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Reduced nontypeable *Haemophilus influenzae* lower airway infection in children with chronic endobronchial suppuration vaccinated with the 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine

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ABSTRACT

Background: Nontypeable *Haemophilus influenzae* (NTHi), the most common bacterial lower airway infection in children with protracted bacterial bronchitis, is associated with progression to bronchiectasis. We determined whether vaccination with 10-valent pneumococcal NTHi protein-D conjugate vaccine (PHiD-CV) reduced NTHi lower airway infection compared to children not PHiD-CV-vaccinated. Our unique childhood vaccination schedule and prospective 9-year bronchoalveolar lavage (BAL) collection provided an exclusive opportunity to examine this hypothesis.

Methods: Paired BAL fluids and nasopharyngeal (NP) swabs were collected from 543 children (2007–2016) undergoing bronchoscopy for chronic cough. Children who received a primary course of ≥2 doses of one pneumococcal conjugate vaccine (PCV) and <2 doses of another PCV were included in each vaccine group. Logistic regression determined associations between NTHi lower airway infection ($\geq 10^4$ colony-forming units/mL BAL) and age, sex, Indigenous status, antibiotic exposure, and PHiD-CV vaccination.

Results: Of 262 PCV7-vaccinated, 53 PHiD-CV-vaccinated and 166 PCV13-vaccinated children (62 had mixed schedules, <2 PCV doses or missing vaccination data), NTHi lower airway infection was detected in 89 (34%), 9 (17%) and 47 (28%), respectively. On multivariate regression, significant independent factors associated with reduced NTHi lower airway infection were PHiD-CV vaccination ($OR_{adjusted}$ = 0.42, 95%CI 0.19–0.93), macrolide use ($OR_{adjusted}$ = 0.57, 95%CI 0.35–0.93) and increasing age ($OR_{adjusted}$ = 0.88, 95%CI 0.80–0.96). PHiD-CV vaccination had no impact on NTHi NP carriage.

Conclusions: PHiD-CV-vaccinated children were significantly less likely to have NTHi lower airway infection than children not PHiD-CV-vaccinated. PHiD-CV is likely an effective intervention for reducing NTHi endobronchial infection in children at risk of chronic suppurative lung diseases.

1. Introduction

Chronic endobronchial suppuration, clinically manifested as chronic cough, is commonly present in children underdoing flexible bronchoscopy; many are diagnosed with protracted bacterial bronchitis (PBB) and bronchiectasis [1,2]. PBB, increasingly recognised as a cause of significant morbidity in children, is a precursor to bronchiectasis in some settings [1,3]. PBB and bronchiectasis cause a high disease burden and bronchiectasis is associated with early death in some populations [2,4].

The two most common bacteria seen in PBB and bronchiectasis are *Haemophilus influenzae* (mostly nontypeable, NTHi) and *Streptococcus pneumoniae* [1,5]. Prospective and retrospective bronchoalveolar lavage (BAL)-based studies undertaken in several centres across different continents found these bacteria in 47–81% and 24–39%, respectively, of children with PBB [1]. Although both bacteria are likely important, only NTHi has been

Abbreviations: BAL, bronchoalveolar lavage; CFU, colony-forming units; CI, confidence interval; CSLD, chronic suppurative lung disease; HR, hazard ratio; HRCT, high-resolution computed tomography; NP, nasopharyngeal; NT, Northern Territory; NTHi, nontypeable *Haemophilus influenzae*; OM, otitis media; OR, odds ratio; PBB, protracted bacterial bronchitis; PCV (7, 13), pneumococcal conjugate vaccine (7-valent, 13-valent); PHiD-CV, pneumococcal NTHi protein D conjugate vaccine; STGGB, skim-milk tryptone glucose glycerol broth.

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associated with poorer clinical outcomes in children [6]. In children with PBB, NTHi lower airway infection ($\geq 10^4$ colony-forming units (CFU)/mL BAL fluid) conferred >7 times higher risk of future bronchiectasis at 2-year follow-up (Hazard Ratio (HR) 7.6, 95% confidence interval (CI) 1.7–34.3, p = 0.009) compared to absence of NTHi [6]. Thus, interventions that reduce lower airway infections, particularly NTHi, are clinically important [1].

Vaccination is one such potential intervention. Currently, the sole licensed vaccine with possible protection against *H. influenzae* is the 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiD-CV, Synflorix®, GlaxoSmithKline Biologicals SA, Belgium). Rats immunised with NTHi protein D had significant protection against *H. influenzae* lung and otitis media (OM) infections [7]. In our region, children vaccinated with PHiD-CV had less suppurative OM than children vaccinated with 7-valent pneumococcal conjugate vaccine (PCV7, Prevnar®, Pfizer, United States) [8], possibly due to a reduction in NTHi infection in the middle ear [9]. Also, our earlier work showed that PHiD-CV vaccination is associated with improved NTHi-specific cell-mediated and humoral immune responses in children with chronic suppurative lung disease (CSLD), including bronchiectasis [10]. Thus, PHiD-CV may be effective in preventing chronic endobronchial disorders through reducing lower airway infection by NTHi. However to date, there are no data on the impact of PHiD-CV in children's lower airways and little data on the effects of the various PCV formulations on pneumococcal lower airway infections in children [11]. This is unsurprising, as obtaining reliable lower airway specimens from young children is difficult. BAL, considered the gold standard specimen type, requires bronchoscopy, an invasive procedure requiring general anaesthesia in most children.

