Impact of protein D-containing pneumococcal conjugate vaccines on non-typeable Haemophilus influenzae acute otitis media and carriage

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ABSTRACT

Introduction: Protein D-containing vaccines may decrease acute otitis media (AOM) burden and nasopharyngeal carriage of non-typeable Haemophilus influenzae (NTHi). Protein D-containing pneumococcal conjugate vaccine PHID-CV (Synflorix, GSK Vaccines) elicits robust immune responses against protein D. However, the phase III Clinical Otitis Media and Pneumonia Study (COMPAS), assessing PHID-CV efficacy against various pneumococcal diseases, was not powered to demonstrate efficacy against NTHi; only trends of protective efficacy against NTHi AOM in children were shown.

Areas covered: This review aims to consider all evidence available to date from pre-clinical and clinical phase III studies together with further evidence emerging from post-marketing studies since PHID-CV has been introduced into routine clinical practice worldwide, to better describe the clinical utility of protein D in preventing AOM due to NTHi and its impact on NTHi nasopharyngeal carriage.

Expert commentary: Protein D is an effective carrier protein in conjugate vaccines and evidence gathered from pre-clinical, clinical and observational studies suggest that it also elicits immune response that can help to reduce the burden of AOM due to NTHi. There remains a need to develop improved vaccines for prevention of NTHi disease, which could be achieved by combining protein D with other antigens.

1. Introduction

Acute otitis media (AOM) is one of the most frequent childhood diseases. In 2005, it had an estimated global incidence of 10.9% of the population, with 51% of the cases occurring in children <5 years of age [1]. Although there is a spectrum of bacterial pathogens that can cause AOM, Streptococcus pneumoniae and non-typeable Haemophilus influenzae (NTHi) are by far the most common causes of bacterial AOM. Pneumococcal conjugate vaccines (PCVs) may help reduce the burden of bacterial AOM due to pneumococci. Three PCVs have been licensed and incorporated in mass vaccination programs: a seven-valent vaccine (7vCRM; Prevenar™, Pfizer) first licensed in 2000; a ten-valent vaccine (PHiD-CV; Synflorix, GSK Vaccines) licensed in 2008; and a 13-valent vaccine (13vCRM; Prevenar13™, Pfizer) licensed in 2009. Of these, PHID-CV is the only one which uses protein D, a non-lipidated form of a 42 kDa cell-surface protein of H. influenzae, as carrier protein for 8 of the 10 pneumococcal serotypes (1, 4, 5, 6B, 7F, 9V, 14, and 23F). Inclusion of protein D in PHiD-CV offered the potential to extend protection against AOM to include NTHi disease, and PHID-CV is licensed in a number of countries for active immunization against AOM caused by NTHi [2-4].

This review focuses on the latest data with protein D-containing vaccines in the context of the previous body of evidence from preclinical, double-blind randomized clinical, and observational studies and aims to better describe the role of this antigen in the prevention of AOM due to NTHi and its impact on NTHi nasopharyngeal carriage.

2. NTHi as a pathogen

H. influenzae, a small Gram-negative coccobacillus with both encapsulated and non-encapsulated strains, is a common commensal that colonizes the nasopharynx of children and adults [5,6]. Among the six types of encapsulated strains (types a–f), type b (Hib) was shown to be responsible for approximately 95% of systemic infections associated with H. influenzae. Over 90% of Hib disease occurs in children below 5 years of age, and the main clinical manifestations of invasive Hib disease are meningitis (frequently associated with severe neurological sequelae), pneumonia, epiglottitis (mainly in industrialized countries), septicemia, cellulitis, and osteoarticular infections [7]. The introduction of Hib conjugate vaccines in child immunization programs resulted in a dramatic decrease in the incidence of invasive Hib infections [8].

Some H. influenzae strains that do not produce a polysaccharide capsule are described as non-encapsulated or NTHi strains. The various phenotypic and genotypic NTHi strains are commonly associated with and implicated as pathogens in respiratory diseases including otitis media (OM), conjunctivitis, sinusitis, pneumonia, cystic fibrosis, persistent bacterial bronchiolitis, and chronic suppurative lung disease (CSDL) [6,9]. Although a causal link has not been established, NTHi...
colonization has frequently been associated with acute exacerbations of respiratory symptoms in patients with chronic obstructive pulmonary disease (COPD) that are often triggered by infection with bacteria or viruses [10,11]. Moreover, NTHi is a commonly identified pathogen in recurrent OM, chronic OM, and cases of antibiotic treatment failure. Although less invasive than the encapsulated types, NTHi can also cause invasive disease, mainly in immunocompromised hosts such as premature infants, elderly persons, or immunosuppressed individuals [6].

The successful implementation of the Hib vaccine caused some concern that other non-type b H. influenzae strains would replace Hib as a cause of invasive disease. In the US and several European countries, a small but steady increase in the incidence of non-Hib invasive infections was reported over the past years and NTHi became the most common cause of these infections in the era after the introduction of Hib vaccination [6]. While the burden of invasive H. influenzae disease shifted toward older adults, most commonly manifesting as bacteremia [12], significant numbers of cases continue to be reported in infants and children.

3. The role of NTHi in AOM

Compared with other manifestations of pneumococcal and NTHi diseases, AOM is usually a mild disease, although it can cause considerable pain and discomfort for the child and can involve multiple visits to the physician. In developed countries, AOM is the most common reason for antibiotics administration in infants and young children [13]. Systematic reviews of the outcomes of antibiotic therapy suggest that in high-income countries, most cases of AOM would eventually resolve without antibiotic treatment [14]. Nevertheless, 5–26% of children with AOM experience recurrent episodes [15,16] and, although rare, serious complications and sequelae can occur [17]. The most frequent of these complications are extracranial complications including chronic suppurative OM, facial nerve paralysis, mastoiditis with subperiosteal abscess, and hearing impairment. Intracranial complications including brain abscess, sigmoid sinus thrombosis, and meningitis, caused by the extension of infection from the middle ear into the central nervous system, are uncommon but life-threatening and potentially followed by permanent neurologic sequelae, including seizures and behavioral disorders. Annually, 21,000 deaths across all age groups have been estimated globally to be due to OM complications; the highest mortality levels are estimated in children below 5 years of age (85.4 and 90.5 per 10 million in the first year of life and in children 1–4 years of age, respectively) and in individuals older than 75 years (160.5 per 10 million) [1].

