Vaccine 37 (2019) 2935–2941

Contents lists available at ScienceDirect

Vaccine

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

Effectiveness of the ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10) against all respiratory tract infections in children under two years of age

Sinikka Karppinen [a,*], Laura Toivonen [a], Linnea Schuez-Havupalo [a], Tamara Teros-Jaakkola [a], Matti Waris [b], Kari Auranen [c,d], Arto A. Palmu [e], Ville Peltola [a]

[a] Department of Paediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku, Finland
[b] Department of Virology and Clinical Virology, University of Turku and Turku University Hospital, Turku, Finland
[c] Department of Mathematics and Statistics, University of Turku, Turku, Finland
[d] Department of Clinical Medicine, University of Turku, Turku, Finland
[e] Department of Public Health Solutions, National Institute for Health and Welfare, Tampere, Finland

ARTICLE INFO

Article history:
Received 5 February 2019
Received in revised form 3 April 2019
Accepted 12 April 2019
Available online 23 April 2019

Keywords:
Acute otitis media
Children
Pneumococcal conjugate vaccine
Respiratory tract infection
Streptococcus pneumoniae

ABSTRACT

Background: Pneumococcal conjugate vaccines reduce the incidence of invasive pneumococcal diseases, pneumonia, acute otitis media (AOM), and antimicrobial prescriptions in children. We investigated the effectiveness of at least one dose of the ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10; GSK) against respiratory tract infections (RTIs) in children under two years of age.

Methods: 424 children enrolled in a cluster–randomized, double-blind Finnish Invasive Pneumococcal disease (FinIP) vaccine trial during the years 2009–2010 were actively followed in a prospective cohort study (STEPS study) for RTIs from birth to two years of age. Children received the PHiD-CV10 vaccine, or a control vaccine (hepatitis A or B vaccine) according to an age-specific schedule. Data on RTIs were collected by symptom diaries, clinic visits, an electronic registry on hospitalizations, and by nasal swab samples analyzed for respiratory viruses. We estimated the vaccine effectiveness against all RTI episodes and RTI episodes with or without AOM by comparing the corresponding incidence rates between PHiD-CV10 vaccinated and control children, adjusted for presence of siblings and cluster as a random effect.

Results: A total of 3193 RTI episodes were documented after the first vaccination in 368 children with all data available. The majority of the illnesses were upper RTIs caused by rhinovirus. The PHiD-CV10-vaccinated children had lower mean annual rates of all RTI episodes (6.4; 95% confidence interval [CI], 6.0–6.8) and RTI episodes with AOM (1.0; 95% CI, 0.9–1.2) as compared to the control children (7.4; 95% CI, 6.8–8.0 and 1.3; 95% CI, 1.1–1.6, respectively). The vaccine effectiveness was 12% (95% CI, 2–22%) against all RTIs, 23% (95% CI, 0–40%) against RTIs with AOM, and 10% (95% CI, 0–19%) against RTIs without AOM.

Conclusions: Vaccination with PHiD-CV10 resulted in lower rates of RTIs in children under two years of age compared to children vaccinated with control vaccine.

1. Introduction

Pneumococcal conjugate vaccines prevent invasive disease, pneumonia, and acute otitis media (AOM) caused by *Streptococcus pneumoniae,* and they reduce prescriptions of antibiotics in children [1–8]. The conjugate vaccines also reduce asymptomatic nasopharyngeal colonization by the vaccine serotypes of pneumococci through protecting against their acquisition [9–11].

Colonization of the nasopharynx serves as a reservoir for transmission of bacteria and constitutes the first step in the development of diseases. Pneumococcal colonization is more common in childhood than in adulthood and up to 87% of children are colonized with pneumococcus before two years of age [12,13]. Higher prevalence of pneumococcal colonization is associated with presence of respiratory symptoms, higher number of siblings, lower

* Corresponding author at: Department of Paediatrics and Adolescent Medicine, Turku University Hospital, 20521 Turku, Finland.
E-mail address: simahe@utu.fi (S. Karppinen).

https://doi.org/10.1016/j.vaccine.2019.04.026
0264-410X/

socioeconomical status, and certain genetic variants in the host immune system [12,14–17].

Development of pneumococcal AOM, pneumonia, and invasive disease during or after influenza or other respiratory virus infection has been well described in clinical studies, and the viral-bacterial synergism has been modelled in small animals [18–22]. The effects of viruses on the epithelium of the respiratory tract make it favorable for bacterial colonization. Viruses may impair the ciliary function, damage the epithelium, and expose receptors for bacterial adhesion [23–27]. Specific alterations occur in the host immune response mechanisms during and after respiratory virus infection, inducing a period of vulnerability to bacterial infections [28]. In turn, bacterial colonization may affect viral infections. Colonizing bacteria increase epithelial cell susceptibility to virus infection and facilitate attachment of viruses to cells [29,30].

In children, pneumococcal colonization is more frequent during respiratory virus infections compared to the healthy state [31]. Rhinovirus infection in children increases acquisition of pneumococci from outside the family and facilitates transmission of pneumococci between family members [15]. In colonized individuals, bacterial density increases in the nasopharynx during viral infections, which may facilitate further spread of the bacteria in the respiratory tract and transmission to other people [32–34]. Bacterial colonization during a viral infection has been related to increased symptoms and longer duration of viral infection [11,35,36]. At the population level, several studies have described temporal associations between circulation of respiratory viruses and increased incidence of pneumonia and invasive pneumococcal diseases [15,37–41].