Thus, we report on a study that hypothesised: 'children vaccinated with PHiD-CV are less likely to have NTHi lower airway infection and/or upper airway carriage compared to children not vaccinated with PHiD-CV'. We tested this hypothesis using paired BAL fluids and nasopharyngeal (NP) swabs collected prospectively from children undergoing bronchoscopy for chronic cough from 2007 to 16. We also examined pneumococcal carriage and lower airway infection. Different vaccination schedules provided the opportunity to examine the impacts of PHiD-CV longitudinally in Australia's Northern Territory (NT) and contemporaneously in Queensland. In the NT, PCV7 was introduced into the childhood vaccination schedule in July 2001, replaced by PHiD-CV in October 2009, which was replaced by the 13-valent PCV (PCV13, Prevnar13®, Pfizer, United States) in October 2011 [9]. In Queensland, PHiD-CV was never scheduled; infants universally received PCV7 from January 2005 followed by PCV13 from July 2011 [12].

2. Methods

2.1. Enrolment

Children (age <18 years) were enrolled in ongoing prospective studies of chronic cough in the NT and Queensland. Children were excluded if they had an underlying cause of bronchiectasis (e.g. primary ciliary dyskinesia, immunodeficiency, cystic fibrosis), current treatment for cancer or diabetes, or a central nervous system or neuromuscular disorder affecting respiratory function. Children with PBB, CSLD or bronchiectasis were included. PBB is characterized by a chronic wet cough (>4-weeks) without signs of an alternative cause and responds to 2-weeks of appropriate antibiotics, such as amoxicillin-clavulanate [13]. CSLD exists where there is a recurrent or chronic wet cough that does not always resolve with prolonged (4-week) courses of oral antibiotics, but responds to intravenous antibiotics, and where despite often having additional symptoms and signs of bronchiectasis present, radiographic evidence for this diagnosis is lacking [14]. Bronchiectasis is defined by the presence of chest high-resolution computed tomography (HRCT) changes in children with a chronic wet or productive cough [2].

Data on pneumococcal vaccinations were collected from the national childhood immunisation register. Antibiotic use data were collected from medical records and directly from the parents/carers. The Human Research Ethics Committees of the NT Department of Health and Menzies School of Health Research (HREC 07/63) and Queensland Children's Health Services (HREC 03/17) approved the studies. The study was registered (ACTRN12614000743662). Written informed consent was obtained from each child's parent or caregiver.

2.2. Specimen collection and processing

Flexible bronchoscopy was performed under general anaesthesia as described previously and when the child was not acutely unwell [6,15]. BAL fluid was obtained from the most abnormal lobe(s), as seen on HRCT scan or during bronchoscopy, in accordance with international guidelines, as per our previous studies [6,15]. The same standard protocol was used by experienced bronchoscopists at both hospitals; 1 mL/kg (maximum 10 mL) sterile saline was instilled during the first lavage and 2 mL/kg (maximum 20 mL) during the second lavage with mean returns of 47% and 53% (standard deviations 17% and 18%, n = 223), respectively, when recorded. NP swabs and BAL fluids were collected during bronchoscopy and stored within 2 h in skim-milk tryptone glucose glycerol broth (STGGB) at -80 °C, as previously described [15]. Queensland specimens frozen in STGGB were transported on dry ice to our research laboratory (Menzies, Darwin) for processing.

Specimens were thawed in batches, 10μL aliquots vortexed and cultured on selective and non-selective media, and respiratory bacteria isolated and identified using standard published methods [15]. Up to four colonies per positive specimen were isolated to aid detection of multiple strains. NTHi was differentiated from *H. haemolyticus* using fucP [16] and/or siaT PCR [17]. Pneumococci were serotyped using the Quellung reaction with antisera from Statens Serum Institute (Denmark). Lower airway infection was defined as $\geq 10^4$ CFU/mL BAL fluid [18] in the first and/or second lavage [19].

2.3. Statistical analyses

We analysed data to answer our primary research question: (a) Is the proportion of children with clinically important NTHi lower airway infection ($\geq 10^4$ CFU/mL BAL) and/or NTHi NP carriage lower in children who received ≥2 doses of PHiD-CV compared to those who did not? Our secondary analyses of NTHi lower airway infection included: (b) NT children only, vaccinated and not vaccinated with PHiD-CV (longitudinal analysis); and (c) PHiD-CV-vaccinated NT children and Queensland children (not PHiD-CV-vaccinated) enrolled during the same time period (contemporaneous analysis). We also analysed data on NP carriage of and lower airway infection ($\geq 10^4$ CFU/mL BAL) by *S. pneumoniae* in children vaccinated with PCV7, PHiD-CV and PCV13.

Stata version 14.2 (StataCorp, College Station, Texas) was used for all analyses. Univariate logistic regression was used to determine associations between NTHi lower airway infection (and NTHi carriage) and age, sex, Indigenous status, exposure to beta-lactam and macrolide antibiotics, and PHiD-CV vaccination. Variables significant in univariate analyses (2-tailed $p < 0.05$) were included in multivariable analysis.