The pathophysiology of AOM typically involves an initial upper respiratory tract viral infection or allergy leading to dysfunction of the Eustachian tube, with subsequent stasis, inflammation, and bacterial infection or invasion of the middle ear [18]. NTHi strains have been isolated from both nasopharynx and middle ear fluid during AOM episodes [19] with identical sequence type in 84% of the studied children 6–30 months of age, suggesting a progression from nasopharyngeal carriage to disease. However, although the concordance between nasopharyngeal and middle ear fluid samples is reported to be high, the positive predictive value of an NTHi-positive nasopharyngeal sample for a paired middle ear fluid sample was lower at around 50% [20]. Thus, NTHi nasopharyngeal carriage does not appear to correlate well with disease.

Prospective studies on the etiology of uncomplicated AOM are uncommon since tympanocentesis is not a routine procedure in clinical practice and is recommended mainly in complicated cases and treatment failures. Etiology is therefore mostly available for more severe AOM or AOM with complications such as otorrhea or requiring tympanostomy tube placement [21,22]. Although there is a spectrum of bacterial pathogens that can cause AOM, S. pneumoniae and NTHi have been isolated in approximately 20–70% of bacterial AOM cases prior to introduction of vaccines that target these pathogens [13]. A systematic review including four studies performed in the US, one in Finland, and one in the Netherlands that assessed pathogens causing AOM in children up to 12 years of age found that S. pneumoniae and H. influenzae were isolated in a similar proportion of cases in the pre-PCV era (33–48% for S. pneumoniae and 41–43% for H. influenzae) [23]. The findings of a systematic review of AOM epidemiology in children <6 years of age from Latin America and the Caribbean were similar, reporting that S. pneumoniae was isolated in 32.4% of the cases and H. influenzae in 26.0% of the cases (including NTHi in 18.3%) [24]. However, prevalence of NTHi appears to be higher in certain populations. For example, in Indigenous Australian children, a population with high incidence of AOM, H. influenzae was the dominant pathogen, being detected by quantitative PCR in 89% of the analyzed paired ear discharge swabs and representing up to 68% of the estimated total bacterial load [25].

The presence of NTHi in AOM cases is associated with recurrence, treatment failure, and middle ear effusion [6]. A number of virulence factors helping NTHi to evade the host immune system and to opportunistically invade host sites have been described. NTHi has been shown to have the capacity to resist the humoral immune system and to colonize respiratory epithelial cells by adhesion followed by invasion and intracellular localization [26–30]. Another virulence determinant implicated in the pathogenesis of NTHi infections is its capacity to form biofilms, as has now been demonstrated both in vitro and in vivo [31,32]. This mechanism contributes to the resistance of NTHi to antibiotics, due to the limited capacity of antibiotics to penetrate the biofilm, reduced bacterial metabolism within the biofilm, or limited oxygen availability [33–35]. Additional factors involved in the evasion of the host immune system are genetic polymorphism, heterogeneous gene expression, and variable phenotype (including the diversity of its lipooligosaccharides [36] and virulence factors critical for defense against complement-mediated killing) [37,38].

4. Impact of 7vCRM on AOM etiology

Vaccines that prevent AOM would offer important clinical and public health benefits due to the high cost of AOM, the disease burden of recurrent and chronic AOM, and costs of antibiotic prescriptions [39,40]. 7vCRM, a 7-valent CRM197-
conjugated PCV, was gradually introduced across the world since 2000, starting in the US. Reductions in the incidence of AOM were anticipated because efficacy of 7vCRM against clinically diagnosed and pneumococcal vaccine-type AOM had been demonstrated in the randomized, double-blind Finnish Otitis Media Vaccine Trial (FinOM) [41]. However, there was also some uncertainty with respect to the magnitude of the reductions in AOM because the FinOM study suggested that reductions in vaccine-type disease could be partly compensated by increases in AOM due to H. influenzae as well as non-vaccine pneumococcal serotypes [41].

After 7vCRM introduction in routine vaccination programs, observational studies in the US have identified increases in the proportions of NTHi among AOM cases. Between two convenience samples (1992–1998 and 2000–2003) of ambulatory patients who required culture of middle ear fluid, the relative proportion of isolates positive for NTHi increased from 41% (137/336 isolates) to 56% (46/83 isolates) [42]. Similar trends were reported in a 9-year prospective study performed in a suburban, community-based private practice in the US. The study enrolled 551 children who had undergone tympanocentesis after experiencing antibiotic treatment failure for AOM, defined as AOM diagnosed within 30 days after 1–2 empiric antimicrobial treatment courses, or nonresponse to antibiotics after 48 h on treatment [22]. Across three time-points (1995–1997, 1998–2000, and 2001–2003) spanning the period of 7vCRM introduction in the US in 2000, there were significant changes in the proportion of both S. pneumoniae, which decreased (50/103 isolates [48%], 50/114 [44%], and 28/89 [31%]), and H. influenzae, which increased over time (39/103 [38%], 49/114 [43%], and 51/89 [57%]) [22]. However, these prevalence rates were likely also affected by other changes in clinical practice in that period, namely the change in antibiotic treatment guidelines: in 1995–1997, a standard dose of amoxicillin was recommended, which was increased to a high dose of amoxicillin in 1998; thus, the 2001–2003 period saw a combination of high-dose amoxicillin and 7vCRM.

In each study, an increase in the proportion of AOM due to β-lactamase-positive NTHi [22,42] was observed after introduction of 7vCRM, but it was not determined whether the increase in prevalence of NTHi AOM translated into an increase in incidence. Similarly, in Costa Rica, H. influenzae (mainly NTHi) became more prevalent post-7vCRM introduction (118/456 episodes [25.9%] post-7vCRM [2009–2010] vs. 181/884 [20.5%] pre-7vCRM [1999–2004]; p < 0.001); 19.5% of H. influenzae isolates were β-lactamase-positive [43].

In contrast, observational studies in Greece and Israel reported decreases in AOM incidences due to NTHi in addition to the expected reductions in pneumococcal AOM and suggested that a causal relationship could be inferred from the temporal association of NTHi AOM reductions and 7vCRM use [44,45]. In children up to 14 years of age in Greece, the number of cases/10,000 hospital admissions of otorrhea due to H. influenzae reduced from 20 to 16 (p < 0.001) between the pre- and post-7vCRM study periods. However, data on potential changes in incidence before PCV introduction were not reported [45]. In Southern Israel, evidence from a population-based study showed that among children <3 years old diagnosed with OM requiring tympanocentesis, reductions in incidence of AOM due to S. pneumoniae following introduction of PCVs were accompanied by reductions in cases due to NTHi [44].

