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Changes in pathogens and pneumococcal serotypes causing community-acquired pneumonia in The Netherlands

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ABSTRACT

Background: In 2006 a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in the immunisation programme for infants in The Netherlands and replaced by PCV10 in 2011. Limited data exist about the impact of PCV on the aetiology of CAP as a whole. The aim of the present study is to describe the overall changes in microbial aetiology, pneumococcal burden (including non-bacteraemic pneumococcal pneumonia) and its serotypes in adult community-acquired pneumonia (CAP) after the introduction of these PCVs.

Methods: Hospitalised adult CAP patients who participated in three consecutive trials were studied (2004–2006 (n = 201), 2007–2009 (n = 304) and 2012–2016 (n = 300) and considered as pre-PCV7, PCV7 and PCV10 period). Extensive conventional microbiological testing was applied for all patients. In addition, patients with a serotype-specific pneumococcal antibody response were diagnosed with pneumococcal CAP. Changes in proportions of causative pathogens and distributions of pneumococcal serotypes were calculated.

Results: The proportion of pneumococcal CAP decreased from 37% (n = 74/201) to 26% (n = 77/300) comparing the pre-PCV7 period with the PCV10 period ($p = 0.01$). For other pathogens, including *Legionella* spp., *Mycoplasma pneumoniae, S. aureus, H. influenzae*, and respiratory viruses, no sustained shifts were observed in their relative contribution to the aetiology of CAP. Within the pneumococcal CAP patients, we observed a decrease in PCV7 and an increase in non-PCV10 serotype disease. PCV10-extra type disease did not decrease significantly comparing the PCV10 period with the pre-PCV7 and PCV7 period, respectively. Notably, PCV7 type disease decreased both in bacteraemic and non-bacteraemic patients.

Conclusions: Our findings confirm that PCV introduction in infants impact the microbial aetiology of adult CAP and suggest herd effects in adults with CAP after introduction of PCVs in children.

1. Introduction

Streptococcus pneumoniae is the most common causative agent of community-acquired pneumonia (CAP) in adults [1,2]. With conventional microbiological methods, *S. pneumoniae* is identified in 12–40% of adults hospitalised with CAP [3–5]. With extensive diagnostics, including the detection of serotype-specific pneumococcal antigens in urine or antibodies in blood, percentages of *S. pneumoniae* as causative agent in up to 54% have been estimated [6].

In June 2006, The Netherlands introduced a 7-valent conjugate vaccine (PCV7) in the national immunisation programme for infants. From May 2011 onwards, PCV7 was replaced by a 10-valent vaccine (PCV10). Vaccine coverage in children has been around 95% since the start of this campaign [7]. In contrast, in

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Dutch adults aged 65 and over the uptake of the 23-valent-pneumococcal-polysaccharide vaccine (PPV23) is less than 1% [8,9]. Since PCV7 and PCV10 introduction, the incidence of vaccine-type invasive pneumococcal disease (IPD) decreased both in infants and adults [10]. Besides these beneficial effects, also a serotype replacement by non-vaccine serotypes has been observed in adults and children [10].

In contrast to IPD, the impact of PCV programmes on the incidence and serotype distribution of non-invasive/non-bacteraemic pneumococcal pneumonia is less well established. This category of pneumococcal disease is the majority (up to 80%) of the pneumococcal disease incidence in adults [11,12] . Recently, Werkhoven et al. observed a reduction in PCV7-type non-bacteraemic pneumococcal pneumonia, parallel to the reduction in PCV7-type IPD [13] . In a UK surveillance study using a similar approach, also a decline in PCV13-type non-bacteraemic pneumococcal disease was observed after PCV13 introduction [14] . To our knowledge, no such data are available after the introduction of PCV10. Furthermore, limited data exist about the impact of PCV on the aetiology of CAP as a whole. Shifts in nasopharyngeal ecology, for example, may impact the risk of other pathogens to cause pneumonia [15] . To the best of our knowledge, there is only one study that reported an overall decrease in proportion of pneumococcal CAP in adults after introduction of childhood vaccination with PCV7 [16] . The latter study, however, identified patients based on ICD coded hospital discharge records, which have limited reliability regarding aetiology [17] . Studies using extensive microbiological diagnostics are needed to be able to assess this in more depth.

The aim of the present study was to describe the changes in overall microbial aetiology, pneumococcal burden and its serotypes in hospitalised adult CAP over the pre-PCV7, PCV7, and PCV10 periods in The Netherlands.

