American Indians and to type 14 in NCKP infants. The NCKP infants had the lowest antibody concentrations in the aggregated serotypes and the South African infants, the highest. Although antibody levels were low in the control groups, significant differences were observed for all serotypes among the control study populations as well. It should be noted, however, that the younger age of the South African control group (18 weeks versus 7 mos) may have resulted in a higher level of residual maternal antibody in that group. In addition varying frequencies of exposure to pneumococci may also contribute to the differences.

Table 3
Geometric mean IgG anticapsular antibody concentrations to individual serotypes and aggregated serotypes in infants immunized with Prevenar[a]

	Geometric mean concentration (μg/mL) with 95% confidence intervals							
	Immunized population			*p*-value[b]	Un-immunized population			*p*-value[b]
Serotype	NCKP	Am Indian	SA		NCKP	Am Indian	SA	
N[a]	190	445	256		189	481	302	
4	**1.36** (1.20, 1.56)	**2.96** (2.68, 3.27)	**4.37** (3.83, 4.99)	<0.001	**0.03** (0.02, 0.03)	**0.03** (0.03, 0.03)	**0.04** (0.04, 0.05)	<0.001
6B	**3.34** (2.75, 4.05)	**3.19** (2.74, 3.72)	**4.54** (3.84, 5.36)	0.011	**0.08** (0.07, 0.09)	**0.13** (0.12, 0.15)	**0.19** (0.16, 0.22)	<0.001
9V	**1.60** (1.41, 1.83)	**1.67** (1.53, 1.84)	**2.37** (2.12, 2.65)	<0.001	**0.05** (0.05, 0.06)	**0.11** (0.10, 0.12)	**0.12** (0.10, 0.13)	<0.001
14	**4.68** (4.07, 5.40)	**3.76** (3.27, 4.32)	**3.99** (3.37, 4.71)	0.174	**0.05** (0.04, 0.06)	**0.03** (0.03, 0.04)	**0.19** (0.16, 0.22)	<0.001
18C	**1.96** (1.71, 2.25)	**1.99** (1.81, 2.20)	**2.50** (2.21, 2.82)	0.010	**0.04** (0.04, 0.05)	**0.06** (0.05, 0.07)	**0.07** (0.06, 0.08)	<0.001
19F	**1.44** (1.26, 1.65)	**1.88** (1.70, 2.09)	**3.91** (3.48, 4.39)	<0.001	**0.11** (0.09, 0.13)	**0.24** (0.21, 0.27)	**0.29** (0.25, 0.33)	<0.001
23F	**1.44** (1.22, 1.70)	**1.80** (1.59, 2.04)	**2.23** (1.91, 2.59)	0.002	**0.05** (0.04, 0.06)	**0.08** (0.07, 0.09)	**0.10** (0.08, 0.11)	<0.001
Aggregated serotypes	**2.02** (1.90, 2.15)	**2.35** (2.24, 2.46)	**3.28** (3.10, 3.46)	<0.001	**0.05** (0.05, 0.06)	**0.08** (0.07, 0.08)	**0.12** (0.11, 0.13)	<0.001

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).
[a] Samples used from each population differ from those reported for NCKP [13], South African [14] and American Indian studies [15].
[b] Based on one-way ANOVA using log-transformed antibody concentrations with study population as the classification variable.

