Prediction of Pneumococcal Conjugate Vaccine Effectiveness against Invasive Pneumococcal Disease Using Opsonophagocytic Activity and Antibody Concentrations Determined by Enzyme-Linked Immunosorbent Assay with 22F Adsorption[▽]

L. Schuerman,[1]* J. Wysocki,[2] J. C. Tejedor,[3] M. Knuf,[4,5] K.-H. Kim,[6] and J. Poolman[1]#

GlaxoSmithKline Biologicals, Wavre/Rixensart, Belgium[1]; University School of Medical Sciences & Regional Medical Centre for Mother and Child, Poznan, Poland[2]; Neonatology Service, Hospital de Mostoles, Madrid, Spain[3]; Zentrum für Kinder- und Jugendmedizin, Johannes Gutenberg-Universität, Mainz, Germany[4]; Children's Hospital, Dr. Horst Schmidt Klinik, Wiesbaden, Germany[5]; and Department of Pediatrics, College of Medicine, Ewha Womans University, Seoul, South Korea[6]

We compared the abilities of two serological readouts, antipolysaccharide IgG antibody concentrations and opsonophagocytic activity (OPA) titers, to predict the clinical effectiveness of the 7-valent pneumococcal conjugate vaccine (7vCRM) against invasive pneumococcal disease (IPD). We also assessed the accuracy of the previously established thresholds for GlaxoSmithKline's enzyme-linked immunosorbent assay with 22F adsorption (22F-ELISA) (≥0.2 μg/ml) and OPA assay (titer, ≥8) in predicting effectiveness. We showed that following a 3-dose 7vCRM primary vaccination, the serological response rates as determined using thresholds of ≥0.2 μg/ml IgG and an OPA titer of ≥8 corresponded well with overall effectiveness against IPD. In addition, the OPA assay seemed to better predict serotype-specific effectiveness than enzyme-linked immunoassay. Finally, when applied to post-dose-2 immune responses, both thresholds also corresponded well with the overall IPD effectiveness following a 2-dose 7vCRM primary vaccination. These results support the importance of the OPA assay in evaluating immune responses to pneumococcal conjugate vaccines.

Diseases caused by *Streptococcus pneumoniae* are an important public health problem worldwide, especially in young children and the elderly (43). Bacterial polysaccharides are T-cell-independent antigens that have little or no immunogenicity in children under 2 years of age. To enhance the immune response, pneumococcal vaccines for use in infants and young children require conjugation of the *Streptococcus pneumoniae* capsular polysaccharide to a carrier protein.

The first pneumococcal conjugate vaccine (PCV) to be licensed in children younger than 2 years of age was a 7-valent vaccine (7vCRM; Prevenar/Prevnar; Pfizer, Inc.). 7vCRM contains capsular polysaccharides from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, each conjugated to the nontoxic diphtheria CRM$_{197}$ protein. This vaccine was included in the childhood immunization program in the United States in 2000 (1) and has been implemented since then by many other countries. When administered according to a 3-dose primary schedule, 7vCRM is effective for the prevention of invasive pneumococcal disease (IPD) caused by the seven vaccine serotypes as well as by the vaccine-related serotype 6A (3, 41). In the United States and many other countries, 7vCRM is administered according to a 4-dose schedule (3-dose primary vaccina-

tion followed by a booster dose in the second year of life [3+1 schedule]) (1). However, in the United Kingdom and several other countries, 7vCRM was introduced according to a 3-dose schedule (2-dose primary vaccination followed by an early booster dose [2+1 schedule]) (6, 36).

Since the introduction of 7vCRM in 2000, new PCVs are licensed on the basis of immunological noninferiority compared to a licensed vaccine with demonstrated efficacy (14, 42, 44). Robust and standardized assays, whose results correlate with efficacy, are therefore needed for correct evaluation of the antipneumococcal immune responses. Currently, the World Health Organization (WHO) Expert Committee on Biological Standardization recommends measuring antipneumococcal IgG concentrations 4 weeks after a 3-dose primary vaccination using a reference enzyme-linked immunosorbent assay (ELISA) (45). They further state that the percentage of subjects reaching a reference antibody concentration as determined by ELISA should be used for statistical noninferiority comparisons between PCVs (44). An analysis of pooled data from three efficacy studies with 7vCRM and the related 9-valent (9vCRM) vaccines indicated that a threshold IgG antibody concentration of 0.35 μg/ml correlates with protection at a population level and was therefore recommended for comparing immune responses between different PCVs (44). In 2001, Concepcion and Frasch (5) described a new-generation ELISA that includes adsorption of the sera with serotype 22F heterologous polysaccharide in addition to adsorption to cell wall polysaccharide. This addition improves the specificity of the

* Corresponding author. Mailing address: GlaxoSmithKline Biologicals, Avenue Fleming 20 (W23), 1300 Wavre, Belgium. Phone: 32 10 85 4738. Fax: 32 10 85 3317. E-mail: lode.schuerman@gskbio.com.
# Present address: Crucell, Leiden, The Netherlands.

