Care for community-acquired pneumonia and emerging pneumococcal serotypes

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Care for community-acquired pneumonia and emerging pneumococcal serotypes

Zorg voor thuis-opgelopen longontstekingen en opkomende pneumokokkenserotypen

(met een samenvatting in het Nederlands)

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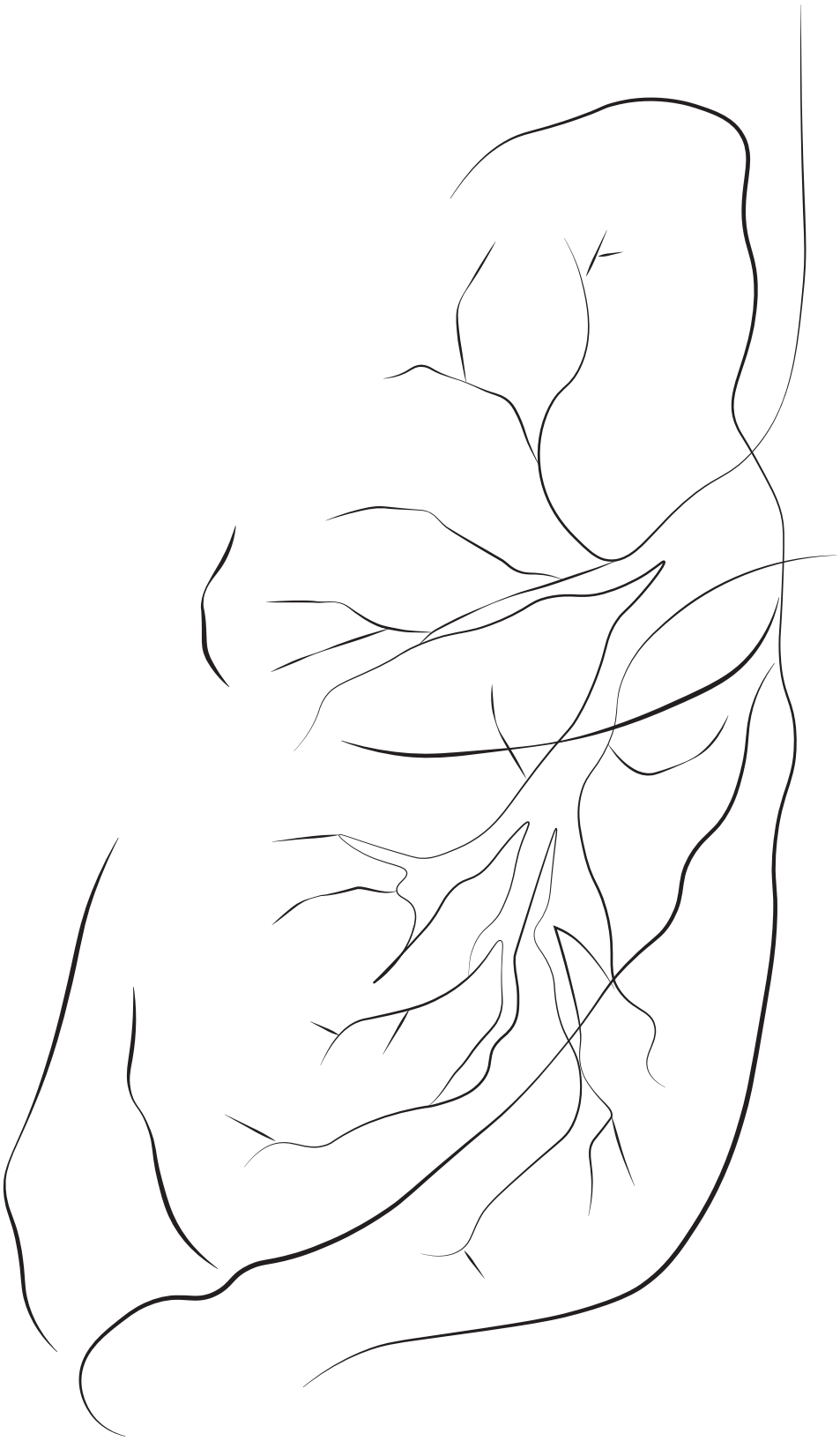

CHAPTER 1

General introduction

Community-acquired pneumonia (CAP) and *Streptococcus pneumoniae*

Infectious pneumonia is an acute inflammation of the lung parenchyma, which can be caused by various micro-organisms (figure 1).[1] Pneumonia is the leading cause of death due to infectious diseases worldwide and the second most common cause of death overall.[2] In the Netherlands, its incidence is especially high in persons ≥65 years of age.[3] Given the ageing population, the number of hospital admissions for pneumonia is expected to rise, as will the associated disease burden and costs.[4]

Empirical treatment of pneumonia is based on the distribution of pathogens, which are linked to the place of acquisition. Therefore, pneumonia is further classified by place of acquisition. Community-acquired pneumonia (CAP) is mainly caused by respiratory viruses, or bacteria susceptible to most early-generation antibiotics. In contrast, nosocomial or healthcare associated pneumonia is often cause by more resistant bacteria. Ventilator-associated pneumonia is more frequently caused by opportunistic bacteria taking advantage of the dysfunction of the ciliated epithelium of the respiratory tract or other factors impairing host defense. Pneumonia due to aspiration is mostly associated with anaerobic bacteria.

Part I and III of this thesis focus on CAP. Part II focuses on CAP caused by *Streptococcus pneumoniae* (or pneumococcal CAP) and on invasive pneumococcal disease (IPD).

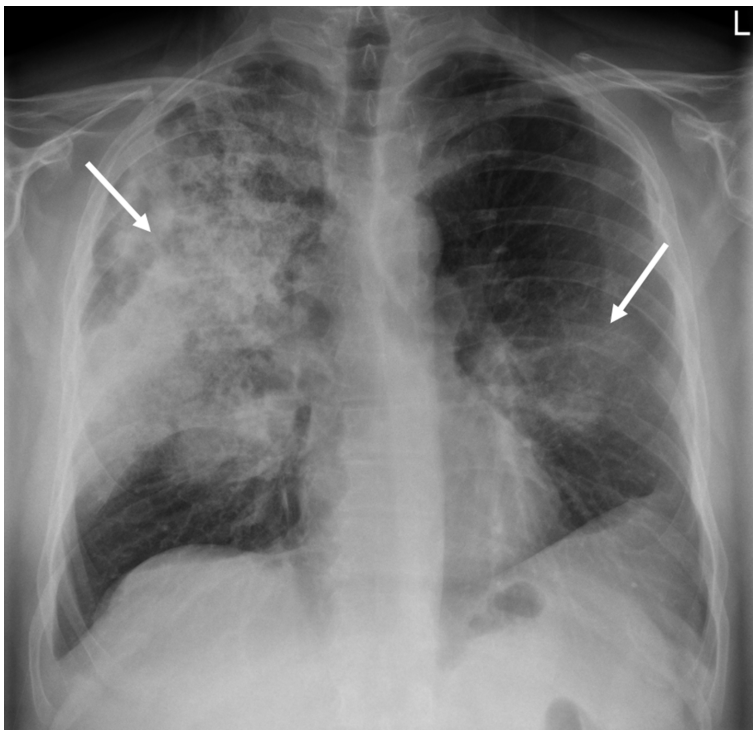

Figure 1. Bilateral pneumonia on chest X-ray (right upper lobe and left lower lobe consolidations).

The vast majority of CAP is considered non-severe and thus can be treated at home.[5] The decision to refer a patient to the emergency department will often be based on the general practitioner's clinical assessment or prior therapy failure.[6] Once referred to a hospital, various clinical scoring systems are available to guide clinical decision making. The Pneumonia Severity Index (PSI)[7] and the CURB-65 (acronym for: confusion, urea, blood pressure and [an age of] ≥65 years)[8] were originally designed to predict 30-day mortality and thereby CAP severity. These scores are also used to guide empirical antimicrobial therapy.[7–9]

Streptococcus pneumoniae is a Gram-positive, lancet shaped bacterium, usually found in pairs when viewed under a microscope (duplococci) (figure 2). The genus and species name are often combined to form the word 'pneumococcus'. The pneumococcus is the most commonly detected pathogen in patients hospitalized with CAP in the Netherlands (up to 24% by extensive conventional microbiological testing).[10] In the Netherlands resistance of *S. pneumoniae* against penicillin is extremely rare. Besides respiratory viruses, other frequently encountered bacterial pathogens in Dutch adults hospitalized with CAP are *Haemophilus influenzae* and the intracellular (or atypical) bacteria: *Mycoplasma pneumoniae, Legionella pneumophila* serogroup 1, *Chlamydophila pneumoniae* (psittacosis) and *Coxiella burnetii* (also known as cause of Q fever). Yet, in over 50% of hospitalized patients with CAP, no pathogen is identified using conventional microbiological diagnostic tests.[10,11] However, based on serological antibody testing it has been proposed that pneumococci are involved in approximately 60% of cases in whom no causative agent is found.[12] In the current Dutch national guideline on the management of CAP, beta-lactam antibiotics (amoxicillin or a 2nd or 3rd generation cephalosporin) form the backbone of empirical treatment. Depending on CAP severity, amoxicilline (monotherapy) or a cephalosporin (monotherapy or dual therapy) are adivesed, a treatment covering the most frequently identified community-acquired pathogens (*S. pneumoniae, H. influenzae* and Enterobacterales) in the majority of cases. Recently, a Dutch clinical trial found that empirical beta-lactam monotherapy is non-inferior to broader spectrum antimicrobial therapy in treating patients with CAP admitted to a general hospital ward, likely because of the high prevalence of *S. pneumoniae* and its low resistance against beta-lactam antibiotics.[11]

The effect of (antibiotic) treatment can be monitored by measuring clinical and/or biological markers, or so called biomarkers (e.g. C-reactive protein and body temperature). Body temperature is a biomarker that is used to monitor short-term recovery. A patient admitted with CAP is considered clinically stable and fit for discharge after resolution of abnormalities in vital signs (body temperature, blood pressure, heart rate, respiratory rate and oxygen saturation), ability to eat, and mental status to a pre-morbid level.[13] Time to clinical stability has been used in trials as a clinical endpoint for recovery. The switch from intravenous to oral antibiotics can generally be made if a patient admitted with CAP has reached clinical stability.[9]

In the last decade the research field in CAP has seen large interest in the use of adjunctive corticosteroids in the treatment of CAP. Worldwide, adjunctive corticosteroid therapy for

patients hospitalized with CAP has been studied in several clinical randomized placebo-controlled trials.[10,14–16] The hypothesis is that more rapid clinical recovery can be reached by reducing the excessive inflammatory response. Even though a more rapid clinical recovery in patients treated with corticosteroids has been observed in multiple studies, side effects were also common.[15,16] It has been suggested that the potential beneficial effects of systemic corticosteroids in patients hospitalized with CAP in fact lay in their antipyretic (or fever reducing) properties, rather than in a faster recovery from an excessive inflammatory response.[17]

Part I - Proportionality of diagnostic testing

The vast majority of healthcare consultations for CAP are with general practitioners.[5] Even though only a small fraction of patients with CAP is admitted to a hospital (<15%), the costs of second line CAP care are substantial.[3] Multiple diagnostic tests are utilized in the hospital management of patients with CAP and the median costs of a hospitalization of CAP are estimated at €4,000 per patient.[4] Mean costs have been shown to be at least twice as high in elderly, and increase with age.[18] Nursing costs represent the largest share of in-hospital CAP care (>50%).[4,18]

Optimal resource utilization is pursued in every hospital. Yet, differences in healthcare use exist for various reasons such as differences in patient populations, local guidelines and logistics. However, an X-thorax, is likely to be utilized in every patient with suspected pneumonia as a part of the diagnostic process. Likewise, many other tests will be ordered in the management of inpatient CAP care. Despite the existence of guidelines, proportionality of care utilization is hard to capture, because of patient heterogeneity and inter-hospital variation.

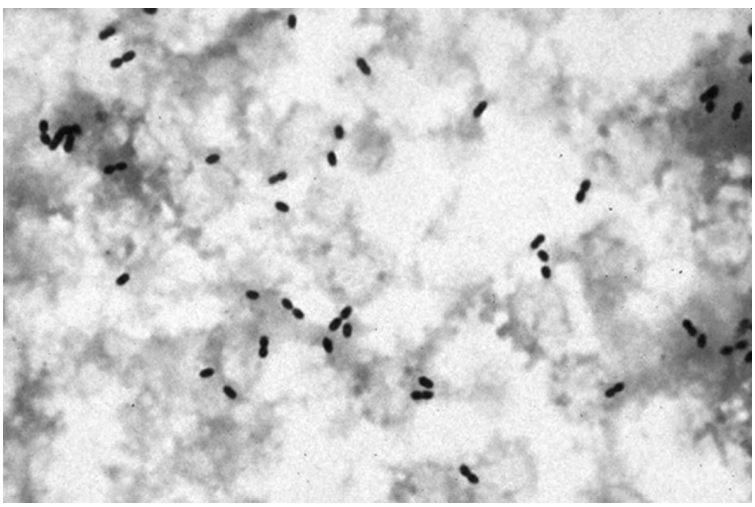

Figure 2. *Streptococcus pneumoniae* Gram-stain microscopically observed at 1000X.

However, it is assumed that alteration of antibiotic therapy, after empirical treatment, is largely dependent of microbiological testing. Rapid de-escalation of antibiotic therapy is desirable to prevent bacterial antibiotic resistance development and drug-related side effects.

Proportionality of diagnostic testing

Given the rise in healthcare related costs in the last decades, proportional use of resources should be pursued. Extensive microbiological testing might be beneficial from a antimicrobial stewardship perspective, but the question is whether this really leads to more frequent alteration of antibiotic therapy, without compromising patient outcomes in clinical practice. On the other hand, society will benefit from low rates of antibiotic resistance.[19] From this perspective, extensive microbiological testing may be useful in the majority of patients admitted with CAP as well. In **chapter 2**, we explore the extent of overall CAP care utilization in the first two days of admission comparing results between four Dutch hospitals, focusing on diagnostic testing. The costs of microbiological testing are compared with the rate of antibiotic therapy de-escalation.

Value of microbiological testing

It is assumed that extensive microbiological testing leads to a maximum diagnostic yield, and subsequently facilitates adjustment of antibiotic therapy. There are many data supporting the first part of the hypothesis, yet, and surprisingly, very limited are available to support the latter assumption.[20–22]

Dutch guidelines on the management of CAP recommend obtaining blood and sputum specimens for culturing (and the latter also for Gram-staining) before starting antimicrobial therapy in all patients with CAP.[9] Because empirical treatment is based on CAP severity, in every patient admitted with severe CAP, urinary antigen testing for *S. pneumoniae* and *L. pneumophila* serogroup 1 should be performed in order to enable rapid adjustment of empirical antibiotic therapy (either de-escalation to penicillin monotherapy or targeted monotherapy directed at *Legionella pneumophila*). These rules are in place, because of the low expected rates of antibiotic resistance in the most frequently identified causative pathogens. Routine use of other microbiological tests is not recommended as standard. In **chapter 3**, we assess the association between individual microbiological tests on alteration of antibiotic therapy and clinical outcomes in one large non-academic teaching hospital.

Part II – Pneumococci, pneumococcal vaccination and serotype-specific antibodies

S. pneumoniae is a major cause of bacterial pneumonia, but also of meningitis and, in children, otitis media. Its major virulence factor is its polysaccharide capsule, which protects the micro-organism from phagocytosis. Based on differences in the composition of the capsular polysaccharides, over 90 pneumococcal serotypes have been identified (figure

3). This capsule is an important trigger for the human immune response and, as an antigen, provokes the production of antibodies.

After encountering (parts of) the bacterium, humans can produce serotype-specific antibodies directed at the capsular polysaccharide. This anti-capsular immune response forms the basis of the current pneumococcal vaccines. The 7-valent pneumococcal conjugate vaccine (PCV7, Prevenar®) covers serotype 4, 6B, 9V, 14, 18C, 19F and 23F and was introduced in the Dutch national immunization program for infants in 2006. PCV7 was replaced in 2011 by the 10-valent conjugate vaccine (PCV10, Synflorix®), which covers serotype 1, 5 and 7F in addition to the serotypes covered by PCV7. The PCV program was initiated in order to reduce the burden of invasive pneumococcal disease (IPD) in young children. The 23-valent polysaccharide vaccine (PPV23, Pneumovax23®) is used in the Netherlands for protection of adults at risk of pneumococcal infections, and as of fall 2020 will be offered to all persons >60 years.

Invasive pneumococcal disease in the Netherlands

Invasive pneumococcal disease (IPD) is defined as *S. pneumoniae* infecting a normally sterile bodily fluid, typically blood or cerebrospinal fluid (CSF), with clinical syndromes including septicemia, invasive pneumonia or meningitis. After the introduction of PCV7 in the Dutch childhood immunization program, the incidence of IPD decreased by more than half in children <2 years, but also in adults ≥65 years via indirect protection.[23]

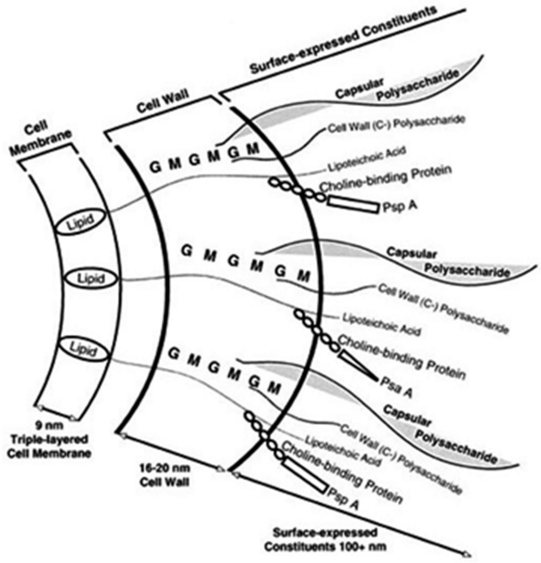

Figure 3. A representation of the cell membrane, cell wall, and capsule of *S. pneumoniae*. (Abbreviations: PsaA, pneumococcal surface adhesin A; PspA, pneumococcal surface protein A) (From: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 7th ed).

The incidence of IPD and the distribution of underlying clinical syndromes varies greatly per age group. While the proportion of pneumonia as presenting syndrome is high among older adults, invasive (or bacteremic) pneumococcal pneumonia is relatively uncommon in young children, in whom the proportion of meningitis is relatively high.

After the introduction of PCV7, the incidence of meningitis decreased in under-fives, but a subsequent increase in the incidence of empyema was observed in elderly ≥65 years. Still, the overall case fatality rate (CFR) decreased, despite replacement by non-PCV7 serotypes.[24] Two years after the switch to PCV10, a further reduction in IPD incidence was observed in children <2 years, but the overall IPD incidence plateaued due to a steady rise of non-PCV10 serotype IPD.[23] In **chapter 4** we describe the effects of replacing PCV7 by PCV10 on clinical syndromes and outcomes which were thus far unknown.

Changes in pathogens and pneumococcal serotypes in adults with CAP

The impact of PCV programs on the proportion of *S. pneumoniae* in Dutch adults hospitalized with CAP, and its influence on the pneumococcal serotype distribution in non-invasive (or non-bacteremic) pneumococcal CAP is less well established. Previous data has shown evidence for shifts in serotypes in Dutch patients hospitalized with non-bacteremic pneumococcal CAP after PCV7 introduction.[25] It remains unclear whether these shifts in the numbers of circulating *S. pneumoniae* influence nasopharyngeal ecology, thereby potentially leading to a higher carriage rates of other pathogens such as *Haemophilus influenza*e type B. This could lead to a shift in pathogens causing CAP.

Therefore, we study the overall changes in microbial etiology and pneumococcal burden, including non-bacteremic pneumococcal pneumonia in adults with CAP before and after introduction of PCVs in **chapter 5**.

Proportion and serotype distribution of *S. pneumoniae* in Bangladeshi children with CAP

Bangladesh has introduced PCV10 in their national immunization program for children <1 year-old in 2015. Even though it has been estimated that approximately 60% of serotypes causing IPD would be covered by PCV10 In Bangladesh, the burden of pneumococcal disease before the introduction of PCV10 was largely unknown.[26–29] In contrast to Western countries, the mortality rate of pneumonia (also non-invasive) in young children in Bangladesh is extremely high due to the poor standard of living. For this reason, Bangladeshi children might also benefit largely from a high vaccine-coverage of serotypes causing non-invasive pneumonia.

For this reason, we serologically assess the proportion of *S. pneumoniae* as pathogen in CAP in over 1500 Bangladeshi children <5 years-old diagnosed with pneumonia in **chapter 6**, by measuring serotype-specific pneumococcal capsular polysaccharide antibodies by using a multiplex immunoassay. Secondly, we estimated the potential PCV10-coverage.

Part III - Adjunctive corticosteroid therapy and prognostic biomarkers

It has been hypothesized that corticosteroids dampen an excessive inflammatory response in patients with CAP, which might result in earlier resolution of pneumonia. In several studies adjunctive corticosteroid therapy indeed reduced the length of hospital stay of patients hospitalized with CAP to a general hospital ward by approximately 1 day.[10,15] Also clinical stability was reached faster in the experimental treatment arms vs. placebo.[15,16] However, doubts have been raised whether the beneficial effects of adjunctive corticosteroid therapy in CAP might be the result of a more rapid defervescence, merely masking the underlying illness, instead of faster clinical recovery. Moreover, in a recent individual patient data meta-analyses, a statistically significantly but only marginally higher rate of CAP-related rehospitalizations in the experimental treatment group was found.[30] Still, the same meta-analysis found that adjunctive corticosteroids reduce time to clinical stability and length of hospital stay by approximately 1 day, without having a significant effect on overall mortality. So far, it remains unclear what specific group of patients hospitalized with CAP benefits most from adjunctive corticosteroid therapy.

Oral dexamethasone as adjunctive therapy

In the search for a subgroup of patients that benefit most from corticosteroids, it was found earlier that adjunctive dexamethasone treatment was associated with a significant decrease in mortality/ICU admission in CAP patients presenting with a high pro-inflammatory cytokine response in combination with a discrepantly low cortisol.[31] In addition, the recent individual patient data meta-analysis found a stronger effect of corticosteroids on in length of hospital stay in patients with a high PSI class.[30] From a cost-effectiveness perspective, CAP with a high PSI score is an interesting subgroup, since PSI-based severity showed to be a cost-driving factor, mainly due to the longer length of hospital stay.[4]

We performed a multicenter placebo-controlled trial that was designed to investigate the beneficial effects of adjunctive oral dexamethasone therapy in patients admitted with CAP. With the aim to assess which patients benefit most from dexamethasone treatment, randomization was stratified by CAP severity based on PSI classification (class I-III vs. IV-V). The results are described in **chapter 7**.

Body temperature as clinical marker and dexamethasone

The resolution of fever and normalization of body temperature is regarded as a sign of recovery in patients with infectious disease. In adults hospitalized with CAP, stable defervescence is one of the criteria for the switch of antibiotics from intravenous to oral towards hospital discharge.[32,33]

Concerns have been raised that the reduction in length of hospital stay in patients treated with adjunctive corticosteroids might be (solely) the result of a faster clinical stability due to the antipyretic effects of corticosteroids, instead of a true clinical recovery.[17] Using data from the study of Meijvis et al[10], these concerns are addressed in **chapter 8**.

Biomarkers for cardiac damage in CAP

Since CAP has been associated with an elevated risk of cardiac events, there has been increased interest in biomarkers predicting cardiac mortality during and after an episode of CAP. One of these biomarkers is cardiac troponin T (cTnT).[34–36] Cardiac complications frequently occur during hospitalization, and within 30 days after hospitalization with CAP.[37] It has been shown that mortality rates are relatively high in patients recovering from an episode of CAP, especially from cardiovascular causes, in comparison with the general population.[38–40] Measurement of serum cTnT concentrations is widely performed in clinics to predict myocardial damage and might therefore contribute in identifying myocardial damage in patients admitted with CAP. In **chapter 9**, we study the prognostic value of the cTnT concentrations at admission with CAP in predicting short- and long-term mortality, also compared to PSI.

