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Serological criteria and carriage measurement for evaluation of new pneumococcal vaccines

Nicola Principi and Susanna Esposito*

Pediatric Highly Intensive Care Unit; Department of Pathophysiology and Transplantation; Università degli Studi di Milano; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy

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The best method of evaluating the efficacy of a vaccine is to compare the incidence of the disease against which it is prepared in randomized, placebo-controlled clinical trials involving vaccinated and unvaccinated subjects. In the case of *Streptococcus pneumoniae*, the proposed alternatives are evaluations of the so-called "correlates of protection" (i.e. markers of the vaccine-induced immune response that predict protection from infection and disease) and nasopharyngeal carriage. The aim of this paper is to discuss the most important limitations of the immunological criteria suggested for licensing new pneumococcal vaccines, and comment on the use of carriage as an endpoint for evaluating efficacy. Data showed why the use of a single serological correlate of protection cannot be considered the best means of evaluating pneumococcal vaccines and highlighted the importance of using carriage for efficacy evaluation but in the meantime the need to develop new sensitive and specific methods.

Introduction

The best way of evaluating the efficacy of a vaccine is to measure clinical outcomes, which is mainly done by means of randomized, placebo-controlled clinical trials comparing the incidence of the disease against which the vaccine is prepared in vaccinated and unvaccinated subjects.[1] However, these trials are complicated, time-consuming and expensive because they involve the enrolment of a large number of subjects who have to be continuously and carefully followed-up for a long time. The problem is even greater when it is wanted to evaluate a new vaccine whose efficacy is presumed to be at least equivalent to one that is already routinely and effectively administered in the same age group against the same pathogen not only because the number of subjects is enormous, but also because it is unethical to compare the new vaccine with placebo as this would expose a significant number of subjects to the risk of an otherwise preventable disease.

In attempt to overcome these issues, it has been suggested to use so-called "correlates of protection" (i.e., markers of a vaccine-induced immune response capable of predicting protection)[2] because it was thought this would significantly simplify the evaluation of new vaccines, avoid the need for large-scale trials, and facilitate the approval of new products and formulations. Among conjugate vaccines, this approach was first used in 1993 for the approval of a *Haemophilus influenzae* type b (Hib) conjugate vaccine[3] and, subsequently in 2003, for the licensing of the meningococcal C conjugate vaccine.[4]

The same method was used for the licensing of the higher valency pneumococcal conjugate vaccines (PCVs) that were specifically prepared to overcome the limitations of the heptavalent preparation (PCV7). After evaluating the immune response of children given PCV7 and the correlations between the specific anticapsular antibody concentrations evoked by the vaccine and protection against invasive pneumococcal disease (IPD), a World Health Organization (WHO) working group proposed that a $\geq$0.35 μg/mL concentration of IgG anticapsular polysaccharide antibodies measured by means of an enzyme-linked immunosorbent assay (ELISA) one month after primary immunisation could be considered as a correlate of efficacy against disease and used to evaluate all new PCVs.[5] The 10- and 13-valent pneumococcal vaccines (PCV10 and PCV13) were consequently licensed only on the basis of this immunological criterion, and clinical effectiveness was simply inferred from the efficacy data relating to PCV7.[6] However, it was immediately pointed out that the method may have a number of limitations,[7] and that its systematic application in the licensing process could obstruct the approval of new and very effective vaccines or favor the licensing of a preparation that actually has little or no impact on public health. Moreover, the method cannot be used to evaluate the vaccines based on protein and other novel mechanisms that are currently being developed.[8]

The aim of this paper is to discuss the most important limitations of using immunological criteria for licensing new pneumococcal vaccines, and to comment on the recently suggested use of carriage as an efficacy endpoint. Discussion will be limited to the

*Correspondence to: Susanna Esposito; Email: susanna.esposito@unimi.it
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problems of evaluating PCVs efficacy in children because several differences exist between children and adults for pneumococcal disease's manifestations (e.g., incidence, morbidity and mortality) and serotypes isolated in nasopharyngeal carriage and diseases. Moreover, there is no evidence that the immune response translates to clinical efficacy in adults as seen in children.[9]

Limitations of the serological correlate of protection for pneumococcal vaccines

In order to determine the serological correlate of protection for PCVs against IPD, 3 double-blind, controlled efficacy trials were considered: 2 of PCV7 and one of 9-valent conjugate vaccine (PCV9), which contains serotypes 1 and 5 in addition to the 7 serotypes contained in PCV7. In the PCV7 trials, the vaccine was administered at 2, 4, 6 and 12 months of age to respectively 37,868 infants at Northern California Kaiser Permanente trial[10] and 8,292 American Indian infants in South-western USA;[11] in the third study, 19,992 infants living in South Africa received PCV9 at the ages of 6, 10 and 14 weeks.[12] The 3 studies recorded different efficacy estimates, and different correlates of protection were calculated: in the Kaiser Permanent trial, global efficacy was 97.3% and the estimated correlate of protection was 0.20 μg/mL,[9] whereas global efficacy in the other trials was respectively 76.8% and 90%, and the estimated correlate of protection was respectively 1.0 and 0.68 μg/mL.[11,12] Consequently, the estimated protective concentration of 0.35 μg/mL was calculated by pooling the data of the 3 studies.