Separate hypotheses have been developed in attempt to explain either the increases or decreases in incidence of NTHi AOM observed in each of these studies. Increases in NTHi have been considered to be the evidence that eliminating vaccine-type S. pneumoniae not only provides the opportunity for replacement with non-vaccine-type S. pneumoniae but also other potential pathogens. In settings where NTHi decreases have occurred, it has been proposed that S. pneumoniae infections predispose children to subsequent infections by NTHi and that preventing episodes of pneumococcal AOM would also reduce NTHi AOM [46]. These two hypotheses appear to be mutually exclusive but it has been difficult to corroborate either possibility, largely due to the observational nature of post-marketing studies. A post-hoc analysis of the FinOM trial, which as a randomized controlled trial is not subject to the inherent biases of observational studies, attempted to resolve this question. A first key finding was that NTHi AOM was more often reported in the 7vCRM-vaccinated group than in the control group regardless of the age at onset, but particularly in the second year of life. Secondly, there was a trend toward decreasing vaccine efficacy (VE) against overall AOM with each additional recurring episode of AOM [47]. Each of these post-hoc analyses findings points toward disease replacement and seems to demonstrate that preventing first episodes of pneumococcal AOM does not reduce the susceptibility of children to disease caused by other pathogens.

Regardless of changes in incidence of NTHi AOM in the 7vCRM era, a consistent theme from all studies in various settings was that NTHi remained a key pathogen in AOM.

5. Protein D as a vaccine antigen

Protein D is a surface-exposed outer membrane lipoprotein identified in all H. influenzae strains [48], including NTHi, with an enzymatic activity similar to Escherichia coli’s glycerophosphodiester phosphodiesterase [49,50]. It is highly conserved among the various H. influenzae strains [48], with >99% identical sequence on both nucleotide and deduced amino acid levels, and relatively evenly distributed substitutions across the gene [51]. Surface exposure and sequence conservation made protein D a promising candidate as for a vaccine antigen.

The phosphodiesterase activity of protein D decorates NTHi with phosphorylcholine scavenged from the host epithelial cells [52,53]. Lipooligosaccharides with incorporated phosphorylcholine bind the platelet-activating factor of bronchial epithelial cells, triggering a host cell signaling cascade and bacterial invasion [54,55]. Evidence that protein D can act as a virulence factor, both participating in the disease process and promoting evasion of immune responses, further supports its use in vaccines as it implies that neutralizing protein D function will interfere with the disease process and selecting for variants lacking expression will render them less fit.

A protein D-negative mutant of NTHi has been shown to be overall less fit than its parental strain [56]. The NTHi strain with a nonpolar deletion of the hpd gene was less efficient at
assimilating phosphorylcholine into biofilms, its growth was sensitive to the availability of L-glycerophosphocholine (a common lipid catabolite), and the strain was less adherent to epithelial cells than the parent strain when assessed in vitro. The adherence deficit of the *hpd*-lacking strain was reproduced in the middle ear epithelium of a chinchilla model of OM but not in the nasopharynx, suggesting that there could be compartmental differences in adherence factors [56]. Additionally, more inflammation was observed in the middle ears of animals challenged with the mutant NTHi strain compared to the parental strain. The authors suggested that (vaccine-elicited) antibodies against protein D could have a similar effect as deleting the *hpd* gene: reducing bacterial adherence and fitness and rendering NTHi more susceptible to host immune responses [56].

Early signs that protein D was immunogenic were the detection of serum IgG and IgA antibodies to protein D in the sera of rats recovering from AOM induced by aural inoculation with NTHi and the detection of salivary anti-protein D IgA antibodies after intranasal inoculation with recombinant protein [57]. Moreover, this immune response appeared functional as the sera from rats which had been immunized with a non-acylated form of recombinant protein D not only contained IgG and IgA antibodies against protein D, but also bactericidal activity against NTHi [58]. The potential protective effect of antibodies against protein D was further studied in rat models of middle ear clearance and pulmonary clearance of NTHi. For both models, the rats received protein D on day 0 via intestinal Peyer’s patches with a tracheal booster on day 14 [59] and an intra-bulbar or pulmonary challenge (according to the rat model and the study) on day 21. In each case there tended to be increased clearance of NTHi in the vaccinated group compared with the control group [59]. Furthermore, a highly reproducible chinchilla model of viral–bacterial super-infection for the study of middle ear infections such as OM that can mimic the natural course of the disease in children was developed [60]. In two separate active-immunization studies, Bakalez et al. showed that the administration of a protein D antigen increased the clearance of NTHi from the middle ear after intranasal challenge with NTHi [60]. In the same model, clearance of NTHi from the nasopharynx was only observed when protein D was administered in combination with P5-fimbriin, but not when given as a single antigen. This suggests that the requirements for prevention of nasopharyngeal carriage could be different from those for preventing disease.

Protein D also elicits an immune response in humans, as antibodies against protein D can be observed after natural exposure to NTHi: IgM antibodies against protein D were found in the sera of 6-month-old infants, while IgG and IgA antibody levels increased after the age of 1 year and maintained an ascending trend until the age of 20 years, after which they decreased [58]. Pichichero et al. prospectively studied the prevalence of natural antibodies to three NTHi outer membrane proteins [61]. In non-otitis-prone children without previous AOM episodes, serum antibodies to protein D increased gradually from 6 to 30 months of age (p < 0.001), with significantly higher levels being detected in sera of children colonized with NTHi at the time of tympanostomy as compared to those not colonized. During both the acute and convalescent phase of AOM, both IgM and IgG antibodies were identified, suggesting that exposure to NTHi may have occurred in these children prior to episodes of AOM. Anti-protein D antibodies from sera of a proportion of children who had experienced an episode of NTHi AOM had bactericidal activity against NTHi strains homologous to the infecting strain (observed for 7 of the 11 tested sera) [61]. A potentially interesting observation was that bactericidal activity in otitis-prone children was to a lesser degree directed to protein D, with bactericidal activity for anti-protein D antibodies observed for only 3 of 21 tested sera [62]. Wiertsema et al. also studied immune responses in children <36 months of age with recurrent AOM in a population of West Australian children. The otitis-prone group of children assessed in this study had significantly higher naturally acquired IgG antibody levels against protein D as well as other NTHi antigens, compared to healthy controls [63]. This suggests that the immune response to NTHi in children with recurrent AOM is not impaired, and that a vaccine containing NTHi antigens might be beneficial for these children [63].

