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Pneumococcal Vaccine and Opsonic Pneumococcal Antibody

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Abstract

Streptococcus pneumoniae is a major human pathogen responsible for a majority of bacterial pneumonia as well as invasive pneumococcal diseases (IPD) with high mortality and morbidity. Use of conjugate vaccines targeting pneumococcal capsule has dramatically reduced the incidence of invasive diseases and there are active efforts to further improve the conjugate vaccines. However, in children new pneumococcal vaccines can no longer be tested with placebo-based clinical trials since effective vaccines are currently available. Thus, vaccine studies must depend on surrogate markers of vaccine efficacy. Although traditional antibody levels (e.g., ELISA) are useful as a surrogate marker of protection, they have limitations and a bioassay measuring the capacity of antibodies to opsonize pneumococci has been developed. This opsonophagocytosis assay (OPA) replicates the *in vivo* mechanism of antibody protection and should therefore better reflect protection by vaccine-induced antibodies. Technical improvements of OPA have made this bioassay rapid, multiplexed and practical for analyzing small samples including those from children. Strong correlations between ELISA and OPA have been observed in many studies of young children. However, poor correlations have been found in some important clinical situations (such as determination of protection by cross-reactive antibodies) and populations (such as elderly adults and immune-deficient patients). In these settings, OPA has become a useful supplementary measure of pneumococcal vaccine immunogenicity. Current efforts to standardize OPA will further expand its uses.

Keywords

Opsonophagocytic killing assay; Pneumococcal vaccine; Immunogenicity; *Streptococcus pneumoniae*

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Conflict of interest

UAB owns intellectual property on the various reagents used for pneumococcal vaccine studies, and MHN and RLB are UAB employees.

Introduction

The worldwide disease burden from *Streptococcus pneumoniae* remains both significant and widespread. The clinical implications of infection span a wide spectrum from mild illnesses, such as otitis media and sinusitis, to various invasive pneumococcal diseases (IPDs). Although the incidence of IPDs has decreased in recent years with the introduction of protein-conjugated vaccines, child deaths caused by *S. pneumoniae* still range from 700,000 to 1 million every year worldwide [1] and account for about 11 % of all deaths in children aged 1–59 months [2]. In the United States, *S. pneumoniae* was responsible for 22,000 deaths, 4 million disease episodes, and direct medical care costs of $3.5 billion in 2004 [3], a year when the conjugate vaccine had significantly reduced IPDs among children [4]. Most of the deaths and medical costs were found to be associated with adult patients who accounted for only half of the disease episodes.

A major reason for such a high disease burden is the polysaccharide (PS) capsule of pneumococci which shields pneumococci from host phagocytes and dramatically increases its virulence [5]. However, antibodies to the capsule can neutralize the shielding effect by opsonizing pneumococci for ingestion and killing by phagocytes. Although *S. pneumoniae* can express more than 90 different capsule types [6, 7], certain capsule types are more often associated with IPDs than others [8]. Thus, antibodies against 10 or 20 different serotypes can provide protection against a great majority of IPDs [8].

Despite antibiotic usage, morbidity and mortality from IPD remain high, in part because antibiotic resistance has become common. Therefore, vaccination has emerged as a key strategy against pneumococcal infections. A polysaccharide vaccine containing capsular PS from 23 different serotypes (PPV23, Pneumovax®) has been available since 1983. PPV23 is immunogenic and is widely used among adults, although its efficacy against IPD and pneumococcal pneumonia is somewhat controversial. The primary limitation of PPV23 is its lack of immunogenicity in young children. Efforts to develop vaccines for young children led to development of a protein-PS conjugate vaccine that included PSs from 7 serotypes (PCV7, Prevnar®) which was introduced in 2000 in the US. Clinical use of this vaccine dramatically reduced the incidence of IPDs in children [4, 9]. Later, PCV10 (Synflorix®) and PCV13 (Prevnar-13®) were licensed for children in 2008 and 2010, respectively. PCV13 was licensed for adults in the US in 2012.

In the wake of these successes, there is a significant effort to improve the currently available conjugate vaccines. For various practical reasons, evaluation of the new vaccines will require the use of surrogate markers of vaccine efficacy rather than efficacy trials measuring clinical endpoints. First, since the conjugate vaccines are clinically efficacious, it would be unethical to randomize patients at risk for pneumococcal disease to placebo. Second, the use of the conventional vaccine would drastically lower the incidence of pneumococcal infections in the control group. Thus, an efficacy study would require a very large sample size which would be prohibitive in terms of costs and logistics.

Surrogate markers of pneumococcal vaccine efficacy

One widely used surrogate marker of protection is the anti-capsular PS antibody levels determined by ELISA. Since the ELISA requires no special equipment and is amenable to scaling up for high throughput needs, it was widely used in developing conjugate vaccines. Based on efficacy data from the Northern California study, an antibody level of 0.2 mg/L was established as the threshold that loosely correlates with protection [10]. Later, immunogenicity data from Native American children and South African children were added to the Northern California data and a meta-analysis was performed. This analysis resulted in a threshold value of 0.35 mg/L for children [11], which was later accepted by

WHO. Additional studies suggested that the threshold may be closer to 0.2 mg/L when the ELISA developed by GlaxoSmithKline (GSK) is used [12]. It should be noted that the GSK ELISA is slightly different from the WHO ELISA [12].