Because of the important role of viral-bacterial interactions in the pathogenesis of respiratory tract infections (RTIs), the impact of pneumococcal conjugate vaccines can be hypothesized to reach beyond strictly-defined pneumococcal infections. Supporting this hypothesis, pneumococcal vaccination reduced not only bacterial pneumonia hospitalizations but also virus-positive pneumonia hospitalizations in a randomized trial [42]. Furthermore, a reduction in the overall rate of RTIs after immunization of children with a pneumococcal conjugate vaccine has been described [8].

The vast majority of RTIs are caused by respiratory viruses, rhinovirus being the most frequently detected virus. Nevertheless, pneumococcal conjugate vaccines may affect the rates of noncomplicated RTIs because of interactions between respiratory viruses and colonizing pneumococci. Because of the high disease burden of RTIs in children, it is important to estimate the overall impact of pneumococcal conjugate vaccines on RTIs. We assessed the total effectiveness of the ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10; GSK) on all RTIs during the first two years of life in children who received at least one dose of the vaccine before 19 months of age in a cluster-randomized trial.

2. Methods

2.1. Study design and conduct

A total of 923 children were followed for RTIs and their viral etiology from birth until two years of age in a prospective cohort study (STEPS Study) from April 2008 through April 2012. No selection criteria other than knowledge of the Finnish or Swedish language by the parent(s) were applied with regard to the recruitment of children to the STEPS Study. Data on RTIs were collected by daily symptom diaries, study clinic visits, and from the electronic registry of the Hospital District of Southwest Finland. Parents were asked to document all symptoms of RTIs as well as all physician visits and associated diagnoses in the diary. If an evaluation by a physician was needed, parents were encouraged to bring the child to a special study clinic, where clinical findings were examined and documented. Data on hospitalizations were collected from the electronic registry. Nasal swab samples were collected from both nostrils for virus diagnostics at the onset of an RTI either by a physician at the study clinic or at home by trained parents who sent them to the study clinic by mail [43,44].

Of the 923 children, 424 were enrolled in a separate Finnish Invasive Pneumococcal disease (FinIP) vaccine trial in 2009–2010 [2]. These 424 children were the study population of the present study, which was planned in the STEPS Study and the FinIP trial protocols. FinIP was a controlled, cluster-randomized, double-blind field trial designed to assess the effectiveness of PHiD-CV10 against invasive pneumococcal disease. In the FinIP trial, children younger than 19 months were randomized to receive PHiD-CV10 or a hepatitis B or A vaccine as control. The vaccination schedule was based on the child's age at the time of enrollment. Children younger than 7 months of age at first vaccination followed either a 3 + 1 or a 2 + 1 vaccination schedule, those 7–11 months of age followed a 2 + 1 schedule, and those 12–18 months of age followed a two-dose schedule. Hepatitis B vaccine was given to control children under 12 months of age and hepatitis A vaccine to control children over 12 months of age.

Children were recruited to the FinIP trial by nurses working in local well-baby clinics of the public health care centers or by the Tampere University Vaccine Research Centre conducting a parallel AOM trial. The areas of participating study clinics were divided geographically into 78 clusters (52 PHiD-CV10 and 26 control clusters) according to the administrative structures and birth cohort sizes of the centers. The southwest part of Finland (Turku area) where the STEPS study was conducted included 11 clusters. In seven clusters children received the PHiD-CV10 vaccine and in four clusters the control vaccine [2].

The STEPS study was approved by The Ministry of Social Affairs and Health and the ethics committee of the Hospital District of Southwest Finland. The FinIP vaccine trial was approved by relevant ethical review boards and competent authorities and it was registered at ClinicalTrials.gov (numbers NCT00861380 and NCT00839254). The parents of the participating children gave their written, informed consent separately for the STEPS Study and the FinIP trial.

2.2. RTI episodes

An episode of acute RTI was defined as rhinitis, cough, or wheezing, with or without fever, documented in the diary by the parents, or by a physician as a diagnosis of an acute RTI. If the symptoms were continuous and nasal swab samples were collected repeatedly, or if there were repeated diagnoses of pneumonia, AOM, or wheezing illness during continuous symptoms, one RTI episode was defined to last 14 days. If the data from diaries were missing, symptoms and diagnoses 1 day before and 14 days after the nasal swab collection were linked with the virological results [45,46].

2.3. Virus detection

Rhinoviruses, enteroviruses, and respiratory syncytial virus (RSV) were detected by multiplex, quantitative reverse transcription-polymerase chain reaction (RT-qPCR) with dual-labeled locked nucleic acid probes as described earlier [47,48]. All specimens collected during influenza seasons were analyzed for influenza A and B viruses by RT-qPCR [49]. Eighty-nine percent of all samples were also analyzed for influenza A and B viruses, parainfluenza type 1, 2 and 3 viruses, adenovirus, human

metapneumovirus, and RSV by a laboratory developed antigen detection test.

