

Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*: a randomised double-blind efficacy study

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Summary

Background Acute otitis media is one of the most commonly-diagnosed childhood infections. This study assessed the efficacy of a novel vaccine that contained polysaccharides from 11 different *Streptococcus pneumoniae* serotypes each conjugated to *Haemophilus influenzae*-derived protein D in prevention of acute otitis media.

Methods 4968 infants were randomly assigned to receive either pneumococcal protein D conjugate or hepatitis A vaccine at the ages of 3, 4, 5, and 12–15 months and were followed-up until the end of the second year of life. Middle-ear fluid was obtained for bacteriological culture and serotyping in children who presented with abnormal tympanic membrane or presence of middle-ear effusion, plus two predefined clinical symptoms. The primary endpoint was protective efficacy against the first episode of acute otitis media caused by vaccine pneumococcal serotypes. Analysis was per protocol.

Findings From 2 weeks after the third dose to 24–27 months of age, 333 clinical episodes of acute otitis media were recorded in the protein D conjugate group (n=2455) and 499 in the control group (n=2452), giving a significant (33·6% [95% CI 20·8–44·3]) reduction in the overall incidence of acute otitis media. Vaccine efficacy was shown for episodes of acute otitis media caused by pneumococcal vaccine serotypes (52·6% [35·0–65·5] for the first episode and 57·6% [41·4–69·3] for any episode). Efficacy was also shown against episodes of acute otitis media caused by non-typable *H influenzae* (35·3% [1·8–57·4]). The vaccine reduced frequency of infection from vaccine-related cross-reactive pneumococcal serotypes by 65·5%, but did not significantly change the number of episodes caused by other non-vaccine serotypes.

Interpretation These results confirm that using the *H influenzae*-derived protein D as a carrier protein for pneumococcal polysaccharides not only allowed protection against pneumococcal otitis, but also against acute otitis media due to non-typable *H influenzae*. Whether this approach would also allow improved protection against lower respiratory tract infections warrants further investigation.

Introduction

Acute otitis media is one of the most commonly diagnosed childhood infections, not only in the USA, where it accounts for more than 20 million visits to a paediatrician every year,¹ but also elsewhere in the industrialised world² and in developing countries.³ The disease is most prevalent in children younger than 2 years.^{1,4} Although acute otitis media is usually clinically mild, it can result in complications such as conductive hearing loss. The two leading bacterial pathogens that cause the infection are *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*. These two pathogens are also recognised to be a major cause of lower respiratory tract infections.^{5–8} In the Czech Republic, where part of this efficacy study was done, *S pneumoniae* was shown to represent 46% and *H influenzae* 17% of all bacterial acute otitis media isolates in children younger than 2 years.⁹ Recurrent acute otitis media is also the main indication for ventilation tube placement in the study areas, followed by poor response to antibiotics.

Vaccines containing plain capsular polysaccharides of *S pneumoniae* have been available for several decades, but are not immunogenic or effective in children younger than 2 years.^{10,11} Efficacy against invasive pneumococcal disease in young children was first shown with a 7-valent vaccine (Prevnar, Wyeth, Philadelphia, USA) containing saccharides from *S pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F each conjugated to CRM197 (a non-toxic cross-reacting mutant of diphtheria toxin).¹² This vaccine also showed efficacy against acute otitis media caused by vaccine pneumococcal serotypes.^{12,13}

In our study, we investigated the efficacy against acute otitis media of a novel candidate pneumococcal vaccine containing 11 different polysaccharide serotypes, each conjugated to a recombinant non-lipidated form of protein D as carrier protein. Protein D is a 42 kD cell-surface lipoprotein of *H influenzae* and has induced protection against non-typable *H influenzae* otitis media in rat and chinchilla models.¹⁴ However, protein D is

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highly conserved in both encapsulated and non-encapsulated *H influenzae* strains and therefore has the potential to provide protection against any *H influenzae* strain that causes otitis media.

Methods

Study design and participants

This randomised double-blind study was designed to assess the efficacy of the candidate vaccine in prevention of acute otitis media caused by pneumococci and nontypable *H influenzae*, and the effect on the overall burden of disease. Recruitment was done by local paediatricians during clinic visits of parents. Infants aged between 6 weeks and 5 months, with no acute illness and for whom informed consent had been obtained from a parent or legal guardian, were enrolled for vaccination and follow-up at 27 paediatric centres in the Czech Republic and 23 in Slovakia. Exclusion criteria for enrolment in the study were: use of any investigational or non-registered drug or vaccine other than the study vaccines within 30 days preceding the first dose of study vaccine, or planned use during the study period; previous vaccination against *S pneumoniae*; rectal temperature 38°C or higher, or temperature by other routes of 37.5°C or higher (a temperature greater than or equal to these cutoffs warranted deferral of vaccination pending recovery of the child); history of allergic disease or reactions likely to be exacerbated by any component of the study vaccines; or other conditions that in the opinion of the investigator might have potentially interfered with the interpretation of study outcomes.

The sponsor numbered the vaccine supplies with the use of a computer-generated randomisation list. Randomisation (1 to 1) was done with a study-specific central randomisation system via the internet that, on receipt of the infant's initials and birth date, determined the vaccine number to be used. The study was done according to the Declaration of Helsinki (as amended in Somerset West, South Africa, 1996). The protocol was reviewed and approved by the appropriate independent ethics committees or institutional review boards. An independent data-safety monitoring board consisting of experts in paediatrics, otitis, infectious diseases, and biostatistics oversaw the progress of the study and the safety of the patients.