Table 1 summarizes the main limitations of using serological correlates of protection for pneumococcal vaccines. The first potential issue concerning the use of antibody concentration as a marker of protection is the only slight relationship between it and real protective antibody activity. The serological correlate of protection determined by means of ELISA indicates the amount of capsular polysaccharide antibody that assures a high probability of protection from IPD due to the serotypes included in a vaccine; however, this is only a surrogate measurement of the vaccine's likely protective activity, which can be more precisely estimated by means of other tests of antibody function such as opsonophagocytic titres or antibody avidity.[13] Opsonophagocytic titres are the most widely used and, on the basis of the validated data concerning *Neisseria meningitidis* serogroup C conjugate vaccines,[14] can be considered to be associated with protection when they are 1 in 8 or higher,[12] whereas a high antibody titer does not always indicate protection because antibody function may be suboptimal.[15] Furthermore, the accuracy of ELISAs may be affected by substances in the sera, the quality of the reagents and the steps used in the assay.[16]

Table 1. Main limitations of using serological correlates to evaluate the protection provided by pneumococcal vaccines

Main limitations
Antibody levels are evaluated only one month after completing the priming vaccine doses and not after the booster dose (that is more important for long-lasting protection)
For all the serotypes included in PCVs, the same antibody level is considered protective, although there are data indicating that some serotypes require higher concentrations
Antibody concentrations are influenced by characteristics of the proteins conjugating capsular polysaccharides
Antibody concentrations are only moderately correlated with opsonophagocytic antibody titres
1. Antibody concentrations have limited efficacy in measuring PCVs ability to reduce pneumococcal diseases other than invasive pneumooccal disease

PCVs: pneumococcal conjugate vaccines.

Other problems arise from the fact that the antibody level considered to be a correlate of protection refers to the IgG concentrations measured one month after completing the priming vaccine doses; levels after a booster dose were not considered, although it is highly likely that they play a major role in long-term protection.[17] Furthermore, the serotypes were considered together even though the (not always available) serotype-specific efficacy data varied from serotype to serotype in the studies that led to the currently used correlate of protection. The Kaiser-Permanent trial, which included the highest number of subjects,[10] showed serotype-specific efficacy in relation to only 4 of the 7 serotypes contained in the vaccine which, at least theoretically, means that the suggested correlate of protection for 3 serotypes might be different.

A further limitation relates to the characteristics of the proteins that conjugate the capsular polysaccharides included in PCVs. PCV7 and PCV9 use the same diphtheria CRM_{197} protein, and PCV10 uses protein D derived from non-typeable *Haemophilus influenzae* for serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F, and tetanus toxoid for 18C. As it has been demonstrated that the characteristics of the protein used for polysaccharide conjugation can affect immune response,[18] the correlate of protection vary depending on the carrier.

Another element of discussion is the role played by the schedule of administration in conditioning immune response. The value of 0.35 μg/mL was based on the findings of studies that used different schedules and, furthermore, did not take into account the findings of any study using the simplified schedule of only 2 doses in the first months of life followed by a booster administered at about one year of age.[19] However, it has been reported that differences in administration schedules can lead to variations in antibody response to at least some serotypes,[20] and so it is possible that the suggested correlate of protection may only be valid in some cases and not for all serotypes.

The definition of immune correlates is further complicated by population-based differences in antibody response to the same vaccine antigens, as is demonstrated by the fact that the calculated correlate of protection was significantly lower in the Kaiser Permanent trial, which involved healthy American Indian children, than that observed in a study limited to the White Mountain Apache population, which is significantly more susceptible to IPD[21]. Once again, serological correlates of protection may vary, and the currently used antibody value may not be universally valid.

The limited precision of 0.35 μg/mL as a marker of protection is also highlighted by data recently collected by Andrews

et al.,[22] who evaluated the immunogenicity and effectiveness of PCV13 and found that it was 90% effective in the case of the PCV7 serotypes, 73% effective in the case of the 6 additional serotypes included in PCV13, and ineffective in the case of serotype 3. The calculated serotype-specific correlates of protection were $\geq$0.35 μg/mL for serotypes 1, 3, 7F, 19A and 19F, and <0.35 μg/mL for serotypes 6A, 6B, 18C, and 23F, with the highest values for serotypes 3 and 19F (2.83 and 1.17 μg/mL) and the lowest for serotypes 18C and 6B (0.14 and 0.16 μg/mL).[22] The very high IgG value needed for protection against serotype 3 is rarely attained as a result of vaccination, which explains why the study concluded that PCV13 was ineffective against it.[23] In agreement with *in vitro* data showing that high IgG concentrations are needed to achieve complement deposition and kill this serotype is the high antibody concentrations required for protection against serotype 19F.[24] Interestingly, opsonophagocytic antibody titres of 1 in 8 or higher did not predict protection, thus suggesting that the validity of this marker should probably be revised and further reducing the importance of immune criteria when evaluating the potential protection provided by new PCVs.