2.4. Statistical analysis

Categorical variables were compared with the Chi-square test. The Mann-Whitney test was used to compare the duration of infection episodes between the PHiD-CV10-vaccinated and control-vaccinated children. Incidence rates of RTI episodes were estimated as the total number of RTI episodes divided by the follow-up time. The vaccine effectiveness (VE) was defined as (1-relative incidence rate) × 100. The VE determined in this cluster randomized trial is the total VE comparing PHiD-CV10-vaccinated and control-vaccinated children from the corresponding vaccination clusters. To account for possible heterogeneity across children in the rate of RTI episodes, VE was estimated using negative binomial regression with a log link and the follow-up time as the offset. Because of cluster-randomization, cluster identifier was included as a random effect in the regression model. As the number of vaccination clusters was small we considered potential confounding and added background variables that differed between the groups (older siblings, living in urban vs. non-urban residential area, and socioeconomic status of the family) to the model one-by-one. The adjustment for older siblings had minor effect and adjustments for residential area and socioeconomic status had no impact on the VE estimates. These variables correlated with each other. We have earlier reported from the STEPS Study that of these background variables only the older siblings is a risk factor for RTIs and acute otitis media [45]. Therefore, only older siblings were included in the final model as a confounder. Combined data from the 3 + 1 and 2 + 1 vaccination schedules were used in the analyses. We report point estimates and 95% confidence intervals (CI). The data were analysed using SPSS version 23 and R version 3.4.3.

3. Results

3.1. Study population

Of the 424 children who participated in both the STEPS Study and the FinIP trial, 25 were excluded because of an error in the randomization and further 31 children were excluded because of missing information on RTIs after the first study vaccination. Of the 368 children included in the analysis, 47% were female and 40% had older siblings. Most study families lived in an urban area and 73% of parents had higher education level (Table 1). The PHiD-CV10 and control vaccine groups differed from each other in terms of type of residential area (urban vs. non-urban), presence of older siblings, and the socioeconomic status of the family, reflecting differences in the social structure between the geographical locations of the FinIP clusters within the STEPS study area.

3.2. Vaccinations and follow-up

The PHiD-CV10 vaccine was administered to 236 children and the control vaccine to 132 children. The first vaccination was given at the time of recruitment to the FinIP trial; 266 children received their first vaccine at the age of 6 weeks to 6 months, 38 at the age of 7–11 months, and 64 at the age of 12–18 months. The median duration of the follow-up for RTIs after the first vaccination was 1.4 (interquartile range [IQR], 1.0–1.7) years. After stratification into three groups by age at the first vaccination, the median durations of follow-up were 1.7 (IQR, 1.2–1.7), 1.2 (0.7–1.3), and 0.9 (0.5–1.0) years, respectively.

3.3. Characteristics of RTI episodes

During the follow-up, which started from the first FinIP trial vaccination, a total of 3193 episodes of RTI were documented in the 368 children included in the analysis (median 9, IQR 4–12). Eight children (2%) did not have any reported RTIs during the follow-up. The median duration of RTIs was 7 (IQR 4–12) days. The median duration between the first vaccination and the first RTI episode was 0.17 (IQR, 0.07–0.31) years. In 59 RTI episodes, symptoms started within two weeks after the first vaccination. The majority of illnesses were upper RTIs. AOM was detected in 521 (16%), wheezing in 93 (3%), and pneumonia in 12 (0.4%) episodes. Antibiotics were prescribed in 629 (20%) episodes. Outpatient visits at physician offices were documented in 1289 (40%) episodes. Twelve children were hospitalized during 26 RTI episodes. Wheezing illnesses were the most common reason for hospital treatment.

3.4. Viral etiology of RTIs

Nasal swab samples for virus analysis were taken during 1679 (53%) RTI episodes. Samples were routinely collected at study clinic visits but during episodes without a study clinic visit the parents collected the samples with a variable activity, which explains why all RTI episodes could not be analyzed for virus etiology. Virus was detected in 1227 (73%) of tested episodes. There were no significant differences in the detection rate of respiratory viruses or in the distribution of viral etiologies of RTIs between the PHiD-CV10 and control groups (Fig. 1). A respiratory virus was detected in 72% (809/1123, 95% CI, 0.69–0.75) of RTI episodes in PHiD-CV10-vaccinated and in 75% (418/556, 95% CI 0.71–0.79) of episodes in control children. Rhinovirus was detected most frequently, in 59% (661/1123, 95% CI, 0.55–0.62) of RTI episodes in PHiD-CV10 vaccinated and in 63% (349/556, 95% CI, 0.59–0.67) of episodes in control, followed by RSV in 5% (59/1123, 95% CI, 0.04–0.07) and 6% (32/556, 95%, CI, 0.04–0.08) of RTIs, respectively. Data on virus diagnostics were available for 267 (51%) of AOM related episodes. A virus was detected in 73% of samples collected during RTIs with AOM, and there were no differences in virus findings between children who had received the PHiD-CV10 or control vaccine (Fig. 2).

3.5. VE for RTI episodes

RTI episodes were less common in children who received PHiD-CV10 than in children who received the control vaccine. The respective incidence rates (95% CI) were 6.4 (6.0–6.8) and 7.4

Table 1
Characteristics of the 368 study subjects.

Characteristic	Total,n/N (%)	PHiD-CV10, n/N (%)	Control, n/N (%)	p
Female	172/368 (47)	113/236 (48)	59/132 (45)	0.56
Older siblings	148/368 (40)	82/236 (35)	66/132 (50)	0.004
Parent́s education level professional	24/6/337 (73)	147/212 (69)	99/125 (79)	0.049
Living in the urban area	304/358 (85)	208/228 (91)	96/130 (74)	<0.001
Mother smoking during pregnancy or during the first 4 months	18/327 (6)	14/210 (7)	4/117 (3)	0.22
Length of pregnancy ≥ 37 weeks	350/368 (95)	224/236 (95)	126/132 (95)	0.82

N = number of subjects with data available.