Aims and outline of the thesis

The specific aims of this thesis were:

1. to assess the associations between the extent of diagnostic testing, antibiotic therapy alteration and clinical outcome in the management of community-acquired pneumonia (CAP);
2. to characterize pneumococcal disease epidemiology and serotype distribution before and after the introduction of pneumococcal conjugate vaccines;
3. to evaluate the effect of adjunctive dexamethasone treatment and value of biomarkers in CAP.

Part I – Proportionality of diagnostic testing

Hospitalization with CAP is accompanied with high costs. Utilization of diagnostic procedures is the second largest cost driver. Thus proportional use of diagnostics should be pursued. In **chapter 2** the inter-hospital variation in the total costs of diagnostic testing in the management of CAP in relation to antibiotic alteration are systematically assessed, comparing multiple hospitals.

It is assumed that extensive microbiological testing results in the largest diagnostic yield and as a result facilitates more frequent alteration of antibiotic therapy. In **chapter 3** using data from one non-academic teaching hospital, the relationship between the extent of microbiological testing and the frequency of antibiotic alteration is studied in adults hospitalized with CAP, and the association between the extent of microbiological testing and clinical outcomes is investigated.

Part II – Pneumococci, pneumococcal vaccination and pneumococcal antibodies

In 2011 the 10-valent pneumococcal conjugate vaccine (PCV10) replaced PCV7 in the Dutch childhood national immunization program. In **chapter 4** the impact of PCV on invasive

pneumococcal disease (IPD) in Netherlands is studied, up to twelve years after PCV7 was introduced, focusing on the overall incidence, clinical syndromes and outcomes.

Limited data exist about the impact of PCV on pneumococcal serotypes causing non-bacteremic CAP and on other pathogens of CAP. In **chapter 5** changes in microbial etiology and pneumococcal serotypes are investigated, also in patients hospitalized with non-bacteremic CAP before and after introduction of PCV7 and PCV10.

Bangladesh has introduced PCV10 in their national immunization program in 2015. Still, the burden of *S. pneumoniae* in children ≤5 years with CAP has remained largely unknown. This also applies to its serotype distribution. The proportion of *S. pneumoniae* and its serotype distribution in Bangladeshi children with non-bacteremic CAP, before introduction of PCV10 is studied in **chapter 6**.

Part III – Adjunctive corticosteroid therapy and prognostic biomarkers

Adjunctive corticosteroids therapy for patients hospitalized with CAP has been subject of study for more than a decade. In **chapter 7**, the results of a multicenter randomized placebo-controlled trial are presented. The study was designed to evaluate whether the effect of oral dexamethasone is different in patients with Pneumonia Severity Index (PSI) class I-III vs. IV-V.

Body temperature is an easy to obtain clinical marker. In **chapter 8**, the course of body temperature in patients admitted with CAP and treated with or without adjunctive dexamethasone is studied, to explore if suppression of fever by dexamethasone influenced the length of hospital stay in an earlier conducted placebo-controlled trial.

Both short- and long-term mortality are relatively high after hospitalization with CAP, compared with age-matched controls. A strong association with cardiovascular disease has been found. **Chapter 9** shows the prognostic value of high-sensitivity cardiac troponin T for mortality in hospitalized patients with CAP.

Chapter 10 presents a summary, discussion and perspectives of the research work of this thesis.

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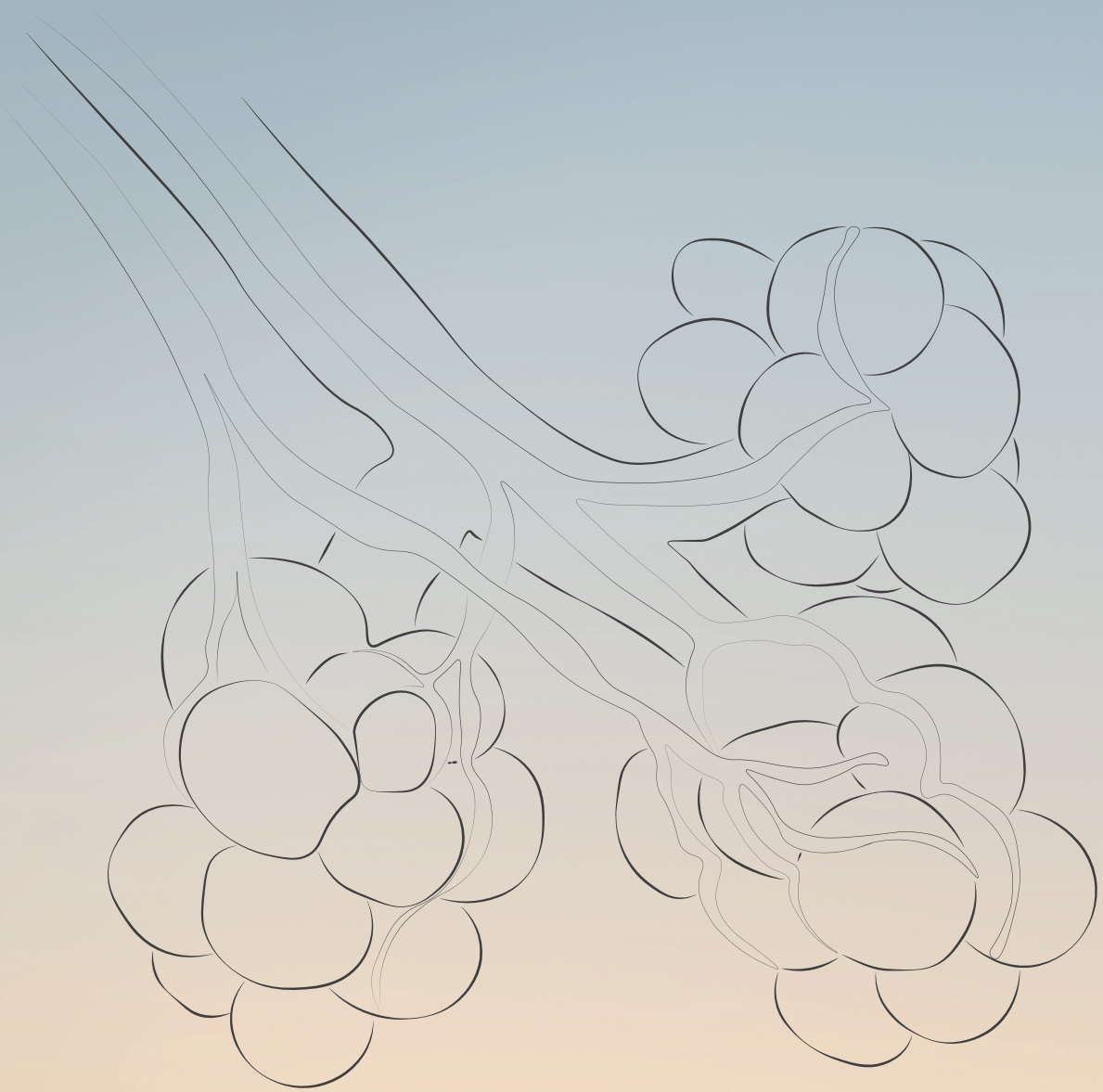
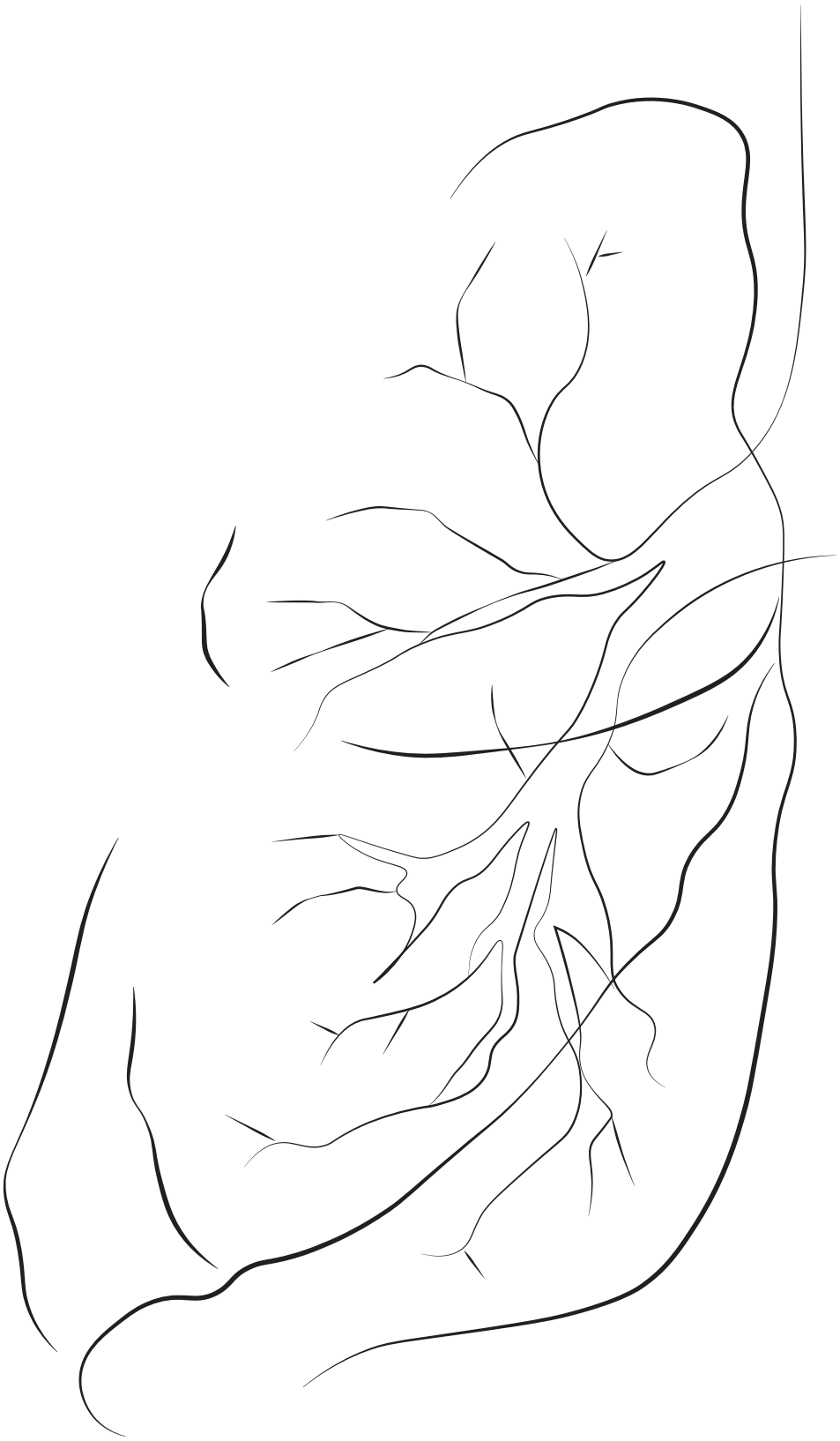


PART I

Proportionality of diagnostic testing



CHAPTER 2

Inter-hospital variation in the utilization of diagnostics and their proportionality in the management of adult community-acquired pneumonia

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Abstract

Background

Utilization of diagnostics and biomarkers are the second largest cost drivers in the management of patients hospitalized with community-acquired pneumonia (CAP). The present study aimed to systematically assess the inter-hospital variation in these cost drivers in relation to antibiotic use in CAP.

Methods

Detailed resource utilization data from 300 patients who participated in a multicenter placebo-controlled trial investigating dexamethasone as adjunctive treatment for community-acquired pneumonia was grouped into 3 categories: clinical chemistry testing, radiological exams, and microbiological testing. Based on the identified top 5 items per category, average costs were calculated per category and per hospital. Antibiotic de-escalation at day 3 and secondary ICU admission were assessed as outcomes for proportionality of diagnostics use.

Results

The mean costs for diagnostics varied between hospitals from 350 (SD 31) to 841 (SD 37) euro per patient ($p<0.001$). This difference was primarily explained by variation in costs for microbiological testing (mean 195 vs. 726 euro per patient, $p<0.001$). There was no difference in number of secondary ICU admissions but there was an inverse association between the costs of microbiological testing and level of antibiotic de-escalation. De-escalation occurred most frequently in the hospital with the lowest cost for microbiological testing (48% vs. 30%; $p=0.018$). The latter hospital had an automated physician alert system in place to consider a timely iv-to-oral switch of antibiotics.

Conclusions

Large inter-hospital variation exists in resource utilization, mainly in microbiological diagnostics in the management of adult patients with community-acquired pneumonia. A counterintuitive inverse association between the magnitude of these costs and the amount of antibiotic de-escalation was found. Future studies about the optimal cost-effective set of microbiological testing for antimicrobial stewardship in pneumonia patients should acknowledge the interaction between testing, way of communication of results and triggered physician alert systems.

Background

Community-acquired pneumonia (CAP) is a frequent cause of hospital admission in the Western world.[1] Total costs for inpatient CAP care are estimated to be approximately €2,5 billion per year in Europe alone.[2] A previous Dutch multicenter study reported that average hospitalization cost were €4000 per patient admitted with CAP.[3] More recently, another Dutch multicenter study in adults >65 years calculated average costs that were almost twice as high.[4] The majority of costs in both studies were nursing costs, which are directly related to length of hospital stay.[3,4]

Concepts like 'Choosing Wisely' target the best patient outcomes with optimal use of resources.[5] Because length of stay is an outcome, in other words the consequence of a multifactorial process, it cannot be modified nor standardized directly. In contrast, utilization of diagnostic tools and biomarkers, being the second largest cost driver in CAP care[3], potentially could be. Because narrowing antibiotic treatment when possible is considered an important outcome from an antimicrobial stewardship perspective, the present study aimed to systematically assess inter-hospital variation in the costs of diagnostics in the management of CAP in relation to antibiotic use.

Methods

Data of adult patients hospitalized with CAP, who participated in a multicenter placebo-controlled trial investigating dexamethasone as adjunctive treatment were studied.[6] The present cohort (n=300) is the mid-trial population. Patients were enrolled between October 2012 and October 2016 in four large Dutch teaching hospitals (Catharina Hospital Eindhoven, Canisius-Wilhelmina Hospital Nijmegen, Onze Lieve Vrouwe Gasthuis Amsterdam and the St. Antonius Hospital Nieuwegein/Utrecht). In short, the trial included immunocompetent patients aged ≥18 years, who were hospitalized with radiologically confirmed CAP, and who did not receive systemic corticosteroids at or shortly before admission and did not require direct intensive care unit (ICU) admission. The study protocol of the original investigation requested participating hospitals to perform pathogen identification including (but not restricted to) sputum cultures, blood cultures and urinary antigen testing (*Legionella pneumophila* serogroup 1 and *Streptococcus pneumonia*). The study was approved by the Medical Ethical Committee of the St. Antonius Hospital and all patients provided written informed consent.

Hospital administration systems of the participating hospitals were consulted to determine resource utilization regarding diagnostics (laboratory testing and radiology) on a patient level. All care items billed to the individual patient's health care insurance were captured. First, the captured items were grouped into three categories:1) clinical chemistry testing, 2) radiological exams, and 3) microbiological testing. Second, only items from the period between admission and hospital discharge or ICU admission were retained. Next, for every hospital and every category, items were ranked based on mean number utilized per patient. After this step, we retained up to 5 items per category and only items that were

utilized more than 0.1x on average per patient in one or more of the hospitals. Lastly, based on the identified top 5 items per category, average costs were calculated per category and per hospital. Standard item list prices from the Dutch Healthcare Authority (edition 2016) were used for all cost calculations.[7]

In order to be able to link possible variations in resource use to relevant outcomes we captured the level of antibiotic de-escalation by day 3 of hospital stay. To assess antibiotic de-escalation at day 3, information on all prescribed antibiotics from day 1 to 3 was obtained from the Santeon Farmadatabase.[8] Whether antibiotics were de-escalated was determined per patient, where de-escalation was defined as narrowing of spectrum, switch to a different class of antibiotics, or switch from dual therapy to monotherapy. During the period in which patients were enrolled, the Dutch national guideline on the management of CAP advised an empirical antibiotic treatment based on the severity of disease. The antimicrobial spectrum varied from penicillin for mild pneumonia to a cephalosporin plus atypical coverage for severe CAP.[9]

Besides antibiotic de-escalation, level of pathogen identification and secondary ICU admission rate were assessed. The latter as proxy for a complicated course of disease possibly caused by an insufficient diagnostic work-up. Descriptive statistics were used providing numbers (%), means (standard deviation (SD)) or medians [range] where appropriate. Categorical data were compared applying the Chi-square test. Differences in costs were analyzed with the Mann-Whitney U test.

Results

Patient characteristics

Mean age of the 300 studied patients was 64 years and 41% of the participants had a high PSI class (4-5). Both age and PSI class were significantly higher in hospital 4, as was the proportion of patients with heart failure and impaired renal function. This, together with the low number of patients from hospital 4 (n=9 of 300), led to the decision to exclude hospital 4 from further analyses. Of the 291 remaining patients, the number with a PSI class of 4-5 at 45% was higher in hospital 1, compared to 30% and 28% in hospital 2 and 3, respectively. Other patient characteristics, including comorbidities and prior outpatient antibiotic treatment were similar in the hospitals.

Resource utilization

Overall, 39% of all diagnostic items were ordered on day of admission and 59% up until day 2. Table 1 shows the items most frequently utilized per category per hospital. The mean amount of radiological exams performed was similar between hospitals. The number of clinical chemistry items was the lowest in hospital 1. In contrast, microbiological testing costs were highest in hospital 1, mainly due to performing the most polymerase chain reaction (PCR) hybridization tests (pathogen level). Microbiological testing was responsible for the majority of costs in every hospital. The mean difference in costs of microbiological

Table 1. Ranking of mean number of items and mean costs per patient plus outcomes.

Resource use	**Hospital 1 (n=201)**	**Hospital 2 (n=50)**	**Hospital 3 (n=40)**
Clinical chemistry testing	Creatinine 2.90; 3 [0-22] Potassium 2.73; 2 [0-48] Sodium 2.72; 2 [0-54] CRP 2.21; 2 [0-13] Leucocytes 1.92; 2 [0-16]	Creatinine 4.62; 5 [0-13] CRP 3.62; 3 [0-8] Glucose 3.48; 2 [0-29] Hemoglobin 3.46; 3 [0-13] Sodium 3.32; 3 [0-12]	Glucose 5.83; 2 [0-55] Creatinine 4.95; 4 [0-12] CRP 4.90; 5 [0-11] Sodium 4.35; 4 [0-13] Potassium 4.35; 4 [0-11]
Mean costs (SE)	€25 (1.5)	€37 (2.9)	€49 (4.5)
Radiological exams	CXR 1.49; 1 [1-8] Chest CT 0.11; 0 [0-1] Abdominal sonography 0.07; 0 [0-1] Echocardiography 0.02; 0 [0-2]	CXR 1.40; 1 [0-6] Echocardiography 0.24; 0 [0-3] Chest CT 0.16; 0 [0-1] Abdominal sonography 0.04; 0 [0-1]	CXR 1.33; 1 [0-4] Chest CT 0.13; 0 [0-1] Abdominal sonography 0.13; 0 [0-1] Echocardiography 0.08; 0 [0-1]
Mean costs (SE)	€89 (7.5)	€118 (20)	€87 (17)
Microbiological testing	PCR – hybridization* 10.06; 9 [0-37] Antigen detection 2.22; 2 [0-6] AMR detection 1.9; [0 0-32] Agar culturing 1.66; 1 [0-9] Gram staining 1.41; 1 [0-8]	AMR detection 3.66; [0 0-57] Antigen detection 2.52; 2 [0-5] Blood culturing 2.40; 2 [0-6] Agar culturing 2.18; 2 [0-10] Gram staining 1.6; 1 [0-5]	PCR – hybridization* 5.33; 6 [0-15] Serologic antibody detection 4.25 5 [0-7] Blood culturing 2.2; 2 [0-6] AMR detection 1.93; [0 0-44] Antigen detection 1.58; 2 [0-4]
Mean costs (SE)	€726 (34)	€195 (17)	€476 (44)
Overall mean costs (SE)	€841 (37)	€350 (31)	€612 (46)
Outcomes	**Hospital 1**	**Hospital 2**	**Hospital 3**
Microbiological diagnostic yield, *n* (%) [$]	91 (45)	15 (30)	15 (38)

Table 1. (continued)

Outcomes	Hospital 1	Hospital 2	Hospital 3
Antibiotic de-escalation, *n* (%) #	57 (30)	21 (48)	13 (33)
Secondary ICU admissions, *n* (%)	10 (5)	3 (6)	1 (3)

A maximum of 5 items per category per hospital are listed. Items are only listed if the mean number per patient was ≥0.1 in at least one hospital. Overall mean costs per patient per hospital per category are shown. *counted on pathogen level. Data are presented as mean; median [minimum-maximum], unless stated otherwise.
$ p=0.050 for difference between hospital 1 and hospital 2.
p=0.018 for difference between hospital 1 and hospital 2.
Abbreviations: AMR, antimicrobial resistance; CRP, C-reactive protein; CT, Computed Tomography; CXR, Chest X-ray; ICU, Intensive Care unit; PCR, Polymerase Chain Reaction; SE, Standard Error.

testing was €531 (p<0.001) per patient between hospital 1 and 2. Overall, the average costs per patient (€841) were highest in hospital 1 and lowest in hospital 2 (€350), p<0.001.

Outcomes

The level of pathogen identification varied from 45% in hospital 1 to 30% in hospital 2 (p=0.050 comparing hospital 1 and hospital 2, see Table 1). Regarding antibiotic de-escalation, the de-escalation rate was highest in hospital 2 (48%), compared to 30% and 33% in hospital 1 and 3, respectively (p=0.018 for difference between hospital 1 and 2). The most frequent de-escalations were from a penicillin plus atypical coverage to penicillin monotherapy (n=20), from a cephalosporin plus atypical coverage to cephalosporin monotherapy (n=17) and from a cephalosporin plus atypical coverage to atypical coverage only (n=11). De-escalation numbers were calculated from 278 patients because at day 3 eight patients had been admitted to the ICU and five patients had been discharged. Percentages of patients receiving empirical dual therapy also varied between hospitals (1: 38%, 2: 30%, 3: 53%). Within the patients with dual therapy, the de-escalation rates were 51%, 100%, and 62% respectively. Overall, secondary ICU admission rates were 5%, 6% and 3% in hospital 1, 2 and 3, respectively.

Discussion

We showed large inter-hospital variations in utilization of diagnostics for in-hospital management of CAP between large Dutch teaching hospitals, mainly in microbiological testing. Considering an annual rate of 35,000 CAP-related hospital admission in the Netherlands[10], this suggests that considerable cost savings could be achieved when the most cost-effective panel of diagnostic tests could be identified.

In studies in pediatric patients with CAP, large inter-hospital variations in diagnostic testing have already been demonstrated.[11,12] The present study now extends this finding to

the management of adults with CAP. The observed large variation not only provides food for thought about cost-effective diagnostic work-up, but also shows that calculations of costs from single center settings need to be interpreted with caution because of limited external validity.