Vaccines and vaccinations

All study vaccines were manufactured by Glaxo-SmithKline Biologicals, Rixensart, Belgium. The candidate protein D conjugate vaccine contained 1 µg each of capsular polysaccharide of pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F conjugated individually to protein D.

Whenever possible, vaccine studies with active control groups rather than placebo groups are preferred. The administration of the concomitant hexavalent combination vaccine (see below) limited the choice of control

vaccines. Hepatitis A vaccine was chosen, since it is not included in the immunisation calendars in the participating countries and therefore would provide a benefit to the control group. The hepatitis A vaccine (Havrix) contained 720 ELISA units of inactivated hepatitis A virus antigen (strain HM 175). Participants were randomly assigned into two groups to receive four doses of either protein D conjugate vaccine or hepatitis A vaccine by intramuscular injection at ages of about 3, 4, 5, and 12–15 months.

The concomitant hexavalent diphtheria-tetanus-3-component acellular pertussis-hepatitis B-inactivated poliovirus types 1, 2, and 3-*H influenzae* type b (DTPa-HBV-IPV/Hib) vaccine (Infanrix hexa) was offered free of charge to all study participants, followed by a booster dose at age 15–18 months.

Procedures

Vaccination visits were scheduled for all participants at around the ages of 3, 4, 5, 12–15, and 15–18 months. Efficacy follow-up started on the day of the first dose of study vaccine (for the intention-to-treat cohort analysis) or 2 weeks after the third vaccine dose (for the per protocol analysis) and continued until 24–27 months of age. There was no active surveillance, and unscheduled sick visits could take place any time during the efficacy follow-up period when parents, according to standard local clinical practice, consulted their local paediatrician if their infant was ill.

Adverse events arising within 31 days of vaccination and serious adverse events occurring throughout the study were recorded. Data on risk factors for acute otitis media (breastfeeding, number of children in household, and exposure to children outside the household) were obtained at the 12–15 month visit.

Immunogenicity assessment was only done for a random subset of participants in selected centres and on blood samples obtained before the first study vaccine dose, 1 month after completion of the primary series, immediately before the booster dose, and 1 month after the booster dose. Nasopharyngeal carriage of *S pneumoniae* and *H influenzae* was assessed in another set of selected centres.

Parents were advised to consult their paediatrician if their child was sick, had ear pain, or had spontaneous ear discharge. As per routine practice in the study area, children with suspected acute otitis media were immediately referred to ear, nose, and throat specialists, who also participated in the study as investigators. A clinical diagnosis of otitis media was evidenced by either the visual appearance of the tympanic membrane (ie, redness, bulging, loss of light reflex) or the presence of middle-ear effusion (as shown by simple or pneumatic otoscopy or by microscopy). The presence of at least two of the following signs or symptoms was also required: ear pain, ear discharge, hearing loss, fever, lethargy, irritability, anorexia, vomiting, or diarrhoea. To be

regarded as acute otitis media, these signs or symptoms had to have started within the 14 days preceding the clinical diagnosis.

If the ear, nose, and throat specialist confirmed the clinical diagnosis, a specimen of middle-ear fluid was obtained by tympanocentesis (tympanostomy being a routine procedure in the management of acute otitis media in the study areas) for bacteriological testing. An episode of acute otitis media was regarded as bacterial if any of the following pathogens was identified in the middle-ear fluid: *S pneumoniae*, *H influenzae*, *Moraxella catarrhalis*, group A Streptococci or *Staphylococcus aureus*.

For patients with repeated sick visits, a new episode of otitis media was judged to have started if more than 30 days had elapsed since the beginning of the previous episode. Additionally, for categories defined according to bacterial pathogen or serotype, a new episode was judged to have started if any interval had elapsed since the beginning of an episode caused by a different bacterial pathogen or serotype. Episodes of acute otitis media with multiple samples of middle-ear fluid might be counted several times, for example as culture negative first and as culture-confirmed bacterial episode subsequently, or as different (mixed or subsequent) bacterial episodes, depending on the results of the different middle-ear fluid cultures.

Recurrent acute otitis media was defined as at least three episodes confirmed by an ear, nose, and throat specialist within 6 months or four episodes within 1 year irrespective of the cause.

Laboratory methods

Microbiology

All specimens of middle-ear fluid and nasopharyngeal swabs were sent within 24 hours in Amies transport medium to four regional laboratories for bacteriological culture. *S pneumoniae* and *H influenzae* isolates underwent further testing for confirmation and serotype identification at the National Reference Laboratory and WHO Collaborating Centre for Streptococci and *H influenzae* at the National Institute of Public Health in Prague, Czech Republic.