TABLE 1. Overview of clinical studies comparing 7vCRM and PHiD-CV

Study	Country	Vaccine groups[a]	Primary vaccination schedule	No. of infants in 7vCRM group	Reference
A	Finland, France, Poland (primary study 001)	PHiD-CV + DTPa-HBV-IPV/Hib[b] 7vCRM + DTPa-HBV-IPV/Hib	2-3-4 months	415	37
B	Germany, Poland, Spain (primary study 011)	PHiD-CV + DTPa-HBV-IPV + Hib-MenC-TT 7vCRM + DTPa-HBV-IPV + Hib-MenC-TT	2-4-6 months	390	46
C′	Poland (primary study 012)	PHiD-CV + DTPw-HBV/Hib + IPV 7vCRM + DTPw-HBV/Hib + IPV	2-4-6 months	103	2
C″	Philippines (primary study 012)	PHiD-CV + DTPw-HBV/Hib + OPV 7vCRM + DTPw-HBV/Hib + OPV	6-10-14 weeks	100	2
D	Korea (primary study 036)	PHiD-CV + Hib 7vCRM + Hib	2-4-6 months	129	18

[a] DTPa-HBV-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated poliovirus/*Haemophilus influenzae* type b vaccine; DTPw-HBV/Hib, diphtheria-tetanus-whole-cell pertussis-hepatitis B virus/*Haemophilus influenzae* type b vaccine; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; Hib-MenC, *Haemophilus influenzae* type b-*Neisseria meninigitis* group C conjugate vaccine; Hib, *Haemophilus influenzae* type b vaccine. All vaccines were manufactured by GSK Biologicals, Rixensart, Belgium, except 7vCRM (Pfizer, Inc.).
[b] In France, the second PCV dose was co-administered with DTPa-IPV/Hib.

ELISA and is therefore now widely used. We have shown that an IgG concentration of 0.35 μg/ml, as determined using the WHO reference ELISA without 22F adsorption (non-22F-ELISA), is equivalent to 0.2 μg/ml using GlaxoSmithKline (GSK) Biologicals' 22F-ELISA (12, 26).

However, ELISAs measure only anticapsular polysaccharide antibody concentrations and may not reflect the functional potential of these antibodies. The functionality of the vaccine-induced antibodies can be assessed by opsonophagocytic activity (OPA) assay, an alternative to ELISA. Indeed, the primary mechanism of protection against *S. pneumoniae* infections is antibody-induced opsonophagocytosis, which is known to correlate well with protection by pneumococcal vaccines (15, 40). OPA assays measure the ability of serum samples to opsonize pneumococci *in vitro* (28). A recent blinded multilaboratory study showed that different OPA assays give robust and reproducible results (30). The lowest serum dilution routinely used in OPA assays is 1:8. Detection of opsonic antibodies at this dilution had been proposed to be predictive of protection against disease (42, 44).

Which assay—ELISA or OPA—is most predictive of effectiveness remains to be determined. Therefore, in this study, we compared the abilities of the 22F-ELISA and OPA assays to predict 7vCRM clinical effectiveness against IPD. We also assessed the accuracy of the previously established thresholds for OPA (1:8 dilution) and GSK's 22F-ELISA (≥0.2 μg/ml) in predicting the clinical effectiveness of PCVs.

MATERIALS AND METHODS

Serum samples. Serum samples from infants who received 7vCRM (Prevenar/Prevnar; Pfizer, Inc., Pearl River, NY) were obtained from four randomized controlled studies comparing 7vCRM and the novel 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV; Synflorix; GSK Biologicals, Rixensart, Belgium) (Table 1) (2, 18, 37, 46). The serum samples from children vaccinated with PHiD-CV were not used in the present analysis. All studies were conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines, and protocols were reviewed by the appropriate ethics committees. Written informed consent was obtained from the parents or legal guardians of all children before enrolment. Serum samples were taken 4 weeks after the third vaccine dose and stored at −20°C until blinded analysis in GSK Biologicals' laboratories (Rixensart, Belgium). In addition, in a random subset of children in study B, serum samples were obtained 2 months after the second primary dose of 7vCRM (46).

Immunological assays. Serotype-specific IgG antibody concentrations were measured using GSK's 22F-ELISA as previously described (12). This assay includes a dual adsorption with cell wall polysaccharide and serotype 22F heterologous polysaccharides and it uses GSK's serotype-specific capsular polysaccharides as coating antigens. The assay cutoff was set at 0.05 μg/ml for all serotypes, which is greater than the limit of quantitation for each serotype.

GSK's OPA assay was adapted from the method originally described by Romero-Steiner et al. (29) and was performed as previously described (11). Briefly, the serotype-specific *S. pneumoniae* OPA was measured in serum samples after incubation with differentiated HL-60 cells (ATCC, Manassas, VA) and baby rabbit complement. Pneumococcal colonies were counted using an automated image analysis system (KS 400; Zeiss, Oberkochen, Germany). Serum samples were 2-fold serially diluted between 1:8 and 1:1,024. The OPA titer was defined as the reciprocal of the lowest serum dilution that induces ≥50% bacterial cell death compared to the control wells (i.e., with complement and without antibodies) (29). Positive control samples with known OPA titer for the specific pneumococcal serotype were included to validate each assay run. The assay cutoff is an OPA titer of 8 (serum dilution of 1:8).

Statistical methods. The percentages of serum samples with IgG concentrations of ≥0.2, ≥0.35, ≥0.5, and ≥1.0 μg/ml and the percentages of serum samples with OPA titers of ≥8, ≥16, ≥32, ≥64, and ≥128 1 month following three primary 7vCRM doses were calculated with 95% confidence intervals (95% CIs) for the according-to-protocol cohorts for immunogenicity. Aggregate reverse cumulative distribution curves of the antibody concentrations and OPA titers were plotted for the combined seven polysaccharides included in 7vCRM. Antibody concentrations and OPA titers below the assay cutoff were assigned an arbitrary value of half the assay cutoff (i.e., 0.025 μg/ml for 22F-ELISA and 4 for OPA assay) when drawing the reverse cumulative distribution curves.