Based on our findings the variation in microbiological testing looks like a good candidate to start identification of the optimal set of diagnostics (e.g. PCR testing was ordered sporadically in hospital 2, whereas, in hospitals 1 and 3 it was ordered frequently). One would expect that the extent of microbiological testing would increase the chances of pathogen identification (which was the case in this study) and as such that there would be a positive association with de-escalation of antibiotic therapy. In the present study, comparing hospitals, however, an inverse association between testing costs and antibiotic de-escalation was observed. Discussions between the hospitals about this finding identified the fact that in hospital 2 an automated advisory message was sent to the treating physicians, alerting them as to when parenteral antibiotics had been administered for >48 hours and that clinical and pharmacological data did not preclude a switch from intravenous to oral therapy.[13] It was apparent that such an intervention along with microbiological investigation increased the likelihood that antibiotics would be de-escalated. In fact no patients in hospital 2 were still on dual therapy on day 3 probably because of earlier notification of microbiological results. In this approach physicians are actively directed to negative test results as well, in some cases also warranting antibiotic de-escalation (e.g. a negative *L. pneumophila* urinary antigen test[9]). This is in contrast to settings where only positive microbiological findings are actively communicated by the clinical microbiologist to the treating physician. We believe that further studies on optimal arrays of microbiological testing in CAP should take into account the potential interaction by automated trigger alerts, optionally present in many modern electronic health records. The approach of the present study can also be seen as an illustrative example for initiatives aiming to reduce health care costs. Our observed large variation in resource utilization and related costs led to discussions with unexpected novel insights eventually.

A limitation of our study is that the number of patients was relatively small and consisted of patients that participated in a clinical trial. Consequently, this could mean that the results may not be generalizable to all patients admitted with CAP. Nevertheless, this phenomenon applied equally to all hospitals and is therefore less likely to be the reason for the observed large inter-hospital variation. Second, in the present study, we chose to focus on variations in those tests that were most frequently utilized. This could imply an underestimation of the total variation in costs. On the other hand, these commonly utilized tests would be the main cost drivers and are likely to have the most impact if taken into account when altering the content of clinical protocols. Third, costs were calculated using price listing of the Dutch Health care Authority for 2016 (maximum prices), while true costs per item will be different and differ per hospital (due to specific agreements made between individual hospitals and health insurers). Lastly, antibiotic de-escalation was scored based

on antibiotic prescription-based criteria solely. However, this should not have biased our results because the same criteria were applied for each hospital.

Conclusions

Large inter-hospital variation exists with regard to diagnostic testing in the management of adult patients with community-acquired pneumonia. We found a counterintuitive inverse association between the costs for microbiological testing and antibiotic de-escalation rates, most likely due to an interactive electronic trigger tool designed to optimise intravenous to oral antibiotic switch in one of the hospitals. Future studies investigating the most cost-effective sets of microbiological testing that would optimize antimicrobial stewardship programs specific to patients admitted with CAP, should acknowledge the interaction between testing, and ways of communicating results to physicians.

Source of support

This study originated from an investigator-initiated research project about pathogens, clinical outcomes and health care costs for community-acquired pneumonia in the Netherlands. That project received financial support from GlaxoSmithKline (GSK). GSK played no role in the study design, data-analyses, and preparation and approval of this manuscript.

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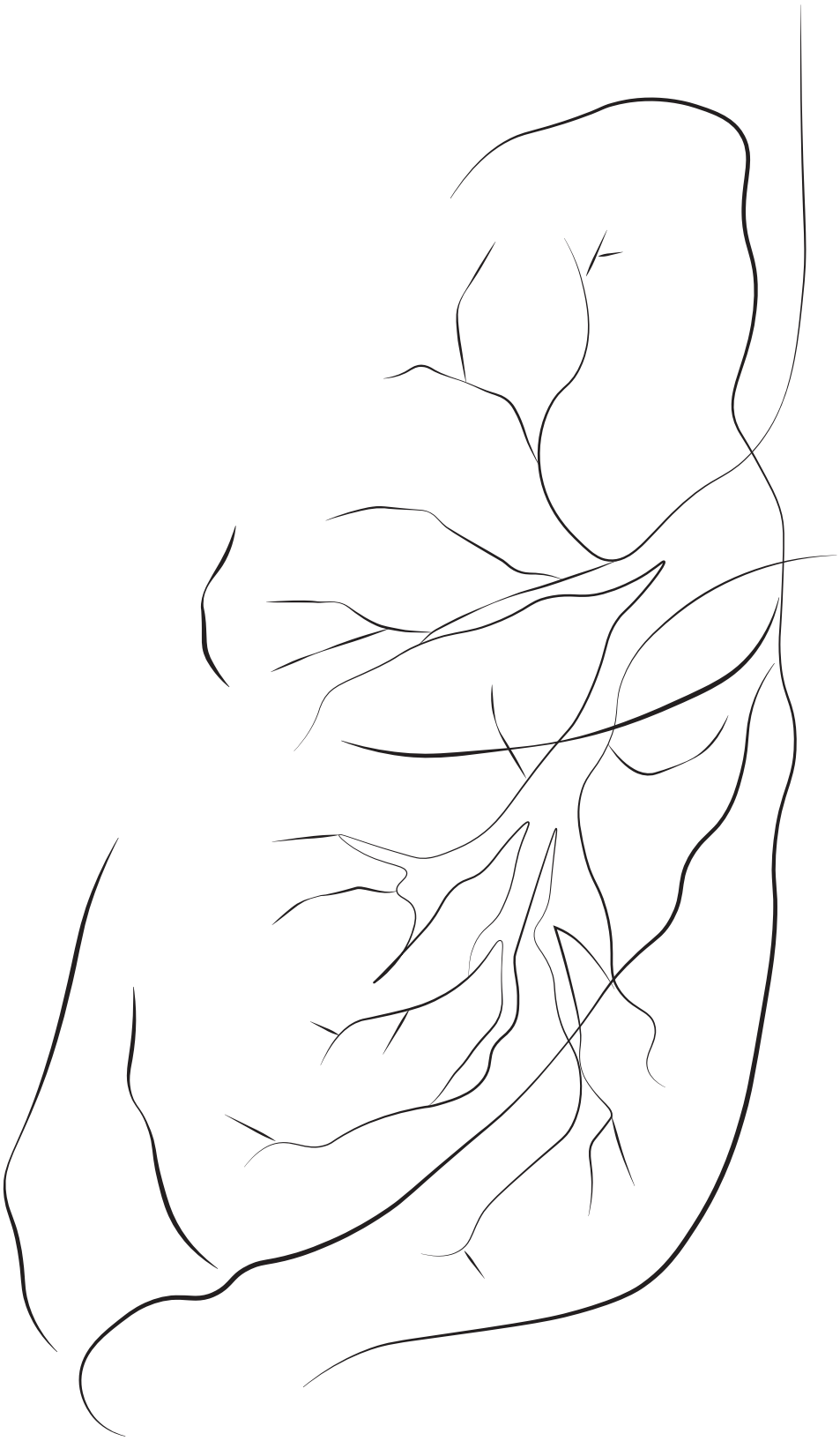

CHAPTER 3

The extent of microbiological testing is associated with alteration of antibiotic therapy in adults with community-acquired pneumonia

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Abstract

Purpose

The aim of this study was to explore the relationship between the extent of microbiological testing and the frequency of antibiotic alteration in adults hospitalised with CAP.

Methods

We retrospectively studied 283 immunocompetent patients hospitalised with CAP. Information on microbiological testing and prescribed antibiotics was obtained. Patients were grouped according to the number of different microbiological tests performed within the first two days of admission (0-5 tests). Alteration rates were compared between these groups. Antimicrobial alteration was defined as a switch at day 3 of hospital stay to 1) a narrower spectrum antibiotics, or 2) a different class of antibiotics or 3) a switch from dual therapy to monotherapy 4) or discontinuation of antibiotic treatment because the indication for antibiotic treatment did no longer exist.

Results

For each additional test performed a stepwise increase in percentage of patients with altered antibiotic regimen ranging from 0% to 59% (p=0.001) was found. Multivariate logistic regression analyses showed that performing PCR assay for atypical pathogens was most strongly associated with any alteration of antibiotic treatment (OR 2.6, 95%CI 1.4-4.9) and with changes in atypical coverage specifically (OR 3.1, 95% CI 1.6-6.0).

Conclusions

The extent of microbiological testing was positively associated with antibiotic alteration in adults hospitalised with CAP. Antibiotic treatment was most likely to be altered in patients in whom a PCR assay for atypical pathogens was performed.

Introduction

Antimicrobial stewardship aims at encouraging appropriate antibiotic use, which should not only be effective but also limit toxic effects, induction of resistance and microbial selection.[1] This is of particular concern in the treatment of community-acquired pneumonia (CAP), which is one of the most common infectious diseases.[2]

Studies have shown that inappropriate therapy is associated with unfavourable outcomes.[3,4] One of the quality indicators of appropriate antibiotic use is alteration of antimicrobial treatment based on microbiological tests results.[1] This may lead to reduced selective pressure for resistance and improved outcomes.[5–8]

Timely and adequate alteration of empiric antibiotic is only possible when actionable microbiological test results are available. However, in day-to-day clinical practice no causative pathogen is found in over 60% of patients hospitalised with CAP. This is partially due to the limited yield of conventional diagnostics.[9] Newer and more rapid testing methods like urinary antigen tests (UAT) and PCR assays have been introduced in the past years.[10] It has been shown in a research setting that combining traditional sputum and blood cultures with these newer diagnostic tests, can increase diagnostic yield up to 67% in patients with CAP.[11–13]

It is assumed that extensive microbiological testing results in an increased diagnostic yield and thereby facilitates more frequent alteration of antibiotic therapy. The aim of this study was to explore the relationship between the extent of microbiological testing and alteration of antibiotic therapy in adults hospitalised with CAP. The secondary objective was to assess the association between the extent of microbiological testing and clinical outcomes.

Methods

Study Design and Patients

Adult patients who were hospitalised with CAP at the St. Antonius Hospital (an 850-bed non-academic teaching hospital in The Netherlands) between January 2013 and January 2017 were assessed.

CAP was defined as a new pulmonary infiltrate on chest X-ray in combination with two of the following findings: cough, sputum production, findings at auscultation indicative of pneumonia, body temperature >38 °C or <35 °C, C-reactive protein concentration >15mg/L and a white blood cell count >$10x10^9$ cells/L or a leftward shift. Immunocompromised patients, either due to acquired or congenital immunodeficiencies or due to the use of immunosuppressive medication within 6 months of admission, were excluded, as were patients participating in a placebo controlled trial evaluating the effectiveness of adjunctive dexamethasone therapy in patients admitted with CAP (NCT01743755) for whom the diagnostic procedures were specified by the trial protocol. Furthermore, we excluded patients with empyema at admission, patients who were directly admitted to the intensive care unit and patients who died within 24-hours of emergency room (ER) presentation.

Eligibility for inclusion was based on radiology reports, laboratory results and patient history and physical examination as reported by the treating physician on the day of ER presentation. The study was approved by the Medical Ethics Committee of the St. Antonius Hospital (Nieuwegein).

Data Collection

Patient medical records were checked to confirm that inclusion criteria were met, to collect data on any antibiotic use prior to hospital admission, to identify patients with a history of COPD and to determine the CURB-65 score (one point for each of the following criteria: confusion, urea >7 mmol/l, respiratory rate >30/min, blood pressure <90mmHg systolic or <60 diastolic, age over 65 years) at time of hospital admission (day 1).[14]

Microbiological tests performed on day 1 and day 2 were selected for analyses using the laboratory information system GLIMS. The following five microbiological tests were included: (1) PCR assays on throat or nasal swabs for detection of respiratory viruses including influenza A, influenza B, parainfluenza viruses 1, 2 and 3, respiratory syncytial viruses type A and B, human metapneumovirus and rhinovirus, (2) PCR assays on throat swabs or on sputum samples for detection of atypical respiratory pathogens including *Coxiella burnetii*, Legionella species, *Chlamydophila psittaci* and *Mycoplasma pneumoniae,* (3) sputum cultures, (4) blood cultures and (5) UAT for the detection of *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* (BinaxNOW®).

Information on prescribed antibiotics was obtained using the Farmadatabase, a database in which all drugs prescribed during admission are registered.[15] All antibiotic prescriptions between January 2013 and January 2017 were extracted. Antibiotics prescribed during hospital admission were identified by matching admission dates to the date that the patient was screened for trial participation. A similar procedure was used to obtain data on all microbiological tests performed from GLIMS. All data were anonymised before analyses were performed.

Outcomes measures

The primary outcome was the percentage of patients whose initial antibiotic regimen had been altered by day 3 of hospital admission. Diagnostic yield was determined according to the number of microbiological tests performed. As secondary outcomes 30-day mortality, secondary intensive care unit (ICU) admission and length of hospital stay (LOS) were determined. Secondary ICU admission was defined as admission to the ICU after 24-hours of hospital admission.

Data analyses

Alteration was defined as one of the following changes in antibiotic regimen: (1) switch to narrower spectrum antibiotics or (2) switch to a different class of antibiotics or (3) switch from dual therapy to monotherapy or (4) discontinuation of antibiotic treatment because

the indication for antibiotic treatment did no longer exist. During the period in which patients were enrolled, the Dutch national guideline on the management of CAP advised to guide empirical antibiotic treatment according to the severity of disease. The antimicrobial spectrum varied from amoxicillin monotherapy for mild CAP to dual therapy with a cephalosporin plus atypical coverage for severe CAP (e.g. erythromycin or ciprofloxacin).[16]

To explore the association between the extent of microbiological testing and alteration of antibiotic treatment, patients were first divided into six groups according to the number of different microbiological tests performed within the first two days of hospital admission. The first group consisting of patients in whom no diagnostic tests were performed (0-test group), up to the last group consisting of patients in whom all five different tests were performed (5-tests group). Subsequently, antibiotic regimens on the day of hospital admission and antibiotic regimens at day 3 of hospital admission were divided into six groups according to antibiotic classification: (1) a small spectrum penicillin with or without β-lactamase inhibitor, (2) a cephalosporin, (3) dual therapy combining a small spectrum penicillin with coverage of atypical pathogens (e.g. macrolide or fluoroquinolone), (4) dual therapy combining a cephalosporin with coverage of atypical pathogens, (5) monotherapy covering atypical pathogens and (6) other antibiotic classes or other combinations of antibiotic classes. Patients with altered antibiotic regimens by day 3 of admission were identified. The number and percentage of patients with altered antibiotic regimens on day 3 were calculated for each diagnostic test group.

Furthermore, we calculated the percentage of patients with at least one positive microbiological test result for the whole study population and for each of the diagnostic groups separately (0-5 tests). The diagnostic yield was compared between groups to determine its relationship with the extent of microbiological testing. A positive microbiological tests was defined as (1) a positive PCR assay for respiratory viruses or atypical pathogens or a positive UAT or (2) a pathogen identified by blood culture except for contamination as noted in the microbiology report or (3) a clinically relevant pathogen identified by sputum culture (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*). To explore the relationship between the number of microbiological tests performed by the end of day 2 and 30-day mortality and secondary ICU-admission, patients were divided into two groups: one group in which limited microbiological testing was performed (0-2 tests) and one group in which extensive testing was performed (3-5 tests). For both groups, the number and percentage of patients who died within 30-days of admission or were admitted to the ICU was calculated.

Overall descriptives are stated as number (%) for categorical data and mean (standard deviation (SD)) or median (interquartile range[IQR]) for continuous data. Categorical data was compared using Chi-squared tests or Fischer's exact tests and continuous data was compared using an independent samples t-test or a Mann-Whitney U test as deemed appropriate. A p-value <0.05 was considered significant.

Multivariable logistic regression analyses were performed to assess the association of each individual microbiological test with the outcomes: 1) any alteration of antibiotic therapy, and 2) alterations in atypical coverage (discontinuation of or a switch to atypical coverage) adjusted for pneumonia severity (CURB-65 score).

Results

Patient selection and baseline characteristics

A total of 390 patients with CAP were screened, of which 283 patients were found eligible for inclusion. The flowchart with reasons for exclusion is shown in Figure 1.

In Table 1, baseline characteristics are shown. The median CURB-65 score was 1 [IQR 1-2]. Antibiotics were prescribed to 32% of patients prior to admission. Baseline characteristics per group (0-test group to 5-tests group) are shown in supplement Table 1.

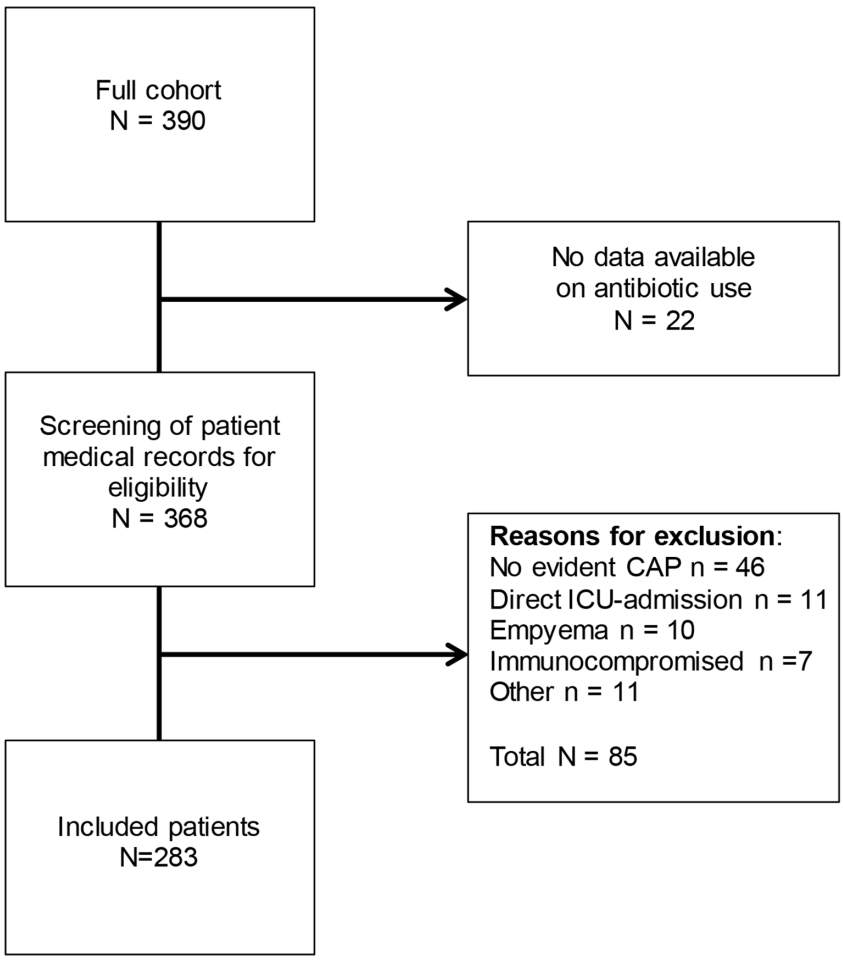

Figure 1. Patient selection.

Table 1. Baseline characteristics.

Characteristic	
Median Age [IQR]	70 [57-81]
Male N (%)	151 (53)
History of COPD N (%)	29 (10)
Antibiotic use prior to admission N (%)	90 (32)
CURB-65 score N (%)	
0	57 (20)
1	87 (31)
2	77 (27)
3	52 (18)
4	8 (3)
5	2 (1)
Initial antibiotic regimen N (%)	
Small spectrum penicillin	130 (46)
Cephalosporin	35 (12)
Small spectrum penicillin with coverage of atypical pathogens	45 (16)
Cephalosporin with coverage of atypical pathogens	53 (19)
Antibiotics for atypical pathogens	11 (4)
Other	9 (3)

Microbiological testing

Blood cultures were performed in 224 (79%) patients, sputum culture in 109 (39%) patients, UAT in 231 (82%) patients, PCR for atypical pathogens in 70 (25%) and PCR for respiratory viruses was performed in 192 (68%) patients.

A pathogen was identified in 104 (37%) patients. There was a clear trend towards a higher pathogen identification rate in patients that did not use antibiotics prior to admission compared to those who did (40% vs. 29%, respectively, p=0.06). As shown in Figure 2, there was a stepwise increase in the pathogen identification rate for each additional test performed (p<0.001, chi-squared test for trend). In descending order the diagnostic yield of individual tests, if performed, was 33% for sputum cultures, 21% for PCR-assay for respiratory viruses, 15% for UAT, 11% for PCR for atypical pathogens and 8% for blood cultures. The most frequently identified pathogen was *S. pneumonia* (17%) followed by *H. influenzae* (5%), influenza A virus (6%), *S. aureus* (3%) and *M. pneumoniae* (2%).

Antimicrobial alteration

Antibiotic regimens on day 1 and day 3 of admission are depicted in Figure 3. At day 3, twelve patients had already been admitted to the ICU, died or had been discharged. For these patients, no reliable data available on antibiotic use on day 3 could be retrieved. We therefore excluded them from further analyses concerning antibiotic alteration. Information on antibiotic use on day 3 of admission was available for 271 (96%) patients.

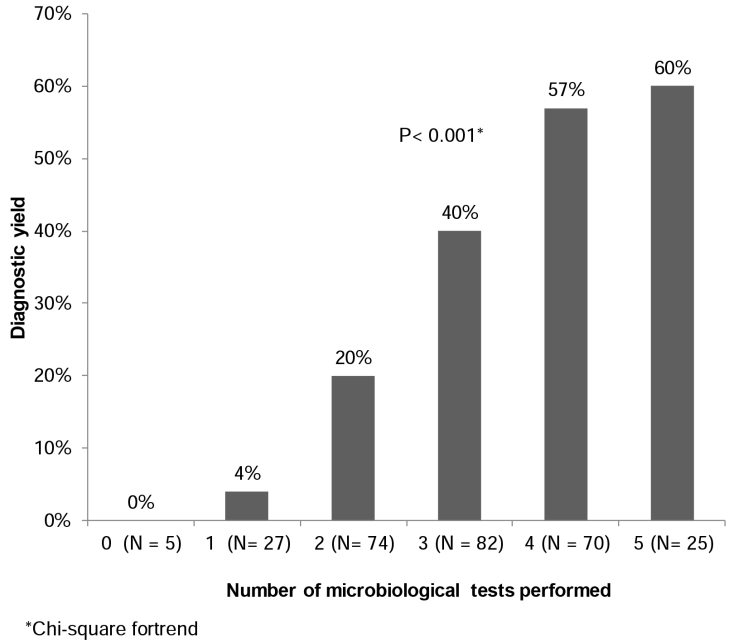

Figure 2. Number of performed microbiological tests and diagnostic yield.

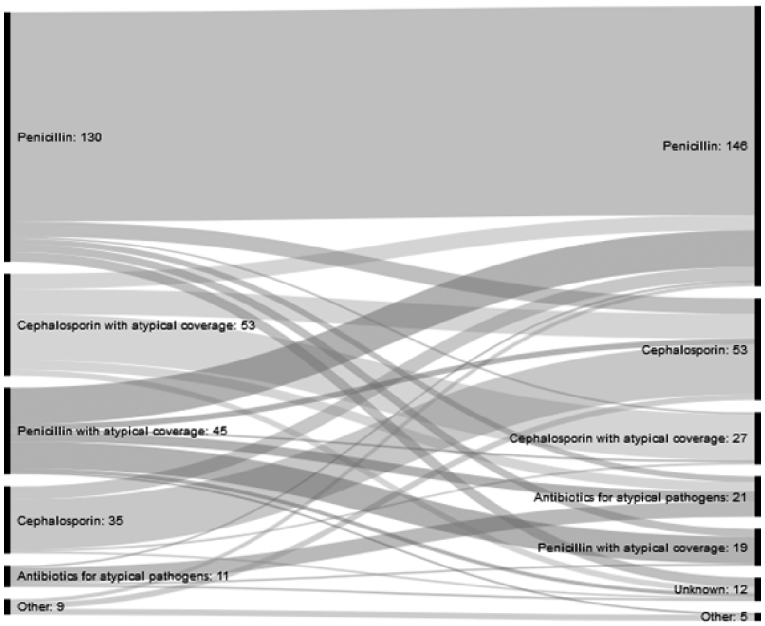

Figure 3. Antibiotic treatment and alterations. The first bar shows antibiotic treatment at time of hospital admission and the second bar shows antibiotic treatment at day 3 of hospital admission. The lines between both bars represent alteration in antibiotic regimens. Numbers represent the number of patients receiving a certain type of antibiotic.