Samples for analysis of *S pneumoniae* were plated onto blood agar or, for nasopharyngeal swabs, selective blood agar with gentamicin. Identification of pneumococci was based on inhibition by optochin and bile solubility.¹⁵ Capsular typing of recovered pneumococci was done by use of the quellung reaction with antiserum obtained from the Statens Serum Institut, Copenhagen, Denmark.¹⁶ Samples for analysis of *H influenzae* were plated onto chocolate agar and identified by factors X and V growth requirements and biochemical tests.¹⁵ The absence of slide agglutination in the presence of a to f antisera (*H influenzae* agglutinating sera MUREX ZM 20-25) was used to identify non-encapsulated (non-typable) strains. The

identification of other organisms was done with standard bacteriological procedures.¹⁵

Immunology

Serum samples were stored at -20°C until masked analyses were done at GlaxoSmithKline Biologicals' laboratory in Rixensart, Belgium. Serum anti-pneumococcal IgG concentrations to each of the serotypes included in the vaccine were measured by an ELISA method, which included a pre-adsorption step with serotype 22F polysaccharide to increase the specificity of the assay.^{17,18} The assay cutoff was 0.05 μg per mL. The antibody concentrations were measured by calibration with the standard reference serum 89-SF.¹⁹ Comparison of GlaxoSmithKline Biologicals' 22F ELISA results to the WHO reference non-22F ELISA for paediatric post vaccination sera²⁰ has established that the antibody concentration equivalent to the WHO 0.35 μg per mL threshold value²⁰ is 0.2 μg per mL for GlaxoSmithKline Biologicals' 22F ELISA.¹⁸

IgG antibodies to the *H influenzae* protein D were measured by a classic ELISA with the non-lipidated protein D as coating material and were expressed in ELISA units per mL. The assay cutoff was 100 ELISA units per mL. Hepatitis A antibodies were measured with a commercially available ELISA kit (Enzymun Boehringer-Mannheim, Mannheim, Germany, or equivalent). The cutoff of the assay is 15 mIU/mL.

Statistical Analysis

Analysis of this trial strictly adhered to a detailed report and analysis plan that was established before unmasking. The primary endpoint was protective efficacy against the first episode of acute otitis media caused by vaccine pneumococcal serotypes. The main secondary endpoint was protective efficacy against the first episode of acute otitis media caused by non-typable *H influenzae*. The primary efficacy analysis was based on the per protocol analysis of the time since entry in the efficacy follow-up until the first acute otitis media event. Vaccine efficacy and its 95% CI were estimated as 1 minus the hazard ratio obtained from a Cox regression model including treatment group as only regressor. Time-to-event was defined as the number of days between entry into the efficacy follow-up and the event. Participants without events were censored on the last day of the efficacy follow-up. The robustness analyses of vaccine efficacy based on all recurrent events were also provided with a generalised Cox regression model with a robust sandwich variance estimate (Anderson and Gill model).²¹ The primary objective was reached at a 4.8% significance level of the two-sided p value for the null hypothesis that vaccine efficacy for acute otitis media attributed to vaccine serotypes was equal to 30%. This p value is subsequently referred to as p value₃₀ to distinguish it from other p values that consider the null hypothesis of 0% difference.

An analysis of vaccine efficacy adjusted for the following covariates (risk factors for acute otitis media) was done: breastfeeding for less than 1 month, mean number of children in the household, and exposure to children outside the household for at least 2 days per week.

In agreement with the sponsor, an interim analysis was done to obtain recommendation from the independent monitoring board for data-safety on stopping or continuing enrolment on the basis of prespecified criteria. No other results were available to the sponsor at the time of the interim analysis. As a result of the interim analysis the enrolment was stopped and the significance level for the final analysis was set at 4.8% after adjustment by the O'Brien and Fleming method.²²

The sample size had 90% power to meet the primary objective on the assumption of an anticipated attack rate of 15% and a true vaccine efficacy of 55%. The analysis was complemented by an intention-to-treat analysis of the first event, and by analyses of the multiple time to events.²¹

Vaccine efficacy calculated for the analysis of carriage was estimated as 1 minus the relative risk, and percentages were compared with a two-sided Fisher's Exact test. The analysis of immunogenicity and safety was descriptive without statistical comparison between groups.

Role of the funding source

The study was funded by GlaxoSmithKline Biologicals. The sponsor was involved in all stages from study design to analysis of the data. The corresponding author had full access to the data and had final responsibility for submission for publication.

Results

4968 infants were enrolled between Oct 30, 2000, and Sept 6, 2002 (figure 1). Table 1 shows demographic characteristics and risk factors for the two groups.

A total of 366 clinical episodes of acute otitis media were reported in the protein D conjugate group and 553 in the control group. Of these, 333 in the protein D conjugate group and 499 in the control group were recorded during the per-protocol follow-up. The overall incidence of acute otitis media was 83.3 episodes per 1000 person-years of follow-up in the protein D conjugate group versus 125.2 episodes per 1000 person-years of follow-up in the control group.

Table 2 shows the data for the primary endpoint and the first secondary endpoint of the study. The vaccine efficacy against the first episode of acute otitis media caused by vaccine pneumococcal serotypes was 52.6% in both the per-protocol (p value₃₀=0.0156) and the intention-to-treat (p value₃₀=0.0104) cohorts. The vaccine efficacy against the first episode of acute otitis media caused by non-typable *H influenzae* was above 30% in both cohorts, but was only significant in the intention-to-treat cohort (p =0.0456). The efficacy against the first episode of acute otitis media caused by vaccine