ELISA has some important limitations, however. First, early generations of the assay were not specific [13]. The specificity was improved by pre-absorbing immune serum with capsular PS from serotype 22F, which is not clinically relevant. The improved ELISA procedure was adopted by WHO and a detailed protocol is at www.vaccine.uab.edu. Second, most adults have antibody levels above 0.35 mg/L prior to vaccination and these high background levels make interpretation of ELISA results difficult in this population. Third, ELISA typically measures only IgG, minimizing any contributions by other isotypes (such as IgM) to overall immunity. Recent evidence has shown that older adults have a lower capacity to opsonize pneumococci despite normal IgG levels, owing to a lack of anti-pneumococcal IgM antibodies [14]. This may be a result of deficient IgM-producing memory B cells, which decline with aging. A Finnish study demonstrated that IgM antibodies contribute to the post-vaccination opsonophagocytic activity in infants as well [15]. This phenomenon (i.e., a poor correlation between opsonic activity and IgG levels) has also been shown in patients who underwent bone marrow transplants [16] and emergency splenectomies [17]. It is unclear if these populations produce subnormal levels of IgM antibodies or produce non-opsonic antibodies.

Another limitation of the ELISA is that it does not measure the functional capacity of antibodies but rather their capacity to bind to PSs immobilized on a plastic surface. For example, PCV7 includes 19F PS but not the structurally related 19A PS. Although PCV7 can be shown to elicit antibodies cross-reactive with 19A by WHO ELISA, the antibodies do not cross-opsonize 19A [18]. Epidemiology data has shown that PCV7 does not provide cross-protection against 19A, thus supporting the results of the opsonophagocytosis assay (OPA). Also, studies of vaccine failures indicated that OPA predicts vaccine failures better than ELISA [19].

Unlike ELISA, OPA is an ideal surrogate marker for vaccine efficacy as it mimics the natural host defense responses (reviewed below). For these reasons, there is a recognized need for a functional assay that measures the ability of antibodies to effectively opsonize bacteria, leading to killing. This review focuses on OPAs as surrogate markers of pneumococcal vaccine efficacy.

Review of bacterial opsonophagocytosis

Phagocytosis can be simply defined as “the process by which a white blood cell envelopes and digests debris and microorganisms …” [20]. Despite the fairly succinct definition, the phagocytic process is both complex and universally important in normal host defense.

The process begins when phagocytes, which are typically neutrophils but can be macrophages or other cell types, encounter foreign targets. These targets are usually pathogens, such as pneumococcus, but can also be apoptotic host cells and/or cellular debris generated from routine metabolism [21]. As phagocytes encounter a foreign target, they can bind directly to the target via receptors on their surface, such as the mannose receptor. Alternatively, they can bind to the host opsonins that have been deposited on the target in a process known as opsonization. There are several varieties of opsonins in the human host including surfactant proteins, such as SP-A and SP-D, which have recently been implicated as effective opsonins in the lung [22]. However, the most important opsonins are immunoglobulins and proteins of the complement system.

Opsonization by complement mainly involves activation of C3, which can occur by three different pathways. Antibodies bound to the target can activate the classical pathway, by activating C1, C2/C4 and C3 in sequence. More recently described is the lectin pathway, which involves the mannose binding lectin (MBL) and ficolins. MBL and ficolins resemble C1q in structure, recognize certain PS structures, and sequentially activate C2/C4 and C3. Finally, the alternative pathway can deposit C3 independent of antigen recognition and can also enhance C3 deposition triggered by the classical or lectin pathways.

Activation of C3 results in deposition of iC3b, a powerful opsonizer. The fragment avidly binds to the complement receptor 3 expressed on the phagocytes resulting in engulfment of the pathogen [23]. After internalization of the target, intracellular phagosomes fuse with granules or lysosomes, leading to killing of the pathogen. The entire process from target recognition to intracellular killing is rapid, often completed in less than 20 minutes [24].

Studies of immunodeficient patients have confirmed that the *in vivo* host defense against pneumococcal infections is critically dependent on antibody-mediated opsonophagocytosis. First, the importance of phagocytes is evidenced by the higher incidence of IPD in neutropenic patients [25]. Second, a need for antibodies against the capsular PS is shown by the fact that patients with an inability to generate antibody against capsular PS are highly susceptible to pneumococcal infection [26]. These patients are protected when given gamma globulin treatments. Finally, the critical role played by the complement system, especially C3, is highlighted by the observation that complement deficiencies, acquired or inherited, render individuals highly susceptible to pneumococcal infections (reviewed in [27]).