However, the most important limitation of using the serological correlate of protection is that it does not really measure a vaccine's ability to reduce pneumococcal diseases other than IPD. *Streptococcus pneumoniae* has a considerable epidemiological, clinical, social and economic impact in relation to a number of conditions, including acute otitis media (AOM),[25] non-bacteremic community-acquired pneumonia (nbCAP),[26] and naso- and oropharyngeal carriage,[27] and knowing whether a vaccine can influence their epidemiology and severity may be very important when deciding whether it should be licensed. It has been demonstrated that all of the currently marketed PCVs are significantly less effective in preventing pneumococcal carriage, AOM, and nbCAP[28] than in preventing IPD, thus suggesting that the effectiveness of the immune response depends on the site of the pathogens to be eradicated and that higher antibody levels than that currently considered a correlate of protection are probably needed for mucosal disease protection. Unfortunately, there are no definite data showing the serological correlate of protection against nbCAP, but studies of subjects with AOM confirm that protective antibody levels against some serotypes are higher than 0.35 μg/mL. Eskola et al. reported that the efficacy of PCV7 against AOM when administered at 2, 4, 6 and 12 months of age was statistically significant in the case of serotypes 6B, 14 and 23F, but very poor in the case of 19F, even though polysaccharide-specific antibody concentrations at 7 and 13 months were higher than 1 μg/mL in more than 90% of the cases.[29] This finding is in line with the finding of Jokinen et al.[30] that the incidence of AOM due to serotype 19F was significantly higher than that of cases due to serotype 6B in children who had received PCVs even though the antibody titres against the former were several times higher than those against the latter, and significantly higher than 0.35 μg/mL in almost all of the patients. Other authors have shown that, although serotypes 6B and 23F elicit similar antibody titres, the concentration required for the 50% killing of the first was 2–10 times higher that required for the 50% killing of the second.[31]

In conclusion, the serological correlate of protection currently used to evaluate new PCVs does not adequately reflect the real efficacy of the new preparations, which is why a number of experts suggested that the licensing and use of new PCVs should be based also on other variables such as their impact on nasopharyngeal carriage.[32]

Measuring Pneumococcal Vaccine Efficacy on the Basis of the Carriage of *S. pneumoniae*

The idea of using pneumococcal carriage as a marker of PCV efficacy is mainly based on the fact that carriage is a prerequisite for the development of both IPD and mucosal pneumococcal diseases, and that the administration of all of the currently available PCVs significantly reduces pharyngeal colonisation by the serotypes they contain.[33] Although carriage itself does not pose a risk for pneumococcal disease, various human and experimental animal studies have shown that it is the first step in its pathogenesis.[33] Most of the data refer to AOM,[34-37] although there are similar data available relating to other diseases. Gray et al. monitored pneumococcal carriage and the development of pneumococcal diseases in a group of children who were followed up from birth to the age of 2 years, and found that diseases (mainly AOM) were very frequently associated with the acquisition of a new pneumococcal serotype.[34] Other authors have confirmed the close relationship between newly acquired serotypes and the development of disease, but found that prolonged carriage is not associated with an increased risk of infection, thus suggesting that this is actually a protective factor.[35-37] It has also been demonstrated that the serotype found at the site of infection can be simultaneously cultured in the pharynx in children with AOM or IPD.[35,38,39]

It is still unclear why colonising pneumococcal strains only sometimes cause disease, although it is supposed that both the pathogen and host factors contribute to the outcome of carriage. The virulence of the pneumococcal strain and the characteristics of the subjects (such as their immunological status, the presence of severe chronic underlying disease, and simultaneous colonisation with other bacteria or viruses) may play a role increasing the risk of the transition from simple carriage to disease.[33] However, not all pneumococcal serotypes have the same tendency to colonise the pharynx and, although being important causes of disease, some (such as serotypes 1, 5, 7 and 12F) are rarely found in pharyngeal samples taken from healthy children. The main reasons for this seem to be the short duration of carriage and difficulties in identifying these serotypes when several others are present, but it is also thought that only some of the steps in the transition may be short-lasting.[32]

It has been found that PCVs are very effective in reducing the incidence of pneumococcal diseases due to the serotypes they contain regardless of schedule of administration, although the proportion of avoided IPDs is greater than that of avoided mucosal diseases. The rate of prevention of diseases due to PCV7

serotypes is higher than 90% in the case of IPD,[40] 30–70% in the case of pneumonia,[41] and about 50% in the case of AOM.[42] A similar or only slightly lower reduction than that reported for AOM has been found in the case of carriage: a number of studies of PCV7[43] and more recent studies of the higher valency preparations[44,45] have shown that vaccination is followed by a sharp reduction in carriage, and that the proportion of vaccinated children carrying vaccine-type pneumococci 6 months after receiving the primary series was significantly lower than that of unvaccinated controls.[46-50] For example, Mbelle et al.[49] in South Africa found that the prevalence of carriage of vaccine-type serotypes was 17.8% in the vaccinated children and 36.0% in controls. Similarly, O'Brien et al.[50] reported that among American Indians in the USA the percentages of carriers were 10.6% in the vaccinated group and 25.0% among unvaccinated children.