RESULTS

Comparison of the ELISA and OPA assays at predicting vaccine effectiveness following three doses of 7vCRM. A previous study compared 7vCRM IPD effectiveness data from a U.S. postlicensure case-control study (41) with GSK's 22F-ELISA and OPA assays using 140 serum samples from German children receiving a 3-dose primary vaccination with 7vCRM (11). In the current study, we extended this analysis to a larger population by combining 7vCRM results from four clinical studies conducted in Europe and Asia that compared 7vCRM and PHiD-CV using different primary vaccination schedules (Table 1).

We first analyzed the distribution of antibody concentrations and OPA titers using aggregate reverse cumulative distribution curves combining the immune responses to the seven vac-
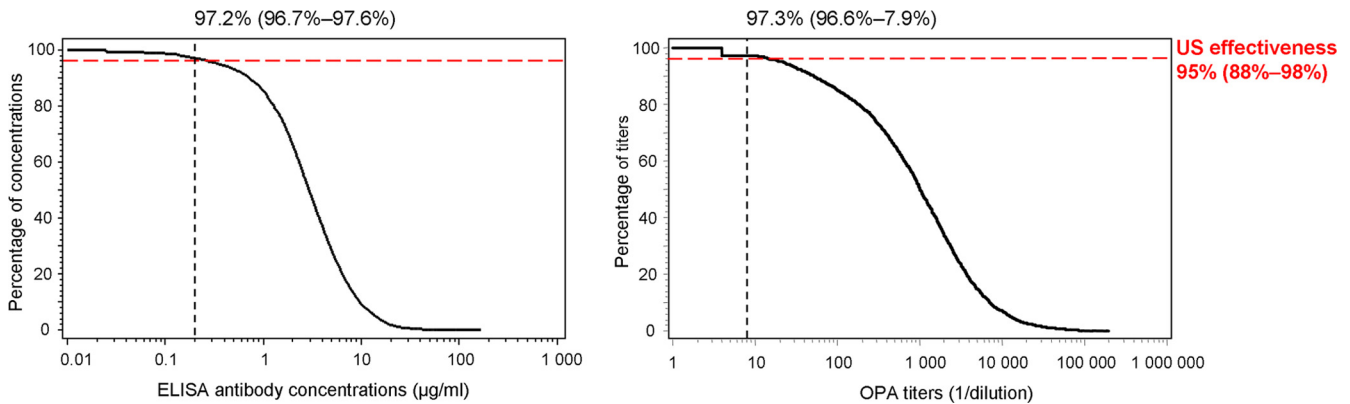
FIG. 1. Aggregate reverse cumulative distribution curves (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) of antipolysaccharide antibody concentrations (left) and osponophagocytic activity (right) following three doses of 7vCRM. Serum samples were obtained from studies A to D 4 weeks after a 3-dose primary series with 7vCRM (Table 1) (2, 18, 37, 46). The vertical dotted lines show the ELISA (≥0.2 μg/ml) and OPA (titer, ≥8) thresholds. The horizontal dotted lines show the effectiveness of 7vCRM against vaccine-serotype IPD (% [95% CI]) estimated in the United States (3+1 schedule) in a postlicensure study (41).

cine pneumococcal serotypes (Fig. 1). The percentage of antibody concentrations of ≥0.2 μg/ml (97.2%; 95% CI, 96.7% to 97.6%) and the percentage of OPA titers of ≥8 (97.3%; 95% CI, 96.6% to 97.9%) were both in line with the effectiveness of three primary doses of 7vCRM against vaccine-serotype IPD in American infants (95%; 95% CI, 88% to 98%) (41).

We next analyzed the 22F-ELISA and OPA response rates against the individual serotypes. For most serotypes, both the percentage of serum samples with an antibody concentration of ≥0.2 μg/ml (Table 2) and the percentage of serum samples with an OPA titer of ≥8 (Table 3) corresponded well with the U.S. effectiveness data. For serotype 4, both ELISA and OPA responses overestimated vaccine effectiveness. However, for some serotypes, there were differences between the results of the two assays. For serotype 6B and the cross-reactive serotype 6A, the ELISA response rate underestimated the vaccine effectiveness against IPD, whereas for serotype 19F, it overestimated vaccine effectiveness. In contrast, the OPA response rate more accurately reflected the observed serotype-specific

vaccine effectiveness values. The OPA response rate slightly overestimated vaccine effectiveness for serotype 19F but underestimated it for the cross-reactive serotype 19A.

To determine whether modification of the threshold for both assays would allow better prediction of protection, we compared the serotype-specific effectiveness of 7vCRM in the United States with the serological response rates determined using different thresholds. For three serotypes (4, 14, and 19F), there was a better correspondence between the serological response rate and vaccine effectiveness when using an ELISA threshold of ≥1.0 μg/ml instead of ≥0.2 μg/ml (Table 2). In contrast, for the other serotypes, the correspondence between response rate and vaccine effectiveness was not improved using an ELISA threshold of ≥1.0 μg/ml. For three vaccine serotypes (6B, 9V, and 23F) and for the cross-reactive serotypes 6A and 19A, the previously established 22F-ELISA threshold of 0.2 μg/ml corresponded best with the serotype-specific vaccine effectiveness in the United States.