Opsonophagocytic killing assays

The need for OPA data in vaccine studies spurred the development of multiple OPA formats. One format (“uptake assays”) utilizes fluorescently labeled bacteria, or fluorescently labeled spheres coated with capsular PS [28, 29]. These assays monitor the uptake of fluorescent bacteria/particles by the granulocytes with flow cytometry. Another format (“killing-type assays”) measures the actual killing of bacteria by enumerating the number of surviving bacteria. For various reasons (including high equipment costs), the uptake assay is rarely used for pneumococcal vaccine studies, with the killing-type OPA being the preferred format [30]. The remainder of this review will therefore focus solely on the killing-type assay.

In 1997, Romero-Steiner and colleagues described in detail a killing-type OPA that incorporated two important innovations: the use of frozen aliquots of target bacteria and the use of cultured cells (HL60) as phagocytes [31]. Following the discovery that only the HL60 cell line from ATCC is suitable for use in OPAs [32], HL60 cells were widely adopted as the phagocytes of choice. Despite these innovations, the 1997 procedure still required tedious manual colony counting which limited its use. However, colony counting became automated with the use of a chemical (triphenyltetrazolium chloride) that colorizes pneumococcal colonies when added to the agar plate [33].

Since PCVs are polyvalent, testing their immunogenicity requires performing OPA against many serotypes and, as vaccine studies are often performed among infants, with limited sample volume. For these reasons, it is highly desirable to have multiplexed OPAs that significantly reduce serum requirements. Using target bacteria that are antibiotic resistant, 2-fold [33] and 7-fold [34] multiplexed OPAs (MOPAs) have been developed. For various operational reasons including the desire to avoid using antibiotics that are used clinically to treat pneumococcal infections, a 4-fold MOPA was originally developed for the serotypes included in PCV13 [35] and was recently expanded to include all of the serotypes in the

PPV23 [36]. With these improvements in OPA, the analytical throughput of the OPA is now comparable to that of the ELISA.

Current clinical experience with opsonophagocytosis assay

Along with technological improvements in OPA, recent studies of pneumococcal vaccines often include OPA results. These studies confirmed that the results of OPA and ELISA generally correlate well, at least in children sera, except in certain situations. In the following sections, we review various infant and adult studies that used the two assays and examine how OPA has been useful as a surrogate marker of vaccine efficacy.

Evidence among infants and young children

Immunogenicity studies of young children that were published before 2008 and included OPA results are summarized in Supplementary Table 1 (reproduced with permission from a previous review [37]). In most of the studies presented in Supplementary Table 1, ELISA results correlated well with the OPA results and the functionality (i.e., ELISA result/OPA result) was comparable for most serotypes. However, in most of these studies, the functionality of anti-19F antibodies was at least 10-fold lower than the functionality of antibodies against other serotypes included in the studies. Additional studies are needed to determine the cause of this phenomenon.

PCV10 development produced several studies which compared PCV10 with PCV7 using ELISA and OPA among infants (Table 1) and toddlers (Table 2). These studies used GSK ELISA and detected low 19A antibody levels in response to PCV7 and PCV10 (Table 1). In one study [38], PCV10 generally elicited lower anti-capsular IgG antibody levels than PCV7 after a 3-dose primary series and the non-inferiority criteria was not satisfied for serotypes 6B and 23F (Table 1). However, the OPA titers for these 2 serotypes were high, and the percentages of subjects with an OPA titer ≥ 1:8 were similar between PCV7 and PCV10 recipients. This OPA data suggested that PCV10 is as immunogenic as PCV7 in these two serotypes. This conclusion was further supported by the fact that PCV11, which was the precursor of PCV10 and included serotype 3 PS, was effective against acute otitis media (AOM) caused by serotypes 6B and 23F, and PCV10 was as immunogenic as PCV7 for all serotypes after the toddler dose. Also, a study in Korean children showed PCV10 to be as immunogenic as PCV7 by both ELISA and OPA [39]. Thus, this experience further showed usefulness of OPA in immune status assessments.

Like PCV10, PCV13 development resulted in several studies that compared PCV13 with PCV7 using both ELISA and OPA (Tables 3 and 4) [40, 41]. When immunogenicity was assessed by ELISA, PCV13 was inferior to PCV7 for two serotypes (6B and 9V) in one study [41] and one serotype (6B) in the other [40], as determined by the responder proportion (percentage of subject with antibody level ≥ 0.35 mg/L) (Table 3). When the GMCs of the antibody levels were compared, non-inferiority criteria was met for all vaccine serotypes although the GMCs were generally lower for PCV13 than PCV7.

When the samples from the aforementioned studies were assessed by OPA, PCV13 and PCV7 elicited comparable functional antibody responses against each of the common serotypes (Table 3). The proportions of subjects with an OPA antibody titer ≥ 1:8 were similar between PCV13 recipients (90%–100%) and PCV7 recipients (93%–100%). As with previous studies, there was a significant increase in antibody levels (determined by both ELISA and OPA) after the toddler dose in both studies (Table 4) [40, 41]. Yeh et al. concluded that “Collectively, these data [ELISA and OPA] support that PCV13 is immunogenic against the 7 common serotypes, including 6B and 9V” [41].

