Vaccine 29 (2011) 7595–7598

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Brief report

Effect of 7-valent pneumococcal conjugate vaccine on nasopharyngeal carriage with *Haemophilus influenzae* and *Moraxella catarrhalis* in a randomized controlled trial

Elske J.M. van Gils[a,b,*], Reinier H. Veenhoven[b], Gerwin D. Rodenburg[a], Eelko Hak[c,d], Elisabeth A.M. Sanders[a]

[a] Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands
[b] Research Center Linnaeus Institute, Spaarne Hospital, Hoofddorp, The Netherlands
[c] Department of PharmacoEpidemiology & PharmacoEconomics, University Medical Center, Groningen, The Netherlands
[d] Department of Epidemiology, University Medical Center, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 30 April 2011
Received in revised form 1 August 2011
Accepted 8 August 2011
Available online 3 September 2011

Keywords:
Pneumococcal conjugate vaccine
Carriage
Colonization
Replacement
Otitis media
Haemophilus influenzae
Moraxella catarrhalis
Infants

ABSTRACT

Seven-valent CRM197-conjugated pneumococcal conjugate vaccine ($PCV7_{CRM197}$) reduces both vaccine serotype nasopharyngeal colonization and vaccine serotype acute otitis media by 50–60%. However, overall pneumococcal carriage and impact on otitis media are partly offset by concomitant increase of nonvaccine serotypes. We investigated in a randomized controlled trial the impact of 2-doses and 2 + 1-doses of $PCV7_{CRM197}$ on carriage of *Streptococcus pneumoniae* and of other nasopharyngeal commensals and well-known otitis media pathogens, *Haemophilus influenzae* and *Moraxella catarrhalis,* in children. Nasopharyngeal swabs were collected at the age of 6 weeks and at 6, 12, 18 and 24 months. We observed high carriage rates up to 68% for *S. pneumoniae*, 71% for *H. influenzae* and 68% for *M. catarrhalis* at the age of 18 months. Reduced dose $_{CRM197}$ schedules induced a slight reduction in overall pneumococcal carriage but no increases in the presence of *H. influenzae* and *M. catarrhalis*.

1. Introduction

The 3 + 1-dose schedule with seven-valent CRM197-conjugated pneumococcal vaccine ($PCV7_{CRM197;}$ Pfizer) not only provides high protection of 97% against vaccine serotype invasive pneumococcal disease (IPD), but also against pneumococcal vaccine serotype nasopharyngeal carriage and acute otitis media (AOM) by 50–60% [1–3]. The vacant nasopharyngeal ecological niche following PCV7 seems to become immediately occupied by nonvaccine serotype pneumococci either as a result of unmasking or of true replacement and leading to only a limited or no net reduction in overall pneumococcal carriage [3–5]. Also for clinical disease, in a randomized controlled trial investigating the impact of 2 different PCVs ($PCV7_{CRM197}$ and $PCV7_{OMPC}$) on AOM in infants, indeed the efficacy was limited due to an increase in nonvaccine serotype pneumococcal AOM. Simultaneously an increase in *Haemophilus influenzae* and *Moraxella catarrhalis* in middle ear fluids was observed [2,6]. This points to a potential replacement phenomenon in respiratory disease not only by nonvaccine pneumococci but also other commensals of the nasopharynx, like *H. influenzae* and *M. catarrhalis* that are frequently involved in respiratory disease in childhood [7,8].

No randomized controlled trial investigating the effect of $PCV7_{CRM197}$ on nasopharyngeal colonization with non-pneumococcal respiratory pathogens has been performed. We explored the effects of reduced-dose $PCV7_{CRM197}$ schedules on nasopharyngeal colonization with *H. influenzae* and *M. catarrhalis* in infants, well before the introduction of PCV7 in the Dutch national infant immunization program in June 2006.

2. Materials and methods

2.1. Study design and participants

This study exploring the effects of a 2-dose and 2 + 1-dose $PCV7_{CRM19}$ schedule on nasopharyngeal colonization with *H. influenzae* and *M. catarrhalis* was part of a randomized controlled

* Corresponding author at: University Medical Centre Utrecht/Wilhelmina Children's Hospital, Department Paediatric Immunology and Infectious Diseases, HP: KB.03.023.3, P.O. Box 85090, 3508 AB Utrecht, The Netherlands,
Tel.: +31 (0)6 17674542; fax: +31 (0)88 7555351.
E-mail address: e.j.m.vangils@umcutrecht.nl (E.J.M. van Gils).

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doi:10.1016/j.vaccine.2011.08.049

trial on the effect of reduced-dose PCV-7 schedules on pneumococcal carriage (NCT00189020). Written informed consent was obtained from both parents. The study received approval by an acknowledged national ethics committee (STEG) from the Netherlands and was undertaken in accordance with the European Statements for Good Clinical Practice, which includes the provisions of the Declaration of Helsinki. Parents did not receive financial compensation.

The trial methodology has been previously described and results have been published for pneumococcal carriage efficacy [9]. In short, infants were randomly allocated (1:1:1) to receive (1) $PCV7_{CRM197}$ at the age of 2 and 4 months (2-dose group), (2) $PCV7_{CRM197}$ at 2, 4, and 11 months (2 + 1-dose group), or (3) no $PCV7_{CRM197}$ (unvaccinated control group).