For most vaccine serotypes and for the cross-reactive serotype 6A, the functional OPA response rates corresponded well

TABLE 2. Antibody concentrations above the indicated thresholds 1 month after a 3-dose 7vCRM primary vaccination[a]

Serotype	% Vaccine effectiveness (95% CI) in the U.S. (41)	% of subjects (95% CI) with ELISA-determined antibody concn of:			
		≥0.2 μg/ml	≥0.35 μg/ml	≥0.5 μg/ml	≥1.0 μg/ml
Vaccine serotypes					
4	93 (65–99)	100 (99.6–100)	99.5 (98.8–99.9)	99.4 (98.6–99.8)	93.7 (91.9–95.2)
6B	94 (77–98)	87.1 (84.7–89.3)	80.8 (78.0–83.4)	75.1 (72.0;78.0)	55.2 (51.8–58.6)
9V	100 (88–100)	99.4 (98.6–99.8)	98.6 (97.6–99.3)	97.7 (96.4–98.6)	93.6 (91.7–95.1)
14	94 (81–98)	99.6 (99.0–99.9)	98.1 (97.0–98.9)	96.7 (95.3–97.8)	93.2 (91.3–94.8)
18C	97 (85–99)	99.2 (98.3–99.7)	98.1 (97.0–98.9)	97.0 (95.6–98.0)	91.7 (89.7–93.5)
19F	87 (65–95)	99.4 (98.6–99.8)	98.5 (97.4–99.2)	98.1 (97.0–98.9)	94.5 (92.8–96.0)
23F	98 (80–100)	95.3 (93.7–96.6)	91.1 (89.0–92.9)	88.1 (85.7–90.2)	76.8 (73.8–79.6)
Vaccine-related serotypes					
6A	76 (39–90)	55.5 (51.3–59.6)	41.4 (37.3–45.5)	33.0 (29.1–37.0)	17.6 (14.6–21.0)
19A	26 (−45 to 62)	31.0 (27.2–35.0)	14.5 (11.7–17.6)	9.6 (7.3–12.3)	2.4 (1.3–4.1)

[a] Shown are percentages of subjects (and 95% CI) with antibody concentrations above the indicated thresholds 1 month after a 3-dose 7vCRM primary vaccination for the seven pneumococcal vaccine serotypes (n = 859) and the vaccine-related serotypes 6A and 19A (n = 574) compared with serotype-specific vaccine effectiveness calculated for 7vCRM in the United States. n = number of subjects with available results pooled from studies A to D (2, 18, 37, 46). The actual number of subjects can slightly vary for the different serotypes depending on the available sera.

TABLE 3. OPA activity above the indicated thresholds 1 month after a 3-dose 7vCRM primary vaccination[a]

Serotype	% Vaccine effectiveness (95% CI) in the U.S. (41)	% of subjects (95% CI) with OPA titer of:				
		≥8	≥16	≥32	≥64	≥128
Vaccine serotypes						
4	93 (65–99)	100 (99.1–100)	100 (99.1–100)	100 (99.1–100)	100 (99.1–100)	98.2 (96.4–99.3)
6B	94 (77–98)	95.9 (93.5–97.7)	95.9 (93.5–97.7)	95.7 (93.2–97.5)	95.2 (92.6–97.1)	94.7 (92.0–96.7)
9V	100 (88–100)	99.5 (98.2–99.9)	99.5 (98.2–99.9)	99.5 (98.2–99.9)	99.0 (97.4–99.7)	98.0 (96.0–99.1)
14	94 (81–98)	97.8 (95.8–99.0)	97.8 (95.8–99.0)	97.8 (95.8–99.0)	97.3 (95.1–98.6)	97.0 (94.8–98.4)
18C	97 (85–99)	98.2 (96.4–99.3)	97.5 (95.4–98.8)	93.9 (91.1–96.1)	85.0 (81.1–88.4)	67.8 (62.9–72.4)
19F	87 (65–95)	91.1 (87.8–93.7)	82.1 (78.0–85.8)	65.1 (60.1–69.8)	43.4 (38.4–48.4)	27.3 (22.9–32.0)
23F	98 (80–100)	98.7 (97.0–99.6)	98.7 (97.0–99.6)	98.7 (97.0–99.6)	98.7 (97.0–99.6)	98.7 (97.0–99.6)
Vaccine-related serotypes						
6A	76 (39–90)	80.0 (75.3–84.2)	80.0 (75.3–84.2)	79.1 (74.4–83.3)	74.6 (69.6–79.2)	67.2 (61.9–72.2)
19A	26 (−45 to 62)	3.5 (1.8–6.1)	2.9 (1.4–5.4)	2.1 (0.8–4.2)	1.5 (0.5–3.4)	0.9 (0.2–2.6)

[a] Shown are percentages of subjects (and 95% CI) with OPA titers above the indicated thresholds 1 month after a 3-dose 7vCRM primary vaccination for the seven pneumococcal vaccine serotypes (n = 401) and the vaccine-related serotypes 6A and 19A (n = 339) compared with serotype-specific vaccine effectiveness calculated for 7vCRM in the United States. n = number of subjects with available results pooled from studies A to D (2, 18, 37, 46). The actual number of subjects can slightly vary for the different serotypes depending on the available sera.

with vaccine effectiveness when using thresholds of ≥8 to ≥128 (Table 3). For serotypes 18C and 19F, increasing the OPA threshold (above ≥8 for 19F and above ≥16 for 18C) resulted in an underestimation of effectiveness. For the cross-reactive serotype 19A, regardless of the threshold used, the OPA response rate was very low following three doses of 7vCRM. This very low response rate could reflect the lack of statistically significant vaccine effectiveness estimated for serotype 19A with this vaccine.