An assessment of the concurrent effects of PCV7 on carriage and disease showed that both were exclusively related to the reduction in the importance of the vaccine-type serotypes, which simultaneously disappeared (albeit at different rates) as causes of disease and as carried pathogens.[33]

Together with this direct effect, the administration of PCV7 to children is followed by 2 indirect effects: herd immunity[51] and serotype replacement,[52] both of which are mediated by the vaccine's direct effect on carriage. It has been found that the reduction in carriage in children who have received PCV7 significantly reduces the circulation of vaccine-type serotypes, limits their colonisation of unvaccinated subjects and significantly reduces the development of pneumococcal diseases. However, the disappearance of PCV7 serotypes as causes of disease and colonisation in both vaccinated and unvaccinated subjects led to them being replaced by non-vaccine strains in the pharynx, and consequently the etiology of the developed diseases. This obviously reduced the benefits of PCV7 itself, which was the most important reason for the development of new vaccines containing a larger number of serotypes.[53]

It was this that led to the suggestion that carriage may be a good candidate surrogate of protection on the grounds that a vaccine capable of eliminating or reducing carriage is really effective because this not only protects vaccinated subjects from disease, but also simultaneously prevents them from transmitting the pathogens, and those avoids colonisation and disease in unvaccinated individuals.[8] It has also been pointed out that, unlike the currently used serological criterion, serotype-specific colonisation endpoints can be directly estimated and may allow a more precise evaluation of the true efficacy of a vaccine.[8]

In terms of colonisation, it is possible to study the ability of a vaccine to interfere with pneumococcal acquisition, the duration of carriage, and pharyngeal bacterial load. Moreover, evaluation of these primary endpoints can be combined. If acquisition and duration of carriage are evaluated together, a feasible measure of an individual's capacity to transmit the pathogen can be obtained.

Furthermore, the effects can be measured in relation to a single serotype or in an aggregate manner, and in individual subjects or large groups of people. It is also easy to study interactions with factors that may influence acquisition and persistence of carriage. A number of studies have tried to directly estimate PCV efficacy against acquisition using longitudinal data from repeated nasopharyngeal samples, showing that different schemes of administration of PCV can have different impact on carriage, at least for same serotypes.[54,55]

However, to estimate rates of acquisition and clearance of colonization repeated active sampling of the same individual is required overtime, an expensive and invasive undertaking. Thus, investigators have turned to using cross-sectional data, and several mathematical models have been developed[8]. A method for defining susceptibility to acquisition of colonization starting from published data that reported vaccine effects against all vaccine types was proposed by Rinta-Kokko et al.[56] Studies simulating randomized controlled trials and leading to mathematical models useful to evaluate the best timing for pharyngeal sampling and estimating strain-specific and overall vaccine efficacy were recently reported by Scott et al.[57] and by Auranen et al.[58], respectively. However, even if all these studies have laid the basis for a possible new approach to the evaluation of pneumococcal vaccines, none of the suggested methods was till now adequately validated even because several potential problems that can question real effectiveness of using carriage as a means of evaluating the efficacy of pneumococcal vaccines can be raised (**Table 2**). First of all, the absence of carriage after vaccine administration does not always mean that a vaccine is really effective because, as mentioned, some rarely carried pneumococcal serotypes are significant causes of disease. Secondly, carriage is greatly influenced by various factors including co-colonisation with other bacteria and viruses, the use of antibiotics, and breastfeeding.

Furthermore, it needs to be remembered that the method used to identify *S. pneumoniae* and the site at which pharyngeal secretions are collected are both important for evaluating carriage precisely. The identification of *S. pneumoniae* can be significantly improved by using molecular biology methods which, albeit with some exceptions, have been found to be significantly more reliable than the traditional non-enriched cultures used in routine practice[59] and, although *S. pneumoniae* is bet sampled nasopharyngeally in infants and young children,[60] recent studies have shown that oropharyngeal sampling is better in older children, adolescents and adults.[61,62]

Finally, it is still unclear exactly when the effect of a vaccine on carriage should be evaluated. Most studies measuring the changes in carriage secondary to vaccine use have been carried out after only a few months or years after vaccination, when antibody levels against the different serotypes are at their peak and the effect

Table 2. Main limitations of using carriage to evaluate the efficacy of pneumococcal vaccines

Main limitations
Influenced by various factors, including co-colonisation with other bacteria and viruses, the use of antibiotics, and breastfeeding
Influenced by methods used to identify *S. pneumoniae*
Influenced by the site of pharyngeal swabbing
Carriage is usually evaluated only some months after vaccine administration and does not indicate long-term protection

of a vaccine is greatest. However, evaluations made some years after the last vaccine dose can lead to different conclusions, and may indicate more precisely the duration of the effect. One recent study found that most of the sample of school-age children who had been fully vaccinated with PCV7 some years before were colonised by some of the vaccine's serotypes.[63] As 19F was the most frequently identified serotype and it is known that its eradication requires high antibody levels[64] it can be presumed that the antibody levels evoked by PCV7 significantly decline over time and are no longer sufficient to eliminate carriage.