### 5.1. Protein D as a carrier protein in PCVs: protection against NTHi infection in animal models

The first vaccine formulations containing protein D that were selected for assessment in clinical development programs were PCVs developed by GSK Vaccines, in which protein D was utilized as a carrier protein for pneumococcal polysaccharide antigens. The predominant basis for choosing protein D as the carrier for the 11-valent predecessor formulation 11Pn-PD and for PHID-CV (licensed formulation) was its ability to stimulate T-cell immunity with minimized risk of interference from concomitantly administered vaccines. Interference is defined as a decrease in the antibody response to a vaccine antigen through concomitant exposure to a similar antigen from another vaccine. This was an important consideration in designing PHID-CV, as interference had been observed during development of PCVs using another (tetanus toxoid) carrier [64]. However, as the preclinical evidence described above suggested that the protein D carrier could itself act as a vaccine antigen, it was also considered that such a strategy could potentially help to prevent NTHi infections.

In an OM chinchilla model, the administration of a protein D antigen had been shown to increase the clearance of NTHi from the middle ear after intranasal challenge with NTHi [60]. This chinchilla model was also used for initial assessments of NTHi protection using sera from children vaccinated with a protein D-containing vaccine. Serum samples were obtained from toddlers immunized with an investigational four-valent pneumococcal protein D conjugate vaccine (treta-Pn-PD) and a booster of 11Pn-PD, or from infants immunized with MenAC-Hib-PD (containing capsular polysaccharides from *Neisseria meningitidis* conjugated to protein D, and Hib capsular polysaccharide conjugated to tetanus toxoid). Passive immunization with pools of these serum samples protected against NTHi-induced AOM in a chinchilla viral–bacterial co-infection
model; overall protective efficacy was ~34% for tetra-Pn-PD and ~38% for MenAC-Hib-PD (Figure 1(a)) [65]. This same animal model was later used to show that the levels of protection after passive transfer of human sera obtained after immunization with 11-Valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; MenAC-Hib-PD, serum from infants immunized with MenAC-Hib (containing capsular polysaccharides from Neisseria meningitidis conjugated to protein D and *H. influenzae* type b capsular polysaccharide conjugated to tetanus toxoid); Anti-PHiD-CV, serum from children immunized with the licensed 10-valent pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine; Anti-11Pn-PD, serum from children immunized with 11-valent pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine. NTHi, non-typeable *H. influenzae*. a: data from [67]; b: data from [66]. a: data reprinted from Vaccine, Vol 28/Issue 1, Roman Prymula, Pavla Kriz, Eva Kaliskova, Thierry Pascal, Jan Poolman, Lode Schuerman, Effect of vaccination with pneumococcal capsular polysaccharides conjugated to *Haemophilus influenzae*-derived protein D on nasopharyngeal carriage of Streptococcus pneumoniae and *H. influenzae* in children under 2 years of age /p 71–88, 2009, with permission from Elsevier. b: data reprinted from Vaccine, Vol 24/Issue 22, Laura A. Novotny, Joseph A. Jurcisek, Fabrice Godfroid, Jan T. Poolman, Philippe A. Denoël, Lauren O. Bakaletz, Passive immunization with human anti-protein D antibodies induced by polysaccharide protein D conjugates protects chinchillas against otitis media after intranasal challenge with *Haemophilus influenzae* /p 4804–4811, 2006, with permission from Elsevier.
5.2. Clinical efficacy of the pneumococcal NTHi protein D conjugate vaccines against AOM caused by NTHi

In clinical trials, both 11Pn-PD [68,69] and PHiD-CV [70,71] vaccines were shown to have acceptable safety profiles and induce robust immune responses against pneumococcal serotype-specific polysaccharides and protein D. In certain studies, efficacy of these formulations against NTHi AOM [72,73] and nasopharyngeal carriage [67,74–77] was evaluated.

The Pneumococcal Otitis Efficacy Trial (POET) was a large randomized double-blind AOM efficacy trial that for the first time in humans documented the potential of protein D to prevent disease due to NTHi [73]. Totally, 4968 infants (aged 6 weeks to 5 months) were vaccinated with either 11Pn-PD or control (hepatitis A) vaccine, at 3, 4, and 5 months of age and a booster dose between 12 and 15 months of age. Middle ear fluid for bacteriological testing was collected per routine practice by tympanocentesis if the clinical diagnosis of AOM was confirmed by a specialist. The primary end point of the study was efficacy against the first episode of AOM due to vaccine-type S. pneumoniae, and it was met (VE: 52.6%; 95% confidence interval (CI): 35.0, 65.5). A trend toward efficacy against first episodes of AOM caused by NTHi (assessed as confirmatory secondary end point) was observed in the per-protocol cohort (VE: 31.1% [95% CI: –3.7, 54.2]), with efficacy in the intent-to-treat cohort being 32.7% (95% CI: 0.77, 54.3). Efficacy against any NTHi AOM episode was 35.3% (95% CI: 1.8, 57.4) (Table 1) [73].

The Clinical Otitis Media and PneumoniA Study (COMPAS) was a large phase III, double-blind, randomized controlled trial designed to assess PHiD-CV efficacy against various pneumococcal diseases. Infants were randomized to receive either PHiD-CV or control vaccines at 2, 4, 6, and 15–18 months of age [72]. AOM outcomes were assessed in a subset of 73S9 children enrolled in Panama, using clinical case definitions similar to those of POET. Tympanocentesis was performed if the AOM diagnosis was confirmed by a specialist and the presence of middle-ear fluid suspected. Unlike in POET,

### Table 1. Vaccine efficacy against non-typeable *Haemophilus influenzae* acute otitis media and nasopharyngeal carriage.