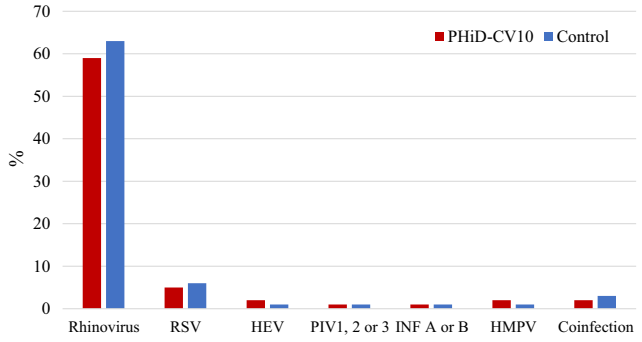

Fig. 1. Viruses detected in nasal swab samples collected during symptomatic RTIs in children who received PHiD-CV10 or the control vaccine. Rhino- and enteroviruses (HEV) were detected with PCR, respiratory syncytial virus (RSV) and influenza (INF) A and B viruses with PCR and/or antigen tests, and parainfluenza virus (PIV) 1, 2, and 3, human metapneumovirus (HMPV), and adenovirus with an antigen test. There were no significant differences by the Chi-square test in the proportion of detected viruses between the PHiD-CV10-vaccinated and control children. Coinfection means here simultaneous detection of 2 or more viruses.

(6.8–8.0) per person-year (Table 2). The vaccine effectiveness (VE) of PHiD-CV10 against all RTI episodes was 12% (95% CI, 2–22%).

The VE was 23% (95% CI, 0–40%) against RTI episodes with AOM and 10% (0–19%) against episodes without AOM (Table 2). Children in the PHiD-CV10 group also had fewer antibiotic prescriptions for RTIs than children in the control group. The median duration of RTI episodes was 8 days (IQR, 5–12) in PHiD-CV10-vaccinated and 7 days (4–11) in control children (p = 0.078).

The VE against rhinovirus-positive RTIs was 8% (95% CI, −13 to 25%). A higher VE was found against RTI episodes from which a nasal swab sample was not available for virus analysis (23%; 95% CI, 9–35%). The VE against episodes with AOM and without a nasal swab sample was 49% (95% CI, 27–64%). Durations of episodes with nasal swab samples were significantly longer compared to episodes where the swab was not collected (median, 9; IQR, 6–13 vs. 6; IQR 3–10, p < 0.001).

In children who received the first vaccine at the age of 6 weeks to 6 months, the mean annual incidence rate of all RTI episodes was 6.4 (95% CI, 6.0–6.8) in the PHiD-CV10 group and 7.3 (6.7–8.0) in the control group (Table 3). The VE was thus 12%

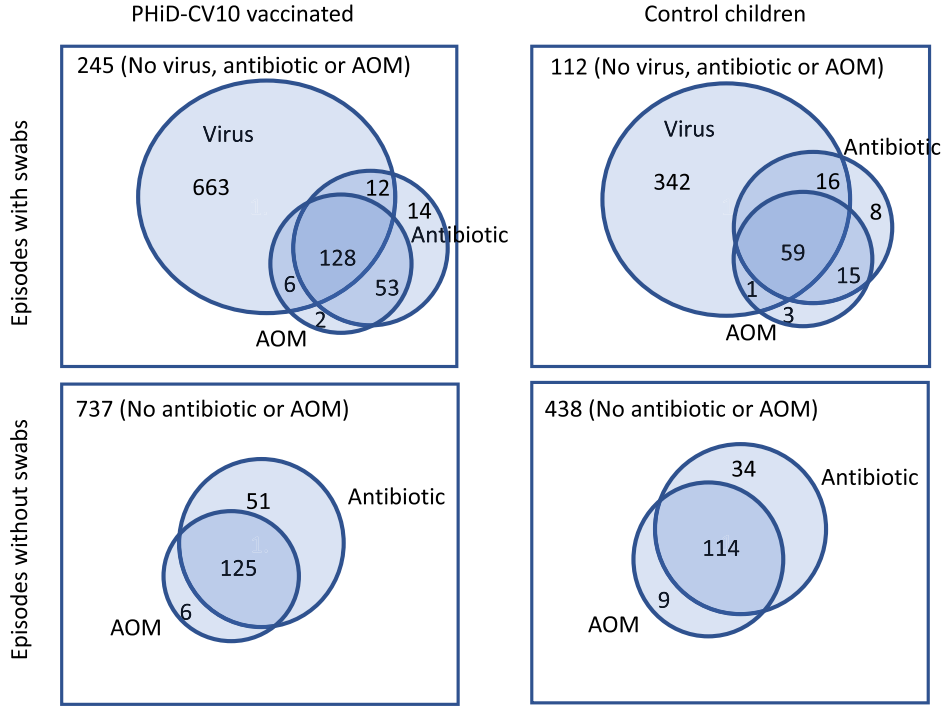

Fig. 2. Venn diagrams of the numbers of episodes with acute otitis media (AOM), antibiotic prescription, and/or viral finding in children vaccinated with PHiD-CV10 or control vaccine (upper boxes), and of the numbers of episodes with AOM and/or antibiotic prescription in children without nasal swab samples (lower boxes).

Table 2
The effectiveness of PHiD-CV10 against RTIs. The effectiveness is the relative reduction in the incidence rate of RTI episodes, based on comparing the PHiD-CV10-vaccinated and control children. The effectiveness estimates are adjusted for the effect of vaccination cluster and siblings.