Together, these results showed that following three doses of 7vCRM, both antibody and OPA responses aggregated for all seven serotypes contained in 7vCRM corresponded well with the overall clinical protection against IPD due to vaccine pneumococcal serotypes. However, an OPA titer of ≥8 was better than an ELISA-determined antibody concentration of ≥0.2 μg/ml at predicting the individual serotype-specific IPD effectiveness for 7vCRM as reported in the United States.

Comparison of the ELISA and OPA assays at predicting vaccine effectiveness following two doses of 7vCRM. We also compared immune responses following 2-dose priming with 7vCRM with vaccine effectiveness against vaccine-serotype IPD for children who received two doses of 7vCRM before 1 year of age. The percentages of antibody concentrations of ≥0.2 μg/ml (85.4%; 95% CI, 83.3% to 87.4%) for the combined seven vaccine serotypes corresponded well with the effectiveness of 7vCRM in the United Kingdom (87%; 95% CI, 71% to 94%) and Germany (89.8%; 95% CI, 20.6% to 100.0%) (Fig. 2) (8, 31). In contrast, the percentage of OPA titers of ≥8 (79.7%; 95% CI, 77.2% to 82.1%) slightly underestimated vaccine effectiveness in these countries and slightly overestimated the observed Norwegian effectiveness (74%; 95% CI, 57% to 85%) (39). Results from both assays corresponded poorly with divergent vaccine effectiveness estimated in Canada (99%; 95% CI, 90% to 100%) (7). Finally, we were unable to evaluate how serotype-specific antibody and OPA responses correspond
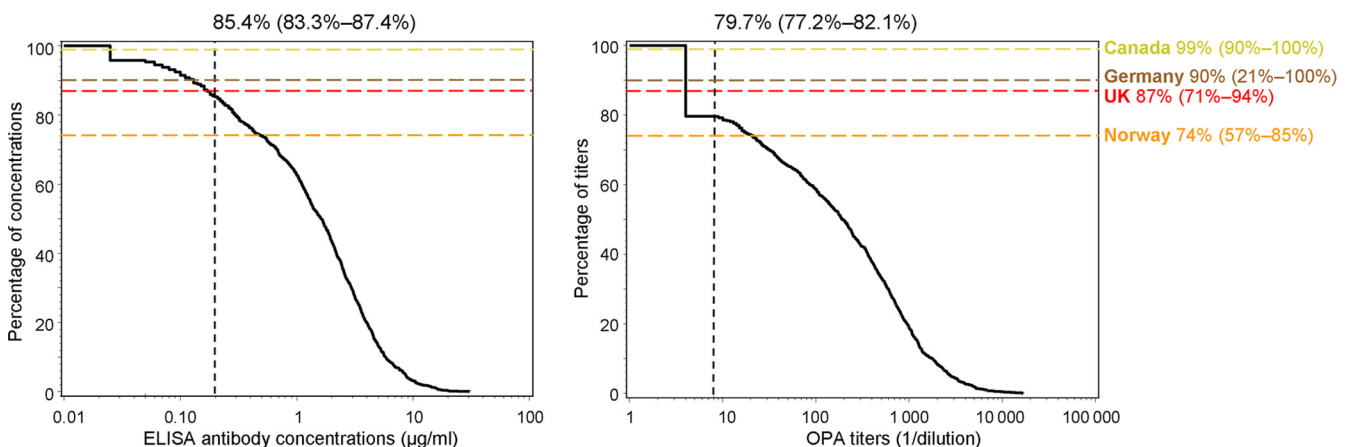

FIG. 2. Aggregate reverse cumulative distribution curves (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) of antipolysaccharide antibody concentrations (left) and osponophagocytic activity (right) following two doses of 7vCRM. Serum samples (n = 166 for the ELISA and n = 156 for the OPA assay) were obtained from a random subset of children two months after the second primary dose in study B (46). The vertical dotted lines show the ELISA (≥0.2 μg/ml) and OPA (titer, ≥8) thresholds. The horizontal dotted lines show the effectiveness of 7vCRM against vaccine-serotype IPD (% [95% CI]) estimated in various countries (2+1 schedule) in postlicensure studies (7, 8, 31, 39).

with effectiveness following 2-dose 7vCRM priming because data were not available.

DISCUSSION

Post-dose-3 serum response rates using both ELISA and OPA corresponded well with overall vaccine effectiveness after three doses of 7vCRM in the United States. However, using the previously established thresholds, OPA titers predicted vaccine effectiveness against the individual serotypes better than ELISA did. Our results also confirm previous reports that an IgG threshold of 0.2 μg/ml using GSK's 22F-ELISA is appropriate for predicting vaccine effectiveness (12, 26). Increasing the threshold as suggested by some (34, 42) would not improve the correspondence of the serological response rate with vaccine effectiveness but would, on the contrary, result in an underestimation of effectiveness for some serotypes.