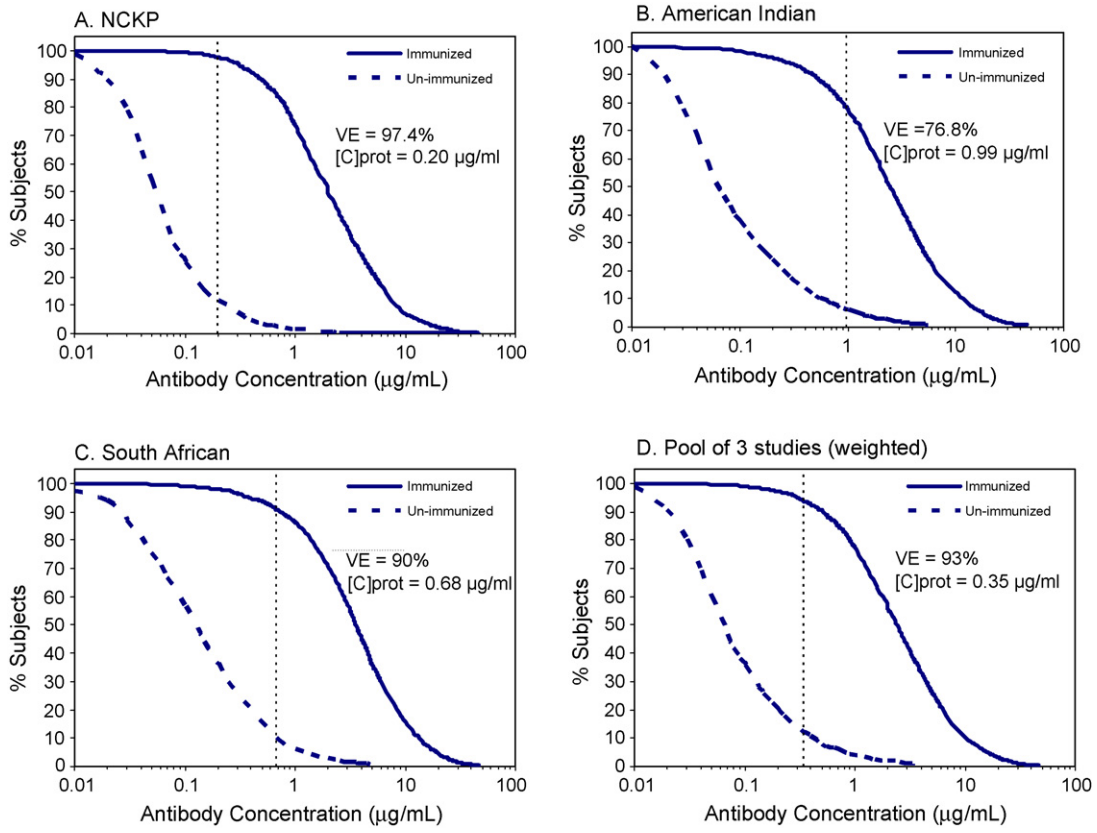

Fig. 2. Reverse cumulative distribution curves of IgG anti-pneumococcal capsular polysaccharide antibody concentrations aggregated for the 7 vaccine types in three controlled PnC efficacy studies and the pooled studies weighted for number of study subjects.

The reverse cumulative distribution curves (RCDC) of the aggregated antibody concentrations for all 7 Prevenar serotypes for each of the three populations are summarized in Fig. 2 (Panels A, B & C) and the pooled population, in Panel D.

3.2. Estimated Protective Pneumococcal Antibody Concentrations

Using serologic results from the single absorption ELISA and applying the step function model described in Methods to the RCDC curves of each population, the concentration which corresponds to the observed VE in each trial was determined (Table 4). The estimated $[C]_{prot}$ was lowest for NCKP at 0.20 μg/ml, higher for South Africa (0.68 μg/ml) and highest for American Indians (1.0 μg/ml.

In addition, pooled estimates are given, determined from the pooled efficacy (93%), either by simple pooling of all available antibody measurements or by weighted pooling in which the available antibody measurements were weighted according to the number of infants in each study. The $[C]_{prot}$ estimate was 0.35 μg/ml whether or not antibody concentrations were weighted. (Table 4) As a check on these pooling procedures, the method of weighting the $[C]_{prot}$ from each trial according to its variability yields a very similar estimate of 0.38 μg/ml indicating that the estimate is robust.

Table 4
Estimated protective antibody concentration to 7 vaccine type pneumococcal capsular polysaccharides

Study	VE observed	Estimated $[C]_{prot}$ (μg/ml, 95% CI)	
NCKP	97.4%	**0.20**	(0.03, 0.67)
American Indian	76.8%	**1.00**	(0.25 > 50.00)
South Africa	90%	**0.68**	(0.03, 6.00)
Pooled (unweighted)	93%	**0.35**	(0.09. 0.89)
Pooled (weighted)	93%	**0.35**	(0.11, 0.85)

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).

3.3. Effects of double absorption on antibody concentrations and estimated protective antibody concentrations based on NCKP Study

Table 5 summarizes the change in IgG anticapsular antibody concentrations with double absorption (C-Ps and 22F) as compared to single absorption ELISA (C-Ps only) using 368 sera from the NCKP study.