Even if very expensive and difficult to organize, most of these problems could be solved planning clinical trials in which putative correlates of protection are evaluated simultaneously with the impact of PCV on carriage.

Conclusions

There are a number of reasons for the difficulty in evaluating the real impact of PCVs. First of all, the various serotypes may not only be different in terms of their ability to cause disease, but may also be cleared from the pharynx before giving rise to any clinical problems. Furthermore, it is not clear precisely what causes the development of disease.

The currently available vaccines are based on capsular polysaccharides and contain only a relatively small number of serotypes. When the polysaccharides are conjugated with carrier proteins, they induce a strong immune response against the proteins that is capable of preventing both colonisation and disease, but this response is neither quantitatively nor qualitatively uniform. Moreover, the antibody concentrations needed to eradicate pathogens vary from serotype to serotype, and from site to site of infection. Higher concentrations are required to avoid colonisation than those necessary to prevent IPD.

All of these limitations explain why the use of a single serological correlate of protection cannot be considered the best means of evaluating potential efficacy of the polysaccharide-based pneumococcal vaccines or the vaccines based on selected bacterial proteins, and this has led to the proposal of using carriage for purposes of evaluation. However, at the moment, because there are still no concrete data indicating how the monitoring of carriage can be used to evaluate vaccine efficacy, the use of putative correlate of protection for the different diseases and for pneumococcal carriage remains the only possible means to evaluate potential efficacy of conjugated vaccine. However, to overcome the limitations of this method and to favor a precise evaluation of the new pneumococcal protein vaccines, evaluation of carriage has to be further developed. Trials comparing immunologic evaluation and carriage are needed to solve at least in part the problems presently not solved by carriage evaluation alone.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Chen RT, Orenstein WA. Epidemiologic methods in immunization programs. Epidemiol Rev 1996; 18:99-117; PMID:9021306; http://dx.doi.org/10.1093/oxfordjournals.epirev.a017931
2. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol 2010; 17:1055-65; PMID:20463105; http://dx.doi.org/10.1128/CVI.00131-10
3. Ellis RW, Granoff DM, Frasch CE. Regulatory perspectives in vaccine licensure. In: Ellis RW, Granoff DM, editors. Development and Clinical uses of *Haemophilus* b Conjugate Vaccines. New York: Marcel Dekker; 1994:435-53.
4. Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. Clin Diagn Lab Immunol 2003; 10:780-6; PMID:12965904
5. World Health Organization. Recommendations for the production and control pf pneumococcal conjugate vaccineess. WHO Tech Rep Ser 2005; 927:1-154; PMID:16454167
6. Vila-Corcoles A, Ochoa-Gondar O. Pneumococcal conjugate vaccination: correlates of protection. Lancet Infect Dis 2014; 14:784-6; PMID:25042757; http://dx.doi.org/10.1016/S1473-3099(14)70849-7
7. Jódar L, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H, Klugman K, Plikaytis B, Siber G, Kohberger R, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine 2003; 21:3265-72; http://dx.doi.org/10.1016/S0264-410X(03)00230-5
8. Auranen K, Rinta-Kokko H, Goldblatt D, Nohynek H, O'Brien KL, Satzke C, Simell B, Tanskanen A, Käyhty H; Pneumococcal Carriage Group (PneumoCarr). Colonisation endpoints in Streptococcus pneumoniae vaccine trials. Vaccine 2013; 32:153-8; PMID:24016803; http://dx.doi.org/10.1016/j.vaccine.2013.08.061
9. Weil-Olivier C, Gaillat J. Can the success of pneumococcal conjugate vaccines for the prevention of pneumococcal diseases in children be extrapolated to adults? Vaccine 2014; 32:2022-6; PMID:24565755; http://dx.doi.org/10.1016/j.vaccine.2014.02.008
10. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, Elvin L, Ensor KM, Hackell J, Siber G, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J 2000; 19:187-95; PMID:10749457; http://dx.doi.org/10.1097/00006454-200003000-00003
11. O'Brien KL, Moulton LH, Reid R, Weatherholtz R, Oski J, Brown L, Kumar G, Parkinson A, Hu D, Hackell J, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. Lancet 2003; 362:355-61; PMID:12907008
12. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med 2003; 349:1341-8; PMID:14523142; http://dx.doi.org/10.1056/NEJMoa035060
13. Nahm MH, Olander JV, Magyarlaki M. Identification of cross-reactive antibodies with low opsonophagocytic activity for Streptococcus pneumoniae. J Infect Dis 1997; 176:698-703; PMID:9291318; http://dx.doi.org/10.1086/514093
14. Gomez-Lus ML, Giménez MJ, Vázquez JA, Aguilar L, Anta L, Berrón S, Laguna B, Prieto J. Opsonophagocytosis versus complement bactericidal killing as effectors following Neisseria meningitidis group C vaccination. Infection 2003; 31:51-4; PMID:12590334; http://dx.doi.org/10.1007/s15010-002-3128-4
15. Lee LH, Frasch CE, Falk LA, Klein DL, Deal CD. Correlates of immunity for pneumococcal conjugate vaccines. Vaccine 2003; 21:2190-6; PMID:12706710; http://dx.doi.org/10.1016/S0264-410X(03)00025-2
16. Balloch A, Licciardi PV, Leach A, Nurkka A, Tang ML. Results from an inter-laboratory comparison of pneumococcal serotype-specific IgG measurement and critical parameters that affect assay performance. Vaccine 2010; 28:1333-40; PMID:19932671; http://dx.doi.org/10.1016/j.vaccine.2009.11.011
17. Deloria Knoll M, Park DE, Johnson TS, Chandir S, Nonyane BA, Conklin L, Fleming-Dutra KE, Loo JD, Goldblatt D, Whitney CG, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on immunogenicity. Pediatr Infect Dis J 2014; 33 Suppl 2:S119-29; PMID:24336054; http://dx.doi.org/10.1097/INF.0000000000000079
18. Pichichero ME. Protein carriers of conjugate vaccines: characteristics, development, and clinical trials. Hum Vaccin Immunother 2013; 9:2505-23; PMID:23955057; http://dx.doi.org/10.4161/hv.26109
19. Esposito S, Pugni L, Bosis S, Proto A, Cesati L, Bianchi C, Cimino C, Mosca F, Principi N. Immunogenicity, safety and tolerability of heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 11 months post-natally to pre- and full-term infants. Vaccine 2005; 23:1703-8; PMID:15705475; http://dx.doi.org/10.1016/j.vaccine.2004.09.029