| Study name and author | Study design | Investigational vaccine | Cohort | Number of children | Number of episodes in the pneumococcal vaccine group | Number of episodes in the control vaccine group | VE against NTHi AOM |
|-----------------------|-------------|-------------------------|--------|-------------------|-----------------------------------------------|---------------------------------|-------------------|
| **NTHi AOM**          |             |                         |        |                   |                                               |                                 |                   |
| POET                  | Randomized  | 11Pn-PD                 | PP     | 4907              | 39<sup>a</sup>                             | 56<sup>a</sup>                  | 31.1%             |
|                      |             |                         | ITT    | 4968              | (N = 2455)                                | (N = 2452)                       | (95% CI: –3.7, 54.2) |
|                      |             |                         | PP     | 4907              | (N = 2489)                                | (N = 2479)                       | (95% CI: 0.77, 54.3) |
|                      |             |                         |        |                   | 41<sup>b</sup>                             | 35.3%                          |                   |
|                      |             |                         |        |                   |                                               |                                 |                   |
| COMPAS                | Randomized  | PHiD-CV                 | PP     | 5989              | 12<sup>a</sup>                             | 14<sup>a</sup>                  | 15.0%             |
|                      |             |                         | ITT    | 7214              | (N = 3010)                                | (N = 2979)                       | (95% CI: –83.8, 60.7) |
|                      |             |                         | PP     | 5989              | 19<sup>a</sup>                             | 24<sup>a</sup>                  | 21.5%             |
|                      |             |                         | ITT    | 7214              | (N = 3602)                                | (N = 3612)                       | (95% CI: –43.4, 57.0) |
|                      |             |                         |        |                   | 12<sup>b</sup>                             | 15.0%                          |                   |
|                      |             |                         |        |                   |                                               |                                 |                   |
| COMPAS                | Randomized  | PHiD-CV                 | PP     | 5989              | 14<sup>b</sup>                             | 25<sup>b</sup>                  | 24.5%             |
|                      |             |                         | ITT    | 7214              | (N = 3602)                                | (N = 3612)                       | (95% CI: –38.3, 58.8) |

### Table 2. NTHi carriage 3 months after booster dose of a pneumococcal protein D conjugate vaccine

| Author                | Study design | Investigational vaccine | Number of children (ITT cohort for NPC) | Colonization rate in the pneumococcal vaccine group | Colonization rate in the control vaccine group | VE against NTHi nasopharyngeal carriage |
|-----------------------|-------------|-------------------------|----------------------------------------|-----------------------------------------------|---------------------------------|-------------------------------------|
| Prymula et al. [67]   | Randomized  | 11Pn-PD                 | 376                                    | 9.0%                                         | 15.4%                           | 41.4%                               |
|                      |             |                         |                                        | (N = 177)                                    | (N = 175)                        | (95% CI: –4.9, 67.3)                |
|                      |             |                         |                                        | 9.0%                                         | 14.3%                           | 36.7%                               |
|                      |             |                         |                                        | (N = 177)                                    | (N = 175)                        | (95% CI: –14.3, 65.0)               |
| Sáez-Llorens et al. [80]| Randomized | PHiD-CV                 | 1921                                   | 4.0%                                         | 5.5%                            | 27.0%                               |
|                      |             |                         |                                        | (N = 696)                                    | (N = 690)                        | (95% CI: –22.2, 56.8)               |
| Vesikari et al. [77]  | Randomized  | PHiD-CV                 | 5093                                   | 6.5%                                         | 4.9%                            | 34.4%                               |
|                      |             |                         |                                        | (N = 1289)                                   | (N = 1897)                       | (95% CI: –84.0, 2.0)                |
|                      |             |                         |                                        | 3+1 schedule                                 | 4.9%                            | 35.0%                               |
|                      |             |                         |                                        | (N = 1803)                                   | (N = 1897)                       | (95% CI: –80.4, –1.2)               |
| Van den Bergh et al. [75] | Randomized | PHiD-CV /7vCRM           | 780                                    | Colonization                                 | 58.9%                           | 0.5%                                |
|                      |             |                         |                                        | (N = 513)                                    | (N = 257)                        | (95% CI: –21.8, 18.4)               |
|                      |             |                         |                                        | Acquisition                                  | 59.1%                           |                                     |
|                      |             |                         |                                        | (N = 257)                                    |                                 |                                     |
|                      |             |                         |                                        | 15.6%                                         | 17.5%                           | 10.9%                               |
|                      |             |                         |                                        | (N = 513)                                    | (N = 257)                        | (95% CI: –31.3, 38.9)               |
| Prymula et al. [74]   | Open        | PHiD-CV                 | 750                                    | 13.4%                                         | 10.4%                           | 29.3%                               |
|                      |             |                         |                                        | (N = 403)                                    | (N = 328)                        | (95% CI: –93.5, 13.7)               |

<sup>a</sup>First episode/occurrence of AOM; <sup>b</sup>All AOM; <sup>c</sup>After *Haemophilus haemolyticus* discrimination.

AOM: acute otitis media; CI: confidence interval; COMPAS: Clinical Otitis Media and PneumoniA Study; ITT: intent-to-treat cohort; N: total number of children for whom AOM or nasopharyngeal carriage was assessed; NPC: nasopharyngeal carriage; NTHi: non-typeable *Haemophilus influenzae*; PHiD-CV: 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; 11Pn-PD: 11-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; PP: per-protocol cohort; POET: Pneumococcal Otitis Efficacy Trial; 7vCRM: 7-valent pneumococcal conjugate vaccine; VE: vaccine efficacy.
tympanocentesis required additional specific informed consent. Efficacy assessed against first episodes of clinically confirmed AOM was assessed as a first secondary confirmatory end point (VE for per-protocol cohort: 16.1% [95% CI: −1.1, 30.4]); VE for the intent-to-treat cohort was 19.0% (95% CI: 4.4, 31.4). In this study, VE against NTHi AOM was only assessed as a descriptive end point; efficacy against first NTHi AOM episodes was 15.0% (95% CI: −83.8, 60.7) for the per-protocol cohort and 21.5% (95% CI: −43.4, 57.0) for the intent-to-treat cohort (Table 1) [72]. Thus, unlike the POET study which was powered to assess VE against NTHi AOM, the COMPAS trial was not powered for this end point and the limited number of cases captured was a contributing factor to the wide confidence intervals and non-significant outcome.

5.3. Impact of vaccination with pneumococcal NTHi protein D conjugate vaccine on nasopharyngeal carriage of NTHi

The effect of protein D-containing vaccines on nasopharyngeal carriage of NTHi is of interest because, as previously shown with Hib and PCVs, reduction of carriage in immunized persons can prevent the transmission of the pathogen to nonimmunized individuals and would likely amplify vaccine impact via a herd protection effect [8,78]. However, although nasopharyngeal colonization with NTHi is a risk factor for AOM [79], it may not be necessary to prevent colonization in order to have an impact on disease in vaccinees.