In 7vPnC vaccine immunized infants at 7 months, GMC antibody declines were ≤5% for 5 of the serotypes which are not statistically significant. The decline for 19F was 11% and for 23F, 10% which are statistically significant. Control

Table 5
Comparison of GMC IgG antibodies to 7 vaccine type capsular polysaccharides with and without 22F Absorption in infants immunized with Prevenar

Serotype	Randomized treatment groups[a]	GMC Antibody Concentration (μg/ml)				Ratio (95%CI) of Antibody concentrations with/without 22F absorption	
		With 22F absorption		Without 22F absorption			
4	7vPnC	**0.92**	(0.81, 1.04)	**0.94**	(0.83, 1.05)	**0.98**	(0.96, 1.01)
	MnCC	**0.01**	(0.01, 0.01)	**0.03**	(0.02, 0.03)	**0.43**	(0.38, 0.48)
6B	7vPnC	**2.75**	(2.21, 3.41)	**2.90**	(2.40, 3.50)	**0.95**	(0.90, 1.00)
	MnCC	**0.06**	(0.06, 0.07)	**0.15**	(0.13, 0.17)	**0.44**	(0.40, 0.49)
9V	7vPnC	**1.08**	(0.97, 1.20)	**1.11**	(1.01, 1.23)	**0.97**	(0.95, 0.99)
	MnCC	**0.05**	(0.05, 0.06)	**0.09**	(0.08, 0.11)	**0.59**	(0.54, 0.63)
14	7vPnC	**4.54**	(3.98, 5.18)	**4.48**	(3.94, 5.10)	**1.01**	(0.98, 1.05)
	MnCC	**0.03**	(0.02, 0.04)	**0.03**	(0.03, 0.04)	**0.85**	(0.77, 0.95)
18C	7vPnC	**1.23**	(1.10, 1.39)	**1.27**	(1.13, 1.43)	**0.97**	(0.94, 1.01)
	MnCC	**0.02**	(0.02, 0.03)	**0.05**	(0.05, 0.06)	**0.44**	(0.39, 0.50)
19F	7vPnC	**1.18**	(1.04, 1.34)	**1.33**	(1.18, 1.49)	**0.89**	(0.85, 0.93)
	MnCC	**0.05**	(0.05, 0.06)	**0.13**	(0.11, 0.15)	**0.43**	(0.39, 0.48)
23F	7vPnC	**1.12**	(0.906, 1.31)	**1.25**	(1.08, 1.44)	**0.90**	(0.86, 0.94)
	MnCC	**0.03**	(0.03, 0.04)	**0.07**	(0.06, 0.08)	**0.50**	(0.45, 0.55)
Aggregated serotypes	7vPnC	**1.54**	(1.44, 1.63)	**1.61**	(1.52, 1.71)	**0.95**	(0.94, 0.97)
	MnCC	**0.03**	(0.03, 0.04)	**0.07**	(0.06, 0.07)	**0.51**	(0.49–0.53)

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).
[a] The number of subjects in the 7vPnC group was 188, and in the control group was 180.

subjects had very low anticapsular antibody concentrations which showed significant declines after 22F absorption ranging from 15 to 57% (Table 5).

The proportion of infants responding to ≥0.35 μg/ml was also not changed significantly for 5 of the 7 types and was reduced by 2.7% for 19F ($p < 0.05$) and 5.3% for 23F ($p < 0.01$) (Table 6). Very few control infants had antibody levels ≥0.35 μg/ml.

The RCDC of the antibody concentrations to all 7 types combined, with and without 22F absorption, are shown in Fig. 3. Using the single absorbed RCDC (−22F), the estimated $[C]_{prot}$ of the NCKP population corresponding to the 93% pooled efficacy observed in the three controlled trials is 0.35 μg/ml. This value is identical to the value obtained by the WHO committee using the pooled RCDCs from all 3 trial populations (Fig. 2, Panel D). This observation together with the similar effect of 22F absorption in the 3 populations (Section 3.4) supports the use of the RCDC from the NCKP infants to assess the impact of 22F absorption on the $[C]_{prot}$. Using the double absorbed RCDC curves (+22F, Fig. 3), the $[C]_{prot}$ corresponding to the 93% pooled efficacy observed in the three trials was determined to be 0.32 μg/ml. Thus the impact of 22F absorption on the estimate of protective concentration is small.