	Incidence rate of RTI episodes (per person-year) (95% CI)		Vaccine effectiveness, % (95% CI)
	PHiD-CV10-vaccinated children (n = 236)	Control children (n = 132)	
All RTI episodes	6.4 (6.0–6.8)	7.4 (6.8–8.0)	12 (2–22)
RTIs with AOM	1.0 (0.9–1.2)	1.3 (1.1–1.6)	23 (0–40)
RTIs without AOM	5.4 (5.0–5.7)	6.1 (5.6–6.6)	10 (0–19)
RTIs with antibiotic use	1.2 (1.0 – 1.4)	1.6 (1.3 – 2.0)	24 (3 – 40)
RTIs with any virus	2.5 (2.3 – 2.8)	2.7 (2.3 – 3.1)	4 (-15 – 21)
RTIs with rhinovirus	2.1 (1.8 – 2.3)	2.3 (1.9 – 2.6)	8 (-13 – 25)
RTIs without any virus	1.0 (0.9 – 1.1)	0.9 (0.7 – 1.1)	−11 (-40 – 13)
RTIs without nasal swab sample	2.9 (2.6 – 3.1)	3.7 (3.3 – 4.3)	23 (9 – 35)

A total of 3193 RTI episodes were documented and 1679 (53%) of these episodes were tested for viruses.
AOM, acute otitis media; CI, confidence interval; PHiD-CV10, ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine; RTI, respiratory tract infection.

Table 3
The effectiveness of PHiD-CV10 against RTIs in children < 7 months of age and children 7 to 18 months of age at the time of vaccination. The effectiveness is the relative reduction in the incidence rate of RTI episodes, based on comparing the PHiD-CV10-vaccinated and control children. The effectiveness estimates are adjusted for the effect of vaccination cluster and siblings.

	Incidence rate of RTI episodes (per person-year) (95% CI), children < 7 months of age at the time of first vaccination			Incidence rate of RTI episodes (per person-year) (95% CI), children 7–18 months of age at the time of first vaccination		
	PHiD - CV10 – vaccinated (n = 182)	Control children (n = 84)	Vaccine effectiveness % (95% CI)	PHiD - CV10 – vaccinated (n = 54)	Control children (n = 48)	Vaccine effectiveness % (95% CI)
All RTI episodes	6.4 (6.0–6.8)	7.3 (6.7–8.0)	12 (0–21)	6.5 (5.6–7.6)	7.6 (6.5–8.9)	15 (−7 to 31)
RTIs with AOM	1.0 (0.8–1.1)	1.2 (0.9–1.5)	19 (−9 to 39)	1.3 (0.9–1.8)	1.6 (1.1–2.4)	22 (−32 to 54)
RTIs without AOM	5.4 (5.0–5.8)	6.1 (5.5–6.8)	10 (−2 to 20)	5.2 (4.5–6.2)	6.0 (5.1–7.1)	13 (−9 to 31)
RTIs with antibiotic use	1.0 (0.8–1.1)	1.2 (0.9–1.5)	19 (−9 to 39)	1.3 (0.9–1.8)	1.6 (1.1–2.4)	22 (−32 to 54)
RTIs with any virus	2.5 (2.2–2.8)	2.6 (2.2–3.1)	0 (−21 to 19)	2.6 (2.0–3.3)	2.8 (2.2–3.6)	23 (−32 to 55)
RTIs with rhinovirus	2.1 (1.8–2.3)	2.2 (1.8–2.6)	3 (−21 to 23)	2.1 (1.6–2.7)	2.4 (1.9–3.2)	19 (−32 to 50)
RTIs without any virus	1.0 (0.9–1.1)	0.8 (0.6–1.0)	−24 (−69 to 8)	1.0 (0.7–1.3)	1.2 (0.8–1.6)	13 (−34 to 43)
RTIs without nasal swab sample	2.8 (2.6–3.2)	3.8 (3.3–4.4)	26 (11–38)	2.9 (2.2–3.8)	3.6 (2.7–4.8)	13 (−43 to 47)

A total of 3193 RTI episodes were documented and 1679 (53%) of these episodes were tested for viruses.
AOM, acute otitis media; CI, confidence interval; PHiD-CV10, ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine; RTI, respiratory tract infection.

(95% CI, 0–21%). In children vaccinated with the catch-up schedules, there were no statistically significant differences in the rate of all RTI episodes or RTI episodes with or without AOM between the PHiD-CV10 and control groups. These groups of children were relatively small.

4. Discussion

In this analysis, we utilized the unique setting of the FinIP vaccine trial and the STEPS observational birth cohort study conducted within the same region and period of time. We combined data of children participating in both studies and found that vaccination with the ten-valent pneumococcal conjugate vaccine (PHiD-CV10) was associated with a 12% reduction (95% CI, 2–22%) in the rate of RTIs in children under two years of age. The effectiveness of pneumococcal vaccination was seen in RTI episodes both with and without AOM. These findings suggest that the PHiD-CV10 vaccine may prevent part of noncomplicated RTIs (i.e., without concomitant AOM, sinusitis, or pneumonia) in children, although these infections are primarily caused by viruses. Our study can be regarded as a vaccine probe study that increases understanding of the disease burden preventable by the PHiD-CV10 vaccine [50].