Antibiotic treatment was altered in 70 (26%) patients. Discontinuation of dual therapy (switch to monotherapy) was the most frequent change in antibiotic regimen (n= 53, 76%), followed by narrowing a cephalosporin to a small spectrum penicillin (n=7, 10%). In 58 (21%) of the patients the alteration involved removal or addition of atypical coverage. There was a stepwise increase in percentage of patients with an altered antibiotic regimen for each additional test performed (p=0.001, chi-squared test for trend) (Figure 4). In the multivariable analyses, performing a PCR assay for atypical pathogens was independently associated with both any alteration of antibiotic treatment on day 3 (OR 2.6, 95%CI 1.4-4.9) and with an alteration regarding atypical coverage (OR 3.1, 95% CI 1.6-6.0) (Table 2, Table 3).

Secondary outcomes

There was no significant difference between patients in whom 0-2 or 3-5 diagnostic tests were performed in either LOS nor negative outcomes (death within 30-days of admission and secondary ICU admission combined, due to low numbers) (Table 4).

Discussion

The main finding of this study is the positive association between the number of microbiological tests performed within the first two days of hospital admission and the rate of antibiotic regimen alteration by day 3 in adults hospitalised with CAP. The antibiotic

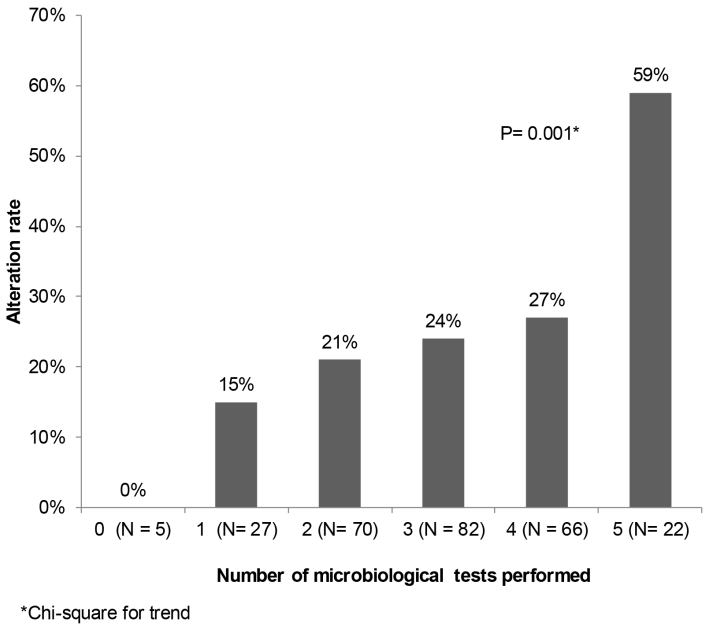

Figure 4. Number of microbiological tests performed and alteration rate.

Table 2. Odds ratio for performing each individual microbiological test and any alteration of antibiotic treatment by day 3 of hospital admission.

	Odds ratio (95% CI)	P-value
Blood culture*	2.2 (1.0-4.9)	0.06
Sputum culture*	1.5 (0.8-2.8)	0.18
Urinary antigen test*	1.7 (0.7-4.0)	0.22
PCR for respiratory viruses*	0.8 (0.4-1.6)	0.53
PCR for atypical pathogens*	2.6 (1.4-4.9)	0.003
CURB-65 (≥2)**	1.7 (1.0-3.1)	0.06

* Reference category: test not performed within two days of admission.
** Reference category: CURB-65 <2.

Table 3. Odds ratio for performing each individual microbiological test and alteration in atypical coverage by day 3 of hospital admission.

	Odds ratio (95% CI)	P-value
Blood culture*	1.8 (0.8-4.2)	0.18
Sputum culture*	1.5 (0.8-2.9)	0.21
Urinary antigen test*	2.2 (0.8-6.1)	0.13
PCR for respiratory viruses*	0.9 (0.5-1.9)	0.87
PCR for atypical pathogens*	3.1 (1.6-6.0)	0.001
CURB-65 (≥2)**	1.0 (0.5-1.8)	0.95

* Reference category: test not performed within two days of admission.
** Reference category: CURB-65 <2.

Table 4. Number of microbiological test performed and secondary endpoints.

Number of tests	N	30-day mortality and/or secondary ICU admission N (%)	Median LOS in days [IQR]
0-2	106	13 (12)	5 [3-8]
3-5	177	13 (7)*	6 [4-8]**
Total	283	26 (9)	5 [3-8]

* p=0.158 (chi-square): Group with 0-2 performed tests compared to the group with 3-5 performed tests.
** p=0.126 (Mann-Whitney U): Group with 0-2 performed tests compared to the group with 3-5 performed tests.

treatment alteration rate was almost three times higher by day 3 in patients in whom a PCR assay for atypical pathogens was performed. A change in atypical coverage was the most frequent alteration.

Regarding specific diagnostic tests, Oosterheert et al. investigated the addition of PCR assays for atypical pathogens and respiratory viruses to standard microbiological testing in day-to-day clinical practice in patients admitted with lower respiratory tract infections

including, but not limited to, pneumonia.[17] The addition of these PCR assays to conventional diagnostics did increase diagnostic yield from 21% to 43%, however antibiotic treatment was only modified based on PCR results in 11% of patients. We found a 26% overall alteration rate. A likely explanation for this difference is the higher frequency of dual therapy in our cohort (35% vs 16%) providing more opportunities for alterations.

More recently, Vestjens et al. retrospectively studied the association between the total costs of diagnostic testing and antimicrobial de-escalation in patients with CAP in three Dutch non-academic teaching hospitals.[18] It was demonstrated that the mean costs for microbiological testing per patient was highest in the hospital where PCR assays were performed most frequently. In the study by Vestjens et al., the de-escalation rate was highest in the hospital with the lowest costs for testing. It was concluded that this seemingly counterintuitive finding could be explained by the presence of an automated iv-to-oral trigger alert in that specific hospital, guiding physicians to reconsider antibiotic regimens by drawing their attention to microbiological test results (including negative results). No such antibiotic stewardship intervention was in place in the hospital where the present study was performed.

However, to assess the potential added value of such an automated antibiotic stewardship intervention, we checked the medical records of the 15 patients receiving dual therapy at admission and in whom a PCR assay for atypical pathogens was performed and whose antibiotic regimen was not altered, to assess the reasons for not switching antibiotic therapy. In the charts of 4 patients the reason for continuing dual therapy was argued. However, in the 11 remaining patients there was no note by the treating physician on the result of the PCR assay for atypical pathogens nor was the reason for continuing dual therapy argued. Considering that all these 11 patients had a negative PCR assay and did not have a positive UAT for Legionella implies that our observed frequency of alteration based on PCR is an underestimation of its true potential. It also supports the conclusion from Vestjens et al. that apart from ordering a specific test also the way of communicating the results to physician is relevant towards the purpose of the test. Still, although its relatively (but decreasing) costliness compared to longer existing microbiological test methods, performing PCR assays for atypical pathogens clearly contributed to antibiotic therapy alteration in this single center study.

This study does have limitations, mainly due to its retrospective and single centre design. First, we included the microbiological tests ordered on the day of hospital admission and the day after hospital admission. Inaccuracy of recorded time of sampling rendered it impossible to use a more exact time-frame (e.g. within 24 or 48 hours) in which microbiological tests were performed for every patient.

Second, we grouped patients receiving amoxicillin/clavulanic acid into the small spectrum penicillin group. As a result we did not identify switches from amoxicillin/clavulanic acid to amoxicillin or penicillin as alteration. However, this only involved 4 patients with this scenario, making the impact on our findings rather small.

Third, due to low rates of antibiotic resistance in The Netherlands, guidelines for antibiotic treatment of CAP differ from countries with higher rates of resistance. As the antimicrobial resistance rates of *S. pneumoniae* for penicillin are low in The Netherlands, a small spectrum penicillin is the first line of treatment in patients with mild to moderate CAP.[16] Therefore the number of patients receiving monotherapy with a small spectrum penicillin in our study is higher than it would be in other countries, thereby limiting the external validity of our findings for these countries. However, a strength of this study is that it reflects day-to-day clinical practice. Furthermore, our study included a well-defined cohort of patients with mainly mild to moderate-severe CAP. Median age, antibiotic use prior to hospital admission, level of pathogen identification, and 30-day mortality were also very similar to those found in another large and recent Dutch cohort including non-ICU patients with CAP).[19]

In conclusion, for each additional microbiological test performed we found a stepwise increase in alteration of antimicrobial therapy in patients admitted with CAP. Performing a PCR assay for atypical pathogens was most evidently associated with antibiotic alteration, most often being a switch from dual therapy to monotherapy.

Sources of support

This work was supported by a grant from the St. Antonius Research Fund via an earmarked donation from Verwelius Construction Corporation for research on CAP.

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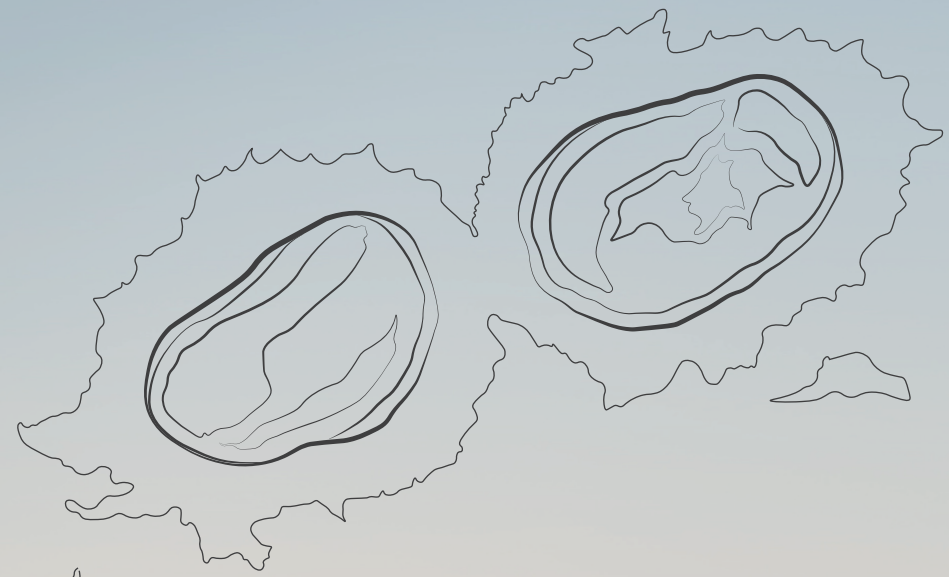
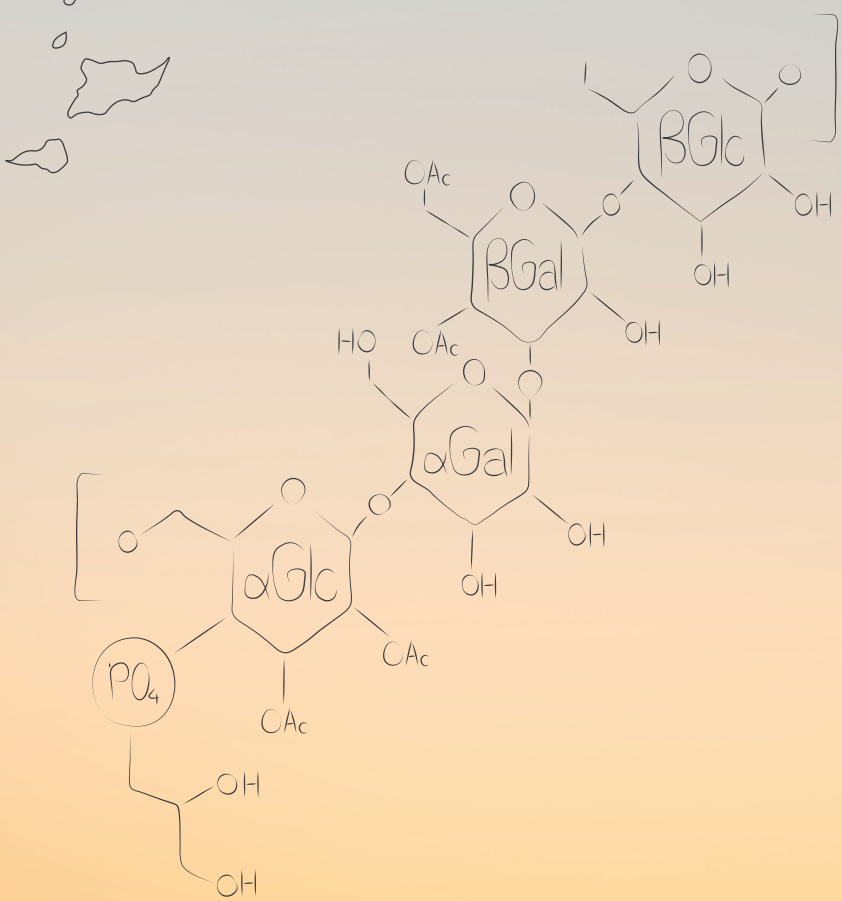
Supplementary material

Supplementary Table 1. Baseline characteristics per group (0-5 tests).

Characteristic	0 tests	1 test	2 tests	3 tests	4 tests	5 tests
Median Age [IQR]	80 [50-89]	70 [60-81]	72 [62-84]	70[62-82]	68 [49-79]	66 [48-77]
Male N(%)	3 (60)	15 (56)	42 (57)	38 (46)	40 (57)	13 (52)
History of COPD N(%)	0 (0)	2 (7)	8 (11)	8 (10)	6 (9)	5 (20)
Antibiotic use prior to admission	1 (20)	9 (33)	20 (27)	27 (33)	22 (31)	11 (44)
Curb-65 score						
0	1 (20)	6 (22)	12 (16)	13 (16)	17 (24)	8 (32)
1	1 (20)	8 (30)	24 (32)	29 (35)	18 (26)	7 (28)
2	1 (20)	8 (30)	17 (23)	25 (31)	18 (26)	8 (32)
3	2 (40)	4 (15)	16 (22)	13 (16)	15 (21)	2 (8)
4	0 (0)	1 (4)	4 (5)	2 (2)	1 (1)	0 (0)
5	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)	0 (0)
Initial Antibiotic regimen N(%)						
Small spectrum penicillin	4 (80)	17 (63)	40 (54)	34 (42)	27 (39)	8 (32)
Cephalosporin	0 (0)	2 (7)	12 (16)	11 (13)	9 (13)	1 (4)
Small spectrum penicillin with coverage of atypical pathogens	1 (20)	2 (7)	6 (8)	13 (16)	16 (23)	7 (28)
Cephalosporin with coverage of atypical pathogens	0 (0)	3 (11)	11 (15)	17 (21)	13 (19)	9 (36)
Antibiotics for atypical pathogens	0 (0)	3 (27)	2 (18)	4 (36)	2 (18)	0 (0)
Other	0 (0)	0 (0)	3 (4)	3 (4)	3 (4)	0 (0)

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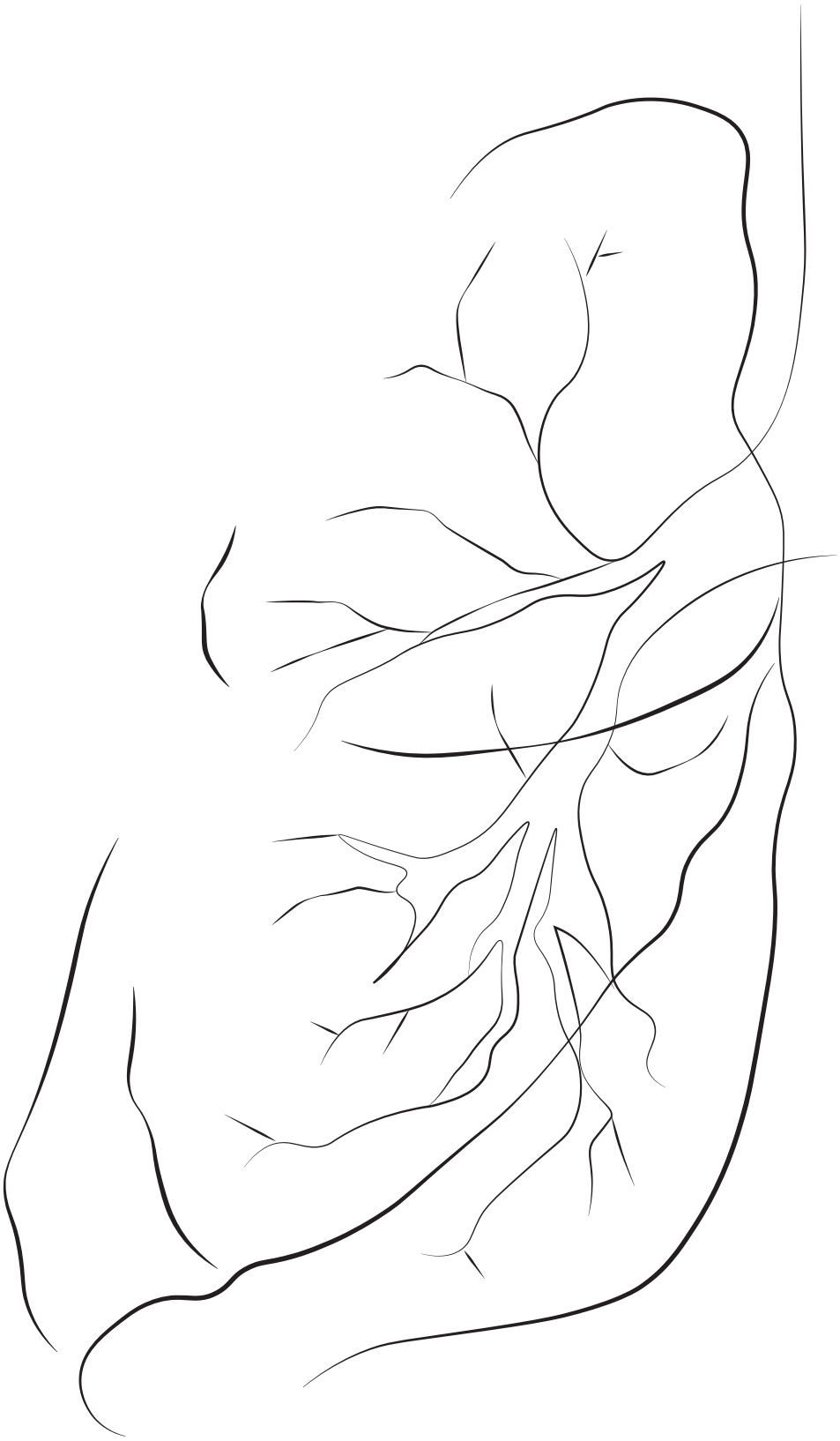
PART II

Pneumococci, pneumococcal vaccination and serotype-specific antibodies



CHAPTER 4

Twelve years of pneumococcal conjugate vaccination in the Netherlands: impact on incidence and clinical outcomes of invasive pneumococcal disease

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* Authors contributed equally

Vaccine, 2019

Abstract

Introduction

In 2006, the Netherlands introduced the 7-valent pneumococcal conjugate vaccine (PCV7) in their national immunisation programme. In 2011, PCV7 was replaced by the 10-valent vaccine (PCV10). We report on the impact of PCV on invasive pneumococcal disease (IPD) incidence, clinical syndromes and patient outcomes.

Methods

Pneumococcal isolates of hospitalised IPD patients between June 2004 and May 2018 were obtained from nine sentinel laboratories, covering 25% of the Dutch population. All isolates were serotyped. IPD incidence and clinical outcome were determined before and after introduction of PCV7 and after the switch to PCV10, stratified by age and serotype.

Results

Compared to before PCV7 introduction, significant declines in IPD incidence were observed in 2016-2018 in children <5 years (69%), 18-49 year olds (31%) and ≥65 year olds (19%). Compared to before PCV10 introduction, the IPD incidence in 2016-2018 declined in children <5 years (RR:0.68, 95%CI:0.42-1.11), 5-17 year olds (RR:0.58, 95%CI:0.29-1.14) and 18-49 year olds (RR:0.72, 95%CI:0.57-0.90), but not in 50-64 year olds (RR:0.94, 95%CI:0.81-1.10) and ≥65 year olds (RR:1.04, 95%CI:0.0.93-1.15). While the case fatality rate (CFR) decreased from 16.2% pre-PCV to 13.4% post-PCV10 (RR:0.83, 95%CI:0.70-0.99), the switch to PCV10 had no further impact on CFR (RR:1.14, 95%CI:0.96-1.36).

Conclusion

Twelve years of PCV in the Netherlands has resulted in a sustained reduction of IPD incidence in children and younger adults. The switch from PCV7 to PCV10 did not have additional impact on the IPD incidence in older adults and CFR due to emerging non-vaccine serotypes.

Introduction

Invasive pneumococcal disease (IPD) is associated with high morbidity and mortality worldwide.[1,2] IPD is defined as an infection of a normally sterile body fluid, typically blood or cerebrospinal fluid (CSF), by the gram-positive bacterium *Streptococcus pneumoniae*, with clinical syndromes including septicaemia, invasive pneumonia or meningitis.

Currently available vaccines are the 10- and 13-valent pneumococcal conjugate vaccines (PCV) and the 23-valent pneumococcal polysaccharide vaccine (PPV23). As of September 2018, 145 countries worldwide have implemented PCV in their national immunisation programme for infants.[3,4] In the Netherlands, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2006, and was replaced by PCV10 in 2011. PCV coverage in children at age 2 years has been 93-95% since the introduction of PCV7.[5] Up to 2018, PPV23 has been recommended only for those at high risk for IPD, and PPV23 use has been very low in the Netherlands (<0.5% of persons ≥65 years in 2017).[6,7]

Four years after the introduction of PCV7, the overall IPD incidence in Dutch children under two years had decreased by 57% and, due to indirect protection, by 22% in adults ≥65 years.[8] Three years after the switch to PCV10, a further reduction in IPD incidence of 30% was observed in children under 2 years. In adults ≥65 years, a decline in IPD caused by the additional PCV10 serotypes 1, 5 and 7F of 25% was observed, but the overall IPD incidence plateaued; this was attributed to a steady rise of non-PCV10 serotypes.[8,9] In England and Wales, replacement disease from non-PCV13 serotypes, in particular serotypes 8, 12F and 9N, has progressively eroded the IPD reduction in older adults; in 2016-2017 almost no net benefit was observed from the PCV7 to PCV13 switch.[10]

The aim of the current study was to describe the overall impact since PCV7 introduction in 2006, and in particular the impact of the switch from PCV7 to PCV10 in 2011 on IPD incidence, clinical syndromes and patient outcomes in the Netherlands.

Methods

Study population and data collection

Since 2004, national IPD surveillance in the Netherlands is based on data from nine sentinel clinical microbiological laboratories which submit pneumococcal isolates to the Netherlands Reference Laboratory for Bacterial Meningitis for serotyping by agglutination and subtyping by the Quellung method.[11] IPD was defined as identification of *S. pneumoniae* isolated from the patient's blood or CSF. The sentinel laboratories cover ~25% of the Dutch population and coverage has not changed since 2004.[12,13] Clinical information on IPD patients, including clinical syndrome and course of disease, was extracted retrospectively from hospital medical records using a standardised form with study procedures as previously reported. IPD serotype data from June 2004 to May 2018 (i.e. using epidemiological years) are included in the current analysis. Clinical data were available from June 2004 to May 2016. The independent Medical Research Ethics Committee of the UMC Utrecht has determined

that the study (protocol number 16-256/C) is not subject to the Medical Research Involving Human Subjects Act (WMO).