After three primary doses of 7vCRM, both the serological response rates and vaccine effectiveness are high for most vaccine serotypes. Because the threshold crossed the reverse cumulative distribution curves in their upper "plateau," modification of the threshold would have only modest impact on the estimation of effectiveness. Immune responses measured following a 3-dose primary vaccination are therefore not very sensitive for predicting vaccine effectiveness. However, for those serotypes with lower efficacy (19F, 6B, or 6A), OPA is better than ELISA at predicting post-dose-3 vaccine effectiveness, and effectiveness is accurately predicted by an OPA threshold of ≥8.

Effectiveness and immunogenicity are generally lower after a 2-dose primary vaccination course and could therefore be useful in identifying a threshold to predict effectiveness. Previous studies indicated that 7vCRM administered in a 2+1 schedule effectively protects against vaccine-serotype IPD, with effectiveness values ranging from 74% to 99% (7, 8, 31, 38, 39). We excluded the most recent Norwegian estimate from our comparison because of a strong emergent herd effect that increased vaccine effectiveness against IPD from 74% in 2007 to 95% in 2008 (38, 39). We found here that post-dose-2 aggregate ELISA and OPA response rates corresponded well with vaccine effectiveness after two doses of 7vCRM in the United Kingdom and Germany (8, 31), although OPA results tended to underestimate overall effectiveness in these countries and to overestimate it in Norway (39). Also, our data suggest that the use of higher thresholds for both ELISA and OPA assays would result in an underestimation of effectiveness for most studies. We could not compare the serological response rates with serotype-specific effectiveness because, for most serotypes, the number of IPD cases currently reported are insufficient to accurately measure vaccine effectiveness in the context of a 2+1 vaccination schedule (7, 38). Therefore, whether post-dose-2 serological response rates are more sensitive than post-dose-3 measurements at predicting serotype-specific vaccine effectiveness remains to be determined.

For the individual serotypes, the OPA response rates following three doses of 7vCRM reflected the observed effectiveness better than the ELISA response rates. This is not surprising because the OPA assay measures the functionality of the produced antibodies, whereas ELISA measures only their concentration irrespective of their biological activity. The OPA

results also suggested that the relationship between the antibody concentration and the clinical protection differs according to the serotype. Similar conclusions were drawn by Madhi et al. (20). This implies that, for antibody responses, the same threshold may not be relevant for all pneumococcal serotypes. In particular, whereas antibody concentrations below 0.35 μg/ml (or 0.2 μg/ml for GSK's 22F-ELISA) might be sufficient to provide protection against IPD caused by serotype 6B, higher concentrations might be needed for serotypes 19F and 19A. In addition, comparing the aggregated immunogenicity results and the overall effectiveness against IPD is complicated by differences in the relative proportions of the vaccine serotypes. Indeed, although the seven vaccine serotypes are equally represented in the aggregated immunogenicity data, vaccine effectiveness for serotypes that more frequently cause IPD may have a larger impact on the overall effectiveness estimates.

The OPA response rate to serotype 19F was lower than the ELISA response rate. This suggests that functional activity against serotype 19F could not be measured in some sera with antibody concentrations of ≥0.2 μg/ml and, therefore, that some antibodies induced by 7vCRM against this serotype are poorly functional. This production of nonfunctional antibodies could be due to the conjugation method used to link the 19F polysaccharides to the CRM$_{197}$ carrier protein in 7vCRM (25). For the cross-reactive serotype 19A, only 31% of samples had antibody concentrations of ≥0.2 μg/ml, and few sera had measurable OPA titers, which reflects the poor effectiveness of the 7vCRM vaccine against serotype 19A IPD in the United States. Indeed, despite an effectiveness of 26% (95% CI, −45% to 62%) as estimated by U.S. Centers for Disease Control and Prevention (41), the vaccine was not able to prevent the increase in 19A IPD (16, 21, 24). Previous studies by Pfizer, Inc. showed that over 80% of subjects vaccinated with three doses of 7vCRM had antibody concentrations against serotype 19A that reached the antibody threshold, whereas only approximately 17% of the subjects had functional antibodies when measured using the OPA assay (4, 17, 47). These and our results support the conclusion that a large fraction of the cross-reactive antibodies induced by 7vCRM against serotype 19A are nonfunctional. It also suggests a higher specificity of GSK's 19A-ELISA. A similar conclusion was drawn when GSK's 22F-ELISA was compared to the WHO reference ELISA (26). A recent review of the literature indicated that 7vCRM might provide some cross-protection against 19A but that this may be masked in the field by other confounding factors such as clonal expansion of an antibiotic-resistant 19A strain and no effect of 7vCRM on 19A nasopharyngeal carriage (10). Our hypothesis is that the modest efficacy of 7vCRM against serotype 19A was not sufficient to prevent the emerging wave of 19A disease observed in the United States (10, 13, 21, 23, 35). Also, our results suggest that the OPA assay predicts vaccine effectiveness against serotype 19A better than 22F-ELISA does.