3.4. Effect of 22F absorption in American Indian and South African infants

The effect of 22F absorption in American Indian infants was assessed in parallel assays in 76 Prevenar recipients and 86 controls but the new sample differed from the original sera assayed because of insufficient available volumes. The new sample of Prevenar immunized infants had higher IgG ELISA antibody concentrations without 22F absorption

Table 6
Comparison of percent of subjects achieving IgG antibody concentration ≥0.35 μg/mL to 7 vaccine type capsular polysaccharides with and without 22F absorption in infants immunized with Prevenar

Serotype	% responding at ≥0.35 μg/ml (95% CI)				Difference (%)	*p*-value[b]
	With 22 F absorption[a]		Without 22F absorption[a]			
4	**88.3**	(82.8, 92.5)	**87.8**	(82.2, 92.1)	0.5	0.564
6B	**89.4**	(84.0, 93.4)	**91.5**	(86.5, 95.1)	−2.1	0.102
9V	**95.2**	(91.1, 97.8)	**95.7**	(91.8, 98.1)	−0.5	0.564
14	**97.9**	(94.6, 99.4)	**98.4**	(95.4, 99.7)	−0.5	0.317
18C	**93.6**	(89.1, 96.7)	**94.7**	(90.4, 97.4)	−1.1	0.317
19F	**92.0**	(87.2, 95.5)	**94.7**	(90.4, 97.4)	−2.7	0.025
23F	**85.1**	(79.2, 89.9)	**90.4**	(85.3, 94.2)	−5.3	0.002

The bold number is Geometric Mean followed by 95% CI (GM is bold, 95% CI).
[a] Exact 2-sided confidence interval based upon the observed proportion of subjects.
[b] *p*-value computed using the Exact McNemar Test for paired samples.

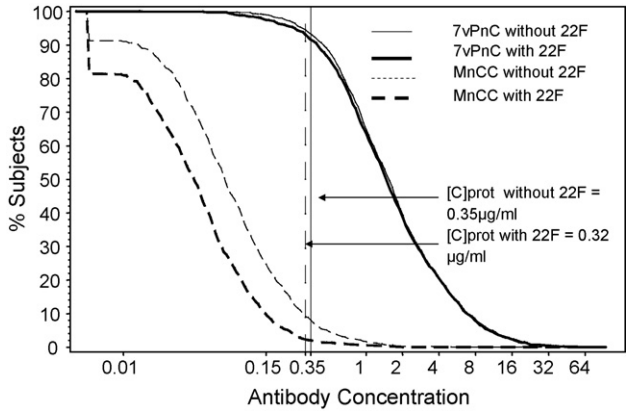

Fig. 3. RCDC of IgG pneumococcal capsular polysaccharide antibodies aggregated for 7 vaccine types with and without type 22F absorption in NCKP infants.

(GMC: 2.97 μg/ml) for the pooled serotypes compared to 2.35 μg/ml in the original WHO sample (Table 3). The controls were similar for the new sample: 0.09 μg/ml versus 0.08 μg/ml in the WHO sample. After 22F absorption of the new post-immunization samples the pooled GMC declined from 2.97 μg/ml to 2.84 μg/ml (ratio 0.96, $p < .01$) and of controls from 0.09 μg/ml to 0.06 μg/ml (ratio 0.63 $p < .001$). These declines are of similar magnitude as in NCKP infants who had ratios of 0.95 and 0.51 in immunized and unimmunized infants, respectively. (Table 5)

When the protective concentration was calculated from the new American Indian sample, it increased from 1.00 μg/ml with the original sample utilized by WHO to 1.41 μg/ml with the new sample when the new samples were assayed without 22F absorption. When the new samples were absorbed with 22F, the protective estimate declined from 1.41 μg/ml to 1.37 μg/ml (2.8%) a proportion even less than the decline from 0.35 μg/ml to 0.32 μg/ml (8.6%) observed in the NCKP population. (Fig. 3)

The effect of 22F absorption in South African infants could be evaluated in only 19 infants (7 Prevenar recipients and 12 controls) from whom sera were still available. The pooled GMC for all 7 serotypes declined from 3.97 μg/ml to 3.71 μg/ml (ratio 0.93, NS) in Prevenar immunized infants and from 0.13 μg/ml to 0.07 μg/ml (ratio: 0.52, $p < 0.1$) in controls. Again, the effect of 22F absorption in South African infants was similar to the effects observed in NCKP and American Indian infants.