20. Deloria Knoll M, Park DE, Johnson TS, Chandir S, Nonyane BA, Conklin L. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on immunogenicity. Pediatr Infect Dis J 2014; 33 Suppl 2:S119-29; PMID:24336054; http://dx.doi.org/10.1097/INF.0000000000000079
21. Cortese MM, Wolff M, Almeido-Hill J, Reid R, Ketcham J, Santosham M. High incidence rates of invasive pneumococcal disease in the White Mountain Apache population. Arch Intern Med 1992; 152:2277-82; PMID:1444688; http://dx.doi.org/10.1001/archinte.1992.00400230087015
22. Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, Slack M, Ladhani SN, Miller E, Goldblatt D. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis 2014; 14:839-46; PMID:25042756; http://dx.doi.org/10.1016/S1473-3099(14)70822-9
23. Ruiz-Aragón J, Márquez Peláez S, Molina-Linde JM, Grande-Tejada AM. Safety and immunogenicity of 13-valent pneumococcal conjugate vaccine in infants: a meta-analysis. Vaccine 2013; 31:5349-58; http://dx.doi.org/10.1016/j.vaccine.2013.09.008
24. Melin M, Jarva H, Siira L, Meri S, Kayhty H, Vakevainen M. *Streptococcus pneumoniae* capsular serotype 19F is more resistant to C3 deposition and less sensitive to opsonophagocytosis than serotype 6B. Infect Immun 2009; 77:676-84; PMID:19047408; http://dx.doi.org/10.1128/IAI.01186-08
25. Pichichero ME. Otitis media. Pediatr Clin North Am 2013; 60:391-407; PMID:23481107; http://dx.doi.org/10.1016/j.pcl.2012.12.007
26. Cardinale F, Cappiello AR, Mastrototaro MF, Pignatelli M, Esposito S. Community-acquired pneumonia in children. Early Hum Dev 2013; 89 Suppl 3:S49-52; PMID:23962481; http://dx.doi.org/10.1016/j.earlhumdev.2013.07.023
27. Mehr S, Wood N. Streptococcus pneumoniae – a review of carriage, infection, serotype replacement and vaccination. Paediatr Respir Rev 2012; 13:258-64; PMID:23069126; http://dx.doi.org/10.1016/j.prrv.2011.12.001
28. Weil-Olivier C, van der Linden M, de Schutter I, Dagan R, Mantovani L. Prevention of pneumococcal diseases in the post-seven valent vaccine era: a European perspective. BMC Infect Dis 2012; 12:207; PMID:22954038; http://dx.doi.org/10.1186/1471-2334-12-207
29. Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, Takala A, Käyhty H, Karma P, Kohberger R, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001; 344:403-9; PMID:11172176; http://dx.doi.org/10.1056/NEJM200102083440602
30. Jokinen JT, Ahman H, Kilpi TM, Mäkelä PH, Käyhty MH. Concentration of antipneumococcal antibodies as a serological correlate of protection: an application to acute otitis media. J Infect Dis 2004; 190:545-50; PMID:15243930; http://dx.doi.org/10.1086/422531
31. Ekström N, Väkeväinen M, Verho J, Kilpi T, Käyhty H. Functional antibodies elicited by two heptavalent pneumococcal conjugate vaccines in the Finnish Otitis Media Vaccine Trial. Infect Immun 2007; 75:1794-800; PMID:17261612; http://dx.doi.org/10.1128/IAI.01673-06
32. Goldblatt D, Ramakrishnan M, O'Brien K. Using the impact of pneumococcal vaccines on nasopharyngeal carriage to aid licensing and vaccine implementation; A Pneumocarr meeting report March 27-28, 2012, Geneva. Vaccine 2013; 32:146-52; PMID:23933374; http://dx.doi.org/10.1016/j.vaccine.2013.06.040
33. Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 2012; 11:841-55; PMID:22913260; http://dx.doi.org/10.1586/erv.12.53
34. Gray BM, Converse GM 3rd, Dillon HC Jr. Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980; 142:923-33; PMID:7462701; http://dx.doi.org/10.1093/infdis/142.6.923
35. Sleeman KL, Daniels L, Gardiner M, Griffiths D, Deeks JJ, Dagan R, Gupta S, Moxon ER, Peto TE, Crook DW. Acquisition of Streptococcus pneumoniae and nonspecific morbidity in infants and their families: a cohort study. Pediatr Infect Dis J 2005; 24:121-7; PMID:15702039; http://dx.doi.org/10.1097/01.inf.0000151030.10159.b1
36. Hill PC, Cheung YB, Akisanya A, Sankareh K, Lahai G, Greenwood BM, Adegbola RA. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian infants: a longitudinal study. Clin Infect Dis 2008; 46:807-14; PMID:18279039; http://dx.doi.org/10.1086/528688
37. Syrjänen RK, Herva EE, Mäkelä PH, Puhakka HJ, Auranen KJ, Takala AK, Kilpi TM. The value of nasopharyngeal culture in predicting the etiology of acute otitis media in children less than two years of age. Pediatr Infect Dis J 2006; 25:1032-6; http://dx.doi.org/10.1097/01.inf.0000241097.37428.1d
38. Kamme C, Lundgren K, Märdh PA. The aetiology of acute otitis media in children. Occurrence of bacteria, L forms of bacteria and mycoplasma in the middle ear exudate. Relationship between bacterial findings in the middle ear exudate, nasopharynx and throat. Scand J Infect Dis 1971; 3:217-23; PMID:4399782; http://dx.doi.org/10.3109/inf.1971.3.issue-3.07
39. Eldan M, Leibovitz E, Piglansky L, Raiz S, Press J, Yagupsky P, Leiberman A, Dagan R. Predictive value of pneumococcal nasopharyngeal cultures for the assessment of nonresponsive acute otitis media in children. Pediatr Infect Dis J 2000; 19:298-03; PMID:10783018; http://dx.doi.org/10.1097/00006454-200004000-00007