An interesting observation from these PCV10 and PCV13 studies was that the immunogenicity of serotypes 1, 3, and 5 was lower than that of other serotypes when determined by OPA (Tables 1–4). Although the toddler dose generally increased antibody levels (as measured by both ELISA and OPA), the results were somewhat mixed and the levels for the three serotypes remained relatively low. Nevertheless, the proportions of subjects with serotype-specific OPA antibody titers ≥ 1:8 were high (>90% for serotypes 3 and 5, and 65–98% for serotype 1) after the PCV10 or PCV13 primary series. Preliminary reports indicate that PCV13 may not be protective against serotype 3 colonization [42]. Therefore, the effectiveness of PCV10 and PCV13 against the three serotypes will need to be closely monitored.

Another interesting finding in these studies was that the OPA titer of serotype 7F was typically high (~1:100) in the PCV7 recipients (Tables 1 and 3). Since PCV7 does not include 7F PS and confers no protection against serotype 7F, the reason for the high titer is unclear. As a result, an OPA titer cutoff of 1:2048 was selected for serotype 7F in two studies [40, 41]. Since the higher titers for 7F in PCV7 recipients are unlikely to be vaccine-induced, the high titer raises the possibility of innate opsonins against 7F. Also, this indicates that we may not be able to use a uniform OPA threshold (1:8) to estimate the immunogenicity of pneumococcal vaccines for all serotypes.

Experience among adults

While an opsonic titer of 1:8 may be a useful threshold for evaluating vaccine immunogenicity in children for most serotypes, many unimmunized adults have titers >1:8 for multiple serotypes (Supplementary Table 2). Although a titer of 1:64 has been suggested as a useful threshold for adults [43], this cutoff was based on limited data. Therefore, most of the adult studies described below rely on comparison of GMTs from two experimental groups (e.g., groups using different vaccines), to determine relative vaccine immunogenicity. Also, some studies use the percentage of subjects with a 2- (or 4-) fold increase (post-vaccination to pre-vaccination) in OPA titers as a tool for evaluating immunogenicity in adults.

The correlations between ELISA and OPA results in adults from multiple studies are shown in Supplementary Table 2 (reproduced with permission from [37]). Generally, there is a low correlation between ELISA and OPA for adults, much lower than what is seen in children (compare to Supplementary Table 1). One study included in Supplementary Table 2 [43] found better correlations among young adults than among elderly participants. This study also showed that the OPA result was a better predictor of protection than the ELISA in animal models of passive protection. In these animal studies, a serum sample from an elderly individual with a high OPA titer (1:8192) conferred considerably more protection against serotype 4 IPD than a serum sample also from an elderly individual with a low OPA (1:4), although both sera had comparable anti-serotype 4 IgG levels (2.7 mg/L versus 2.4 mg/L, respectively). Additional studies confirmed that elderly adults have reduced opsonic activity compared to healthy younger adults [44]. The discrepancy may be explained by the fact that ELISA does not measure IgM antibodies that are opsonic but may be deficient in elderly adults [14].

Following the success of conjugate vaccines in children, multiple studies were performed to address the safety and immunogenicity of PCV7 (and later PCV13) in normal adults (Table 5). In one study [45], PCV7 induced significantly higher OPA titers compared to PPV23 in five of seven serotypes. In a second study [46], PCV13 also induced higher mean OPA titers than PPV23, with statistically significant differences for some serotypes. Most of the other studies in Table 5 showed comparable opsonic activity of PPV23 and PCV7 [47–49]. Jackson et al. [47] showed significantly increased OPA titers for five of seven vaccine

serotypes compared to PPV23 when the PCV7 dose was doubled to 1.0 ml (Table 5), suggesting that the pediatric dose (0.5 ml) may not be sufficient for adults. Also, as with children, the OPA titers for serotype 3 were lower in adults than those of other serotypes after vaccination with either PCV13 or PPV23 [46, 50].

However, it should be noted that there were significant differences in the patient populations targeted in the above referenced studies. Musher et al. [49] conducted the study with high risk subjects who recovered from pneumococcal pneumonia, and the study by Miernyk et al. [48] targeted high risk native Alaskan adults. Jackson et al. [47] included previous PPV23 recipients in their study, while de Roux et al. [45] and Scott et al [46] enrolled only PPV23-naive subjects. This is relevant since prior exposure to PPV23 may induce immune hyporesponsiveness (reviewed in [51]). Based on current available data describe above [45–49] as well as in a recent editorial by Dr. Musher [64], PCV13 is not superior to PPV23 in adults previously vaccinated with PPV23. In PPV23-naive adults, PCV13 has a possibility to be more immunogenic than PPV23, but further studies are warranted to clarify the difference.