4. Discussion

Once an effective vaccine has been licensed and placed into widespread use, it is no longer feasible or ethical to perform additional controlled clinical studies to demonstrate the efficacy of a new vaccine against the same pathogen in the age group for which the vaccine is recommended. Immunologic correlates of protection then become critical for predicting the efficacy of new vaccines.

For most vaccines, protective activity is mediated either exclusively or primarily by antibodies, and the correlate of protection is thus a specified concentration of antibody estimated to confer protection in an immunized population. An ideal protective correlate should have a number of characteristics. First it should measure the protective activity directly (e.g. antitoxin, bacterial killing or virus neutralization) or alternatively measure binding antibodies which are correlated with functional protective activity.

Second, it should be a well standardized, validated, reproducible and preferably inexpensive assay which can be conveniently applied to the large numbers of patients that must be studied during clinical development of vaccines and subsequently during post-marketing studies.

Third, the chosen protective concentration should be directly linked to clinical protection observed in controlled efficacy trials. In determining the protective level, it is important to avoid choosing concentrations that are either too high or too low. If the chosen correlate is too high, then new vaccines may be rejected for inadequate efficacy even though they would be highly protective. If the protective correlate is set inappropriately low, then new vaccines may meet this benchmark and yet have lower antibody responses and lower efficacy than the vaccine which was shown to be efficacious in clinical trials.

In this paper we present the antibody concentrations and statistical methods used by a WHO Working Group to recommend the protective concentration of IgG class pneumococcal capsular polysaccharide antibodies measured by ELISA. In addition we present the impact of a significant modification of the IgG ELISA assay, absorption of test sera with type 22F polysaccharide, on antibody concentrations and on the estimated protective concentration.Absorption with type 22F renders the assay more specific for capsular polysaccharide antibodies which are the only known functional and protective antibodies induced by pneumococcal polysaccharide and polysaccharide conjugate vaccines.

WHO conducted an initial consultation in Alaska in 2002, summarized by Jodar et al. [3] and developed recommendations at a second consultation in Geneva in 2003 which were issued in 2005 [4]. The theoretical basis for estimating the protective concentration was to relate the observed antibody concentrations in the populations studied in controlled efficacy trials to the observed point estimates of vaccine efficacy against invasive pneumococcal disease (see Section 2 for details). At the time of the WHO consultations, three controlled efficacy trials had been conducted with PnC vaccines using IPD as a primary outcome. The first and largest trial of 7vPnC vaccine at NCKP, on which licensure of this vaccine in the US was based, had the highest VE (97.3%) and the lowest estimated $[C]_{prot}$ (0.20 μg/ml). The committee made the decision not to base the protective concentration solely on the NCKP trial. Rather, the committee chose a more conservative approach, pooling the NCKP study with the two additional controlled studies then available which had been performed in American Indian and South African infants.

These additional trials showed vaccine efficacies of 76.8% and 90% respectively for the 7 vaccine types in Prevenar and protective concentrations of 1.0 and 0.68 μg/ml respectively. (See Tables 1 and 3)

The committee deemed the pooled efficacy of 93% obtained by combining these trials more representative of the likely efficacy of the vaccine globally. The pooled efficacy estimate is also very close to the intent-to-treat efficacy of 93.9% observed in NCKP Study [6]. The estimated protective antibody concentration from the pooled studies was 0.35 μg/ml. As described in Section 3, this estimate was robust, regardless of whether the measured antibody concentrations were simply pooled or whether they were weighted according to the numbers of patients in each study.