40. Myint TT, Madhava H, Balmer P, Christopoulou D, Attal S, Menegas D, Sprenger R, Bonnet E. The impact of 7-valent pneumococcal conjugate vaccine on invasive pneumococcal disease: a literature review. Adv Ther 2013; 30:127-51; PMID:23397399; http://dx.doi.org/10.1007/s12325-013-0007-6
41. Loo JD, Conklin L, Fleming-Dutra KE, Deloria Knoll M, Park DE, Kirk J, Goldblatt D, O'Brien KL, Whitney CG. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on prevention of pneumonia. Pediatr Infect Dis J 2014; 33 Suppl 2: S140-51; PMID:24336056; http://dx.doi.org/10.1097/INF.0000000000000082
42. Principi N, Baggi E, Esposito S. Prevention of acute otitis media using currently available vaccines. Future Microbiol 2012; 7:457-65; PMID:22439723; http://dx.doi.org/10.2217/fmb.12.23
43. Fleming-Dutra KE, Conklin L, Loo JD, Knoll MD, Park DE, Kirk J, Goldblatt D, Whitney CG, O'Brien KL. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type nasopharyngeal carriage. Pediatr Infect Dis J 2014; 33 Suppl 2:S152-60; PMID:24336057; http://dx.doi.org/10.1097/INF.0000000000000083
44. Cohen R, Levy C, Bingen E, Koskas M, Nave I, Varon E. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. Pediatr Infect Dis J 2012; 31:297-301; PMID:22330166; http://dx.doi.org/10.1097/INF.0b013e318247ef84
45. Loughlin AM, Hsu K, Silverio AL, Marchant CD, Pelton SI. Direct and indirect effects of PCV13 on nasopharyngeal carriage of PCV13 unique pneumococcal serotypes in Massachusetts' children. Pediatr Infect Dis J 2014; 33:504-10; PMID:24670957; http://dx.doi.org/10.1097/INF.0000000000000279
46. Ghaffar F, Barton T, Lozano J, Muniz LS, Hicks P, Gan V, Ahmad N, McCracken GH Jr. Effect of 7-valent pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* in the first 2 years of life. Clin Infect Dis 2004; 39:930-8; PMID:15472842; http://dx.doi.org/10.1086/423379
47. Hennessy TW, Singleton, Bulkow LR, Bruden DL, Hurlburt DA, Parks D, Moore M, Parkinson AJ, Schuchat A, Butler JC. Impact of heptavalent pneumococcal conjugate vaccine on invasive disease, antimicrobial resistance and colonization in Alaska Natives: progress towards elimination of a healthy disparity. Vaccine 2005; 23:5464-73; PMID:16188350; http://dx.doi.org/10.1016/j.vaccine.2005.08.100
48. Huang S, Platt R, Rifas-Shiman SL, Pelton SI, Goldmann S, Finkelstein JA. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics 2005; 116: e408-e413; PMID:16140686; http://dx.doi.org/10.1542/peds.2004-2338
49. Mbelle N, Huebner RE, Wasas AD, Kimura A, Chang I, Klugman KP. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. J Infect Dis 1999; 180:1171-6; PMID:10479145; http://dx.doi.org/10.1086/315009
50. O'Brien KL, Millar EV, Zell ER, Bronsdon M, Weatherholtz R, Reid R, Becenti J, Kvamme S, Whitney CG, Santosham M. Effect of pneumococcal conjugate vaccine on nasopharyngeal colonization among immunized and unimmunized children in a community-randomized trial. J Infect Dis 2007; 196:1211-20; PMID:17955440; http://dx.doi.org/10.1086/521833
51. Centers for Disease Control and Prevention (CDC). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease–United States, 1998-2003. MMWR Morb Mortal Wkly Rep 2005; 54:893-7; PMID:16163262
52. Dagan R. Serotype replacement in perspective. Vaccine 2009; 27(Suppl. 3):c22-4; PMID:19545935; http://dx.doi.org/10.1016/j.vaccine.2009.06.004
53. Principi N, Esposito S. Use of the 13-valent pneumococcal conjugate vaccine in infants and young children. Expert Opin Biol Ther 2012; 12:641-8; PMID:22397739; http://dx.doi.org/10.1517/14712598.2012.670217
54. Dagan R, Givon-Lavi N, Zamir O, Fraser D. Effect of a nonavalent conjugate vaccine on carriage of antibiotic-resistant Streptococcus pneumoniae in day-care centers. Pediatr Infect Dis J 2003; 22:532-40; PMID:12799510
55. Dagan R, Givon-Lavi N, Porat N, Greenberg D. The effect of an alternative reduced-dose infant schedule and a second year catch-up schedule with 7-alent pneumococcal conjugate vaccine on pneumococcal carriage: a randomized controlled trial. Vaccine 2012; 30:5132-40; PMID:22683519; http://dx.doi.org/10.1016/j.vaccine.2012.05.059
56. Rinta-Kokko H, Dagan R, Givon-Lavi N, Auranen K. Estimation of vaccine efficacy against acquisition of pneumococcal carriage. Vaccine 2009; 27:3831-7; PMID:19490983; http://dx.doi.org/10.1016/j.vaccine.2009.04.009
57. Scott P, Herzog SA, Auranen K, Dagan R, Low N, Egger M, Heijne JC. Timing of bacterial carriage sampling in vaccine trials: a modelling study. Epidemics 2014; 9:8-17; PMID:25480130; http://dx.doi.org/10.1016/j.epidem.2014.08.003
58. Auranen K, Rinta-Kokko H, Halloran ME. Estimating strain-specific and overall efficacy of polyvalent vaccines against recurrent pathogens from a cross-sectional study. Biometrics 2013; 69:235-44; PMID:23379663; http://dx.doi.org/10.1111/j.1541-0420.2012.01826.x
59. Cvitkovic Spik V, Beovic B, Pokorn M, Drole Torkar A, Vidmar D, Papst L, Seme K, Kogoj R, Müller Premru M. Improvement of pneumococcal pneumonia diagnostics by the use of rt-PCR on plasma and respiratory samples. Scand J Infect Dis 2013; 45:731-7; PMID:23826792; http://dx.doi.org/10.3109/00365548.2013.804631