In the POET trial, there was a trend toward reduction of culture-confirmed nasopharyngeal carriage of H. influenzae and of NTHi in the 11Pn-PD vaccinated group, with the differences between groups appearing transiently after the booster dose and disappearing by 24 months of age (Table 1 and Figure 2(a)). After discrimination for Haemophilus haemolyticus by PCR, the difference between groups was most evident 3 months after administration of the booster dose (H. influenzae VE: 38.6% [95% CI: −6.3, 64.6]; NTHi VE: 36.7% [95% CI: −14.3, 65.0]) [67]. The occurrence of culture-confirmed nasopharyngeal carriage of H. influenzae and NTHi in Panama for the COMPAS study was much less frequent (4.0–5.7%) than was observed in POET (8.8–16.9%). A trend toward VE against nasopharyngeal carriage of NTHi was observed after the booster dose (Figure 2(b)) and the point estimate was greatest 3 months after the booster dose (NTHi VE: 27.0% [95% CI:

![Figure 2. Effect of an 11-valent (panel a) and a 10-valent (panel b) pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine on the nasopharyngeal carriage of non-typeable Haemophilus influenzae.](http://creativecommons.org/licenses/by/3.0/)
Thus, these two studies share similar trends with respect to nasopharyngeal carriage of NTHi although the difference between groups appeared of smaller magnitude and shorter duration in COMPAS than POET.

The impact of infant vaccination with PHID-CV on nasopharyngeal carriage of NTHi was also evaluated in other clinical trials conducted in Finland, the Netherlands, and the Czech Republic. A large cluster-randomized double-blind invasive pneumococcal disease effectiveness study in Finland (FinIP study) enrolled infants 6 weeks to 6 months of age to receive either PHID-CV or a control (hepatitis) vaccine in either a two-dose (3 and 5 months of age) or three-dose (3, 4, and 5 months of age) primary schedule with a booster dose at 12 months of age [81]. A trial nested within this study assessed the impact of PHID-CV on bacterial nasopharyngeal carriage at timepoints before and up to 12 months after administration of the booster dose [77]. The results suggested that PHID-CV reduced the nasopharyngeal carriage of S. pneumoniae vaccine serotypes regardless of the primary vaccine schedule administered, but there was no difference in occurrence of nasopharyngeal carriage of NTHi between groups 3 months post-booster (VE: 2+1: −34.4% [95% CI: −84.0, 2.0], 3+1: −35.0% [95% CI: −80.4, −1.2]) [77] (Table 1). In another satellite study of FinIP that evaluated indirect effectiveness against nasopharyngeal carriage in older siblings of vaccinated children, indirect effectiveness against NTHi carriage was 9% (95% CI: −25, 34) [82]. No impact of PHID-CV on NTHi carriage was observed in other randomized control trials in the Netherlands (vaccine schedule 2, 3, 4, and 12 months of age) where PHID-CV was compared to 7vCRM in children up to 2 years of age [75], or in the Czech Republic (vaccine schedule 3, 4, 5, and 12–15 months of age) where children were followed up for 12 months after the booster dose [74] (Table 1). Unlike the other studies mentioned here, the Czech Republic study was not randomized for the carriage assessment, as the control group for carriage was age-matched but was only added during the conduct of the trial at the time of the booster.

Lastly, in a double-blind randomized controlled trial in which Kenyan toddlers aged 1–4 years were vaccinated with control vaccine (hepatitis A) or PHID-CV at either month 0 and 2 (group A) or month 0 and 6 (group B) and followed up for 2–6 months after the first dose, there was a small trend toward a reduction in NTHi carriage in both PHID-CV groups compared to the control group from 2 months after the first dose. This reduction was statistically significant in group B at the 6-month timepoint (56% vs. 66%, p = 0.04) [76].

VE against nasopharyngeal carriage of NTHi, when present, has been modest and mostly CIs span zero. Therefore, it can be concluded that if there is a true impact of PHID-CV on NTHi nasopharyngeal carriage, it can only be small and transient in nature. Of note, carriage is currently assessed as presence or absence of the pathogen, but potential changes in density of the pathogen or relative expression of protein D have not yet been investigated. The clinical implications of these observations are not fully understood as the protective effects against AOM due to NTHi by PHID-CV may be independent of the effects on nasopharyngeal carriage. However, a lack of a significant long-lasting effect on nasopharyngeal carriage implies that there might not be an impact on the transmission of NTHi or herd effects; on the flip side, replacement disease is likely to be limited. It is possible that to observe robust reductions in nasopharyngeal carriage, other elements in addition to protein D might be needed, as noted in the chinchilla model in which clearance of NTHi from the nasopharynx was only achieved when protein D was combined with P5-fimbrin [60].

5.4. Post-marketing studies on the impact of pneumococcal NTHi protein D conjugate vaccine on NTHi AOM and carriage

At the time of this review, to our knowledge, one observational study has provided information on the prevalence of NTHi AOM in a PHID-CV-vaccinated population. This cross-sectional survey compared the prevalence of OM in a cohort of PHID-CV-vaccinated Australian Indigenous children with a historical cohort of children who had received 7vCRM. While there was no difference in the overall prevalence of OM between cohorts, the prevalence of the more severe suppurative presentations of disease was significantly lower in the PHID-CV cohort [83,84]. Moreover, for children diagnosed with supplicative disease from whom otopathogens could be cultured, the prevalence of NTHi was significantly lower in the PHID-CV cohort [84]. Although these findings need to be interpreted with the same caution as for other observational studies and cannot be definitively attributed to vaccination, they provide an encouraging sign that protein D-based vaccines may offer some level of protection against disease due to NTHi.

As mentioned previously, population-based studies of the impact of vaccines on AOM are generally based on clinical rather than pathogen-specific diagnoses and this type of study would not be expected to provide further information about the impact of protein D-containing vaccines on AOM due to NTHi. However, the three additional pneumococcal serotypes included in PHID-CV compared to 7vCRM (1, 5, and 7F) do not appear to be major causes of AOM [45,72,85,86], and efficacy against vaccine-type AOM is quite similar between 7vCRM and PHID-CV. Thus, should PHID-CV show additional benefits against AOM in post-marketing studies over and above 7vCRM and if these benefits are not due to its impact on serotypes 1, 5, and 7F, then an alternative explanation could be that protein D is contributing to reductions in clinical AOM.