Pneumococcal conjugate vaccines are highly effective in preventing invasive pneumococcal diseases. Several studies have reported substantial decreases also in the rates of AOM, pneumonia, and antibiotic prescriptions, despite the fact that the pneumococcus is only one of the agents causing AOM or pneumonia [1,7,8]. Any impact of the pneumococcal vaccines on all RTIs, most of which are limited to the upper respiratory tract and are caused by viruses, has remained largely unknown. Sigurdsson et al analyzed the impact of introducing PHiD-CV10 in the childhood immunization program on hospital visits and admissions for overall RTIs, AOM, pneumonia, and bronchiolitis in Iceland [8]. In addition to a 26% reduction in all-cause AOM, there was a significant reduction in all RTIs in children 1–2 years of age. In a randomized, controlled study in Israel, the rate of respiratory tract illness episodes and use of antibiotics were reduced in children who received the nine-valent pneumococcal conjugate vaccine [7]. Our findings are in accordance with these earlier reports.

Increased nasopharyngeal colonization by pathogenic bacteria occurs in both noncomplicated and complicated viral RTIs [51,52]. Almost all AOM cases develop during a viral RTI [46]. In our study, all AOMs were related to symptomatic RTIs, which were mostly caused by rhinovirus or other respiratory viruses. The decrease in the rate of AOM after pneumococcal vaccination has been explained by reduced nasopharyngeal colonization and spread to the middle ear of pneumococci during viral respiratory infection [53,54]. However, the decrease in colonization by pneumococcal vaccine serotypes may also affect the development of symptomatic viral RTIs by preventing attachment of viruses to the host cells [29,30].

We found an effect of the PHiD-CV10 vaccine on the incidence rate of RTIs but not on the duration of symptoms during RTI episodes. The effect on RTIs was seen especially in those episodes, particularly with AOM, from which nasal swab samples were not obtained. We have previously reported that these episodes were milder and shorter in duration than episodes in which nasal swab samples were taken [46]. Our results suggest that prevention of colonization by the pneumococcal vaccine serotypes could inhibit development of symptoms in viral infections. Other studies have documented that symptoms of viral respiratory infection are stronger and last longer in the presence of bacterial colonization [36,55]. Cebey-Lopez et al reported that the severity of viral infection was reduced in children who had received pneumococcal conjugate vaccine [11]. The effects of vaccine-type pneumococcal colonization on symptoms of respiratory virus infection might be due to a stronger immune response during the viral–bacterial coinfection or colonization, even in the absence of pneumococcal complications such as AOM or pneumonia. The inflammatory response increases during RSV infection in cultured human epithelial cells challenged with pneumococci but not in those negative for bacteria, and the virulence of the pneumococcus may also increase [27].

Taken together, earlier findings on viral-bacterial interactions support our findings: the effect of pneumococcal conjugate vaccines may extend beyond specific etiology-proven pneumococcal diseases. This effect would bring substantial benefits for families with children, as well as for the society, because of the high disease burden caused by all-cause RTIs in children. Our results suggest that vaccine-type pneumococci contribute to the development of RTIs, either independently or in combination with viruses, and that the disease burden caused by vaccine-type pneumococci is larger than previously anticipated. Alternatively, protection against *H. influenzae* due to the carrier protein of PHiD-CV10 could have a role in our findings, or there could be nonspecific vaccine effects on other targets than the vaccine-type pneumococci or *H. influenzae*.

The FinIP vaccine trial was a nation-wide cluster-randomized trial and there were too few clusters coinciding with the catchment area of the STEPS Study to ensure complete balance of all potential

confounders between the PHiD-CV10 and control vaccine groups. The families of children in the PHiD-CV10 clusters had on average lower parental educational level, lived more often in the urban area, and had less children than the families of the control children. However, in Finland the differences in living conditions between the urban and non-urban area, and between different socioeconomic levels are rather subtle. The presence of siblings is known to increase the incidence of RTIs [45]. To account for any of these differences across areas, we adjusted the estimates of vaccine effectiveness for cluster effects and siblings. A limitation of this study is that we did not analyze pneumococcal nasopharyngeal colonization or the bacterial etiology of AOM episodes.

In conclusion, we found that vaccination with the ten-valent pneumococcal conjugate vaccine reduced the rate of all symptomatic RTIs and the rates of RTIs with or without AOM in children younger than 2 years. If corroborated by other studies, this would be an additional benefit of PHiD-CV10. Our results suggest that the potentially vaccine-preventable disease burden associated with the pneumococcus in young children may be higher than previously estimated.

Funding

There is no conflict of interest.

The STEPS study was supported by the University of Turku; Abo Akademi University; Turku University Hospital; Academy of Finland grants 123571, 140251, and 277535; the Foundation for Pediatric Research; and research funds from the Specified Government Transfers, Hospital District of Southwest Finland. Dr. Karppinen was supported by the Finnish Medical Foundation and the Foundation for Pediatric Research. The FinIP trial was funded by GlaxoSmithKline Biologicals SA, and the National Institute for Health and Welfare, Finland.

Conflict of interest

There is no conflict of interest.