The results of this study should be interpreted in light of certain limitations. First, some estimates of serotype-specific vaccine effectiveness in the United States, such as 19F and 19A, have wide confidence intervals. This limits the robustness of the comparison of vaccine effectiveness with the serological ELISA or OPA response rates. Second, the immune responses to PCVs vary across different populations and geographical areas (19, 22, 32, 33), so the ELISA and OPA results from the

five countries included in this study may not be representative of other populations or countries, especially when they are compared to vaccine effectiveness generated elsewhere. However, because of insufficient data, we could not assess the correspondence between the serological response rates and vaccine effectiveness for each country. Third, immune responses as measured by ELISA and OPA do not take indirect vaccine effects into account, in contrast to effectiveness estimates. However, the United States postlicensure effectiveness data were derived using a case-control study design that should measure only the direct effects of vaccination (27, 41).

Together, our analysis showed that the OPA assay provides a good estimate of antibody functionality and that it is better than ELISA at estimating vaccine effectiveness after three doses of 7vCRM. Our analysis also suggests that the current thresholds for both OPA (titer, ≥ 8) and GSK's 22F-ELISA (≥ 0.2 µg/ml) accurately predict PCV effectiveness at the population level. This implies that these thresholds can be used in the previously described algorithm that attempts to predict the overall impact of PCVs on IPD (9). Furthermore, we showed that both thresholds also correspond well with overall IPD effectiveness when applied to immune responses after a 2-dose primary vaccination with 7vCRM and that the use of higher thresholds would underestimate post-dose-2 effectiveness. This supports the importance of the OPA assay in evaluating new PCVs in the context of both 3+1 and 2+1 vaccination schedules.

REFERENCES

1. **ACIP.** 2000. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. **49:**1–35.
2. **Bermal, N., et al.** 2009. The 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) co-administered with DTPw-HBV/Hib and poliovirus vaccines: assessment of immunogenicity. Pediatr. Infect. Dis. J. **28:**S89–S96.
3. **Black, S., et al.** 2000. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr. Infect. Dis. J. **19:**187–195.
4. **Bryant, K. A., S. L. Block, S. A. Baker, W. C. Gruber, and D. A. Scott.** 2010. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine. Pediatrics **125:**866–875.
5. **Concepcion, N. F., and C. E. Frasch.** 2001. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol. **8:**266–272.
6. **De Carvalho Gomes, H., M. Muscat, D. L. Monnet, J. Giesecke, and P. L. Lopalco.** 2009. Use of seven-valent pneumococcal conjugate vaccine (PCV7) in Europe, 2001–2007. Euro Surveill. **14**(12):pii=19159.
7. **Deceuninck, G., P. De Wals, N. Boulianne, and G. De Serres.** 2010. Effectiveness of pneumococcal conjugate vaccine using a 2+1 infant schedule in Quebec, Canada. Pediatr. Infect. Dis. J. **29:**546–549.
8. **Goldblatt, D., et al.** 2010. Immunogenicity of a reduced schedule of pneumococcal conjugate vaccine in healthy infants and correlates of protection for serotype 6B in the United Kingdom. Pediatr. Infect. Dis. J. **29:**401–405.
9. **Hausdorff, W. P., R. Dagan, F. Beckers, and L. Schuerman.** 2009. Estimating the direct impact of new conjugate vaccines against invasive pneumococcal disease. Vaccine **27:**7257–7269.
10. **Hausdorff, W. P., B. Hoet, and L. Schuerman.** 2010. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? BMC Pediatr. **10:**4.
11. **Henckaerts, I., N. Durant, D. De Grave, L. Schuerman, and J. Poolman.** 2007. Validation of a routine opsonophagocytosis assay to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. Vaccine **25:**2518–2527.
12. **Henckaerts, I., D. Goldblatt, L. Ashton, and J. Poolman.** 2006. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. Clin. Vaccine Immunol. **13:**356–360.
13. **Hicks, L. A., et al.** 2007. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. J. Infect. Dis. **196:**1346–1354.
14. **Jodar, L., et al.** 2003. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine **21:**3265–3272.
15. **Johnson, S. E., et al.** 1999. Correlation of opsonophagocytosis and passive protection assays using human anticapsular antibodies in an infant mouse model of bacteremia for Streptococcus pneumoniae. J. Infect. Dis. **180:**133–140.
16. **Kaplan, S. L., et al.** 2010. Serotype 19A is the most common serotype causing invasive pneumococcal infections in children. Pediatrics **125:**429–436.
17. **Kieninger, D. M., et al.** 2010. Safety, tolerability, and immunologic noninferiority of a 13-valent pneumococcal conjugate vaccine compared to a 7-valent pneumococcal conjugate vaccine given with routine pediatric vaccinations in Germany. Vaccine **28:**4192–4203.
18. **Kim, K.-H., et al.** 2010. Immunogenicity of primary vaccination with the 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Korean infants, abstr. 472. Seventh Int. Symp. Pneumococci and Pneumococcal Diseases (ISPPD), Tel Aviv, Israel.
19. **Klugman, K. P., et al.** 2008. Meta-analysis of the efficacy of conjugate vaccines against invasive pneumococcal disease, p. 317–326. *In* G. R. Siber, K. P. Klugman, and P. H. Makela (ed.), Pneumococcal vaccines: the impact of conjugate vaccine, ASM Press, Washington, DC.
20. **Madhi, S. A., et al.** 2005. Quantitative and qualitative antibody response to pneumococcal conjugate vaccine among African human immunodeficiency virus-infected and uninfected children. Pediatr. Infect. Dis. J. **24:**410–416.
21. **Moore, M. R., et al.** 2008. Population snapshot of emergent Streptococcus pneumoniae serotype 19A in the United States, 2005. J. Infect. Dis. **197:**1016–1027.
22. **Pavia, M., A. Bianco, C. G. Nobile, P. Marinelli, and I. F. Angelillo.** 2009. Efficacy of pneumococcal vaccination in children younger than 24 months: a meta-analysis. Pediatrics **123:**e1103–e1110.
23. **Pelton, S. I., et al.** 2007. Emergence of 19A as virulent and multidrug resistant Pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. Pediatr. Infect. Dis. J. **26:**468–472.
24. **Pichichero, M. E., and J. R. Casey.** 2007. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. JAMA **298:**1772–1778.
25. **Poolman, J., et al.** 2011. Impact of the conjugation method on the immunogenicity of *Streptococcus pneumoniae* 19F polysaccharide in conjugate vaccines. Clin. Vaccine Immunol. **18:**327–336.
26. **Poolman, J. T., et al.** 2010. Evaluation of pneumococcal polysaccharide immunoassays using a 22F adsorption step with serum samples from infants vaccinated with conjugate vaccines. Clin. Vaccine Immunol. **17:**134–142.
27. **Rodrigues, L. C., and P. G. Smith.** 1999. Use of the case-control approach in vaccine evaluation: efficacy and adverse effects. Epidemiol. Rev. **21:**56–72.
28. **Romero-Steiner, S., et al.** 2006. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. Clin. Vaccine Immunol. **13:**165–169.
29. **Romero-Steiner, S., et al.** 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin. Diagn. Lab. Immunol. **4:**415–422.
30. **Rose, C. E., et al.** 2011. Multilaboratory comparison of *Streptococcus pneumoniae* opsonophagocytic killing assays and their level of agreement for the determination of functional antibody activity in human reference sera. Clin. Vaccine Immunol. **18:**135–142.
31. **Rückinger, S., M. van der Linden, R. R. Reinert, and R. von Kries.** 2010. Efficacy of 7-valent pneumococcal conjugate vaccination in Germany: an analysis using the indirect cohort method. Vaccine **28:**5012–5016.