Clinical characteristics and definitions

We applied the same definitions for clinical syndromes and outcomes as in previous surveillance reports.[9] Five clinical IPD syndromes were defined: meningitis, invasive pneumonia with empyema, invasive pneumonia without empyema, bacteraemia with another and bacteraemia without a focus. Empyema assessment was based on clinical and/or radiographic evaluation. Death was defined as deceased during admission or within 30 days after obtaining the *S. pneumoniae* culture-positive material. Observations with missing values were excluded. Underlying conditions were divided into immunocompromising and non-immunocompromising comorbidities, as reported previously.[9]

Data analyses

Incidence of IPD was calculated as number of cases per 100,000 persons per epidemiological year, taking into account the coverage of the surveillance programme (25% of the Dutch population). IPD incidence in the last two epidemiological years (June 01, 2016 to May 31, 2018) was compared to the two epidemiological years before PCV7 introduction (June 01, 2004 to May 31, 2006) and PCV10 introduction (June 01, 2009 to May 31, 2011). Two-year periods were chosen instead of one year to reduce the impact of temporal variations. Incidence was stratified by age groups (<5, 5-17,18-49, 50–64, ≥65 years) and/or by four serotype groups: PCV7 type (4, 6B, 9V, 14, 18C, 19F, 23F); PCV10-7 type (1, 5, 7F), PCV13-10 type (3, 6A and 19A) and non-PCV13 type (all other serotypes).

Clinical syndromes, ICU admission and case fatality rate (CFR) were compared between three periods: pre-PCV (June 01, 2004 to May 31, 2006), post-PCV7 (June 01, 2008 to May 31, 2011) and post-PCV10 (June 01, 2013 to May 31, 2016). The first two epidemiological years after PCV7 and PCV10 introduction were disregarded, since herd protection was not yet expected to be observed.[13] Note that the periods for clinical data analyses are slightly different from the periods used for the IPD incidence analyses because of data availability and sample size considerations. The same age and serotype group strata as for the incidence data were applied.

Baseline differences in patient characteristics between time periods were tested using independent sample t-tests or $\chi 2$, where appropriate. Differences in incidences and proportions between the time periods were tested with $\chi 2$ tests and RRs with 95% CI were calculated. A *p*-value of <0.05 was considered to represent a statistically significant difference. If considered appropriate, multivariable logistic regression analyses were performed to adjust for age and/or comorbidities when assessing associations between time periods and outcomes.

Results

IPD incidence (June 2004 to May 2018)

A total of 8865 IPD patients were identified between June 2004 and May 2018. Compared with the pre-PCV period, the overall incidence of IPD in 2016-2018 across all age groups had not declined significantly (RR: 0.94, 95%CI: 0.87-1.02) (Table 1, Figure 1). There was, however, a statistically significant decline in IPD incidence in children <5 years (69%), in 18-49 year olds (31%) and in persons ≥65 years (19%). After the switch from PCV7 to PCV10, a continuous decline in overall IPD incidence was observed in age groups below 50 years; this was statistically significant only in the 18-49 years age group, whereas overall IPD incidence showed no (further) reduction in 50-64 year-olds and in persons ≥65 years. Subdividing the IPD cases among persons ≥65 years into smaller groups shows a very similar trend in each group (Supplementary Table S1 and Supplementary Figure S1).

Between 2004-2006 and 2016-2018, PCV7 type IPD has declined by 90-100% in all age groups. After the switch to PCV10, the incidence of PCV10-7 type IPD (serotypes 1, 5 and 7F) also declined in all age groups, with 91% reduction in children under 5 years and 72-76% reduction in adult age groups by 2016-2018 (Table 1). The incidence of PCV13-10 type IPD (serotypes 3, 6A and 19A) increased by 44% after PCV10 introduction, and by 97% over the whole period between 2004-2006 and 2016-2018; this increase was apparent in all adult age groups, though only statistically significant in the age groups 50-64 years and ≥65 years. Serotype 6A IPD incidence declined substantially after PCV7 introduction, due to cross reactivity with PCV7 serotype 6B. Serotype 19A IPD incidence increased significantly from 0.5 per 100,000 in 2004-2006 to 1.4 per 100,000 in 2009-2011 and 2.0 per 100,000 in 2016-2018 (Figure 2). Serotype 3 IPD incidence increased significantly after the switch to PCV10, from 0.9 per 100,000 in 2009-2011 to 1.4 per 100,000 in 2016-2018. In 2016-2018, serotypes 19A and 3 were the second and third most common IPD-causing serotypes in the Netherlands. In 2016-2018, the non-PCV13 IPD incidence had increased by 65%-160% in adult age groups compared with 2004-2006. This was primarily due to the rapid emergence of serotype 8, and to a lesser extent by serotypes 9N, 12F and 6C (Figure 2). Serotype 8 was the main serotype in adult IPD in 2016-2018, and its incidence increased from 1.5 per 100,000 in 2009-2011 to 3.7 per 100,000 in 2016-2018. The incidence of emerging serotype 22F did not increase further after the switch to PCV10, and was the fourth most prevalent serotype in IPD in 2016-2018.

Clinical follow-up (June 2004 to May 2016)

Study population

Both clinical and serotype data were available from 7254 IPD patients of whom 1215 were diagnosed in the pre-PCV period (June 2004-May 2006), 1732 in the post-PCV7 period (June 2008-May 2011) and 1776 in the post-PCV10 period (June 2013-May 2016). The mean patient age increased over the three study periods (60 years (SD 24.1) pre-PCV; 63 years (SD 21.0) post-PCV7; 65 years (SD 18.5) post-PCV10: p<0.001). The percentage of patients with

Table 1. Incidence (per 100,000) and relative risks of IPD incidence comparing June 2016-May 2018 with the two years before PCV introduction (June 2004 to May 2006) and the two years before PCV10 introduction (June 2009 to May 2011) by age group and serotype group.

		2004-2006		2009-2011		2016-2018		2016-2018 vs. 2004-2006		2016-2018 vs. 2009-2011	
Age	Serotype group	Incidence (/100,000)	N	Incidence (/100,000)	N	Incidence (/100,000)	N	RR	95% CI	RR	95% CI
<5 years	PCV7	13.48	68	0.65	3	0.69	3	0.05	0.02-0.16	1.06	0.21-5.25
	PCV10-7	2.38	12	2.59	12	0.23	1	0.10	0.01-0.74	0.09	0.01-0.68
	PCV13-10	2.18	11	1.73	8	1.60	7	0.74	0.28-1.90	0.93	0.34-2.56
	non-PCV13	1.78	9	4.10	19	3.66	16	2.05	0.91-4.65	0.89	0.46-1.74
	total	19.82	100	9.07	42	6.18	27	0.31	0.20-0.48	0.68	0.42-1.11
5-17 years	PCV7	0.85	11	0.46	6	0.00	0	0.00	NA	0.00	NA
	PCV10-7	0.39	5	0.93	12	0.16	2	0.41	0.08-2.10	0.17	0.04-0.76
	PCV13-10	0.00	0	0.23	3	0.16	2	NA	NA	0.68	0.11-4.08
	non-PCV13	0.46	6	0.15	2	0.71	9	1.53	0.55-4.30	4.60	0.99-21.28
	total	1.70	22	1.78	23	1.03	13	0.60	0.30-1.20	0.58	0.29-1.14
18-49 years	PCV7	1.71	63	0.97	35	0.17	6	0.10	0.04-0.23	0.18	0.07-0.42
	PCV10-7	1.87	69	2.17	78	0.52	18	0.28	0.16-0.46	0.24	0.14-0.40
	PCV13-10	0.33	12	0.58	21	0.60	21	1.85	0.91-3.76	1.03	0.56-1.89
	non-PCV13	1.44	53	1.39	50	2.38	83	1.65	1.17-2.34	1.71	1.20-2.43
	total	5.35	197	5.12	184	3.67	128	0.69	0.55-0.86	0.72	0.57-0.90
50-64 years	PCV7	7.88	120	3.19	53	0.45	8	0.06	0.03-0.12	0.14	0.07-0.30
	PCV10-7	2.82	43	4.99	83	1.25	22	0.44	0.26-0.74	0.25	0.16-0.40
	PCV13-10	2.63	40	3.06	51	4.81	85	1.83	1.26-2.67	1.57	1.11-2.22
	non-PCV13	4.60	70	8.35	139	11.95	211	2.60	1.98-3.41	1.43	1.15-1.77
	total	17.92	273	19.59	326	18.47	326	1.03	0.88-1.21	0.94	0.81-1.10

Table 1. (continued)

		2004-2006		2009-2011		2016-2018		2016-2018 vs. 2004-2006		2016-2018 vs. 2009-2011	
Age	**Serotype group**	**Incidence (/100,000)**	**N**	**Incidence (/100,000)**	**N**	**Incidence (/100,000)**	**N**	**RR**	**95% CI**	**RR**	**95% CI**
≥65 years	PCV7	30.09	344	8.85	112	2.55	40	0.08	0.06-0.12	0.29	0.29-0.41
	PCV10-7	10.06	115	9.01	114	2.49	39	0.25	0.17-0.36	0.28	0.19-0.40
	PCV13-10	7.35	84	9.64	122	11.98	188	1.63	1.26-2.11	1.24	0.99-1.56
	non-PCV13	15.22	174	21.65	274	33.97	533	2.23	1.88-2.65	1.57	1.36-1.81
	total	62.72	717	49.16	622	50.99	800	0.81	0.74-0.90	1.04	0.93-1.15
All ages	PCV7	7.44	606	2.52	209	0.67	57	0.09	0.07-0.12	0.26	0.20-0.36
	PCV10-7	2.99	244	3.61	299	0.96	82	0.32	0.25-0.41	0.27	0.21-0.34
	PCV13-10	1.80	147	2.47	205	3.55	303	1.97	1.62-2.40	1.44	1.20-1.71
	non-PCV13	3.83	312	5.84	484	9.99	852	2.61	2.29-2.97	1.71	1.53-1.91
	total	16.06	1309	14.45	1197	15.18	1294	0.94	0.87-1.02	1.05	0.97-1.14

Abbreviations: CI, confidence interval; NA, not applicable; PCV, pneumococcal conjugate vaccine, N, number of cases.

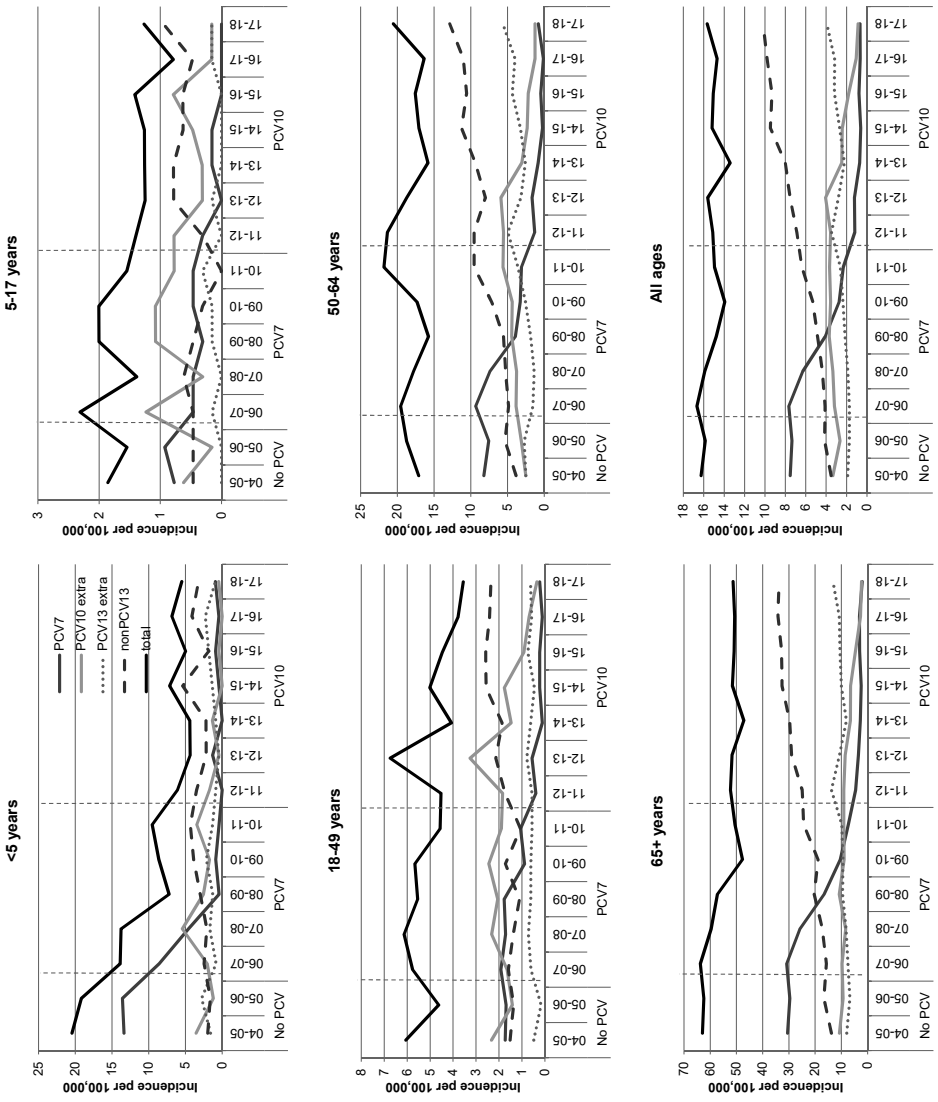

Figure 1. IPD incidence by age group and serotype group from June 2004 to May 2018.

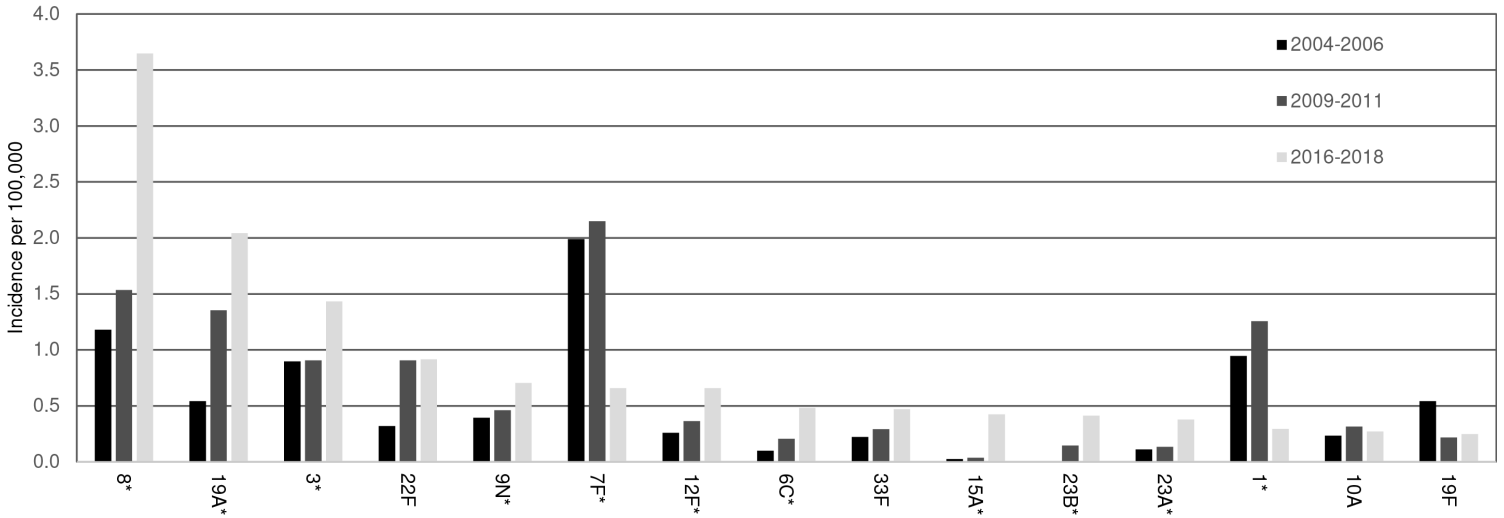

Figure 2. Incidence before PCV introduction (2004 to 2006), two years before PCV10 introduction (2009 to 2011), and the last two years (2016 to 2018) of the top fifteen serotypes causing IPD in 2016-2018. * Indicates a statistically significant difference ($p<0.05$) in incidence between 2009-2011 and 2016-2018.

a non-immunocompromising comorbidity was higher in the post-PCV10 period compared with the other two study periods (p<0.001), but this was no longer statistically significant after adjustment for age (Table 2). There was no difference in the proportion of IPD cases with an immunocompromising condition between study periods (p=0.129).

Clinical syndromes

After the switch to PCV10, the incidence of meningitis decreased by 23% compared to pre-PCV across all ages (RR: 0.77, 95%CI: 0.61-0.96, Table 2) and was most pronounced in children under 5 years (Figure 3 and Supplementary Table S2); no statistically significant further decline was observed in the post-PCV10 period compared to the post-PCV7 period (RR: 0.91, 95%CI: 0.74-1.13). The incidence of pneumonia with empyema increased significantly after introduction of PCV7, but did not increase further after the switch to PCV10 (RR: 0.86, 95%CI: 0.64-1.15) and stabilised at a higher level, comprising 4.7% of all IPD post-PCV10 (vs. 5.5% post-PCV7 and 2.4% pre-PCV). Post-PCV10, the proportion of PCV10 type empyema declined significantly (from 49.5% post-PCV7 to 27.7% post-PCV10, p=0.003). A shift towards non-PCV13 type empyema was observed, mainly serotype 15A (from 0% to 6.7% (n=3)), 22F (from 6.9% (n=2) to 13.3% (n=6)) and 8 (from 44.8% (n=13) to 51.1% (n=23)).

Patient outcomes

The overall CFR was 13.4% in the post-PCV10 period, which was significantly lower than in the pre-PCV period (16.2%, Table 2). The reduction in CFR was most pronounced in 50-64 year olds and persons ≥65 years, the age groups that contribute most to the CFR. The decline in CFR was established after PCV7 introduction and the switch to PCV10 did not affect overall CFR; adjustment for age did not change this. Between 2004 and 2018, serotype 19F had the highest CFR (Figure 4)**.** There was no difference in the proportion of ICU admissions between the three study periods.

Discussion

Since the introduction of PCV in the Dutch national immunisation programme in 2006, the incidence of IPD has declined, particularly among children under 5 years, with 69% reduction, and adults ≥65 years and older, with 19%. After the switch to PCV10 in 2011, a further 30% reduction in overall IPD was observed in those under 50 years of age, but no reduction was seen in those aged 50 to 64 years and ≥65 years. In these older age groups, the ongoing decline in vaccine serotypes was countered by the continuous increase in non-vaccine serotype IPD, that resulted in stabilisation of the overall IPD incidence. Since older adults and the elderly (≥ 65 years) account for almost 90% of reported IPD cases in the Netherlands, no overall net benefit on IPD incidence across all age groups was observed after the switch from PCV7 to PCV10. As the remaining proportion of vaccine-type IPD in 2016-2018 was only 11% compared with 65% in 2004-2006, this suggests that the maximum

Table 2. Relative risks of patient characteristics, clinical syndrome incidences and outcomes comparing the pre-PCV7, PCV7 and PCV10 period.

	Pre-PCV (2004-2006) n=1215	Post-PCV7 (2008-2011) n=1732	Post-PCV10 (2013-2016) n=1776	Post-PCV10 vs pre-PCV		Post-PCV10 vs post-PCV7	
				RR	95% CI	RR	95% CI
Comorbidity, % (n/N)							
Non-immunocompromising	70.0 (850/ 1215)	69.9 (1211/ 1732)	75.4 (1339/ 1776)	1.08	1.03-1.13	1.08	1.04-1.12
Immunocompromising	20.1 (244/1215)	21.0 (364/ 1732)	19.1 (339/ 1776)	0.95	0.82-1.10	0.91	0.80-1.04
Clinical syndrome, /100,000 (N)							
Meningitis	1.68 (137)	1.41 (175)	1.29 (163)	0.77	0.61-0.96	0.91	0.74-1.13
Pneumonia only	10.65 (868)	9.85 (1220)	10.06 (1273)	0.94	0.87-1.03	1.02	0.95-1.11
Pneumonia with empyema	0.36 (29)	0.77 (95)	0.66 (83)	1.84	1.21-2.81	0.86	0.64-1.15
Bact. without focus	1.30 (106)	1.11 (137)	1.23 (156)	0.95	0.74-1.21	1.12	0.89-1.40
Bact. other focus	0.82 (67)	0.81 (100)	0.80 (101)	0.97	0.71-1.32	0.99	0.75-1.30
Patient outcomes, % (n/N)							
ICU admission	21.8 (258/1181)	21.3 (364/1708)	21.9 (389/1776)	1.00	0.87-1.15	1.03	0.91-1.17
Case fatality*	16.2 (194/1201)	11.8 (201/1708)	13.4 (235/1750)	0.83	0.70-0.99	1.14	0.96-1.36
<5 years	5.6 (5/90)	3.6 (2/55)	8.1 (3/37)	1.46	0.37-5.80	2.23	0.39-12.7
5-17 years	4.4 (1/23)	3.1 (1/32)	4.0 (1/25)	0.88	0.06-13.3	1.24	0.98-18.9
18-49 years	4.4 (8/180)	5.1 (14/275)	4.4 (10/227)	0.99	0.40-2.46	0.91	0.41-2.00
50-64 years	12.8 (32/250)	7.7 (33/428)	8.5 (36/424)	0.63	0.40-0.99	1.11	0.71-1.74
≥65 years	22.5 (148/658)	16.5 (151/918)	17.8 (185/1037)	0.75	0.62-0.90	1.10	0.91-1.34
Mortality rate, /100,000 (N)	2.38 (194)	1.62 (201)	1.86 (235)	0.78	0.65-0.94	1.14	0.43-3.08

Abbreviations: Bact., bacteraemia; CI, confidence interval; ICU, intensive care unit; N, number of cases; PCV, pneumococcal conjugate vaccine; RR, relative risk; yr., year.
*Number of missing values/age group (<5, 5-17,18-49, 50–64, ≥65 years, respectively): pre-PCV7 (3,-,2,8,11); post-PCV7 (3,-,2,8,11); post-PCV10 (-,-,2,6,18).

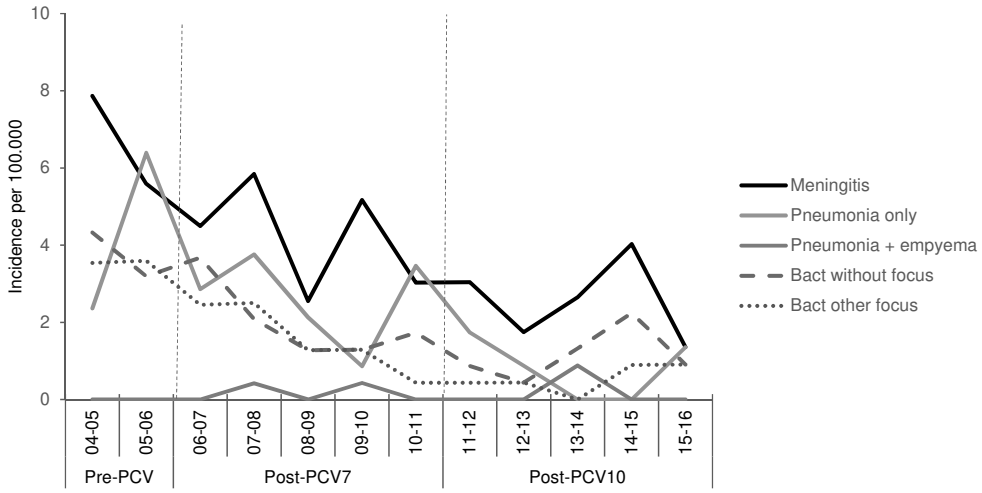

Figure 3. Incidence of clinical syndromes in children under 5 years (2004 to 2016).

impact of PCV10 has almost been achieved. Likewise, while the overall CFR decreased after PCV7 introduction, no further reduction was observed after the switch to PCV10. The incidence of empyema stabilised in the post-PCV10 period, after it had increased in the post-PCV7 period, due to a reduction in PCV10-type empyema.