References

[1] Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001;344(6):403–9.
[2] Palmu AA, Jokinen J, Borys D, et al. Effectiveness of the ten-valent pneumococcal haemophilus influenzae protein D conjugate vaccine (PHiD-CV10) against invasive pneumococcal disease: a cluster randomised trial. Lancet 2013;381(9862):214–22.
[3] Palmu AA, Jokinen J, Nieminen H, et al. Effect of pneumococcal haemophilus influenzae protein D conjugate vaccine (PHiD-CV10) on outpatient antimicrobial purchases: a double-blind, cluster randomised phase 3–4 trial. Lancet Infect Dis 2014;14(3):205–12.
[4] Grijalva CG, Nuorti JP, Arbogast PG, Martin SW, Edwards KM, Griffin MR. Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. Lancet 2007;369(9568):1179–86.
[5] Griffin MR, Zhu Y, Moore MR, Whitney CG, Grijalva CGUS. hospitalizations for pneumonia after a decade of pneumococcal vaccination. N Engl J Med 2013;369(2):155–63.
[6] Palmu AA, Rinta-Kokko H, Nohynek H, Nuorti JP, Kilpi TM, Jokinen J. Impact of ten-valent pneumococcal conjugate vaccine on pneumonia in finnish children in a nation-wide population-based study. PLoS ONE 2017;12(3):e0172690.
[7] Dagan R, Sikuler-Cohen M, Zamir O, Janco J, Givon-Lavi N, Fraser D. Effect of a conjugate pneumococcal vaccine on the occurrence of respiratory infections and antibiotic use in day-care center attendees. Pediatr Infect Dis J. 2001;20(10):951–8.
[8] Sigurdsson S, Kristinsson KG, Erlendsdottir H, Hrafnkelsson B, Haraldsson A. Decreased incidence of respiratory infections in children after vaccination with ten-valent pneumococcal vaccine. Pediatr Infect Dis J. 2015;34(12):1385–90.
[9] Vesikari T, Forsten A, Seppa I, et al. Effectiveness of the 10-valent pneumococcal nontypeable haemophilus influenzae protein D-conjugated vaccine (PHiD-CV) against carriage and acute otitis media-A double-blind randomized clinical trial in finland. J Pediatric Infect Dis Soc 2016;5(3):237–48.
[10] Littorin N, Ahl J. Udden F, Resman F, Riesbeck K. Reduction of streptococcus pneumoniae in upper respiratory tract cultures and a decreased incidence of related acute otitis media following introduction of childhood pneumococcal conjugate vaccines in a swedish county. BMC Infect Dis. 2016;16(1). pp. 407–016-1750-5.
[11] Cebey-Lopez M, Herberg J, Pardo-Seco J, et al. Does viral co-infection influence the severity of acute respiratory infection in children? PLoS ONE 2016;11(4):e0152481.
[12] Regev-Yochay G, Raz M, Dagan R, et al. Nasopharyngeal carriage of streptococcus pneumoniae by adults and children in community and family settings. Clin Infect Dis. 2004;38(5):632–9.
[13] Syrjanen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal carriage of streptococcus pneumoniae in finnish children younger than 2 years old. J Infect Dis. 2001;184(4):451–9.
[14] Karppinen S, Vuononvirta J, He Q, Waris M, Peltola V. Effects of rhinovirus infection on nasopharyngeal bacterial colonization in infants with wild or variant types of mannose-binding lectin and toll-like receptors 3 and 4. J Pediatric Infect Dis Soc. 2013;2(3):240–7.
[15] Karppinen S, Terasjarvi J, Auranen K, et al. Acquisition and transmission of streptococcus pneumoniae is facilitated during rhinovirus infection in families with children. Am J Respir Crit Care Med. 2017;196(9):1172–80.
[16] Bogaert D, de Groot R, Hermans P. Streptococcus pneumoniae colonisation: The key to pneumococcal disease. Lancet Infect Dis 2004;4(3):144–54.
[17] Lee GM, Kleinman K, Pelton S, et al. Immunization, antibiotic use, and pneumococcal colonization over a 15-year period. Pediatrics 2017;140(5):e20170001.
[18] Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis. 2008;46(6):815–23.
[19] Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. N Engl J Med 2006;355(1):31–40.
[20] Heikkinen T, Silvennoinen H, Peltola V, et al. Burden of influenza in children in the community. J Infect Dis 2004;190(8):1369–73.
[21] Sharma-Chawla N, Sender V, Kershaw O, et al. Influenza A virus infection predisposes hosts to secondary infection with different streptococcus pneumoniae serotypes with similar outcome but serotype-specific manifestation. Infect Immun 2016;84(12):3445–57.
[22] McCullers JA. Preventing and treating secondary bacterial infections with antiviral agents. Antivir Ther 2011;16(2):123–35.
[23] McCullers JA, Rehg JE. Lethal synergism between influenza virus and streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. J Infect Dis 2002;186(3):341–50.
[24] Oliver BG, Lim S, Wark P, et al. Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. Thorax 2008;63(6):519–25.
[25] Bosch AA, Biesbroek G, Trzcinski K, Sanders EA, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. PLoS Pathog. 2013;9(1):e1003057.
[26] Ishizuka S, Yamaya M, Suzuki T, et al. Effects of rhinovirus infection on the adherence of streptococcus pneumoniae to cultured human airway epithelial cells. J Infect Dis. 2003;188(12):1928–39.
[27] Smith CM, Sandrini S, Datta S, et al. Respiratory syncytial virus increases the virulence of streptococcus pneumoniae by binding to penicillin binding protein 1a. a new paradigm in respiratory infection. Am J Respir Crit Care Med 2014;190(2):196–207.
[28] Goulding J, Godlee A, Vekaria S, Hilty M, Snelgrove R, Hussell T. Lowering the threshold of lung innate immune cell activation alters susceptibility to secondary bacterial superinfection. J Infect Dis 2011;204(7):1086–94.
[29] Nguyen DT, de Witte L, Ludlow M, et al. The synthetic bacterial lipopeptide Pam3CSK4 modulates respiratory syncytial virus infection independent of TLR activation. PLoS Pathog 2010;6(8):e1001049.
[30] Verkaik NJ, Nguyen DT, de Vogel CP, et al. Streptococcus pneumoniae exposure is associated with human metapneumovirus seroconversion and increased susceptibility to in vitro HMPV infection. Clin Microbiol Infect 2011;17(12):1840–4.
[31] Webster LT, Clow AD. The association of pneumococci, hemophilus influenzae, and streptococcus hemolyticus with coryza, pharyngitis, and sinusitis in man. J Exp Med 1932;55(3):445–53.
[32] Wolter N, Tempia S, Cohen C, et al. High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. J Infect Dis 2014;210(10):1649–57.
[33] Short KR, Reading PC, Wang N, Diavatopoulos DA, Wijburg OL. Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of streptococcus pneumoniae. MBio 2012;3(5):e00255–e312.
[34] Fan RR, Howard LM, Griffin MR, et al. Nasopharyngeal pneumococcal density and evolution of acute respiratory illnesses in young children, peru, 2009–2011. Emerg Infect Dis 2016;22(11):1996–9.
[35] Kloepfer KM, Lee WM, Pappas TE, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. J Allergy Clin Immunol 2014;133(5):1301–7.
[36] Jartti T, Kuneinen S, Lehtinen P, et al. Nasopharyngeal bacterial colonization during the first wheezing episode is associated with longer duration of hospitalization and higher risk of relapse in young children. Eur J Clin Microbiol Infect Dis 2011;30(2):233–41.
[37] Gwaltney Jr JM, Sande MA, Austrian R, Hendley JO. Spread of streptococcus pneumoniae in families. II. relation of transfer of S. pneumoniae to incidence of colds and serum antibody. J Infect Dis 1975;132(1):62–8.