A subsequent controlled study of 9 valent PnC vaccine performed in The Gambia [16], showed a vaccine efficacy point estimate of 86% for IPD. In the per protocol analysis, 28 cases of IPD caused by the 7 serotypes in Prevenar were observed in controls and 4, in the 9vPnC group. The protective antibody estimate in Gambian infants was 1.20 μg/ml measured by ELISA with 22F absorption. These results suggest that the three groups who are at higher risk for pneumococcal diseases may require higher antibody levels to achieve an equivalent protective efficacy than Californian infants. However, even when the three studies in high-risk groups are pooled, the 95% confidence intervals of the IPD VE (85%, Cl: 66, 94) overlap with those of the NCKP (97.4%, Cl: 85, 100). Possible reasons for lower vaccine efficacy in these populations include earlier exposures to pneumococci, more frequent exposure to multiple serotypes shown by more rapid acquisition of pneumococcal carriage in South African, Gambian and American Indian infants, exposure to larger inoculum sizes and the presence of concurrent infections such as endemic malaria or respiratory infections. It is also possible that differences in host factors such as innate immune defenses, functional activity of their antibodies or nutrition play a role. Finally, the severity of the IPD outcomes was greater in the American Indian and South African studies than at NCKP where a large proportion had occult pneumococcal bacteremia without a focus. In any case, lowering the estimate of protective concentration for purposes of assessing vaccine efficacy below 0.35 μg/ml would risk the licensure of vaccines which have lower efficacy in populations with diverse risk factors.

An important simplifying assumption that is required to estimate a single protective level applicable to all serotypes is that the concentration of antibody required for protection is in fact similar for all types. This assumption is necessary because the rates of IPD are low, resulting in estimates of individual serotype efficacy with wide confidence intervals or with point estimates of 100%, which preclude the calculation of protective concentrations. Several observations provide some assurance that this assumption is reasonable. The variation of GMC antibody concentrations to the 7 vaccine types after immunization is relatively narrow, ranging over a 2 to 3 fold range in the three studies (Table 3). The pooled serotype specific efficacies in the three studies for the 7 vaccine types are also quite similar (Table 2) suggesting that the observed differences in immunogenicity among the seven vaccine serotypes do not lead to major differences in protective activity. Post-marketing studies in the US ABC surveillance system have yielded the most precise serotype specific effectiveness data available to date [17]. The post-marketing effectiveness differ from per protocol efficacy in the controlled studies in that children who received only one or more doses of PnC on a variety of schedules were included, which would tend to reduce vaccine effectiveness, and indirect herd immune effects occur, tending to increase effectiveness. Nevertheless, these data confirm that protective activity is similar across all 7 serotypes in the vaccine (Table 2, last column). For each of the 7 serotypes, 90% of individuals with >0.35 μg/ml of IgG antibody have evidence of opsonophagocytic activity in a validated functional assay (3 and unpublished data, Wyeth).

After the WHO Working Group defined the protective pneumococcal antibody concentration of 0.35 μg/ml, a further improvement in the specificity of the IgG ELISA was introduced and has now been widely accepted [9]. The original protocol absorbed non-specific antibodies binding to the type specific polysaccharides used for coating ELISA plates with C-Ps (single absorption). Conception and Frasch found that the use of a second absorbent, type 22F pneumococcal polysaccharide (double absorption), further reduced non-specific antibody binding which still occurred, particularly with sera from adults. [9]

We utilized sera from the pivotal NCKP efficacy trial to directly compare anti-pneumococcal antibodies in the Prevnar immunized and control infants at 7 months. Double absorption induced negligible additional declines in sera from Prevenar immunized infants and somewhat greater reductions in the unimmunized controls (Table 6, Fig. 3). The difference between immunized and control groups may be explained by the fact that the high concentrations of pneumococcal antibody measured in the immunized group is mainly specific anti-capsular antibody induced by PnC vaccine, whereas the low concentrations of pneumococcal antibody in the control group represents either residual pneumococcal antibody acquired transplacentally from mothers or the responses of the infants themselves to natural pneumococcal exposures. These naturally induced antibodies in unimmunized individuals are more likely to contain antibodies to non-capsular pneumococcal antigens.

Using the single absorbed ELISA values and the RCDC from the NCKP study, the 0.35 μg/ml protective concentration established by the WHO working group predicts a VE of 92.7%, which is very close to the estimated pooled VE of 93% utilized by the WHO committee to determine this concentration originally. If double absorbed antibody values are used, the calculated protective concentration is slightly lower at 0.32 μg/ml. This decline is driven not by the slight change in antibody levels in immunized infants but by the larger declines in control infants. Both are used in the formula for calculating $[C]_{prot}$.