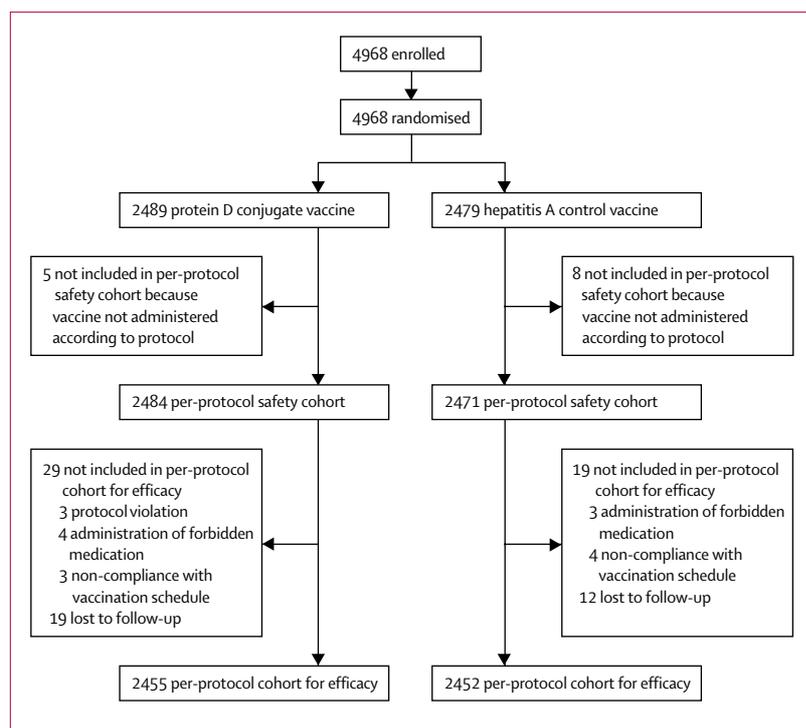


Figure 1: Trial profile

pneumococcal serotypes or non-typable *H influenzae* persisted for at least 18 months (figure 2). Covariate analyses (data not shown) showed no effect of the risk factors for acute otitis media on vaccine efficacy against the first episode caused by vaccine pneumococcal serotypes or non-typable *H influenzae*.

Table 3 shows the protective efficacy of the protein D conjugate vaccine in the per protocol cohort against clinical otitis episodes and episodes caused by different pneumococcal serotypes, *H influenzae*, and other pathogens. Significant protective efficacy was shown against clinical acute otitis media episodes (33.6%) and otitis episodes confirmed by aspiration of middle-ear fluid. More than 60% of the episodes of acute otitis media

	Pneumococcal vaccine (protein D conjugate)	Control vaccine (hepatitis A)
Number of patients	2455	2452
Sex (male)	51.2%	49.8%
Mean age at vaccination (years)		
Dose 1 (weeks, SD)	14.1 (2.75)	14.1 (2.75)
Dose 2 (weeks, SD)	18.9 (3.01)	18.9 (3.03)
Dose 3 (weeks, SD)	23.8 (3.34)	23.7 (3.34)
Dose 4 (months, SD)	12.3 (0.60)	12.3 (0.64)
Breast feeding for >1 month	84.1%	83.7%
Mean number of children in the household	1.4 (0.64)	1.4 (0.67)
Exposure to children outside the household for at least 2 days per week	52.2%	51.4%

Table 1: Demographic characteristics and acute otitis media risk factors for vaccinated infants (per-protocol cohort)

	Pneumococcal vaccine		Control vaccine		Vaccine efficacy* % (95% CI)
	Number of children	Incidence per 1000 person-years	Number of children	Incidence per 1000 person-years	
Per-protocol cohort	2455		2452		
Vaccine pneumococcal serotypes	57	14.4	118	30.4	52.6% (35.0 to 65.5)
Non-typable <i>H influenzae</i>	39	9.8	56	14.2	31.1% (-3.7 to 54.2)
Intention-to-treat cohort	2489		2479		
Vaccine pneumococcal serotypes	64	14.2	132	29.8	52.6% (36.1 to 64.9)
Non-typable <i>H influenzae</i>	43	9.5	63	14.0	32.7% (0.77 to 54.3)

Table 2: Protective efficacy of pneumococcal protein D conjugate vaccine for time to first occurrence of acute otitis media during per-protocol and intention-to-treat follow-up

were associated with a rectal fever of 38.5°C or more, but the efficacy against these more severe episodes remained similar (post-hoc analysis). Significant protective efficacy was also shown against bacterial episodes confirmed by culture and episodes caused by culture-confirmed pneumococci.

The protective efficacy against any acute otitis media episode caused by vaccine pneumococcal serotypes (57.6%) or non-typable *H influenzae* (35.3%) in table 3

is in line with that calculated on the basis of the time to occurrence of the first episode as shown in table 2. The protein D conjugate vaccine also reduced the incidence of otitis episodes caused by vaccine-related cross-reactive pneumococcal serotypes by about two-thirds (table 3).

The number of otitis episodes was not sufficient to assess efficacy for each of the individual vaccine serotypes, but significant protective efficacy was seen for serotypes 6B, 14, 19F, and 23F. However, the vaccine did not provide protection against episodes caused by serotype 3.

No increase in pneumococcal acute otitis media caused by other non-vaccine serotypes or other bacterial pathogens (*M catarrhalis*, group A streptococci, and *S aureus*) was recorded over the study period (table 3), whereas episodes of acute otitis media with negative middle-ear fluid culture were reduced by 23% (post-hoc analysis). Other interesting, but statistically not significant findings were the 60.3% reduction in the number of infants with ventilation tube placement (95% CI -26.7 to 87.5; ten infants given vaccine in the control group vs four in the protein D conjugate group), and the 56% reduction in the occurrence of recurrent episodes of acute otitis media (-1.9 to 80.7; 18 controls vs eight in the protein D conjugate group).