2.2. Laboratory procedures

Deep nasopharyngeal samples were taken transnasally with a flexible, sterile, dry cotton-wool swab (Transwab Pernasal Plain, Medical Wire & Equipment Co. Ltd.) by trained study nurses according to WHO standard procedures at age 6 weeks, and age 6, 12, 18, and 24 months [10]. After sampling, swabs were immediately inoculated in Transwab (modified Amies) transport medium and stored at room temperature. Swabs were plated within 24 h onto chocolate agar, Haemophilus chocolate agar and 5% sheep blood agar, 1 with and 1 without 5 mg/L gentamicin. Agar plates were incubated at 35 °C for 48 h; the blood agar plate without gentamicin aerobically and the blood agar plate with gentamicin and the chocolate agar plates with raised CO_2. Isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were identified using colony morphology and conventional methods of determination. A questionnaire on risk factors for nasopharyngeal bacterial colonization was obtained with each nasopharyngeal swab.

2.3. Sample size and statistical methods

Sample size calculation for the trial was based on the main outcome measure of the study, being vaccine serotype pneumococcal carriage in the second year of life, and resulted in a sample size of 330 infants per group including a 10% dropout rate [9]. The present analyses were planned as secondary outcomes. All statistical analyses were carried out according to the intention-to-treat principle. Determinants for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* carriage in unvaccinated control children from the age of 6–24 months were identified using 3 separate generalized estimating equations (GEE) models, including sex, age, day care attendance, symptoms of upper respiratory tract infection (URTI), presence of siblings in the household, antibiotic consumption during the last 2 months prior to swab, use of pacifier and season of sampling (October–March versus April–September). To investigate the effect of $PCV7_{CRM197}$ on *H. influenzae* and *M. catarrhalis* carriage, we constructed 2 separate repeated measurement models with an unstructured correlation structure (SPSS version 17.0) with *H. influenzae* and *M. catarrhalis* carriage respectively as the dependent variable and age as within-subject variable [12]. Next to the assigned vaccination schedule (controls, 2-dose, or 2 + 1-dose), determinants associated with colonization ($P < 0.10$) were included in the model. *P*-values smaller than 0.05 were considered significant and all reported *P*-values are 2-sided.

3. Results

3.1. Enrolment

As reported previously 1005 infants were enrolled between July 7, 2005 and February 9, 2006, well before the introduction of $PCV7_{CRM197}$ in the Dutch national infant immunization program in June 2006 [9,13]. The study ended when the last child had reached 24 months of age on February 14, 2008. A total of 4939 (99% of planned) nasopharyngeal samples were collected. There were no major differences in demographics or distribution of risk factors (e.g., number of siblings, day care attendance) between the 3 study groups [14].

3.2. Carriage in PCV7 unvaccinated children

Carriage rates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* increased in the first year of life (Fig. 1). Factors associated with carriage in unvaccinated children from the age of 6 months to 24 months are presented in Table 1.

3.3. Carriage in $PCV7_{CRM197}$ vaccinated children

The effects of $PCV7_{CRM197}$ on pneumococcal and *S. aureus* colonization have been previously published [9,14]. Overall (vaccine and nonvaccine serotypes together) pneumococcal carriage was slightly reduced in vaccinated children, being 55% (95% CI 49–60%) and 57% (95% CI 52–62%) in children in the 2-dose group and 2 + 1-dose group, respectively, compared to 66% (95% CI 61–71%) in unvaccinated children. As for the other bacteria, there was no significant effect of PCV7 vaccination in the overall significance test of model effects on *H. influenzae* ($P = 0.20$) or *M. catarrhalis* ($P = 0.39$) carriage from age 6 to 24 months. When investigating separate time points to evaluate possible temporary effects, no significant differences in carriage with *H. influenzae* were observed between vaccinated children of either vaccination schedule and controls (Fig. 1). With respect to *M. catarrhalis*, also no significant changes in carriage were observed. Only at 12 months, the point estimate for children in the 2 + 1-dose group (61%, 95% CI 56–66%), 1 month after receiving the $PCV7_{CRM197}$ booster dose, was significantly lower compared to unvaccinated control children (68%, 95% CI 63–73%; $P = 0.017$) (Fig. 1). Determinants for bacterial carriage in vaccinated children were similar compared to unvaccinated controls (data not shown).

4. Discussion

In this randomized controlled study with a 2-dose or 2 + 1-dose $PCV7_{CRM197}$-schedule, we did not observe substantial changes in carriage of *H. influenzae* and *M. catarrhalis* after pneumococcal vaccination in children in the first 2 years of life. This is despite the fact that we had found significant reductions in vaccine serotype carriage after both $PCV7_{CRM197}$ schedules with a final 60% reduction and concomitant shifts to nonvaccine serotype carriage at 24 months of age [9]. We observed slightly higher point estimates for *H. influenzae* carriage in vaccinated children compared to unvaccinated controls at the age of 12 and 18 months, but these differences were not significant.

Post-licensure studies on AOM incidence following the introduction of the 3 + 1-dose $PCV7_{CRM197}$ schedule in the USA for children do show relative increases in *H. influenzae* positive and *M. catarrhalis* positive middle ear fluid cultures together with a decrease in pneumococcal and vaccine serotype AOM but an increase in nonvaccine serotype AOM, particularly serotype 19A [15–18]. These are ecological and uncontrolled observations, and do not need to represent true increases of otitis due to non-pneumococcal pathogens. However, higher pneumococcal vaccine pressure following the 3 + 1 schedule (not included in this study) and/or nationwide introduction in the routine infant vaccination schedule may nevertheless over time induce bacterial shifts in the population, which cannot be detected in our trial setting. Future