60. O'Brien KL, Nohynek H. World health organization pneumococcal vaccine trials carriage working group. Report from a WHO working group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J 2003; 22:133-40
61. Principi N, Terranova L, Zampiero A, Manzoni F, Senatore L, Rios WP, Esposito S. Oropharyngeal and nasopharyngeal sampling for the detection of adolescent *Streptococcus pneumoniae* carriers. J Med Microbiol 2014; 63:393-8; PMID:24298049; http://dx.doi.org/10.1099/jmm.0.068726-0
62. Trzciński K, Bogaert D, Wyllie A, Chu ML, van der Ende A, Bruin JP, van den Dobbelsteen G, Veenhoven RH, Sanders EA. Superiority of trans-oral over trans-nasal sampling in detecting *Streptococcus pneumoniae* colonization in adults. PLoS One 2013; 8:e60520; PMID:23555985; http://dx.doi.org/10.1371/journal.pone.0060520
63. Principi N, Terranova L. Zampiero A, Montinaro V, Ierardi V, Peves Rios W, Pelucchi C, Esposito S. Pharyngeal colonisation by *Streptococcus pneumoniae* in older children and adolescents in a geographical area characterised by relatively limited pneumococcal vaccination coverage. Pediatr iinfect Dis J 2014; in press; PMID:25760567
64. Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, Slack M, Ladhani SN, Miller E, Goldblatt D. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis 2014; 14:839-46; PMID:25042756; http://dx.doi.org/10.1016/S1473-3099(14)70822-9