Recently Henckaerts et al. proposed that the protective concentration for assessing PnC vaccine efficacy be lowered from 0.35 μg/ml to 0.20 μg/ml when using their ELISA protocol [18]. They based this proposal on reductions in post- immunization antibodies measured in sera of infants immunized with a different PnC manufactured by Glaxo SmithKline utilizing a different protein carrier and different linkage chemistry. The ELISA assay showing the reduction in antibody after 22F absorption also differed in important respects from the WHO ELISA protocol including coating antigens, coating conditions, and absorption of the 89SF standard with 22F Ps. [12]

The data presented by Henckaerts et al. do not support lowering the protective pneumococcal antibody concentration because they did not follow a number of fundamental principles required for valid bridging of this estimate.

The most important principle is that the sera used for bridging must be from infants immunized with the vaccine for which efficacy was clinically demonstrated i.e, Prevenar. The sera evaluated by Henckaerts et al. which showed a decline after 22F absorption were from infants immunized with an 11-valent PnC manufactured by Glaxo SmithKline. The 11-valent vaccine is manufactured by different processes than Prevenar. In particular, a different linkage chemistry and a different protein carrier are used. It is well known that antibody responses induced by different conjugate vaccines may differ in concentrations and quality of antibody [19,20].

It has also been demonstrated that pneumococcal polysaccharide and conjugate vaccines can induce antibodies to C-Ps and protein contaminants [21–24]. Different manufacturing processes may result in different concentrations of these contaminating antigens in the final product. For example, periodate oxidation, a step used in the conjugation process for Prevenar, has been shown to reduce the immunogenicity of the immunodominant phosphorylcholine epitope on C-Ps [24–26]. Thus, pneumococcal antibodies induced by different conjugates may differ in the effects of absorption with C-Ps and 22F. Indeed the Henckaerts report showed that type 6B pneumococcal antibodies after Prevenar did not decline after type 22F absorption [18].

The second principle is that the study population for bridging should be the same or equivalent to the one in which clinical efficacy was demonstrated. Theoretically, different populations may vary in their exposure to pneumococci which may result in differences in their concentrations of antibodies to nonspecific pneumococcal antigens and thus show a greater reduction in anticapsular antibodies with 22F absorption than pertained in the NCKP study.

The third principle is that the ELISA assay used to assess the effect of 22F absorption must be the same as the assay used to establish the protective concentration (ie, the WHO ELISA protocol) [12]. Therefore the effect of an assay modification on protective anticapsular antibody concentrations should be studied by assessing the modification in the WHO method in parallel assays in a single laboratory where the presence or absence of 22F is the only variable. The comparison should include a sufficient number of sera from Prevenar immunized and control infants to give confidence in the result. These conditions were not met by Henckaerts et al.

The data on 22F absorption presented in this report follow the above principles for bridging the pneumococcal protective antibody concentration. Using the NCKP sera, the concentration declined from 0.35 μg/ml estimated by the WHO Working Group to 0.32 μg/ml after 22F absorption, providing confidence that this concentration is based on the measurement of antibodies specific for the capsular polysaccharide which confer protection. Although many of the original serum samples from the American Indian and South African studies were not available in sufficient volumes, we did assess the effect of 22F absorption on a modified serum set from these studies and showed only small declines in antibody concentration, similar to the declines observed in NCKP infants.

Several caveats should be kept in mind about the pneumococcal protective concentration described in this report. This concentration applies only to the prevention of IPD in children who resemble the trial populations. For example children with immuno-compromising conditions such a HIV may require higher antibody levels for protection. Infants with HIV have been shown to produce antibodies to pneumococci [27] and Hib [28] with lower functional activity per μg IgG than healthy infants.Also, circulating anticapsular antibody concentrations required for prevention of pneumonia, otitis media or colonization are likely to be higher [29,30] and have not been established.

Also the protective level is best applied to populations rather than to individuals [1]. Thus, if a high proportion of individuals in a population achieves anticapsular antibody concentration $\geq$ 0.35 μg/ml then we can predict a high level of protection for IPD in that population. In a particular individual the outcome of an encounter with the pneumococcus may vary depending on multiple host and pathogen factors, in addition to the circulating antibody level.

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