Evidence in special populations

Although vaccination is recommended for various immune compromised and high risk groups, only limited information is available for these populations. Since HIV-infected adults are highly susceptible to IPD [52], PPV23 and PCV7 were compared among HIV-infected patients using both ELISA and OPA [53]. In both assays, there were significantly higher antibody levels for multiple serotypes in the patient groups that received PCV7 (PCV7 followed by PCV7 and PCV7 followed by PPV23 groups) compared to the pre-vaccination samples. By ELISA, there were significantly higher antibody levels for serotypes 4, 6B, and 9V in the conjugate-PS group and serotype 23F in the conjugate-conjugate groups after the second dose compared to the group that received only one PS vaccination. By OPA, there were significantly higher levels for serotypes 4, 6B, and 9V in the conjugate-conjugate group and serotypes 9V and 23F in the conjugate-PS group compared to the group that received only one PS vaccination (Table 6).

Due to long-term immunosuppression, solid-organ transplant recipients also have an increased risk of pneumococcal disease [54, 55]. One study [56] compared the single-dose immunogenicity of PCV7 and PPV23 in renal transplant recipients by assessing immunogenicity eight weeks after vaccination by ELISA and OPA. Although anti-capsular IgG levels showed enhanced immunogenicity for some serotypes (6B, 9V, and 23F) after PCV7 vaccination, OPA responses were not different between the two vaccine groups (Table 6).

Patients with chronic obstructive pulmonary disease (COPD) are at higher risk for IPD and non-bacteremic pneumococcal pneumonia—up to 10 times more than age-matched controls [57]. Previous studies with PPV23 in COPD patients failed to demonstrate protection against acute exacerbation, pneumonia, IPD or death though these studies were underpowered for these endpoints [58]. In a 2012 study, the immunogenicity of PCV7 (1.0 mL dose) and PPV23were compared among patients with moderate to severe COPD by both ELISA and OPA [59]. PCV7 induced statistically higher OPA titers for six of seven serotypes (except for serotype 19F) compared to PPV23 when measured at one month post vaccination. These discrepancies persisted for five of seven serotypes (4, 9V, 14, 18C and 23F) at one year (Table 6), and four of seven serotypes (4, 14, 18C and 23F) at two years, post-vaccination. ELISA results showed a similar trend, but the differences were less significant. Considering such superior long-term functional responses of 1.0 mL PCV7 in patients with COPD, clinical trials evaluating PCV13 would be warranted in COPD patients with both clinical and immunogenicity end points.

Future directions

Although OPA techniques have been refined to become faster and more reproducible, inter-laboratory variability is still significant and is an important issue to be resolved [60]. With increasing demands for OPA data in clinical trials, it is becoming more important that we standardize the OPAs. The goal of OPA standardization is not to select one OPA protocol/format, but to have OPA results that are comparable. There are currently efforts to determine if a reference serum pool ("007sp") could be used to normalize OPA data from different laboratories. OPA standardization would require a sustained and active effort to be successful.

Another important point to be investigated is whether we can define a protective threshold for OPA. So far, the 1:8 OPA threshold has been used for all serotypes in studies with children for protection against IPD caused by vaccine serotypes. It is unlikely, however, that the same threshold applies for all additional serotypes, in different diseases, and in varying target populations.

Non-capsular, protein antigens are currently being investigated as potential antigen targets for pneumococcal vaccination. Mouse models have demonstrated pneumococcal protection using choline binding protein A and pneumococcal surface protein A. More recently, pneumolysin, another non-capsular antigen, has been incorporated into a vaccine and is currently in a Phase 1 clinical trial [61]. The immunogenicity data from this trial used antibody measurements determined by ELISA, rather than OPA. Some of these surface proteins are known to interfere with deposition of complement [62, 63], which is critical for opsonization. Also, opsonophagocytosis plays a central role in protecting the host against pneumococcal infection. Thus, OPA should be useful in assessing new protein-based vaccines as well.

Lastly, the basic OPA technologies that were developed for pneumococcal vaccines can be applied to OPAs for other pathogens (e.g., *Streptococcus pyogenes*) or to bactericidal assays for Gram negative bacteria (e.g., meningococci). The widespread use OPA or bactericidal assays in the future would result in a paradigm shift in vaccine evaluations from measuring antibody concentrations to measuring antibody function.

Conclusion

Although ELISA is well established as a measure of immune responses to pneumococcal vaccines, OPA reflects *in vivo* protection by the vaccine-induced antibodies. Thus, the OPA would be an ideal surrogate marker for pneumococcal vaccine efficacy. Recent technical developments has allowed OPA to be used in many vaccine studies and OPA results correlate well with disease protection, particularly in evaluating cross-protection (serotype 19A) and specific populations (e.g., the elderly and immune-compromised patients). As OPA becomes standardized, OPA will be used more widely, supplement ELISA, and greatly facilitate serologic assessment of pneumococcal vaccines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Comparison of immune responses to PCV10 versus PCV7 among infants