High-quality surveillance data on impact of PCV10 is limited. A recent review of observational data on the impact of current childhood PCV programmes included data from four countries using PCV10, of which one had no prior PCV7 use (Finland) and two had recently switched from PCV10 to PCV13 (Quebec/Canada and New Zealand); the Netherlands was the only country in this review that used PCV10 after previous PCV7 introduction.[14] Data from Finland showed a 79% reduction in overall IPD incidence in vaccine eligible children; this appears slightly more than the overall 69% reduction in children under 5 years in the Netherlands.[15] Among the elderly, Finnish data showed that while reductions in PCV10 type IPD were seen after PCV10 introduction, a simultaneous large increase in non-PCV10 type IPD incidence was observed, resulting in no net benefit of PCV10 introduction.[16]

More data have been published on the impact of PCV13. In England and Wales, a continuous reduction in IPD incidence across all ages was observed in the first three years after the switch from PCV7 to PCV13 in 2010.[10] However, from 2015, replacement by non-PCV13 serotypes eroded the previous PCV13 benefit and only a small net benefit was retained in 2016-2017, compared with the two-year period before PCV13 introduction (RR: 0.93, 95%CI: 0.89-0.97). It is noteworthy that England and Wales found a slightly larger reduction in IPD incidence in children <5 years (36-48%) upon PCV13 introduction compared with the 32% reduction in the Netherlands after the switch to PCV10, likely due to reduction of the additional serotypes covered by PCV13, especially 19A. In Dutch adults between 50

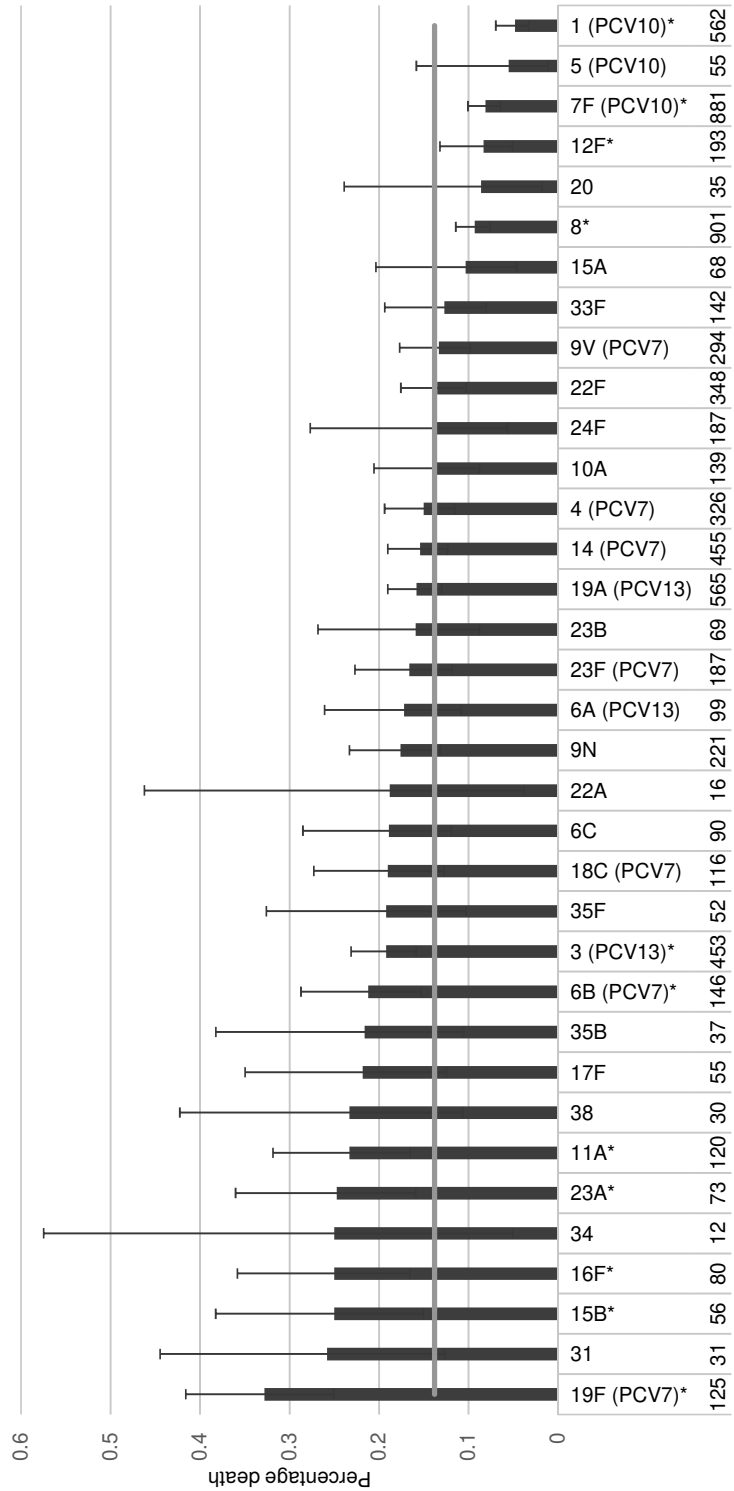

Figure 4. Serotype specific propensity for death. Serotype specific case-fatality with 95% Confidence intervals (Agresti–Coull method) in all patients. Included are all data from June 2004 to May 2018. All (additional) serotypes included in the 7, 10 and 13-valent pneumococcal conjugate vaccines are shown. From non-vaccine serotypes all serotypes with at least 5 cases and 2 deaths are shown. The horizontal line represents overall case-fatality (14%). * indicates a statistically significant difference (p-value <0.05) between serotype specific case-fatality versus all other serotypes tested with Fisher's exact test. The numbers represent the total number of isolates per serotype. Abbreviations: PCV, pneumococcal conjugate vaccine.

and 64 years, the reduction in PCV10-type IPD incidence was countered by non-vaccine-type IPD after the switch to PCV10. Similarly, in England and Wales the initial reduction in IPD incidence in 45-64 year olds after the switch to PCV13 was eroded over time due to the increase of non-vaccine serotypes. In the elderly of ≥65 years, both countries observed a slight increase of around 5% in IPD, mainly due to a rise in non-PCV13 serotypes 8, 9N and 12F.

With respect to the serotypes included in PCV13 but not in PCV10, in the Netherlands 19A IPD increased slightly after the switch to PCV10, and is one of the main serotypes causing IPD across all age groups, whereas in England and Wales an initial decline in 19A IPD was observed after PCV13 introduction. IPD due to serotype 6A had already declined substantially in both countries after PCV7 introduction. Serotype 3 IPD increased in both the Netherlands and England and Wales, likely due to secular trends, despite the fact that serotype 3 is included in PCV13. Although similar impact on overall IPD has been observed after the switch to PCV10 in the Netherlands, and to PCV13 in England and Wales (compared with their respective periods before *PCV7* introduction), England and Wales still have a net benefit of around 40% across all age groups, compared to a statistically non-significant 6% in the Netherlands. This seems to be mainly due to the much larger impact of childhood pneumococcal vaccination in 45-64 year olds in England and Wales, and the greater contribution of this age group to the overall IPD burden in England and Wales, compared with the Netherlands.

A recent meta-analysis including European data assessed the impact of childhood vaccination on IPD incidence in people aged 65 years and older, comparing 2015 data with 2009 data. This study included six sites with universal PCV13 vaccination for infants, including England and Wales, and four sites with universal PCV10 (+/- PCV13) vaccination, including the Netherlands.[16] The study showed a decline in all-type IPD incidence of 14% (-4 to 30%) in PCV13 sites and 1% (-21 to 18%) in PCV10 sites. A major difference was seen between the PCV13 sites and PCV10 sites with respect to serotype 19A incidence, with decreasing trends in PCV13 sites and increasing trends in PCV10 sites. Similar to what was observed in England and Wales, the decrease of serotype 19A incidence in the PCV13 sites halted in 2015.

Comparison of surveillance data between countries remains difficult because of differences in health care practices, pre-vaccine epidemiology and vaccination policies. Interestingly, in Sweden, different counties switched either to PCV10 or PCV13 after PCV7 and therefore the impact of both vaccines could be compared within the country. The study found, as expected, a differential impact of PCV10 and PCV13 on serotype 19A IPD, with an increase of 19A IPD in PCV10 counties. However, the study did not find a difference in the overall impact on IPD incidence and rate of non-vaccine serotype IPD replacement between PCV10 and PCV13.[17] Recent data from Belgium also showed the differential impact of PCV10 and PCV13 on serotype 19A IPD, as an increase in 19A IPD was observed in children ≤2 years of age following the switch from PCV13 to PCV10 in 2015-2016.[18] Longer-term data

on 19A and overall IPD from Belgium, and other countries that switched from PCV13 to PCV10, are needed to confirm this.

The decrease in CFR after PCV7 introduction from 16% to 12% in the Netherlands did not continue after the introduction of PCV10, due to the higher burden of IPD from serotypes associated with higher fatality such as 19A, 9N and 3, and a reduction of PCV10 serotypes 1 and 7F which cause less mortality. In England and Wales, the CFR in children under 5 years was 5.1% in the six years after PCV13 introduction (2010-2016)[19] vs. 4.4% after PCV7 introduction (2006-2010).[20] At the same time, the prevalence of comorbidity among children with IPD increased, indicating that a shift in patient characteristics might influence the CFR. In the Netherlands, we observed a rise in both age and the proportion of cases with non-immunocompromising comorbidities, mainly in the elderly, but adjustment for these variables did not change the association between time period and CFR. To the best of our knowledge, no other reports on changes in CFR in adults in relation to PCV10 or PCV13 introduction have been published.

As stated by the recently published WHO position paper[21], there is at present insufficient evidence of a difference in the net impact of the two vaccines on overall IPD burden. The benefit of vaccination for the elderly with PCV13 depends on the serotype distribution of IPD in this age group, but in many countries, PCV13 likely has a limited effect, because of the large indirect effects of infant PCV10 and PCV13 vaccination on vaccine-type IPD in the elderly. On the other hand, the potential additional benefit of PPV23 vaccination has increased, because of the expanded PPV23 coverage of emerging serotypes like 8, 9N and 12F. A recent cost-effectiveness analysis in the Netherlands showed that PPV23 was superior to PCV13 in adults 60 years and older in all investigated scenarios, despite the relatively moderate effectiveness and the limited duration of protection of PPV23.[22] Routine vaccination with PPV23 every five years, among older adults 60-75 years has been advised by the Dutch Health Council as of February 2018[23], and is planned for implementation at the end of 2019.

Higher-valent vaccines, including 15-valent and 20-valent conjugate vaccines, are currently being evaluated for safety and immunogenicity in phase 3 trials.[24,25] Another current issue is the development of whole cell and recombinant protein vaccines, which could offer serotype-independent protection, although these vaccines are still years from clinical implementation.

The main strength of this study is that we obtained data from a sentinel surveillance system that has not changed its methods over time. This has allowed us to collect reliable clinical and serotype data across vaccination periods. Even though the CAPiTA study, in which Dutch persons of ≥65 years were vaccinated with PCV13, coincided with our post-PCV7 period, its influence on our results is expected to be negligible: despite its large sample size of 85,000 participants, this only accounts for 3% of all persons of ≥65 years in the Netherlands.[26]

Our study had an observational design. Therefore, factors other than vaccination might have affected our results. Even though we adjusted for age and/or comorbidities when

applicable, unknown confounders might still have been present, so no causal relationship between PCV and observed outcomes can be established. Despite our strong sentinel surveillance system with no change in coverage over time, the system only covers 25% of the Dutch population which may affect the generalizability of the results. Also, shifts in long-term secular pneumococcal serotype trends may have influenced the results.

Conclusions

Although we still observe a net benefit of introduction of PCV on IPD incidence in children and elderly, and on the CFR of IPD in the Netherlands, IPD incidence could rebound in the near future as vaccines have nearly reached their maximum effect, serotype replacement is ongoing, and the population is aging. New vaccines, either with broader serotype coverage or focused on the pneumococcus as a whole, might be needed to reduce the burden of IPD in the future. Ongoing monitoring of IPD serotype distribution and clinical outcomes is warranted, to evaluate existing vaccination policies and to inform new vaccination policies.

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Supplementary Table S1. Incidence (per 100,000) and relative risks of IPD incidence comparing June 2016-May 2018 with the two years before PCV introduction (June 2004 to May 2006) and the two years before PCV10 introduction (June 2009 to May 2011) by age group and serotype group.

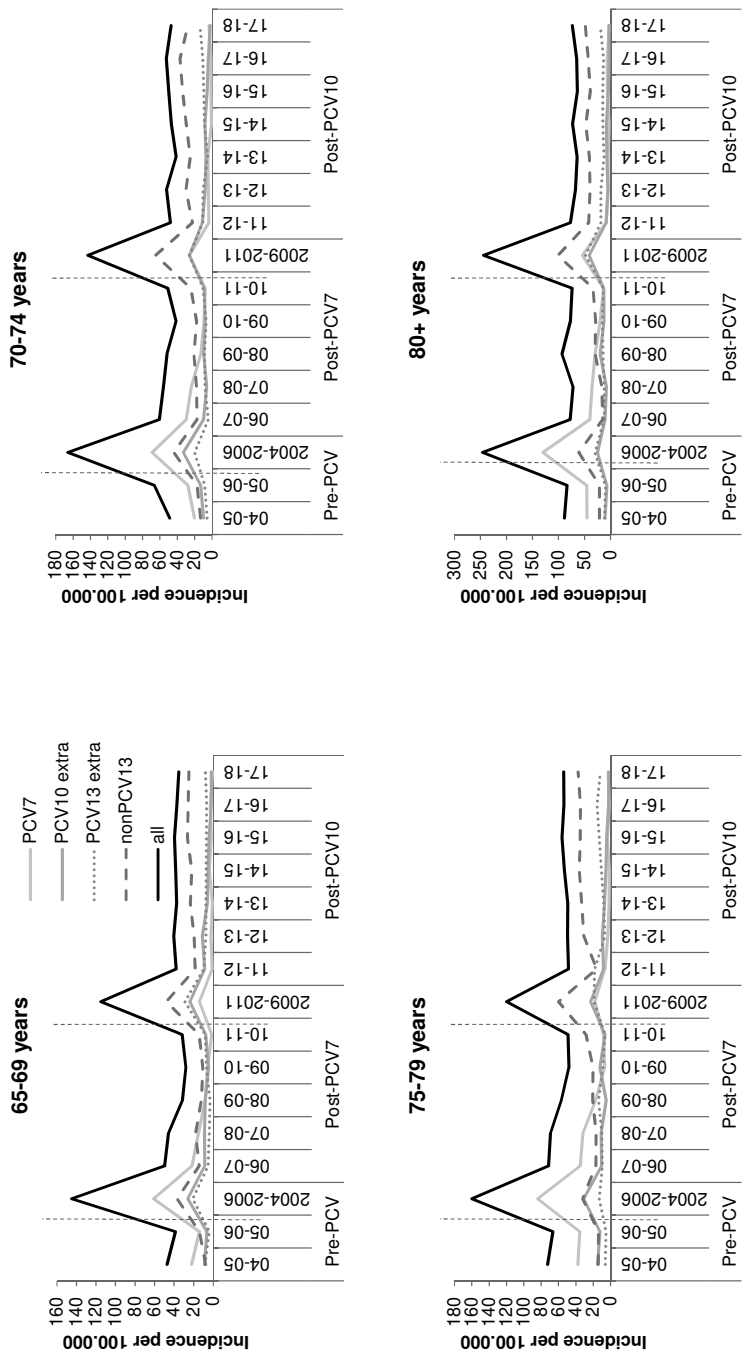
		2004-2006		2009-2011		2016-2018		2016-2018 vs. 2004-2006		2016-2018 vs. 2009-2011	
Age	Serotype group	Incidence (/100,000)	N	Incidence (/100,000)	N	Incidence (/100,000)	N	RR	95% CI	RR	95% CI
65-70 years	PCV7	18.0	61	3.6	14	1.8	9	0.10	0.05-0.20	0.49	0.21-1.12
	PCV10-7	7.7	26	6.2	24	2.0	10	0.26	0.12-0.53	0.31	0.15-0.66
	PCV13-10	6.2	21	7.5	29	7.2	37	1.17	0.68-2.00	0.96	0.59-1.56
	non-PCV13	10.9	37	12.5	48	25.3	129	2.31	1.60-3.33	2.03	1.46-2.82
	total	42.8	145	29.8	115	36.2	185	0.85	0.68-1.05	1.21	0.96-1.53
70-75 years	PCV7	23.9	69	8.4	26	2.3	9	0.09	0.05-0.19	0.27	0.13-0.57
	PCV10-7	11.4	33	8.4	26	3.0	12	0.26	0.14-0.51	0.36	0.18-0.71
	PCV13-10	6.9	20	8.4	26	12.3	49	1.77	1.05-2.98	1.46	0.91-2.35
	non-PCV13	15.3	44	21.0	65	32.1	128	2.10	1.49-2.96	1.52	1.13-2.05
	total	57.5	166	46.3	143	49.6	198	0.86	0.70-1.06	1.07	0.86-1.33
75-80 years	PCV7	36.5	84	7.3	18	2.1	6	0.06	0.03-0.13	0.29	0.11-0.73
	PCV10-7	13.5	31	9.3	23	2.1	6	0.16	0.07-0.37	0.23	0.09-0.55
	PCV13-10	5.6	13	7.7	19	13.6	39	2.41	1.29-4.52	1.78	1.03-3.07
	non-PCV13	13.9	32	24.3	60	36.0	103	2.59	1.74-3.85	1.48	1.08-2.04
	total	69.5	160	48.5	120	53.8	154	0.78	0.62-0.97	1.11	0.87-1.41
≥80 years	PCV7	45.5	130	16.7	54	4.2	16	0.09	0.06-0.16	0.25	0.14-0.44
	PCV10-7	8.7	25	12.7	41	2.9	11	0.33	0.16-0.67	0.23	0.12-0.44
	PCV13-10	10.5	30	14.8	48	16.5	63	1.58	1.02-2.43	1.11	0.77-1.62
	non-PCV13	21.3	61	31.2	101	45.6	174	2.14	1.6-2.86	1.46	1.14-1.87
	total	86.0	246	75.4	244	69.2	264	0.81	0.68-0.96	0.92	0.77-1.09

Abbreviations: CI, confidence interval; PCV, pneumococcal conjugate vaccine, N, number of cases.

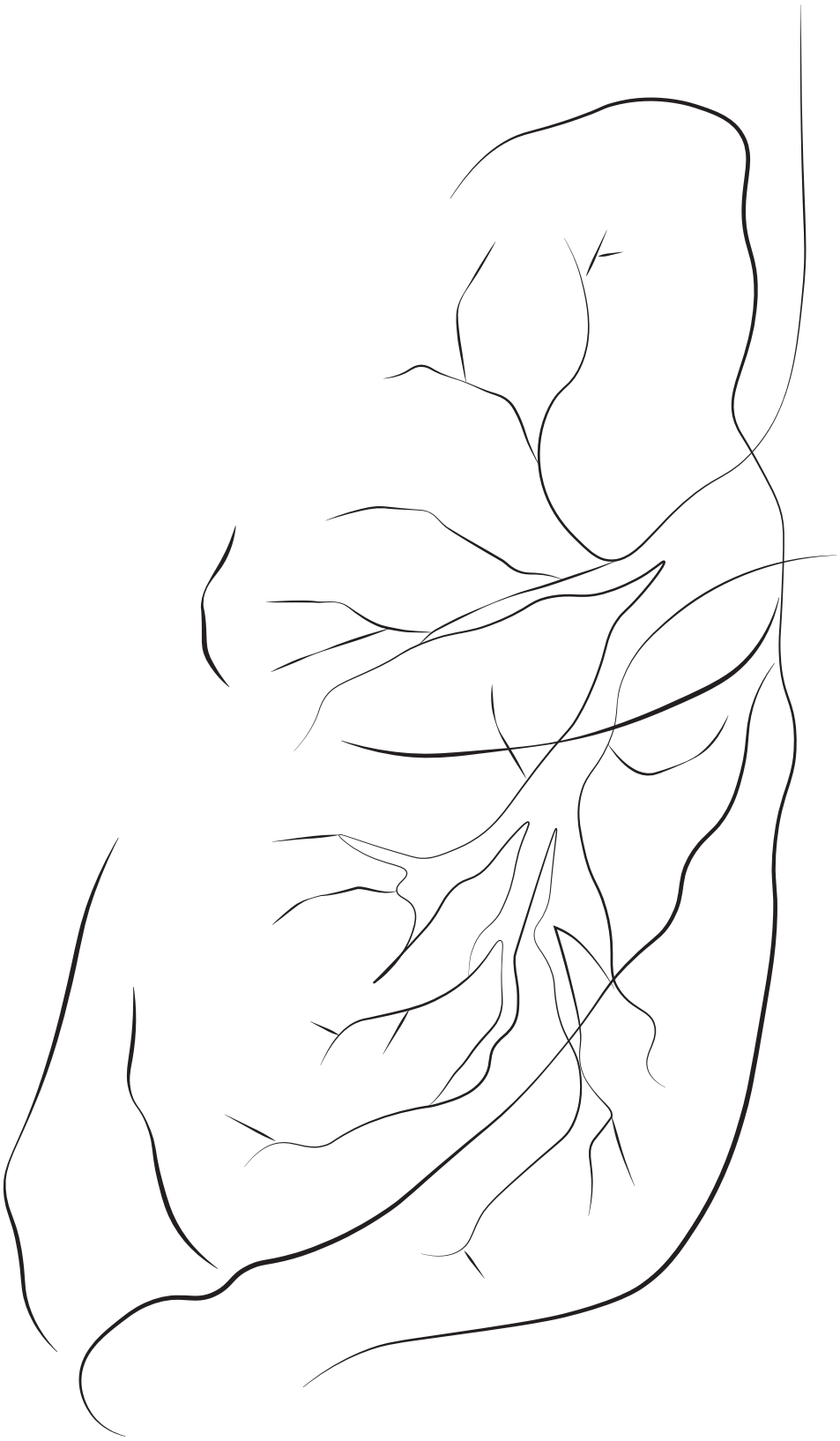
Supplementary Table S2. Incidence (per 100,000) and relative risks of clinical syndrome incidence comparing pre-PCV7, post-PCV7 and post-PCV10 period.