32. **Schuerman, L., et al.** 2010. Population variability in antibody responses following pneumococcal conjugate vaccination: experience with the non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV), Abstr. 523. Seventh Int. Symp. Pneumococci and Pneumococcal Diseases (ISPPD), Tel Aviv, Israel, 14 to 18 March 2010.

33. **Schuerman, L., et al.** 2010. Population variability of opsonophagocytic activity following 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) vaccination more limited than antibody Responses, Abstr. 517. Seventh Int. Symp. Pneumococci and Pneumococcal Diseases (ISPPD). Tel Aviv, Israel, 14 to 18 March 2010.

34. **Siber, G. R., et al.** 2007. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. Vaccine **25:**3816–3826.

35. **Singleton, R. J., et al.** 2007. Invasive pneumococcal disease caused by non-vaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. JAMA **297:**1784–1792.

36. **United Kingdom Department of Health.** 2007. Immunisation against infectious disease—'The Green Book'—2006 updated edition. http://www.dh.gov.uk/en/Publichealth/Healthprotection/Immunisation/Greenbook/DH_4097254.

37. **Vesikari, T., et al.** 2009. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. Pediatr. Infect. Dis. J. **28:**S66–76.

38. **Vestrheim, D. F., E. A. Hoiby, I. S. Aaberge, and D. A. Caugant.** 2010. Impact of a pneumococcal conjugate vaccination program on carriage among children in Norway. Clin. Vaccine Immunol. **17:**325–334.

39. **Vestrheim, D. F., et al.** 2008. Effectiveness of a 2+1 dose schedule pneumococcal conjugate vaccination programme on invasive pneumococcal disease among children in Norway. Vaccine **26:**3277–3281.

40. **Vitharsson, G., I. Jonsdottir, S. Jonsson, and H. Valdimarsson.** 1994. Opsonization and antibodies to capsular and cell wall polysaccharides of Streptococcus pneumoniae. J. Infect. Dis. **170:**592–599.

41. **Whitney, C. G., et al.** 2006. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. Lancet **368:**1495–1502.

42. **World Health Organization.** 2008. Executive Summary—WHO/Health Canada consultation on serological criteria for evaluation and licensing of new pneumococcal vaccines. http://www.who.int/biologicals/publications/meetings/areas/vaccines/pneumococcal/en/index.html.

43. **World Health Organization.** 2007. Pneumococcal conjugate vaccine for childhood immunization—WHO position paper. Wkly. Epidemiol. Rec. **82:** 93–104.

44. **World Health Organization.** 2005. Recommendations for the production and control of pneumococcal conjugate vaccines. WHO Tech. Rep. Ser. **927**(Annex 2)**:**64–98. http://whqlibdoc.who.int/trs/WHO_TRS_927_eng.pdf.

45. **World Health Organization, Expert Committee on Biological Standardization.** 2010. Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines: replacement of: TRS 927, Annex 2. World Health Organization, Geneva, Switzerland. http://www.who.int/biologicals/areas/vaccines/pneumo/Pneumo_final_23APRIL_2010.pdf.

46. **Wysocki, J., et al.** 2009. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) when co-administered with different Neisseria meningitidis serogroup C conjugate vaccines. Pediatr. Infect. Dis. J. **28:**S77–88.

47. **Yeh, S. H., et al.** 2010. Immunogenicity and safety of 13-valent pneumococcal conjugate vaccine in infants and toddlers. Pediatrics **126:**e493–e505.