Reductions in AOM following the introduction of PHID-CV have been reported in Iceland and New Zealand. Of these two settings, 7vCRM had been used previously only in New Zealand. Data from Iceland suggested reductions in all-cause AOM in children <2 years of age during the first 2 years after PHID-CV vaccination, when assessing the mean annual incidence of hospital visits due to AOM (incidence rate ratio: 0.76 [95% CI: 0.67, 0.87]; p < 0.0001) [87]. Preliminary evidence from New Zealand in children <6 years indicated a reduction in OM hospitalizations (incidence rate ratio 0.92 [95% CI: 0.90, 0.95]), when compared to the pre-PCV era [88].

Unlike the situation with NTHi-induced AOM, post-marketing data regarding the efficacy of PHID-CV on against NTHi carriage is available from several countries (i.e. Kenya, Australia, the Netherlands) and showed inconsistent results.
An observational study performed in Kilifi, Kenya, 2 years following the introduction of PHiD-CV in the infant vaccination program in 2011 showed a significant decrease in carriage of NTHi (from 54% to 40%) among children ≤5 years of age in the vaccine period compared with pre-vaccination [89]. VE against NTHi carriage in children ≤5 years of age in the first-year postvaccination was 43% (95% CI: 26, 56). However, VE was 26% (95% CI: 9, 40) by the second-year postvaccination and a post-hoc exploratory analysis showed no difference in prevalence of nasopharyngeal carriage of NTHi between children 1 and 4 years old who had received two doses of PHiD-CV compared to those receiving zero or one dose (odds ratio 1.22 [95% CI: 0.87, 1.70]) [89]. Further study of this population will be of interest to answer questions of sustained reduction in NTHi carriage and the potential for new NTHi strains to emerge through selective pressure.

Preliminary reports of cross-sectional surveys conducted in the Netherlands [90] and Australian Indigenous children [84,91] did not identify any change in the prevalence of nasopharyngeal carriage of H. influenzae or NTHi between the period of PHiD-CV use and previous periods when 7vCRM was used.

5.5. Other evidence of impact of pneumococcal NTHi protein D conjugate vaccine on NTHi disease

Other diseases for which a beneficial effect of protein D-containing vaccines might be anticipated include invasive disease due to NTHi and lower respiratory tract infections. To date, there is little evidence for either of these possibilities, though there have been some interesting observations which are worthy of further discussion.

First, on the assumption that NTHi could be a cause of community-acquired pneumonia (CAP), there was some expectation that inclusion of protein D in PHiD-CV would offer improved efficacy against CAP compared to nonprotein D-conjugated formulations. Consequently, when the point estimate of VE against likely bacterial CAP reported for PHiD-CV in COMPAS (22.0% [95% CI: 7.7, 34.2]) was found to be in the same range as that of other PCVs, it was considered lower than expected for a vaccine effective against both S. pneumoniae and NTHi [72]. Among many possible factors, NTHi may also play a role, i.e. if NTHi is not a prevalent cause of pneumonia in the assessed region. As the etiology of pneumonia in this study is not known, this possibility cannot be further assessed. However, the prevalence of NTHi carriage in this trial suggests that NTHi could be a less prevalent pathogen in Panama than in other studied regions such as Finland.

The second point relates to CSLD. H. influenzae is a common bacterial pathogen identified in the secretions of children with CSLD or cystic fibrosis, as well as in sputum of adult patients with bronchiectasis and COPD; its prevalence as etiological factor of invasive infections in immunocompromised individuals is increasing [6].

Pizzuto et al. [9] compared the responses of peripheral blood mononuclear cells (PBMCs) from CSLD patients and healthy controls to a challenge with live NTHi and found that, while most differences were minor, cells from CSLD patients produced significantly lower levels of interferon gamma (IFN-γ). The authors speculated that this impaired immune response to NTHi challenge could contribute to the susceptibility of these children to chronic respiratory disease [9]. Interestingly, in a second study, the same authors discovered that in response to NTHi challenge, PBMC from CSLD children who had received at least three doses of PHiD-CV produced significantly more IFN-γ (similar to healthy controls), than a comparison group who had been vaccinated with CRM197-conjugated vaccines (7vCRM or 13vCRM) [92]. These results raise the possibility that vaccination with PHiD-CV could have beneficial effects in children at risk of CSLD, and a randomized controlled trial is underway to begin to address this question [93].

6. NTHi identification and its challenges

One of the major challenges for assessing the impact of protein D-containing vaccines has been the limitations in our understanding of the pathogen itself. This includes unambiguous identification of NTHi and differentiation from highly related nonhemolytic H. haemolyticus [94] strains as well as the understanding that distinct clinical isolates may vary in their sensitivity to antibodies against protein D [95].

The differentiation between NTHi and H. haemolyticus was based initially on the hemolytic phenotype of H. haemolyticus, i.e. production of zones of β-hemolysis on horse blood agar. However, this approach can no longer be considered reliable as up to 40% of the H. haemolyticus strains are nonhemolytic [96]. Therefore, molecular typing by PCR targeting several genes became the preferred method of differentiating NTHi and H. haemolyticus [6]. Binks et al. found that no single gene could be used to unequivocally differentiate between NTHi and H. haemolyticus, but proposed hpd, the gene encoding protein D, to be the best option (specificity of 91.7% and a sensitivity of up to 88.9%) [94]. However, subsequently, clinical NTHi strains lacking the hpd gene have been isolated from Indigenous Australian children with non-cystic fibrosis bronchiectasis using whole-genome sequencing of 20 nasal or nasopharyngeal carriage Haemophilus isolates from 16 children [95]. They have shown that five isolates from three children lacked hpd and the isolates from the three children were not phylogenetically related, meaning that these were multiple hpd-negative clones. The identification of these strains has implications for the hpd-based diagnostic tests used to identify NTHi and also for the efficacy of the protein D conjugate vaccine against NTHi disease and carriage [95]. Although numbers are small, the identification of five phylogenetically unrelated isolates from three children illustrates the challenges in developing single-target diagnostic tests to identify NTHi. Previous research had suggested this gene to be highly conserved [48,51], and this is the only study to date that reports NTHi lacking the hpd gene. It suggests further analysis of clinical isolates from different anatomical and geographical locations may be warranted to help interpret the results of clinical studies with protein D-based vaccines.

It is possible that PHiD-CV vaccination could result in replacement of protein D-containing NTHi by protein D-negative
strains or by those in which expression of protein D is down-regulated, resulting in lowering VE against hpd-positive strains. This would elegantly explain the inconsistent results observed in studies of nasopharyngeal carriage. However, as protein D is thought to be a virulence factor, replacing protein D-expressing strains with non-expressing strains is still likely to result in clinical benefits.