		Pre-PCV (2004-2006)		Post-PCV7 (2008-2011)		Post-PCV10 (2013-2016)		Post-PCV10 vs pre-PCV		Post-PCV10 vs post-PCV7	
Age	Syndrome	Incidence (/100,000)	N	Incidence (/100,000)	N	Incidence (/100,000)	N	RR	95% CI	RR	95% CI
<5 years	Meningitis	6.73	34	3.58	25	2.68	18	0.40	0.22-0.70	0.75	0.41-1.37
	Pneumonia only	4.38	22	2.15	15	0.45	3	0.10	0.03-0.35	0.21	0.06-0.73
	Pneumonia+empyema	0.00	0	0.14	1	0.29	2	NA	NA	2.05	0.19-22.6
	Bact. without focus	3.76	19	1.43	10	1.49	10	0.40	0.18-0.85	1.04	0.43-2.50
	Bact. other focus	3.57	18	1.00	7	0.60	4	0.17	0.06-0.50	0.60	0.18-2.05
5-17 years	Meningitis	0.46	6	0.31	6	0.37	7	0.79	0.27-2.35	1.19	0.40-3.54
	Pneumonia only	0.70	9	0.87	17	0.63	12	0.90	0.38-2.15	0.72	0.34-1.51
	Pneumonia+empyema	0.00	0	0.10	2	0.05	1	NA	NA	0.51	0.05-5.62
	Bact. without focus	0.31	4	0.10	2	0.05	1	0.17	0.02-1.51	0.51	0.05-5.62
	Bact. other focus	0.31	4	0.26	5	0.21	4	0.68	0.17-2.71	0.81	0.22-3.03
18-49 years	Meningitis	0.49	18	0.76	41	0.46	24	0.93	0.51-1.72	0.60	0.36-1.00
	Pneumonia only	3.44	127	3.43	185	3.00	158	0.87	0.69-1.10	0.88	0.71-1.08
	Pneumonia+empyema	0.33	12	0.28	15	0.23	12	0.70	0.31-1.56	0.82	0.38-1.75
	Bact. without focus	0.35	13	0.28	15	0.46	24	1.29	0.66-2.54	1.64	0.86-3.13
	Bact. other focus	0.30	11	0.35	19	0.21	11	0.70	0.30-1.61	0.59	0.28-1.24
50-64 years	Meningitis	2.76	42	1.98	49	1.82	47	0.66	0.43-1.00	0.92	0.62-1.37
	Pneumonia only	10.90	166	12.05	299	11.64	301	1.07	0.88-1.29	0.97	0.82-1.13
	Pneumonia+empyema	0.52	8	1.13	28	0.93	24	1.77	0.79-3.93	0.82	0.48-1.42
	Bact. without focus	1.25	19	1.45	36	1.00	26	0.80	0.44-1.45	0.69	0.42-1.14
	Bact. other focus	0.91	14	0.97	24	1.24	32	1.35	0.72-2.53	1.28	0.75-2.17
≥65 years	Meningitis	3.24	37	2.87	54	3.03	67	0.93	0.62-1.39	1.06	0.74-1.51
	Pneumonia only	47.58	544	37.61	704	36.04	799	0.76	0.68-0.84	0.96	0.87-1.06
	Pneumonia+empyema	0.79	9	2.61	49	1.98	44	2.50	1.22-5.13	0.76	0.50-1.14
	Bact. without focus	4.45	51	3.96	74	4.30	95	0.96	0.69-1.36	1.09	0.80-1.47
	Bact. other focus	1.75	20	2.41	45	2.25	50	1.29	0.77-2.17	0.94	0.63-1.40

Abbreviations: Bact, bacteraemia; NA, not applicable; PCV, pneumococcal conjugate vaccine; RR, relative risk; N, number of cases.



Supplementary Figure S1. IPD incidence by age groups ≥65 years and serotype group from June 2004 to May 2018.



CHAPTER 5

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.

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 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.

Methods

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.

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 (>10x10^9 cells per L), more than 10% of bands in leucocyte differentiation or leucopenia (<4x10^9 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.

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 to 3 and 7 to 100 after hospital admission).

Conventional methods

Blood cultures were obtained (drawn before the start of in-hospital antibiotic treatment) at time of admission. Sputum specimens (if applicable) were Gram 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®).

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 100x 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 analysed 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). A positive immune response was defined as at least a 2-fold increase in serotype-specific antibodies between the early and late serum sample (with an end concentration >0.35 µg/ml). The fold increase in antibody concentration against a given single pneumococcal serotype had to be at least two times greater than the fold increase against any other serotype (with exception of a ≥2-fold increase for serotypes within the same serogroup (e.g. 19A/19F), for which the serotype with the highest fold increase was regarded as infecting serotype). Only patients in whom no causative pathogen was detected using conventional methods but did have a positive serotype-specific antibody response, were diagnosed as pneumococcal CAP so mixed infections were not taken into account.

Final microbial aetiology

The information from the conventional work-up plus the serotype-specific antibody measurement (only taking into account the 14 serotypes common to the two multiplex immunoassay panels applied) was used to categorise the patients as 1) pneumococcal CAP, 2) CAP due to an atypical pathogen, 3) CAP due to another identified pathogen or 4) CAP with no causative agent detected by methods used herein. In case multiple

pathogens were detected by conventional methods the main causative agents was determined by a consensus panel, consisting of two medical microbiologists who reviewed all microbiological results. Subsequently, for pneumococcal CAP patients the infecting serotype was determined based on Quellung for isolates cultured from blood and, in a subselection of patients, from sputum (using specific antisera from Statens Serum Institute SSI, Denmark) and based on the serotype-specific antibody measurement in case blood culture was negative (based on the 14-plex assay common for all patients). Within patients with pneumococcal CAP and an infecting serotype identified, we discriminated PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), PCV10-extra (present in PCV10 but not PCV7; 1, 5, 7F), non-PCV10 serotype disease (all serotypes not present in PCV10) and non-PCV7 (all serotypes not present in PCV7).

For pneumococcal CAP patients with known serotype, pneumococcal CAP was divided further in bacteraemic (*S. pneumoniae* cultured from blood) and non-bacteraemic pneumococcal pneumonia (defined as a positive serotype-specific antibody response against a single serotype and/ or *S. pneumoniae* cultured from sputum in the absence of a positive blood culture).

Statistical analyses

Proportions of the causative pathogens in CAP were calculated for comparisons of pre-PCV7 (October 2004 to August 2006, cohort 1), PCV7 (and pre-PCV10, November 2007 to June 2009, cohort 2), and PCV10 (October 2012 to October 2016, cohort 3) periods.

Likewise, distributions of pneumococcal serotypes (according to the PCV7, PCV10-extra, non-PCV10 and non-PCV7 group) were compared for the pre-PCV7, PCV7, and PCV10 period within all CAP patients, within all pneumococcal CAP patients and within all pneumococcal CAP patients with serotype known. Last, proportions of serotypes within bacteraemic and non-bacteraemic pneumococcal pneumonia were compared.

Differences in proportions were tested with X2 or Fisher exact test, where appropriate. Relative risks (RR) and 95% confidence intervals (CI) were calculated using 2x2 tables (z-distribution).[20] Means were compared using Student's t-test. A *p*-value of <0.05 was considered to represent a statistically significant difference. Microsoft Excel and SPSS software (version 22.0) were used for statistical analyses. The data of the 11 additional serotypes from the 25-plex panel were used as a sensitivity analysis for identification of pneumococci in the PCV10 period (because the 14-plex panel does not cover all non-vaccine serotypes which potentially increased due to replacement disease).

Results

Patient characteristics

In total, 805 patients were included in the analyses (201 pre-PCV7, 304 in the PCV7 and 300 in the PCV10 period). Table 1 shows the patient characteristics of the three cohorts. Mean age of the patients varied between 63 and 64 years, and the proportion of patients categorised as PSI class 4-5 ranged from 41 to 47%.

Shifts in pathogens causing CAP

Comparing the PCV7 with pre-PCV7 period, the proportion of patients with pneumococcal pneumonia decreased from 37 to 27% (*p*=0.01) (Fig. 1, Table 2). This coincided with a significant increase in proportion of patients with CAP due to atypical pathogens from 7 to 19%, primarily Q-fever and an increase in patients diagnosed with *Chlamydophila spp*.

In the PCV10 period, the proportion of atypical pathogens decreased back to 11% (similar to pre-PCV7). This, however, did not result in an increase in proportion of pneumococcal pneumonia, which remained significantly lower (26%) compared to pre-PCV7 (*p*=0.01). The proportion of patients with no identified pathogen increased from 44 to 53% comparing the PCV7 and PCV10 periods. For other pathogens, including *Legionella spp., Mycoplasma pneumoniae, S. aureus*, *H. influenzae*, influenza and other respiratory viruses causing CAP, no shifts were observed for their relative contribution over time (Supplementary Table 1). The data from the 11 additional serotypes tested in the 25-plex assay, resulted in 5 additional cases of pneumococcal pneumonia in the PCV10 period. Including these cases in the analysis did not impact the findings (an overall proportion of patients with pneumococcal pneumonia in the PCV10 period (27%) remained significantly lower compared with the pre-PCV7 period (*p*=0.03)).

Shifts in distribution of pneumococcal serotypes

Shifts in pneumococcal serotype distribution are shown in Table 3. The serotype distribution of pneumococcal CAP changed after PCV7 implementation. Overall, within all CAP patients, the proportion of PCV7-serotypes decreased from 12 to 4% (RR 0.34 95% CI: 0.18-0.66) comparing the pre-PCV7 to the PCV7 period. For non-PCV10 and non-PCV7 serotype disease, the overall proportions remained similar (from 7 to 9% and 12 to 13%, respectively).

Within all pneumococcal CAP patients with serotype known (pre-PCV7 n=50, PCV7 n=51 and PCV10 n=44), after PCV7 introduction PCV7-serotypes decreased from 50 to 25% (RR 0.51 95% CI: 0.3-0.88) and-non-PCV10-type serotypes increased from 28 to 51% (RR 1.82 95% CI: 1.08-3.06) whereas PCV10-extra type disease remained stable (from 22 to 24% RR 1.07 95% CI: 0.52-2.2). These relative changes were quite similar for both bacteraemic and non-bacteraemic patients (Fig. 2).

In the PCV10 period, the overall decrease in PCV7-type disease within all CAP patients continued with an additional absolute reduction of 3% in proportion (from 4 to 1%; p<0.01), comparing the PCV7 and PCV10 periods. Within pneumococcal CAP patients with known serotype, the relative decrease in the proportion of PCV7 type disease was from 25 to 5% (RR 0.18 95% CI: 0.04-0.75) whereas non-PCV10 type disease increased from 51 to 77% (RR 1.52 95% CI: 1.11-2.07). PCV10-extra type disease did not decrease significantly (RR 0.77 95% CI: 0.35-1.72). No decrease in potentially cross-reactive PCV10-related serotype 19A was observed (from 1 to 3 to 6 cases in the pre-PCV7, PCV7 and PCV10 period, respectively). The most prevalent serotypes in the PCV10 period in descending order were 3, 8, 19A, 7F, 9N and 12F.

Table 1. Patient characteristics from the three cohorts.

	pre-PCV7 2004-2006 (n=201)	PCV7 2007-2009 (n=304)	PCV10 2012-2016 (n=300)
Male sex	124 (62)	171 (56)	178 (59)
Age (years)	64 (17)	63.7 (18)	64.4 (16)
Comorbidities			
Chronic renal failure	10 (5)	30 (10)	41 (14)
Diabetes mellitus	35 (17)	43 (14)	75 (25)
Liver disease	0 (0)	2 (1)	3 (1)
Neoplastic disease	26 (13)	19 (6)	12 (4)
Chronic heart failure	19 (10)	48 (16)	26 (9)
COPD	64 (32)	34 (11)	60 (20)
PSI class			
Classes 1-3	117 (58)	161 (53)	177 (59)
Classes 4-5	84 (42)	143 (47)	123 (41)
Days ill before admission	5.2 (4.9)	5.7 (5.3)	5.4 (5.4)
Pretreated with antibiotics at home	48 (24)	82 (27)	83 (28)

Data are presented as number (%) or mean (SD). Abbreviations: PSI, pneumonia severity index; COPD, chronic obstructive pulmonary disease.

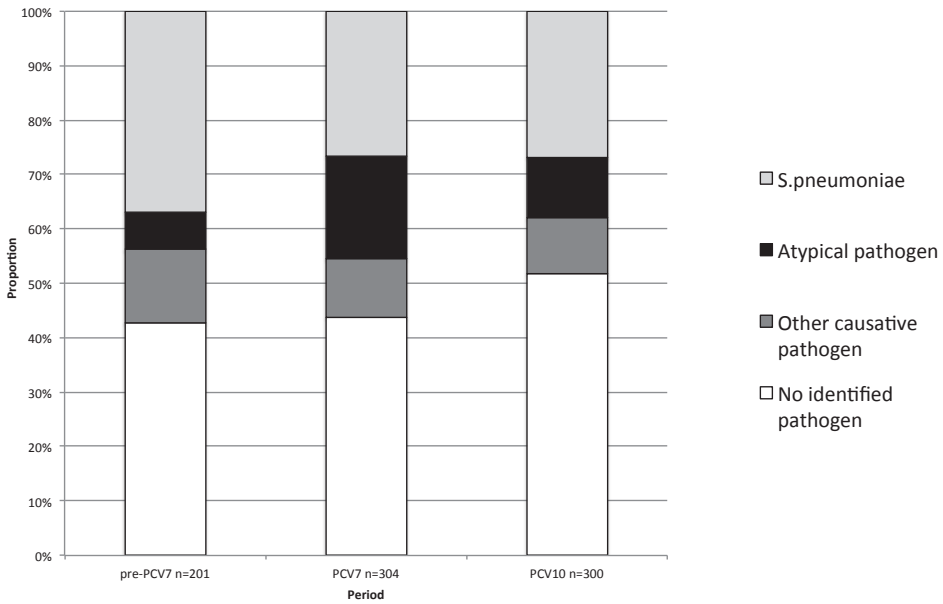

Figure 1. Proportion of causative pathogens in CAP patients in the pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period.

Table 2. Proportions of causative pathogens in CAP patients pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period.

	Pre-PCV7 2004-2006 (n=201) No. (%)	PCV7 2007-2009 (n=304) No. (%)	PCV10 2012-2016 (n=300) No.(%)	PCV7 vs pre-PCV7 RR 95% CI [a]	*p*-value	PCV10 vs PCV7 RR 95% CI [b]	*p*-value	PCV10 vs pre-PCV7 RR 95% CI [c]	*p*-value
S. pneumoniae	74 (37)	81 (27)	77 (26)	0.72 (0.56-0.94)	0.02	0.96 (0.74-1.26)	0.79	0.70 (0.54-0.91)	0.01
Atypical pathogens	14 (7)	57 (19)	34 (11)	2.69 (1.54-4.70)	<0.01	0.60 (0.41-0.9)	0.01	1.63 (0.90-2.95)	0.10
Other causative pathogen	27 (13)	33 (11)	31 (10)	0.81 (0.50-1.30)	0.38	0.95 (0.60-1.51)	0.84	0.77 (0.47-1.25)	0.28
No identified pathogen	86 (43)	133 (44)	158 (53)	1.02 (0.83-1.25)	0.83	1.20 (1.02-1.42)	0.03	1.23 (1.02-1.49)	0.03

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases; RR: Relative risk; 95% CI: 95% Confidence interval. We assessed the proportion of causative pathogens in CAP patients comparing the post-PCV to pre-PCV periods. Differences in proportions were tested with X2 test and relative risks and 95% confidence intervals were calculated.

[a] RR comparing proportion of the PCV7 (and pre-PCV10) to the pre-PCV7 period.

[b] RR comparing proportion of the PCV10 to the PCV7 period.

[c] RR comparing proportion of the PCV10 to the pre-PCV7 period.

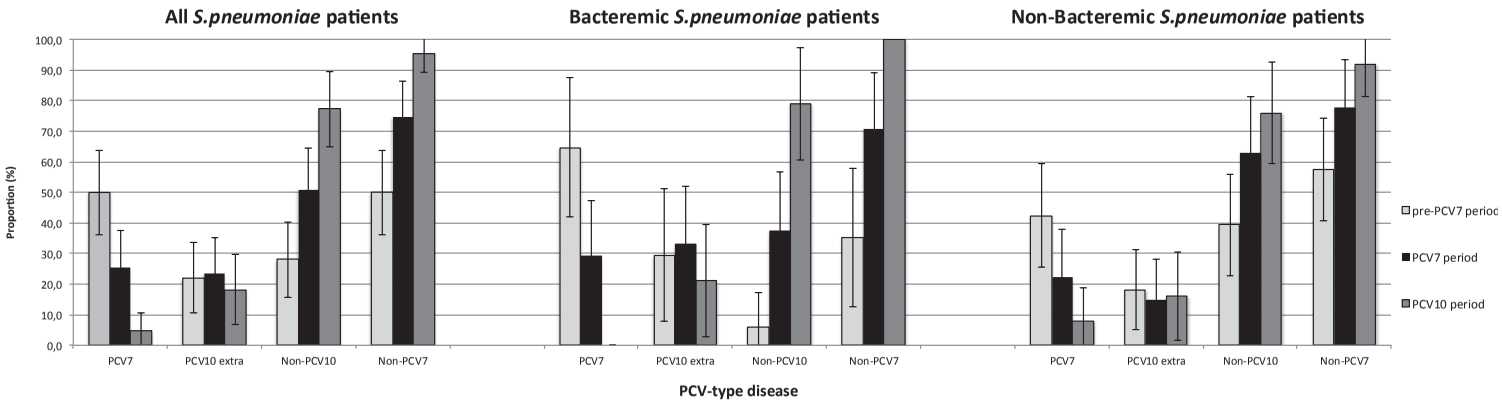

Figure 2. Serotype distribution in pneumococcal pneumonia patients with serotype known. Proportions in the pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period. Bars are divided in serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7); serotypes 1, 5, 7F (PCV10 extra); and all serotypes not included in previous groups (non-PCV10 and non-PCV7 and, respectively) and error bars represent the 95% confidence intervals.

Table 3. Serotype distribution in overall pneumococcal CAP (with serotype available) and bacteraemic vs non-bacteraemic pneumococcal CAP.

	Pre-PCV7 2004-2006 No. (%)	PCV7 2007-2009 No. (%)	PCV10 2012-2016 No. (%)	PCV7 vs pre-PCV7 RR 95% CI [a]	PCV10 vs PCV7 RR 95% CI [b]	PCV10 vs pre-PCV7 RR 95% CI [c]
Within all CAP patients	201 (100)	304 (100)	300 (100)			
PCV7	25 (12)	13 (4)	2 (1)	0.34 (0.18-0.66)	0.16 (0.04-0.68)	0.05 (0.01-0.22)
PCV10 extra	11 (5)	12 (4)	8 (3)	0.72 (0.32-1.6)	0.68 (0.28-1.63)	0.49 (0.2-1.19)
Non-PCV10	14 (7)	26 (9)	34 (11)	1.23 (0.66-2.29)	1.33 (0.82-2.15)	1.63 (0.9-2.95)
Non-PCV7	25 (12)	38 (13)	42 (14)	1.01 (0.63-1.61)	1.12 (0.74-1.69)	1.13 (0.71-1.79)
Within all pneumococcal CAP patients	74 (100)	81 (100)	77 (100)			
PCV7	25 (34)	13 (16)	2 (3)	0.48 (0.26-0.86)	0.16 (0.04-0.69)	0.08 (0.02-0.31)
PCV10 extra	11 (15)	12 (15)	8 (10)	1 (0.47-2.12)	0.7 (0.3-1.62)	0.7 (0.3-1.64)
Non-PCV10	14 (19)	26 (32)	34 (44)	1.7 (0.96-2.99)	1.38 (0.92-2.06)	2.33 (1.37-3.98)
Non-PCV7	25 (34)	38 (47)	42 (55)	1.39 (0.94-2.06)	1.16 (0.85-1.58)	1.61 (1.11-2.36)
Within all pneumococcal CAP patients with serotype known	50 (100)	51 (100)	44 (100)			
PCV7	25 (50)	13 (25)	2 (5)	0.51 (0.3-0.88)	0.18 (0.04-0.75)	0.09 (0.02-0.36)
PCV10 extra	11 (22)	12 (24)	8 (18)	1.07 (0.52-2.2)	0.77 (0.35-1.72)	0.83 (0.37-1.87)
Non-PCV10	14 (28)	26 (51)	34 (77)	1.82 (1.08-3.06)	1.52 (1.11-2.07)	2.76 (1.72-4.43)
Non-PCV7	25 (50)	38 (75)	42 (95)	1.49 (1.08-2.05)	1.28 (1.08-1.52)	1.91 (1.44-2.54)
Within bacteraemic *S. pneumoniae* with serotype known	17 (100)	24 (100)	19 (100)			
PCV7	11 (65)	7 (29)	0 (0)	0.45 (0.22-0.92)	NA	NA
PCV10 extra	5 (29)	8 (33)	4 (21)	1.13 (0.45-2.87)	0.63 (0.22-1.78)	0.72 (0.23-2.24)
Non-PCV10	1 (6)	9 (38)	15 (79)	6.38 (0.89-45.73)	2.11 (1.19-3.71)	13.42 (1.98-91.14)
Non-PCV7	6 (35)	17 (71)	19 (100)	2.01 (1-4.01)	1.41 (1.09-1.82)	2.83 (1.49-5.39)

Table 3. (continued)

	Pre-PCV7 2004-2006 No. (%)	PCV7 2007-2009 No. (%)	PCV10 2012-2016 No. (%)	PCV7 vs pre-PCV7 RR 95% CI [a]	PCV10 vs PCV7 RR 95% CI [b]	PCV10 vs pre-PCV7 RR 95% CI [c]
Within non-bacteraemic *S. pneumoniae* with serotype known	33 (100)	27 (100)	25 (100)			
PCV7	14 (42)	6 (22)	2 (8)	0.52 (0.23-1.18)	0.36 (0.08-1.62)	0.19 (0.05-0.76)
PCV10 extra	6 (18)	4 (15)	4 (16)	0.81 (0.26-2.6)	1.08 (0.3-3.86)	0.88 (0.28-2.79)
Non-PCV10	13 (39)	17 (63)	19 (76)	1.6 (0.96-2.67)	1.21 (0.84-1.74)	1.93 (1.2-3.11)
Non-PCV7	19 (58)	21 (78)	23 (92)	1.35 (0.95-1.93)	1.18 (0.94-1.49)	1.6 (1.17-2.19)

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases; RR: Relative risk; 95% CI: 95% Confidence interval. We assessed the proportion of pneumococcal serotypes (according to the PCV7, PCV10-extra, non-PCV10 and non-PCV7 group) in (pneumococcal) CAP patients comparing the post-PCV to pre-PCV periods. Differences in proportions were tested with X2 test and relative risks and 95% confidence intervals were calculated.

[a] RR comparing proportion of the PCV7 (and pre-PCV10) to the pre-PCV7 period.

[b] RR comparing proportion of the PCV10 to the PCV7 period.

[c] RR comparing proportion of the PCV10 to the pre-PCV7 period.

Discussion

In this study in over 800 hospitalised adults with CAP from 2004 to 2016, we observed a significant reduction in proportion CAP due to *S. pneumoniae* following introduction of PCV in children in The Netherlands. Furthermore, within the pneumococcal pneumonia patients, we observed a continuing decrease in the proportion of cases due to PCV7 serotypes and an increase in non-PCV10 serotypes. These findings confirm that PCV introduction in infants impact the microbial aetiology of adult CAP.

Our study compared pre- and post-vaccine periods for shifts in overall microbial aetiology of adults hospitalised with CAP including pneumococcal serotype-specific antibody detection in serum. In the PCV7 and PCV10 periods, the proportion of patients with pneumococcal CAP clearly decreased coinciding with an increase in proportion of CAP patients without a causative pathogen identified in the PCV10 period. No increase in proportion of CAP due to *H. influenzae* or *S. aureus* was observed. The latter suggests that the potential increases in nasopharyngeal colonisation in adults, as has been observed in parents of vaccinated children[15, 21], does not result in more disease caused by these pathogens in adults. The higher proportion of patients with Q-fever observed in the PCV7 period is likely to be linked to an epidemic in the Netherlands, which ended in 2010 [21]. The transient increase in number of *Chlamydophila* pneumonia cases is possibly related to a to a cluster of *C. psittaci* patients who visited a bird fair (November 2007).[22] The frequencies of pathogens like *Legionella spp., Mycoplasma pneumoniae, S. aureus* and respiratory viruses causing CAP did not change over time. Since serological tests to detect atypical pathogens and respiratory viruses (and viral culturing) were replaced by PCRs, which generally have a higher sensitivity[23, 24], our comparisons are not likely to be biased by decreased detection chances over time. Likewise, the detection probability for *S. pneumonia* did not decrease over time whereas a constant rate of (conventional) diagnostics able to detect *S. pneumoniae was* applied in the three periods (Supplementary Table 2). Furthermore, our sensitivity analyses resulted in only 5 additional pneumococcal pneumonia cases detected through the additional 11 serotypes that were absent in the 14-plex assay applied to the samples of the first two trials.