At age 15–18 months, nasopharyngeal carriage was assessed on swabs obtained from 352 infants (177 in the protein D conjugate group and 175 controls). About 3 months after the protein D conjugate or control vaccine booster dose, vaccine serotype pneumococci were isolated from the nasopharynx of 6% of the infants in the protein D conjugate group versus 11% of controls, and *H influenzae* was isolated in 10% of the infants in the protein D conjugate group versus 18% in the control group (table 4).

Immunogenicity of the protein D conjugate vaccine was assessed in 149 infants included in the per-protocol immunogenicity subset (table 5) and compared with 148 controls (data not shown). 1 month after the third dose of the protein D conjugate vaccine, antibody concentrations of at least 0.2 µg per mL were measured in more than 96% of infants for all the vaccine pneumococcal serotypes except 6B and 23F. At the same time, the percentages of infants with pneumococcal antibody concentrations of at least 0.2 µg per mL in the

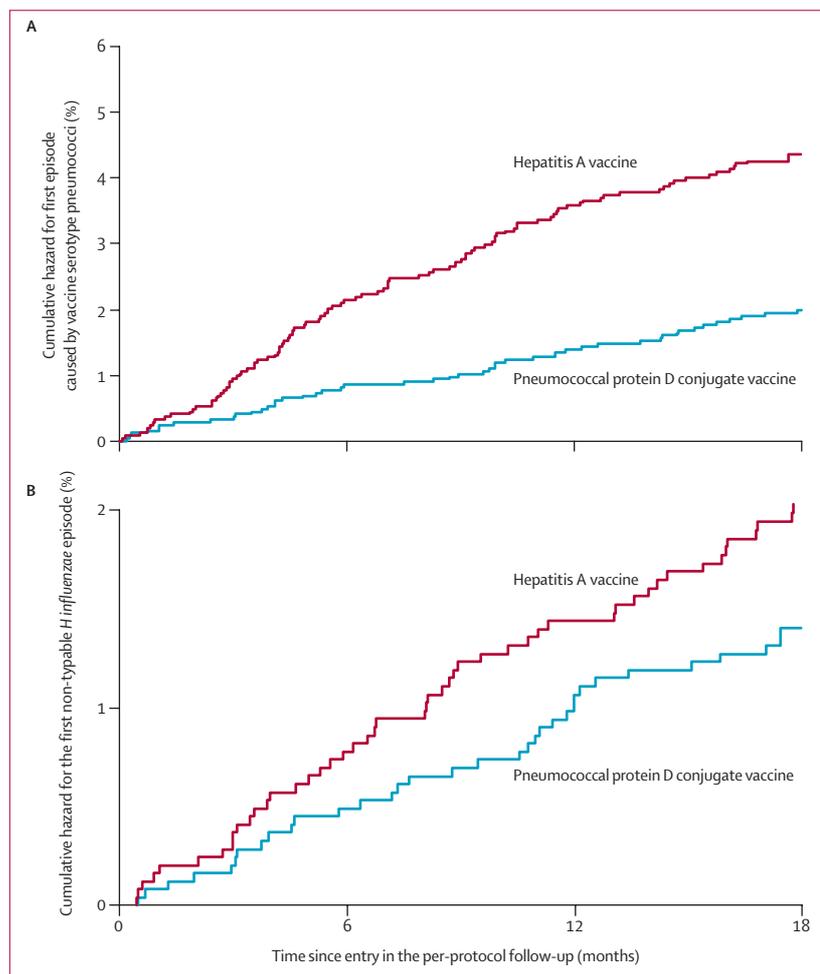


Figure 2: Cumulative hazard curves for the first occurrence of acute otitis media during per protocol follow-up caused by (A) vaccine serotype pneumococci and (B) non-typable *H influenzae*

	Number of episodes		Vaccine efficacy		p (Cox)
	Pneumococcal vaccine (N=2455)	Control vaccine (N=2452)	%	95% CI	
Clinical episodes	333	499	33.6	20.8 to 44.3	<0.001
Episodes with fever of 38.5°C or more*	207	312	33.9	15.9 to 48.0	<0.001
Episodes with MEF obtained by aspiration	322	474	32.4	19.0 to 43.6	<0.001
Episodes with negative MEF culture*	199	256	22.6	3.5 to 38.0	0.023
Culture-confirmed bacterial episodes	178	306	42.1	27.7 to 53.7	<0.001
Culture-confirmed pneumococcal episodes	92†	189	51.5	36.8 to 62.9	<0.001
Vaccine pneumococcal serotypes					
All eleven serotypes combined	60	141	57.6	41.4 to 69.3	<0.001
Serotype 1	1	1	0.2	-149.5 to 93.8	0.999
Serotype 3	20	17	-17.1	-126.5 to 39.5	0.639
Serotype 4	0	3	100.0	-27.8‡ to 100.0	-
Serotype 5	0	0	-	-	-
Serotype 6B	3	24	87.6	58.4 to 96.3	<0.001
Serotype 7F	0	1	100.0	-283.6† to 100.0	-
Serotype 9V	3	8	62.6	-40.8 to 90.1	0.146
Serotype 14	1	22	95.5	66.0 to 99.4	0.003
Serotype 18C	3	5	40.1	-176.6 to 87.0	0.512
Serotype 19F	24	43	44.4	8.3 to 66.3	0.021
Serotype 23F	5	18	72.3	24.8 to 89.8	0.012
Vaccine-related cross reactive pneumococcal serotypes					
All vaccine related cross-reactive serotypes combined	8	23	65.5	22.4 to 84.7	0.010
Serotype 6A	4	11	63.7	-13.9 to 88.4	0.083
Serotype 9N	3	4	25.5	-231.2 to 83.2	0.699
Serotype 18A	0	1	100.0	-283.6† to 100.0	-
Serotype 18B	0	1	100.0	-283.6† to 100.0	-
Serotype 18F	0	1	100.0	-283.6† to 100.0	-
Serotype 19A	1	3	67.4	-208.7 to 96.6	0.328
Serotype 23A	0	2	100.0	-91.8† to 100.0	-
Other non-vaccine pneumococcal serotypes					
All other non-vaccine pneumococcal serotypes combined	23	25	8.5	-64.2 to 49.0	0.766
<i>H influenzae</i> §	44	68	35.6	3.8 to 57.0	0.032
Non-typable <i>H influenzae</i>	41	63	35.3	1.8 to 57.4	0.041
<i>M catarrhalis</i>	31	34	9.4	-52.5 to 46.1	0.711
Group A streptococci	6	8	24.7	-117.3 to 73.9	0.600
<i>S aureus</i>	24	28	14.6	-50.9 to 51.7	0.587