2. Methods

2.1. Study population and data collection

Samples and clinical data were used from adult patients with CAP who required hospitalisation and participated in one of three consecutive clinical trials conducted in The Netherlands. The first trial was a single centre study on polymorphisms in host immune response genes and included patients between October 2004 and August 2006 (n = 201, cohort (1) [18] . The other two trials, both multi-centre placebo-controlled trials investigating dexamethasone as adjunctive treatment in CAP, included patients between November 2007 and June 2009 (n = 304, cohort (2) [4], and between October 2012 and October 2016 (n = 300, cohort (3). The 300 patients from the third trial are the first 50% of patients recruited (ClinicalTrials.gov Identifier NCT01743755 with the aim to include 600 patients). We consider the inclusion periods mentioned above as pre-PCV7, a PCV7 (and pre-PCV10), and a PCV10 period, respectively. All three clinical trials were approved by the medical ethics committee of St. Antonius Hospital and all patients provided informed consent before participation.

2.2. Clinical characteristics

All three clinical trials applied similar inclusion and exclusion criteria providing a homogeneous overall study population. In short, the trials included patients aged ≥18 years hospitalised with CAP that was defined as presence of a new infiltrate on a chest radiograph and at least two of the following criteria (1) cough; (2) sputum production; (3) temperature of >38.0 °C or <35.0 °C; (4) auscultatory findings consistent with pneumonia; (5) elevated C-reactive protein concentration (>15 mg/dl); (6) leucocytosis (>10×10^9 cells per L), more than 10% of bands in leucocyte differentiation or leucopenia (<4×10^9 cells per L). Patients with congenital or acquired immunodeficiency, haematological malignant disease or immunosuppressive treatment in the last 6 weeks were excluded. In addition, patients that required immediate ICU admission were excluded from the trials with exception of the first trial (PCV7 period).

For all patients, the following characteristics were prospectively collected: age, gender, pneumonia severity index (PSI [19]) and two comorbidities not included in the PSI (chronic obstructive pulmonary disease and diabetes mellitus). Besides clinical data, serum samples were collected at day 1 (day of admission), day of discharge and at day 30 in all three trials. All samples were immediately stored at −80 °C.

2.3. Microbial aetiology

A standard microbiological work-up was applied for all patients. This included a set of conventional methods at the time of hospitalisation plus additional measurement of serotype-specific pneumococcal antibodies in serum in persons with an early and a late serum sample available (respectively drawn at day 1–3 and 7–100 after hospital admission).

2.3.1. Conventional methods

Blood cultures were obtained (drawn before the start of in-hospital antibiotic treatment) at time of admission. Sputum specimens (if applicable) were Gramme stained and cultured. In addition, TaqMan real-time PCRs (in-house assay) were performed on sputum to detect DNA of atypical pathogens (*Mycoplasma pneumoniae, Legionella pneumophila*, *Coxiella burnetii*, *Chlamydophila pneumoniae*, and *Chlamydophila psittaci*). Serological testing (in cohort 1 and 2) on day 1–3 and day 10–21, respectively, was used to detect antibodies to *M. pneumoniae*, *C. burnetii*, *Chlamydophila* spp. or respiratory viruses (adenovirus, influenza virus A and B, parainfluenza and respiratory syncytial virus). Pharyngeal samples at time of admission were taken for viral culture on influenza (cohort 1) or PCR for detection of (para)influenza, adenovirus, respiratory syncytial virus (cohort 2 and 3) and PCR for detection *Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae/psittaci* (for cohort 3). Urine antigen tests (UAT) were performed for the detection of *L. pneumophila* serogroup 1 and *S. pneumoniae* (BinaxNOW®).

2.3.2. Serotype-specific pneumococcal antibodies in serum

As an additional indication for the involvement of pneumococci, an early and a late serum sample were tested for development of serotype-specific pneumococcal antibodies as described previously [6]. Samples were diluted 100× in sample buffer composed of phosphate-buffered saline (PBS), pH 7.3, 5% antibody depleted human serum (ADHS) pneumococcal cell wall polysaccharide (CWPS), to inhibit nonspecific binding of anti-cell wall polysaccharides I and II. Diluted sera were incubated with a mixture of microsphere types, each coated with polysaccharides representing the serotype. After incubation, non-bound antibodies were washed away and incubated with phycoerythrin (PE)-conjugated goat anti-human IgG. Bead suspensions were analyzed on a Bio-Plex 200 (IS 2.3). The standard used was calibrated against the 89SF reference serum, were used to generate a standard curve for quantification of antibody concentrations. Three assay controls 007sp (NIBSC) in 3 dilutions were taken along in duplicate with the test samples in each assay as internal control. For the PCV10 period, a 25-plex immunoassay panel was used (including the 14 serotypes mentioned before plus 11 additional serotypes; 2, 5, 6A, 10A, 11A, 12A, 15B, 20, 22F, 33F and 45).

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