Study	Vesikari et al. [38]								Kim et al. [39]							
Country (year)	Finland, France and Poland (2009)								Korea (2011)							
Dosing schedule	3 doses (2, 3, 4 months)								3 doses (2, 4, 6 months)							
Vaccine	PCV7				PCV10				PCV7				PCV10			
Immunogenicity	OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)	
	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)
PCV7 serotypes																
4	1,010	100	2.78	100	735	99.6	1.45	97.1	1,457	100	5.35	100	632	98.1	4.00	99.7
6B	999	95.5	0.59	79.0	457	92.4	0.33	65.9	1,456	100	2.07	98.4	597	93.8	1.40	92.4
9V	1,233	100	2.68	99.5	1,400	100	1.32	98.1	1,901	98.4	5.09	99.2	1,051	99.4	3.39	99.7
14	1,891	98.9	4.49	99.5	1,061	99.6	2.90	99.5	2,169	98.4	8.51	100	1,248	98.8	5.54	99.4
18C	212	95.5	2.46	98.9	130	93.6	1.66	96.0	389	96.8	5.13	100	157	89.3	5.80	99.7
19F	52	92.1	3.42	99.2	149	87.7	1.84	95.4	75	90.2	2.77	100	358	97.0	7.41	98.8
23F	4413	97.7	1.34	94.1	1,010	93.9	0.53	81.4	9,336	98.4	3.94	98.4	1,489	97.6	1.96	96.2
Other serotypes																
1	5	4.5	0.03	4.0	25	65.7	1.05	97.3	6	12.9	0.04	5.7	188	92.6	3.41	100
5	5	3.4	0.03	1.9	60	90.9	1.70	99.0	5	9.7	0.07	14.6	238	97.6	4.52	100
7F	10	18.2	0.04	4.5	2254	99.6	1.72	99.5	152	67.8	0.03	3.3	3,968	100	4.08	100
Cross-reactive serotypes																
6A	83	68.5	0.10	31.2	41	58.0	0.07	22.2	358	90.0	0.48	69.1	189	84.8	0.38	67.4
19A	5	3.4	0.11	28.7	9	19.6	0.08	22.6	5	11.7	0.12	26.8	11	32.7	0.29	59.0

OPA, opsonophagocytosis assay; GMC, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers)

Table 2

Comparison of immune responses to PCV10 versus PCV7 among toddlers

Study	Vesikari et al. [38]								Kim et al. [39]							
Country (year)	Finland, France and Poland (2009)								Korea (2011)							
Dosing schedule	1 dose (12–18 months)								1 dose (12–18 months)							
Vaccine	PCV7				PCV10				PCV7				PCV10			
Immunogenicity	OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)		OPA titer		ELISA(IgG, μg/mL)	
	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)	GMT	≥1:8 (%)	GMC	≥0.20 (%)
PCV7 serotypes																
4	2,813	100	4.40	100	1,856	100	3.35	99.7	3,717	100	12.19	100	1,058	99.3	5.77	100
6B	3,460	98.7	3.53	97.7	981	96.6	1.94	96.5	3,827	97.8	7.09	100	547	94.7	2.78	97.5
9V	5,357	100	6.09	100	2,344	100	3.25	100	5,204	100	12.72	100	1,708	100	4.99	100
14	2,134	100	9.29	100	2,086	100	5.56	99.1	3,958	100	22.22	100	1,815	100	7.73	100
18C	969	100	5.21	100	810	99.7	5.01	100	1,723	100	14.53	100	608	100	13.14	100
19F	288	92.5	3.35	100	624	94.9	6.05	99.4	277	97.7	4.82	100	1,285	98.7	16.89	98.7
23F	13,901	98.8	6.67	98.9	2,830	99.7	2.38	97.4	29,919	97.7	14.81	98.0	2,703	99.3	3.75	98.7
Other serotypes																
1	4	3.6	0.04	4.9	192	91.0	1.53	99.4	5	6.4	0.06	11.0	364	94.6	4.03	99.7
5	4	1.2	0.05	6.1	144	96.3	2.20	99.4	4	0.0	0.19	38.2	234	97.3	5.51	100
7F	25	31.1	0.04	7.1	330	99.7	3.50	100	1,038	93.5	0.06	13.7	5,468	100	5.39	99.7
Cross-reactive serotypes																
6A	873	94.9	1.53	89.5	255	85.0	0.71	84.4	1,416	97.7	2.51	91.2	196	78.8	0.99	85.8
19A	11	27.6	0.47	76.5	29	48.8	0.85	83.8	16	38.6	0.32	59.8	65	69.2	1.54	84.9

OPA, opsonophagocytosis assay; GMC, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers)