N=number of children in the per-protocol cohort for efficacy analysis. *Post-hoc analyses. †Serotyping was not available for one pneumococcal episode of acute otitis media in the protein D conjugate group. This case is therefore counted as a pneumococcal episode, but not included in any of the serotype specific subgroups. ‡Conservative approximation with attack rate definition (standardised asymptotic CI). §In addition to the non-typable *H influenzae* episodes, there were one *H influenzae* type b and two type f episodes in the protein D conjugate group, and two type b and three type f episodes in the control group.

Table 3: Protective efficacy of pneumococcal protein D conjugate vaccine for any episode of acute otitis media during per-protocol follow-up

control group ranged from 0% to 10.2% apart from serotype 14 (31 of 129 analysed [24%]). Geometric mean concentrations of antibodies after the third dose of protein D conjugate vaccine ranged from 0.62 µg per mL for serotype 6B to 3.78 µg per mL for serotype 3.

After primary vaccination with protein D conjugate, all infants apart from one had measurable antibodies against the protein D carrier protein (compared with 23% of infants in the control group). Post-primary, the geometric mean concentration of antibodies was 1995 ELISA units

	Pneumococcal vaccine (N=177)		Control vaccine (N=175)		Vaccine efficacy*		p†
	n	%	n	%	%	95% CI	
<i>S pneumoniae</i>	30	16.9	39	22.3	23.9	-16.6 to 50.4	0.228
<i>S pneumoniae</i> vaccine serotypes	11	6.2	19	10.9	42.8	-16.7 to 71.9	0.130
<i>S pneumoniae</i> non-vaccine serotypes‡	12	6.8	14	8.0	15.3	-78.0 to 59.7	0.689
<i>H influenzae</i>	18	10.2	31	17.7	42.6	1.3 to 66.6	0.046
Non-typable <i>H influenzae</i>	16	9.0	27	15.4	41.4	-4.9 to 67.3	0.075

N=number of swabs collected. n/%=number/percentage of swabs positive for *S pneumoniae* or *H influenzae*. *Vaccine efficacy was estimated as 1 minus relative risk. †Two-sided Fisher's Exact test. ‡Excluding vaccine-related cross reactive serotypes.

Table 4: Carriage of *S pneumoniae* and *H influenzae* in nasopharyngeal swabs taken at 15–18 months of age

	1 month after third primary dose of protein D conjugate vaccine				Booster (fourth) dose of protein D conjugate vaccine			
	Post-primary geometric mean antibody concentration		Serum IgG seropositivity (proportion of infants)		Pre-booster geometric mean antibody concentration		Post-booster geometric mean antibody concentration	
	N	µg/mL (95% CI)	n	% ≥0.2 µg/mL (95% CI)	N	µg/mL (95% CI)	n	µg/mL (95% CI)
Serotype 1	140	1.58 (1.37–1.81)	140	100 (97.4–100.0)	133	0.29 (0.25–0.34)	144	2.55 (2.23–2.93)
Serotype 3	146	3.78 (3.32–4.31)	146	100 (97.5–100.0)	118	0.72 (0.58–0.89)	144	2.84 (2.49–3.24)
Serotype 4	144	2.16 (1.91–2.45)	144	100 (97.5–100.0)	129	0.45 (0.38–0.52)	142	2.50 (2.22–2.82)
Serotype 5	143	1.92 (1.68–2.19)	143	100 (97.5–100.0)	129	0.50 (0.44–0.58)	143	3.11 (2.71–3.56)
Serotype 6B	133	0.62 (0.50–0.77)	107	80.5 (72.7–86.8)	139	0.55 (0.45–0.67)	145	2.17 (1.77–2.67)
Serotype 7F	143	2.34 (2.07–2.65)	142	99.3 (96.2–100.0)	143	0.91 (0.80–1.03)	143	4.66 (4.15–5.24)
Serotype 9V	140	1.60 (1.38–1.86)	137	97.9 (93.9–99.6)	141	0.71 (0.60–0.84)	142	3.63 (3.14–4.20)
Serotype 14	143	3.00 (2.55–3.52)	143	100 (97.5–100)	131	1.62 (1.33–1.96)	145	6.48 (5.67–7.41)
Serotype 18C	140	1.49 (1.27–1.74)	135	96.4 (91.9–98.8)	111	0.48 (0.40–0.57)	144	2.71 (2.40–3.06)
Serotype 19F	143	2.60 (2.20–3.08)	139	97.2 (93.0–99.2)	127	0.77 (0.64–0.93)	145	4.88 (4.29–5.54)
Serotype 23 F	139	0.90 (0.75–1.09)	127	91.4 (85.4–95.5)	120	0.59 (0.48–0.71)	145	3.55 (3.03–4.17)
	N	ELISA units/mL (95% CI)	N	% ≥100 ELISA units /mL (95% CI)	N	ELISA units/mL (95% CI)	N	ELISA units/mL (95% CI)
Protein D	145	1995 (1754–2269)	144	99.3 (96.2–100.0)	124	1317 (1111–1561)	140	5628 (4939–6413)†