7. Expert commentary

Preclinical data and clinical trials with protein D-containing formulations have suggested that this protein not only acts as an effective carrier protein in conjugate vaccines, but is also a viable approach for decreasing the burden of AOM caused by NTHi. Passive transfer of antibodies from children vaccinated with protein D-containing formulations has shown protective effects in experimental models of AOM, and consistent trends toward efficacy were observed in phase III clinical trials. For PHID-CV (the licensed formulation), the point estimates for VE against NTHi AOM in COMPAS ranged between 15% and 22%, although the study was not powered to assess VE against NTHi AOM and the wide CI spans zero, which makes it difficult to draw a conclusion. Nonetheless, the observed point estimates were quite similar to that observed with 11Pn-PD in the POET study, standing in contrast to the negative trend observed in the FinOM study with PCV formulations not containing protein D [41,85].

The effect of protein D-containing vaccines on nasopharyngeal carriage was not very consistent, which is probably not critical for protection against disease but could have implications for herd effects. The relation between carriage and disease may be different for NTHi than for the pneumococcus. Although the observed trends are promising and suggest that a clinical and public health benefit will be observed, the impact of protein D-containing vaccine formulations on NTHi disease has been much lower than that observed for vaccines against other pathogens, such as Hib. Understanding the reasons behind these observations will help in the development of improved formulations. Clinical NTHi strains that do not express protein D have already been identified [95]; any disease caused by such strains and the carriage of these serotypes can therefore not be influenced by protein D conjugate vaccines. Additionally, virulence factors other than protein D, which may be implicated in colonization, have been identified [59]. Therefore, adding other antigens to complement protein D is an obvious next step for prevention of diseases due to NTHi.

In conclusion, efficacy of the 11-valent protein D-conjugated PCV formulation against NTHi AOM was demonstrated in the POET study, which was the only trial specifically designed to assess AOM. Other studies with the licensed PHID-CV formulation are supportive of this finding, but these trials were not powered to assess efficacy against NTHi AOM. Based on the currently known facts and gaps in knowledge, protein D-containing vaccines appear to have a positive impact on the clinical burden of NTHi AOM, but the exact mechanism needs to be further elucidated, and further studies could be useful to provide conclusive evidence.

8. Five-year view

Clinical and preclinical studies have suggested the promise of protein D-containing vaccines for prevention of AOM due to NTHi. However, the long-term public health impact will only become clear as data accumulates over the coming years, allowing to see whether protein D-containing vaccines provide additional clinical benefit in terms of AOM when compared to other PCVs. After introduction of 7vCRM as the first vaccine for prevention of AOM, the prevalence of bacterial AOM cases due to NTHi increased in many settings. It will now be interesting to compare what happens to NTHi AOM in countries that introduced PHID-CV which contains protein D, to those in which 7vCRM was replaced by 13vCRM (without protein D). One possibility is that NTHi AOM will decline in settings where PHID-CV is in use, but remain stable or even increase, in settings where 13vCRM is in use. However, there are very few places where it will be possible to study the incidence of microbiologically defined NTHi AOM and most of the data is likely to describe the prevalence of NTHi AOM. This will be challenging to interpret because the prevalence of NTHi will also be affected by changes in disease caused by S. pneumoniae. For example, if pneumococcal AOM declines much more rapidly than NTHi AOM, the prevalence of NTHi disease could appear to increase. Other issues that may be anticipated are the emergence of protein D-deficient NTHi strains and replacement with other pathogens in response to vaccine pressure. The clinical impact of these effects will depend on the virulence of bacteria circulating after vaccine introduction as compared to the pre-vaccine era. Research on new NTHi candidate vaccine antigens that may complement protein D continues and the clinical utility of second-generation NTHi vaccines will be further informed by these types of data.

Key issues

- Non-typeable Haemophilus influenzae (NTHi) is a bacterial pathogen causing mainly mucosal respiratory diseases but also invasive disease. Together with Streptococcus pneumoniae, NTHi remains the main cause of acute otitis media (AOM). NTHi has been specifically associated with recurrent and chronic otitis media (OM) and with antibiotic treatment failure. Vaccination could be an important strategy for NTHi AOM prevention.
- While several pneumococcal conjugate vaccines are licensed and are recommended for immunization against AOM caused by S. pneumoniae, only PHID-CV contains protein D from NTHi which could potentially extend protection of this vaccine against AOM caused by NTHi.
- In pre-clinical studies, protein D-containing vaccine formulations were capable of protecting against NTHi infection in a chinchilla model of OM.
- Clinical studies with the 11Pn-PD predecessor of PHID-CV (POET) or PHID-CV formulation itself (COMPAS) induced robust immune responses against protein D and positive point estimates against NTHI AOM were observed (35.3% in POET and 15% in COMPAS). This contrasts with the negative
point estimates observed in the FinOM study with PCV formulations not containing protein D.

- Findings from a post-hoc analysis of the FinOM study pointed towards disease replacement, and contradicted the hypothesis that preventing first episodes of pneumococcal AOM may reduce the susceptibility of children to disease caused by other pathogens.
- Post-marketing studies suggested encouraging reductions in OM in children <2 years of age after PHID-CV vaccination. In one study where pathogen-specific data was available, a trend towards a reduction in NTHi OM was observed.
- Evidence of an impact of PHID-CV on chronic suppurative lung disease by improvement of the interferon gamma response in response to NTHi challenge further support the role of the protein D-containing PHID-CV in protecting against NTHi disease.
- In line with AOM efficacy, trends towards vaccine efficacy against nasopharyngeal carriage of NTHi were observed in POET (36.7%) and in COMPAS (27.0%). These trends were transient and were not always apparent in other clinical trials (Finland, the Netherlands, Kenya) or post-marketing studies. The relationship between carriage and disease might be different for NTHi than for the pneumoccus. A limiting factor of these studies is that the fact that the studies rely on culture-confirmed carriage which does not allow to determine impact on density of the pathogen or relative expression of protein D.
- The heterogeneity of NTHi, with some strains recently identified as lacking protein D, complicates the evaluation of the impact of protein D-containing vaccines on NTHi disease and carriage.
- The data available to date suggest that PHID-CV may decrease NTHi AOM and might protect against NTHi carriage, but more evidence including pathogen-specific outcomes is clearly warranted.

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