[38] Peltola V, Heikkinen T, Ruuskanen O, et al. Temporal association between rhinovirus circulation in the community and invasive pneumococcal disease in children. Pediatr Infect Dis J 2011;30(6):456–61.
[39] Diavatopoulos DA, Short KR, Price JT, et al. Influenza A virus facilitates streptococcus pneumoniae transmission and disease. FASEB J 2010;24 (6):1789–98.
[40] Honkinen M, Lahti E, Osterback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. Clin Microbiol Infect 2012;18(3):300–7.
[41] McCullers JA. Insights into the interaction between influenza virus and pneumococcus. Clin Microbiol Rev 2006;19(3):571–82.
[42] Madhi SA, Klugman KP. Vaccine Trialist Group. a role for streptococcus pneumoniae in virus-associated pneumonia. Nat Med 2004;10(8):811–3.
[43] Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypia T. Rhinovirus transmission within families with children: Incidence of symptomatic and asymptomatic infections. J Infect Dis 2008;197(3):382–9.
[44] Waris M, Osterback R, Lahti E, Vuorinen T, Ruuskanen O, Peltola V. Comparison of sampling methods for the detection of human rhinovirus RNA. J Clin Virol 2013;58(1):200–4.
[45] Toivonen L, Karppinen S, Schuez-Havupalo L, et al. Burden of recurrent respiratory tract infections in children: A prospective cohort study. Pediatr Infect Dis J 2016;35(12):e362–9.
[46] Toivonen L, Schuez-Havupalo L, Karppinen S, et al. Rhinovirus infections in the first 2 years of life. *Pediatrics* 2016;138(3). https://doi.org/10.1542/peds.2016-1309.
[47] Osterback R, Tevaluoto T, Ylinen T, et al. Simultaneous detection and differentiation of human rhino- and enteroviruses in clinical specimens by real-time PCR with locked nucleic acid probes. J Clin Microbiol 2013;51 (12):3960–7.
[48] Toivonen L, Schuez-Havupalo L, Rulli M, et al. Blood MxA protein as a marker for respiratory virus infections in young children. J Clin Virol 2015;62:8–13.
[49] Jokela P, Vuorinen T, Waris M, Manninen R. Performance of the alere i influenza A&B assay and mariPOC test for the rapid detection of influenza A and B viruses. J Clin Virol 2015;70:72–6.
[50] Feikin D, Scott J, Gessner B. Use of vaccines as probes to define disease burden. Lancet 2014;383(9930):1762–70.
[51] Glennie S, Gritzfeld JF, Pennington SH, et al. Modulation of nasopharyngeal innate defenses by viral coinfection predisposes individuals to experimental pneumococcal carriage. Mucosal Immunol 2016;9(1):56–67.
[52] Skevaki CL, Tsialta P, Trochoutsou AI, et al. Associations between viral and bacterial potential pathogens in the nasopharynx of children with and without respiratory symptoms. Pediatr Infect Dis J 2015;34(12):1296–301.
[53] Saez-Llorens X, Rowley S, Wong D, et al. Efficacy of 10-valent pneumococcal non-typeable haemophilus influenzae protein D conjugate vaccine against acute otitis media and nasopharyngeal carriage in panamanian children - a randomized controlled trial. Hum Vaccin Immunother 2017;13(6):1–16.
[54] Simell B, Auranen K, Kayhty H, et al. The fundamental link between pneumococcal carriage and disease. Expert Rev Vacc 2012;11(7):841–55.
[55] Mallia P, Footitt J, Sotero R, et al. Rhinovirus infection induces degradation of antimicrobial peptides and secondary bacterial infection in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012;186 (11):1117–24.