Table 3

Comparison of immune responses to PCV13 versus PCV7 among infants

Study	Kieninger et al. [40]								Yeh et al. [41]							
Country (year)	Germany (2010)								USA (2010)							
Dosing schedule	3 doses (2, 3, 4 months)								3 doses (2, 4, 6 months)							
Vaccine	PCV7				PCV13				PCV7				PCV13			
Immunogenicity	OPA titer		ELISA (IgG, μg/mL)		OPA titer		ELISA (IgG, μg/mL)		OPA titer		ELISA (IgG, μg/mL)		OPA titer		ELISA (IgG, μg/mL)	
	GMT	≥1:8 (%)	GMC	≥0.35 (%)	GMT	≥1:8 (%)	GMC	≥0.35 (%)	GMT	≥1:8 (%)	GMC	≥0.35 (%)	GMT	≥1:8 (%)	GMC	≥0.35 (%)
PCV7 serotypes																
4	1,861	100	2.99	98.2	1,573	100	2.18	98.2	536	98.9	1.93	98.0	359	97.8	1.31	94.4
6B	1,161	98.9	1.49	87.1	744	96	0.98	77.5	1,514	100	3.14	92.8	1,055	98.9	2.1	87.3
9V	5,380	100	1.96	96.4	4,938	100	1.65	98.6	3,259	98.9	1.40	98.4	4,035	100	0.98	90.5
14	3,345	100	4.61	97.5	2,140	100	4.14	98.9	1,481	100	5.67	97.2	1,240	100	4.74	97.6
18C	1,780	98.9	2.25	98.6	1,510	100	1.94	97.2	376	100	1.79	98.4	276	100	1.37	96.8
19F	166	93.6	2.86	96.0	150	96	1.73	95.8	45	92.6	2.24	97.6	54	90.4	1.85	98
23F	1,071	95.7	1.44	89.5	1,090	96	1.26	88.7	924	98.9	1.90	94.0	791	98.9	1.33	90.5
Other serotypes																
1	4	4.3	0.03	1.4	50	93	1.83	96.1	4	9.8	0.02	1.6	52	98.9	2.03	95.6
3	6	24.5	0.05	6.3	251	99	1.55	98.2	7	21.3	0.04	4.6	121	96.8	0.49	63.5
5	5	4.3	0.20	31.6	162	99	1.31	93	4	2.2	0.20	31.0	91	92.3	1.33	89.7
6A	122	72.0	0.23	31.6	1,228	96	1.33	91.9	100	77.7	0.25	42.5	980	100	2.19	96
7F	115	78.7	0.04	4.0	11,545	100	2.59	98.6	128	76.4	0.04	2.8	9,494	100	2.57	98.4
19A	7	17.0	0.64	79.2	442	100	3.26	99.3	7	16.3	0.89	86.6	152	91.4	2.07	98.4

OPA, opsonophagocytosis assay; GMC, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers)

Table 4

Comparison of immune responses to PCV13 versus PCV7 among toddlers

Study	Kieninger et al. [40]						Yeh et al. [41]					
Country (year)	Germany (2010)						USA (2010)					
Dosing schedule	1 dose (11–12 months)						1 dose (12–15 months)					
Vaccine	PCV7				PCV13		PCV7				PCV13	
Immunogenicity	OPA titer		ELISA (IgG, μg/mL)		OPA titer	ELISA (IgG, μg/mL)	OPA titer		ELISA (IgG, μg/mL)		OPA titer	ELISA (IgG, μg/mL)
	GMT	≥1:8 (%)	GMC	≥0.35 (%)	GMT	≥1:8 (%) GMC	GMT	≥1:8 (%)	GMC	≥0.35 (%)	GMT	≥1:8 (%) GMC ≥0.35 (%)
PCV7 serotypes												
4	2,885	100		5.07	2,491	100 4.16	1,492	98.9		5.49	1,180	98.9 3.73
6B	2,907	98.9		9.85	2,687	100 9.14	4,066	100		15.63	3,100	98.9 11.53
9V	16,026	100		3.36	13,704	100 2.75	18,032	100		3.63	11,856	98.9 2.62
14	4,925	100		11.01	3,012	100 8.34	2,366	100		12.72	2,002	100 9.11
18C	1,762	100		3.44	1,326	100 2.79	1,722	100		4.70	993	98.9 3.20
19F	265	94.8		4.72	345	99.0 5.99	167	94.8		5.60	200	96.7 6.60
23F	3,738	95.6		4.33	2,854	97.9 3.36	4,982	100		7.84	2,723	98.9 5.07
Other serotypes												
1	5	15.8		0.03	198	99.0 4.25	5	12.0		0.03	164	98.9 5.06
3	8	35.8		0.07	188	98.0 1.02	12	43.8		0.07	380	97.8 0.94
5	4	2.2		0.51	327	99.0 3.56	5	5.2		0.55	300	98.9 3.72
6A	501	92.5		1.74	2,502	99.0 5.88	539	94.8		1.87	2,242	98.9 8.20
7F	182	85.2		0.05	10,213	100 4.79	268	80.4		0.05	11,629	100 5.67
19A	59	68.1		3.79	1,349	100 9.58	268	53.2		3.54	1,024	97.8 8.55

OPA, opsonophagocytosis assay; GMC, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers)