N=number of children from the protein D conjugate vaccine group included in the per-protocol immunogenicity subset and with available results for the specified time point. n%=number/percentage of children with antibody levels above the specified cutoff.

Table 5: Serum concentrations of antibodies against individual pneumococcal vaccine serotypes and protein D one month after the third protein D conjugate vaccine dose and before and 1 month after booster vaccination*

per mL compared with 76 ELISA units per mL in the control group. 1 month after primary vaccination, all infants in the control group had antibodies against hepatitis A, and the booster dose induced a 10-fold increase in hepatitis A antibody concentrations. In the protein D conjugate group only 11% (11 of 101 analysed) of the infants had hepatitis A antibodies after primary vaccination and no booster response was recorded (data not shown).

Safety data for the primary vaccination course co-administered with Infanrix hexa were available for 2489 infants in the protein D conjugate vaccine group and 2479 infants in the hepatitis A control group (intention-to-treat cohort). The percentages of infants for whom unsolicited adverse events were reported within 31 days after vaccination were similar in both groups (48% [1188] of infants in the protein D conjugate group and 50% [1234] of infants in the hepatitis A vaccine group). The percentages of infants with unsolicited symptoms that were judged by the investigator to be causally related to vaccination were also similar (2.5% [61] in the protein D conjugate group and 3.0% [75] in the hepatitis A vaccine group). Safety data were also available for 2461 protein D conjugate vaccine and 2458 hepatitis A vaccine booster doses. Unsolicited adverse events were reported after 21% [515] of the protein D conjugate and 22% [534] of hepatitis A vaccine booster doses. In both groups, unsolicited symptoms that were judged by the investigator to be causally related to vaccination were reported after 0.5% of the booster doses. Apart from administration site conditions, which were noted most frequently in controls, unsolicited symptoms were equally balanced between both groups.

During the whole study (21 months of follow-up per child), serious adverse events were reported for 491

(19.7%) infants given protein D conjugate and 508 (20.5%) given hepatitis A. Of these adverse events, 14 were judged by the investigator as causally related to vaccination. Seven infants reported such events after a dose of protein D conjugate vaccine co-administered with Infanrix hexa (febrile reaction with viral infection [same day], vomiting [same day], allergic reaction [same day], immunisation reaction with viral infection [same day], agitation [at day 1], depressed rate of consciousness [at day 14], and purpura [at day 20]). Three infants reported a serious adverse event after a dose of hepatitis A co-administered with Infanrix hexa. One child reported such an event on the same day as the booster vaccination with protein D conjugate (breath-holding spells), one reported events 2 days after booster vaccination with hepatitis A, and a further two reported events (bleeding disorder and exanthema subitum lymphadenitis) on the same day as booster vaccination with Infanrix hexa. All these events, apart from one case of epilepsy in the hepatitis A group, resolved without sequelae.

During the study, there were four deaths, one in the protein D conjugate group (8 months after receiving the third vaccine dose a diagnosis of epilepsy was made, 25 months after the third dose the child had grand mal epilepsy and died from suffocation) and three in the hepatitis A group. None were regarded as related to the study vaccine by the investigators.

Discussion

We found a reduction of ear, nose, and throat specialist-confirmed episodes of acute otitis media by about a third in infants in the vaccine group compared with controls. Despite the fact that pneumococci are one of the major causes of acute otitis media, prospective analyses of clinical trials have failed so far to show a substantial effect

of infant pneumococcal immunisation on the overall burden of this disease.^{13,23,24} A second important finding of our study is the 35.6% protection provided by the protein D conjugate vaccine against acute otitis media caused by *H influenzae*. This finding is of clinical significance, in view of the importance of this organism as a major cause of acute otitis media and lower respiratory tract infections.^{7,6,25} Although a clear correlation between efficacy and ELISA antibody concentrations against the carrier protein D could not be established, we can reasonably assume that the protein D carrier protein contributed to the induction of the protection against *H influenzae*. We cannot, however, rule out that the effect of the vaccine on acute otitis media episodes due to *H influenzae* was at least in part caused by an indirect effect of the reduction of episodes of pneumococcal acute otitis media that made infants receiving protein D conjugate vaccine less vulnerable to subsequent *H influenzae* infection. This hypothesis is, however, not lent support by the findings from the Finnish otitis media trial (FinOM),^{13,24} in which an increase in episodes of acute otitis media caused by *H influenzae* was recorded for both seven-valent pneumococcal conjugate vaccines.