Table 5

n adults after pneumococcal vaccination

yk et al. [48]		Jackson et al. [47]			de Roux et al. [45]		Musher et al. [49]		Frenck et al. [50]	Scott et al. [46]	
, 55–70 years		Adults[a], 70–79 years			Adults, ≥70 years		Adults[b]		Adults, 50–59 years	Adults, 18–49 years	
L)	PCV7 (0.5 mL)	PPV23 (0.5 mL)	PCV7 (0.5 mL)	PCV7 (1.0 mL)	PPV23 (0.5 mL)	PCV7 (0.5 mL)	PPV23 (0.5 mL)	PCV7 (0.5 mL)	PCV13 (0.5 mL)	PPV23 (0.5 mL)	PCV13 (0.5 mL)
	4 (4–64)	-	-	-	-	-	-	-	264	244 (123–486)	467 (193–1,126)
	-	-	-	-	-	-	-	-	78	13 (9–19)	35 (19–64)
	128 (4–1,024)	45 (16–127)	39 (13–110)	106 (37–308)	663 (454–971)	1,457 (997–2,130)	-	-	2,203	2,353 (1,553–3,564)	2,580 (1,727–3,856)
	-	-	-	-	-	-	-	-	204	323 (117–887)	489 (161–1,483)
	-	-	-	-	-	-	-	-	3,157	140 (43–464)	2,702 (1,148–6,360)
4)	512 (4–2,048)	35 (15–84)	27 (10–74)	48 (19–125)	809 (553–2,284)	1,351 (938–1,946)	159	135	3,215	1,867 (677–5,148)	5,161 (254–10,453)
	-	-	-	-	-	-	-	-	2,691	3,251 (1,767–5,982)	2,964 (1,553–5,659)
	-	17 (8–36)	39 (18–86)	32 (13–79)	985 (710–1,366)	2,915 (2,142–3,967)	-	-	1,749	4,705 (2,066–1,0718)	10,809 (5,762–20,277)
3)	128 (4–2,048)	259 (92–730)	186 (68–515)	235 (95–581)	1,011 (639–1,598)	2,397 (1,606–3,579)	565	379	1,554	8,579 (5,772–12,754)	9,855 (5,570–17,437)
	-	135 (65–281)	119 (54–263)	195 (89–426)	465 (314–688)	1,318 (919–1,889)	-	-	1,978	741 (198–2,776)	2,464 (1,051–5,775)
	-	-	-	-	-	-	-	-	1,022	562 (198–1,597)	741 (416–1,321)
)	1024 (4–2,048)	77 (37–158)	50 (25–102)	80 (40–160)	203 (136–302)	182 (121–273)	54	39	625	708 (383–1,306)	1,351 (629–2,904)
	-	168 (67–418)	235 (91–611)	106 (42–271)	302 (206–444)	1,309 (862–1,990)	51	200	483	588 (328–1,055)	3,566 (1,330–9,559)

(Range) for Miernyk et al; GMT (95% CI) for Jackson et al, de Roux et al, and Scott et al; GMT for Musher et al. and Frenck et al.

occal pneumonia

, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers); CI, confidence interval; -, not reported

Table 6

Comparison of OPA results in special populations after pneumococcal vaccination

Study		Feikin et al. [53]						Kumar et al. [56]		Dransfield et al. [59]	
Target population		HIV-infected subjects with CD4 counts ≥200 cells/μl						Renal transplant recipients		COPD patients	
Vaccine schedule (dosage)		PCV7 (0.5 ml)-PCV7 (0.5 ml) with 8 week interval		PCV7 (0.5 ml)-PPV23 (0.5 ml) with 8 week interval		Placebo-PPV23 (0.5 ml) with 8 week interval		PPV23 (0.5 ml)	PCV7 (0.5 ml)	PPV23 (0.5 ml)	PCV7 (1.0 ml)
Sample collection schedule		8 weeks post 1st vaccine	8 weeks post 2nd vaccine	8 weeks post 1st vaccine	8 weeks post 2nd vaccine	8 weeks post 1st vaccine	8 weeks post 2nd vaccine	8 weeks post vaccine	8 weeks post vaccine	1 year post vaccine	1 year post vaccine
OPA Titer by Serotype	4	40 (23–70)	46 (29–73)	20 (11–39)	16 (9–28)	15 (9–26)	18 (11–31)	173 (32–1,024)	208 (32–2,048)	62	268
	6B	116 (67–204)	169 (94–305)	48 (23–100)	57 (26–124)	45 (25–83)	49 (27–91)	199 (32–4,096)	294 (32–4,096)	229	476
	9V	39 (22–67)	46 (32–67)	24 (12–47)	23 (12–43)	17 (11–28)	20 (12–36)	79 (4–1,024)	83 (4–8,192)	142	444
	14	61 (37–101)	64 (37–112)	61 (23–161)	50 (24–105)	45 (18–117)	83 (35–198)	203 (4–2,048)	239 (4–4,096)	839	1,801
	18C	-	-	-	-	-	-	86 (4–2,048)	119 (4–2,048)	284	665
	19F	-	-	-	-	-	-	111 (16–1,024)	147 (8–2,048)	116	87
	23F	40 (25–66)	39 (22–69)	20 (10–39)	19 (11–34)	18 (13–25)	19 (14–27)	29 (4–512)	64 (4–2,048)	48	407

Note. Results are expressed as: Mean (95% CI) for Feikin et al; Mean (Range) for Kumar et al; and Mean for Dransfield et al.

OPA, opsonophagocytosis assay; GMC, geometric mean concentration; GMT, geometric mean titer (calculated from the reciprocal of the individual titers); CI, confidence interval; -, not reported