Although 3 months after the booster dose of protein D conjugate vaccine the reduction of the nasopharyngeal carriage of *H influenzae* was significant only when all *H influenzae* strains were considered, this finding is still in favour of a direct protective effect of the protein D carrier protein. In view of the post-licensure experience after the introduction of pneumococcal conjugate vaccines in the USA, where non-typable *H influenzae* has become the predominant cause of recurrent or refractory acute otitis media with mostly β -lactamase-producing isolates,^{7,26,27} the additional protection provided by the protein D conjugate vaccine against infection caused by non-typable *H influenzae* could have a major public-health impact.²⁸

The overall vaccine efficacy for acute otitis media episodes caused by vaccine pneumococcal serotypes were remarkably similar for the protein D conjugate vaccine (57.6%) and the two vaccines in the FinOM study (57% for the CRM197 conjugate vaccine and 56% for the OMPC conjugate vaccine^{13,24}). Individual serotype-specific efficacy also seemed similar to those seen in the FinOM study, and were statistically significant for serotypes 6B, 14, 19F, and 23F. As seen for the CRM197 conjugate vaccine¹³ (but not the OMPC conjugate vaccine²⁴) the protein D conjugate vaccine provided significant protection (65.5%) against acute otitis media caused by vaccine related serotypes.

After vaccination with the CRM197 conjugate, an increase in acute otitis media due to non-vaccine pneumococcal serotypes or other bacterial pathogens has been recorded.^{13,24,29} In our study there was no evidence of the occurrence of replacement acute otitis media, although such an event cannot be ruled out

either, since to establish this occurrence would need epidemiological surveillance after large-scale use of the vaccine.

Although most samples of middle-ear fluid were cultured within 12 h after tympanocentesis, bacterial pathogens could not always be isolated. With the positive effect of the vaccine on culture negative acute otitis media episodes, in reality vaccine serotype or cross-reacting pneumococci and *H influenzae* are probably the implicated bacterial pathogens involved in at least some of these (false) culture negative episodes of acute otitis media. As a result, the overall effect of the vaccine exceeded that derived from the prevention of pneumococcal and *H influenzae* acute otitis media episodes.

Comparisons between clinical trials are often hampered by differences in study design, case definitions, or case ascertainment. The FinOM study,^{13,24} for example, did not require referral to an ear, nose, and throat specialist for confirmation of the diagnosis of acute otitis media, and used a more sensitive case definition with only one clinical symptom. Therefore, the FinOM study probably captured a wider range of the disease, including mild episodes. This wide capture resulted in a higher incidence of acute otitis media episodes in the FinOM study, and could also account for the lower percentage of episodes associated with vaccine serotypes and the higher proportion of episodes associated with *H influenzae* or *M catarrhalis*. Determination of whether these differences might have affected the vaccine efficacy estimates is difficult, especially those based on non-bacteriological endpoints. However, in our study, as in the FinOM study,³⁰ the severity of the clinical symptoms did not significantly affect efficacy against clinical episodes of acute otitis media.

The study confirmed, as previously reported,³¹ that the protein D conjugate vaccine is safe in infants and induces antibody responses to all components of the vaccine, including protein D. As noted for other vaccines, there was no clear correlation between efficacy against acute otitis media and antibody concentrations for individual serotypes after primary vaccination.^{13,24,32} Concentrations were lowest for serotypes 6B and 23F despite high levels of efficacy against acute otitis media episodes caused by these serotypes, and were highest for serotype 3 against which no protection was provided.

The absence of protection against serotype 3 was an unexpected finding. Notably, this serotype was the only one in which the post-booster antibody concentration was below that after primary vaccination. Although an immune response could be induced after boosting with plain polysaccharide vaccine at 12–15 months of age, the assessment of immune memory is hampered by the early development of an immune response to plain serotype 3 polysaccharide in non-primed children.³¹ Clearly the pattern of immune response to serotype 3 and the absence of protection merits further investigation.

In summary, this study showed a statistically significant and clinically relevant reduction in episodes of acute otitis media, and confirmed that use of the *H influenzae*-derived protein D as a carrier protein for pneumococcal polysaccharides not only allowed protection against pneumococcal otitis, but also against acute otitis media due to non-typable *H influenzae*. In view of the importance of non-typable *H influenzae* as causal pathogen for lower respiratory tract infections, these findings open interesting perspectives for improved disease control, especially in developing countries.

Contributors

All authors participated in the design, implementation, analysis and interpretation of the study. R Prymula, P Peeters, P Lommel, I Kohl, and J Poolman were involved in all phases of the study. P Peeters and L Schuerman led the clinical team at GlaxoSmithKline Biologicals. P Lommel conducted the data analysis. Roman Prymula was the principal investigator and V Chrobok coordinated the assessments by ear, nose, and throat specialists. P Kriz and E Novakova coordinated the microbiological laboratory work. E Kaliskova and I Kohl coordinated the GlaxoSmithKline Biologicals' study activities in the Czech Republic and Slovakia. J Poolman and J-P Prieels head the bacterial disease vaccine development programme at GlaxoSmithKline Biologicals.

Conflict of interest statement

P Peeters, E Kaliskova, I Kohl, P Lommel, J Poolman, J-P Prieels, and L Schuerman are employees of GlaxoSmithKline (GSK) Biologicals. J Poolman, L Schuerman, J-P Prieels, and P Peeters own shares in GSK. R Prymula is a consultant to GSK and other pharmaceutical companies, and has received travel grants or honoraria paid by health-care companies within the past 3 years.

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