The most likely explanation for the significant reduction in proportion pneumococcal CAP are herd protection effects of PCVs resulting in a decline in vaccine-serotypes and replacement by non-vaccine-serotypes with an overall lower (invasive) disease potential. This has been observed in many previous studies.[10, 25-28] Regarding the serotypes, the present study showed a decline in the relative contribution of vaccine serotypes after PCV7 and after PCV10 introduction. PCV7 type disease decreased both in bacteraemic patients and non-bacteraemic patients. For PCV10-extra serotype disease no decline was observed in non-bacteraemic pneumococcal CAP patients. This finding aligns with Werkhoven et al. who also observed a reduction in PCV7-type non-bacteraemic pneumonia in the Netherlands, but no impact on PCV10 serotypes.[13] The latter study had an observation period of only until ~2.5 years after PCV10 introduction, which is too short to detect possible herd effects (and

take around 2 years to establish for IPD without catch-up campaigns[29, 30]). Our study period, with sampling up to 5 years after introduction of PCV10, should have been long enough to observe potential herd effects on non-bacteraemic pneumococcal pneumonia.

A strength of this study is that we used data from three prospective studies in adults requiring hospitalisation for CAP that was diagnosed by clear and consistent criteria. In all patients, the standard microbial diagnostic workup included an extensive effort to detect the causative pathogen including a reliable serotype-specific immunoassay to diagnose pneumococcal CAP (in only 1 out of 27 cases with serotype identified by both Quelling and serology, there was a discordant result i.e. Quellung of blood isolate; 12F, with a positive (12-fold) immune response against serotype 8). MIA identified 13, 16 and 22 additional pneumococcal CAP cases in the pre-PCV7, PCV7 and PCV10 period, respectively, as compared with the conventional methods (blood cultures, sputum culture, urinary antigen testing). The sensitivity of MIA to detect pneumococcal pneumonia (with conventional methods as gold standard) was 42% (95% CI: 0.34-0.50). Furthermore, our study period extended to over 10 years after PCV7 introduction and over 5 years after PCV10 introduction.

Our study is limited by its observational design. Non-vaccine related factors might have contributed to the shifts in aetiology of CAP, as was illustrated by the Q-fever epidemic. Also the study excluded patients with congenital or acquired immunodeficiency, haematological malignant disease or recent immunosuppressive treatment, which are important risk groups for CAP.[31] The number of patients with a history of chronic obstructive pulmonary disease (COPD) differed significantly between the three periods ($p<0.001$ with a chi-square test). To explore COPD as potential confounding factor, we have therefore conducted a sensitivity analysis by excluding all COPD patients. This analysis, showed very similar findings compared to the original analysis and excludes COPD as important confounding factor (*S. pneumoniae* decreased from 34% to 26% in the sensitivity analysis versus from 37% to 27% in the original analysis). In addition, patients that required immediate ICU admission were excluded from the trials with exception of the first trial (PCV7 period). However, this only concerned 5 patients of whom one had pneumococcal pneumonia so this does not impact our findings. Consequently, these two restrictions might impact the generalisability of our findings to all patients with CAP. Nevertheless, the comparison between periods remains valid since these restrictions were applicable to all three cohorts. In addition, there were no substantial changes over time in (1) resistance patterns for common causative agents of CAP (including *S. pneumoniae* penicillin resistance, which remained <1%) and (2) the empirical treatment of CAP so these factors are not likely to impact our results. Lastly, the study time frame of the post-PCV periods coincided with the CAPiTA trial, in which 42.240 Dutch elderly persons received PCV13.[32] The influence, however, is expected to be negligible, because less than 2% of the Dutch population ≥65 years old participated in that trial. The same applies to PPV23 vaccination of Dutch elderly, in which the uptake is less than 1%.[8, 9]

Conclusions

The proportion of *S. pneumoniae* as causative agent in hospitalised adults with CAP (both bacteraemic and non-bacteraemic) decreased following the introduction of the PCV programme in children in The Netherlands. In addition, there has been a shift to non-vaccine serotypes. These findings suggest herd effects in adults with CAP after introduction of PCVs in the national immunisation programme in children. Ongoing monitoring of CAP aetiology, including *S. pneumoniae* serotype distribution, is needed to evaluate the long-term effects of pneumococcal conjugate vaccination programmes.

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Supplementary material

Supplementary Table 1. Proportions of causative pathogens in CAP patients pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period.

	Pre-PCV7 2004-2006 (n=201) No. (%)	**PCV7 2007-2009 (n=304) No. (%)**	**PCV10 2012-2016 (n=300) No. (%)**
S. pneumoniae	74 (37)	81 (27)	77 (26)
Atypical pathogens	14 (7)	57 (19)	34 (11)
Legionella spp.	7 (3)	12 (4)	19 (6)
Chlamydophila spp.	2 (1)	14 (5)	5 (2)
C. burnetii	1 (0)	26 (9)	1 (0)
M. pneumoniae	4 (2)	5 (2)	9 (3)
Other causative	27 (13)	33 (11)	31 (10)
S. aureus	4 (2)	2 (1)	2 (1)
H. influenzae	8 (4)	7 (2)	10 (3)
Influenza A/B virus	6 (3)	5 (2)	9 (3)
Other	9 (4)	19 (6)	10 (3)
No identified pathogen	86 (43)	133 (44)	158 (53)

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases.

Supplementary Table 2. Amounts of diagnostic testing and different tests used to determine aetiology of CAP.

	Pre-PCV7 2004-2006 (n=201) No. (%)	PCV7 2007-2009 (n=304) No. (%)	PCV10 2012-2016 (n=300) No. (%)
Sputum culture	148 (74)	143 (47)	188 (63)
Sputum PCR [a]	78 (39)	91 (30)	113 (38)
Urinary antigen *L. pneumophila* [b]	186 (93)	289 (95)	287 (96)
Urinary antigen *S. pneumoniae* [b]	183 (91)	288 (95)	287 (96)
Blood culture	182 (90)	259 (85)	271 (90)
Conventional serology [c]	130 (65)	252 (83)	NA
Throatswab viral PCR [d]	NA	225 (74)	220 (73)
Throatswab atypical pathogens [e]	NA	NA	73 (24)
Viral culture [f]	88 (44)	NA	NA
Pneumococcal serology [g]	109 (54)	169 (56)	211 (70)

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases.

[a] Sputum samples for atypical pathogens PCR for detection of *Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae/psittaci.*

[b] Urine antigen testing for the detection of *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* (BinaxNOW®).

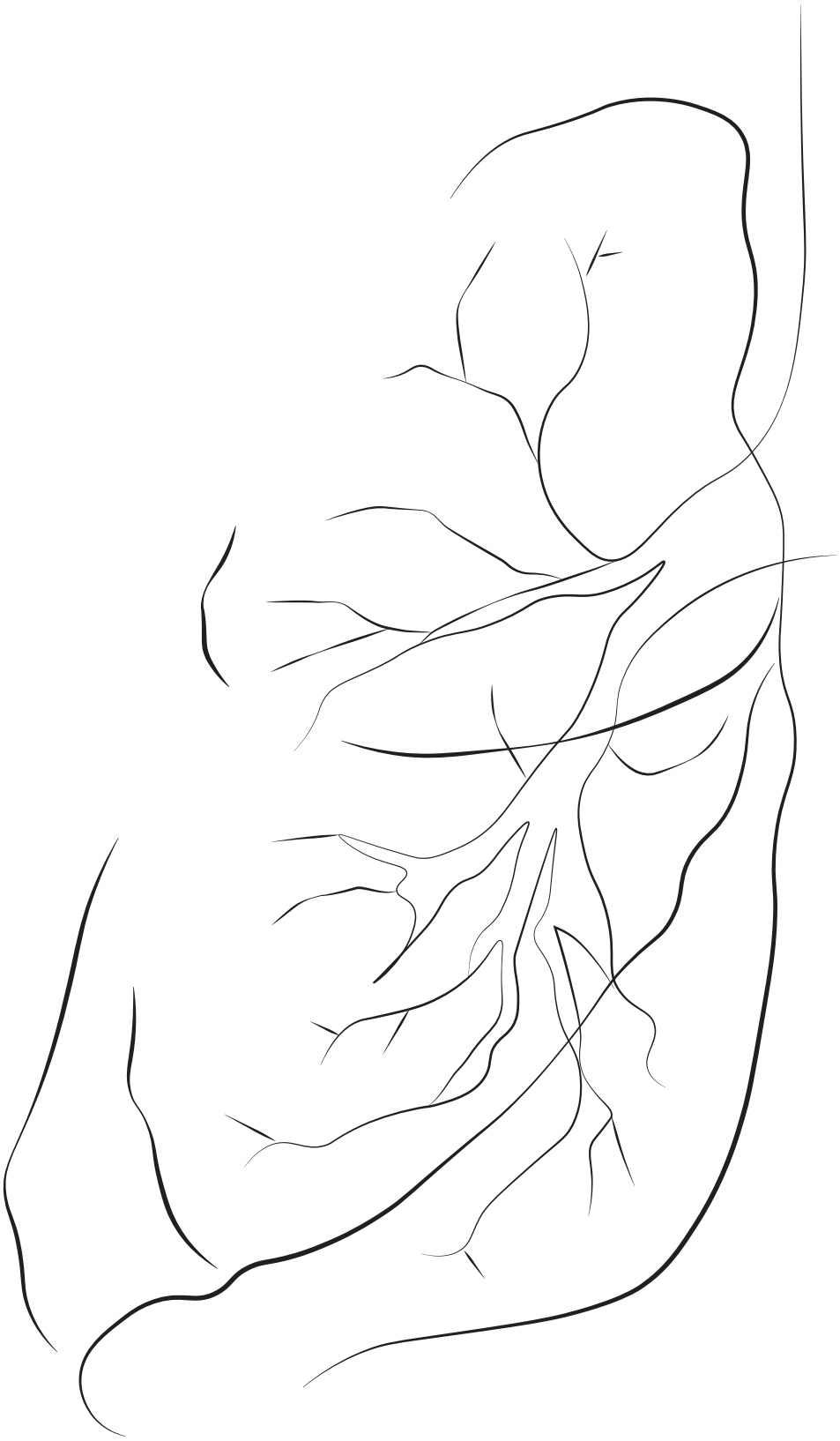
[c] Paired serological testing (day 1-3 and day 10-21, respectively) for detection of antibodies to *Mycoplasma pneumoniae*, *Coxiella burnetii*, *Chlamydophila spp*. or respiratory viruses (adenovirus, influenza virus A and B, parainfluenza and respiratory syncytial virus).

[d] Pharyngeal samples for viral PCR for detection of (para)influenza, adenovirus, respiratory syncytial virus.

[e] Pharyngeal samples for atypical pathogens PCR for detection of *Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae/psittaci*.

[f] Pharyngeal samples for viral culture for detection of influenza.

[g] Serotype-specific pneumococcal antibodies in serum were tested for development of serotype-specific pneumococcal antibodies using a quantitative multiplex immunoassay.



CHAPTER 6

Community-acquired pneumonia in Bangladeshi children under five years: proportion of *Streptococcus pneumoniae*, pneumococcal serotype distribution and polysaccharide antibody dynamics

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Submitted

Abstract

Background

Streptococcus pneumoniae is the most frequent causative pathogen of bacterial pneumonia in children worldwide. In 2015, Bangladesh introduced the 10-valent pneumococcal conjugate vaccine (PCV10) in their national immunization program for infants. We assessed its potential effects in under-fives with community-acquired pneumonia (CAP) in the years before PCV10 was introduced.

Methods

A total of 1502 childhood pneumonia cases (<5 years old and living in the urban section Kamalapur, Dhaka) were enrolled between 2011 and 2013. Early and late (convalescent) serum samples were collected from 1380 cases, in which serotype-specific pneumococcal antibodies were measured using a 25-plex immunoassay panel. Pneumococcal CAP was diagnosed in cases with a single serotype-specific pneumococcal antibody response.

Results

S. pneumonia was determined as cause of pneumonia in 406/1380 (31%) cases that met the inclusion criteria (two of which with invasive pneumococcal disease). The potential PCV10 coverage was 29%.The five most prevalent serotypes were (in descending order) 11A, 22F, 3, 2 and 19F. For these serotypes, the 95th percentile antibody concentrations were relatively high, suggesting early and heavy colonization.

Conclusions

Based on these results, the expected PCV10-coverage of serotypes causing non-bacteremic pneumococcal pneumonia (and potentially invasive pneumococcal disease) is low. Because the sensitivity of the used 25-plex assay has shown to be <50%, the determined *S. pneumoniae* proportion of 31% might in fact be twice as high. Given the high pneumococcal contribution in these vulnerable under-fives, a higher-coverage PCV or recombinant protein vaccine seems optimal for the region.

Introduction

Streptococcus pneumoniae is the most frequent bacterial cause of community-acquired pneumonia (CAP) in children worldwide.[1] The burden of pneumococcal disease is especially high in young children from developing countries.[2] Bangladesh is situated in a region where the vast majority of the world's severe and fatal pneumonia cases in young children occur.[3,4]

Pneumococcal conjugate vaccines (PCV) have been developed for the prevention of severe pneumococcal disease in children, especially invasive pneumococcal disease (IPD). With support of the GAVI Alliance, Bangladesh has implemented the 10-valent conjugate vaccine (PCV10; Synflorix®) in its childhood national immunization program as of March 2015.[5] PCV10 offers protection against 10 pneumococcal serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F.

Based on differences in the composition of the pneumococcal polysaccharide capsule, over 90 serotypes have been identified. The capsular polysaccharide is the single most important trigger to the host immune response, which is serotype specific with limited cross-reactivity.[6,7] Antibodies against the polysaccharide capsule are protective against IPD and form the basis of the available pneumococcal vaccines.[8] Most studies conducted in Bangladesh have focused on the serotype prevalence and distribution in IPD.[9–11] Very limited data is available on the contribution of the *S. pneumoniae* and the pneumococcal serotype distribution in children with CAP before PCV10 introduction in Bangladesh, especially non-invasive pneumonia.[12] Yet, the vast majority of pneumococcal CAP is non-invasive (or non-bacteremic).[13,14]

Given the limited resource setting of Bangladesh, including human resources for health services[15], the additional benefit of PCV on non-invasive pneumococcal CAP in children, in addition to its effects on IPD might be enormous.[16] Serological multiplex analyses of anti-capsular antibodies has shown to be useful in identifying the infecting pneumococcal serotype in adults hospitalized with bacteremic and non-bacteremic CAP.[17,18]

The aim of the present study was to evaluate the proportion of *S. pneumoniae,* and its serotype distribution, in children <5 years from Bangladesh with CAP before the introduction of PCV10, based on serotype-specific pneumococcal antibody dynamics. Furthermore, we assessed if an infecting serotype can be identified from serum drawn when CAP is diagnosed using population characteristics of anti-pneumococcal capsular antibody concentrations.

Methods

Study design and participants

Children between 1 and 59 months old from the urban field site of Kamalapur, Dhaka that were diagnosed with pneumonia were enrolled. This study was locally conducted by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), which has conducted pneumonia disease burden surveillance and intervention trials at this site since

1998.[10,12,19–21] The Research Review and Ethical Review Committees of the icddr,b approved the study.

Data collection, clinical characteristics and definitions

During weekly home visits, vital signs and signs of respiratory illness were evaluated based on a structured calendar questionnaire used by the study site for respiratory and febrile illness surveillance since 2004. All study participants with signs of respiratory and febrile illness were initially referred to the onsite study clinic within 24-48 hours of start of illness signs and definitive CAP diagnosis was made by the study physician based on standard clinical case definitions/. Definitions of CAP and severity of illness can be found in the Supplementary Text. Multiple enrolments of the same child were allowed, but only after the child had recovered from a prior episode of CAP and at least 7 disease free days between two illness episodes. Each unique CAP episode is hereafter referred to as a 'case'. Available outcome measures were: duration of illness (in days) and outcome of illness (full recovery, recovery with disabilities, continues to be ill, lost to follow-up, deceased). Full definitions of outcomes are included as Supplementary Text.

For the current study, two serum samples were collected and stored at the field clinic at 4°C, and transported twice daily in a cool box at 4° - 8°C to the clinical microbiology laboratory of Dhaka and stored at -20°C. The first sample was collected at day 1 at the time of CAP diagnosis. The second sample was obtained during a post-recovery follow-up visit (convalescent sample). Later all samples were aliquoted and stored at -80°C. Aliquots were sent to the St. Antonius Hospital in Nieuwegein, The Netherlands, for batch analyses.

Serotype-specific pneumococcal antibody measurement by multiplex immune assay

Serotype-specific pneumococcal antibodies were measured in paired serum samples. The multiplex immunoassay (MIA) was performed on a Luminex platform (Luminex Corporation, Austin, TX). This method has been described in detail elsewhere[18] and is added as Supplementary Text. A 25-plex immunoassay panel was used including the 10 serotypes covered by PCV10 (4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5 and 7F), and 15 additional serotypes: 2, 3, 6A, 8, 9N, 10A, 11A, 12A, 12F, 15B, 19A, 20, 22F, 33F and 45). Serotypes 12A and 45 are not covered by the 007sp reference serum, hence concentrations are given in arbitrary units (AU/ml). The serotypes in our panel were selected based on serotype prevalence in IPD in the region[9–12], and by potential vaccine-coverage. Further information on selection of sera and details about the assay is added as Supplementary Text.

Pneumococcal pneumonia definition

A positive immune response was defined as at least a 2-fold increase in serotype-specific antibodies between the first and second serum sample and a concentration at convalescence of ≥0.2 µg/ml (≥0.2 AU/ml for serotype 12A and 45). This concentration was chosen as it was well within the range of the lower end of the standard curves for all serotypes. The fold

increase in antibody concentration against a given single pneumococcal serotype had to be at least two times greater than the fold increase against any other serotype, with exception of a 2-fold increase for serotypes within the same serogroup (e.g. 6A/6B), a scenario in which the serotype with the highest fold increase was regarded as infecting serotype. Cases with a positive serotype-specific antibody response were defined as pneumococcal CAP. To prevent false positive and negative results, cases whose convalescent serum was drawn <14 days or >100 days after diagnosing CAP were excluded.

Based on the methods described above, patients were categorized as having (1) pneumococcal CAP or (2) CAP with unknown etiology.

To assess whether pneumococcal CAP can be serologically identified based on antibody concentrations at time of diagnosis, we compared serotype-specific antibody concentrations from early serum with serotype-specific 95th percentile concentrations of the pooled data (from all unique early and late sample concentrations combined), using the MIA as a diagnostic method for pneumococcal CAP.

Conventional microbiological diagnostics

Blood cultures were performed using pediatric FAN bottles. Bottles were sent twice daily (within 4 hours of collection) to the clinical microbiology laboratory at the icddr,b for culture by BactAlert 3D (BioMerieux, France). Pneumococcal isolates were not available for serotyping. In addition, nasopharyngeal washes (NPW) were done. The obtained NPW samples were tested for the presence of influenza virus A or B components using PCR.

Data analyses

Analyses are stratified by disease severity (non-severe vs. severe and very severe CAP) or age group (1-17 months vs. 18-60 months of age). The 18 months cut-off is based on the evidence that newborns and infants up to the age of 18-24 months are unable to produce antibodies to bacterial capsular polysaccharides which may increase susceptibility for infections with pneumococci in cases below 18 months old.[22–24] Proportion of pneumococcal CAP and PCV10 coverage were determined.

Statistics

Descriptives were stated as number (%) and continuous data were presented as mean (SD) or median (interquartile range (IQR)). Differences in proportions between serotype groups were tested with $\chi 2$ tests. Pearson correlation coefficients (PCC) were calculated to determine correlation between anti-polysaccharide antibodies specific for different serotypes. A p-value <0.05 was considered to represent a statistically significant difference. Microsoft® Office Excel for Windows (version 2010) and SPSS software (version 22.0) were used for statistical analyses.

Results

All-cause pneumonia

Between October 2011 and December 2013, two unique serum samples were obtained from 1502 cases. This group consisted of 815/381/142/164 children that participated either once (54%), twice (25%), three times (9.5%) or >3 times (11%), respectively, with a maximum of 7 enrolments (one child). The 122 cases with a delta <14 days (n=41, 2.7%) or >100 days (n=81, 5.4%) between the early and late sample were excluded from further analyses (unless stated otherwise).

The mean age of the remaining 1380 cases was 20.2 months (median 17.2 months, IQR 10.6-27.0). The majority of cases (1274/1380, 92%) was diagnosed with non-severe pneumonia, 105/1380 (7.6%) cases met the criteria of severe CAP. One child had very severe CAP. Baseline characteristics, conventional microbiological test results and outcomes of illness are shown in Table 1.

Proportion of Streptococcus pneumoniae

The definition of pneumococcal CAP was met by 406/1380 (31%) cases (further explored in the next paragraph), the remaining 974 cases were considered CAP with unknown etiology. Twenty-two of 406 cases (5.1%) had severe CAP and 384 (95%) non-severe pneumococcal CAP. The prevalence of pneumococcal CAP was similar in both age groups: 25% (193/769) in 1-17 months old and 29% (213/733) in 18-60 months old, p=0.08). There were no significant differences between pneumococcal CAP and CAP with unknown etiology in duration of illness (7.3 days (SD 5.4) vs. 7.6 days (SD 5.8) or the rate of recovery with disabilities (95/406 (23%) vs. 219/974 (22%)).

Ten out of 406 (2.5%) cases with pneumococcal CAP had a positive blood culture. *S. pneumoniae* was isolated two times, coinciding with a humoral response against serotypes 1 and 8 (no conventional typing performed). In the remaining eight cases the following bacteria were isolated: coagulase-negative *Staphylococci* (n=4), *Salmonella* typhi (n=3) and *Pseudomonas* spp. (n=1). NPW PCR identified influenza virus in 13/397 (3.3%) cases (n=6 H1N1, n=4 H3N2, n=2 influenza type B). No NPW sample for flu testing was collected in the remaining 9/406 cases. Results of blood cultures and NPW flu PCR of all 1380 cases are shown in Table 1.

Pneumococcal serotypes

Figure 1 shows the distribution of serotypes in pneumococcal CAP cases. All 25 serotypes included in the assay were identified at least once. The three most frequently identified serotypes were 11A (n=45, 11%), 22F (n=35, 8.6%) and 3 (n=34, 8.4%). The most prevalent serotype potentially covered by PCV10 was serotype 19F (n=26, 6.4%). Based on the identified serotypes, the potential PCV10-coverage was 29%.

Table 1. Baseline characteristics, results from conventional diagnostic testing and outcomes.

Baseline characteristics (n=1380)	
Age (months (SD))	20.2 (13.1)
Pneumonia by severity	
Non-severe	1274 (92%)
Severe	105 (7.6%)
Very severe	1 (<0.1%)
Blood culture isolates (n=1358)	
*Coagulase-negative Staphylococcus species**	14
Salmonella typhi	7
Streptococcus pneumoniae	4
Streptococcus species	3
Micrococcus species	3
Moraxella species	3
Pseudomonas species	3
Acinetobacter species	2
Enterococcus species	2
Campylobacter species	1
Neisseria meningitidis	1
Nasopharyngeal wash influenza virus PCR	
Type A – H1N1	18 (1.3%)
Type A – H3N2	22 (1.6%)
Type B	10 (0.7%)
No sample collected	22 (1.6%)
Negative	1308 (95%)
Outcomes	
Non-severe pneumonia (n=1274)	
Duration of illness (days)	6 [IQR 4-9]
Outcome of Illness	
Recovered	966 (76%)
Recovered with disability	291 (23%)
Continues to be ill	1 (0.1%)
Deceased	0
Lost to follow-up	16 (1.3%)
Severe pneumonia (n=106)	
Duration of illness (days)	8 [5-13]
Outcome of Illness	
Recovered	83 (78%)
Recovered with disability	22 (21%)
Continues to be ill	0
Deceased	0
Lost to follow-up	0
Very severe pneumonia (n=1)	
Duration of illness (days)	6
Outcome of Illness	Recovered with disability

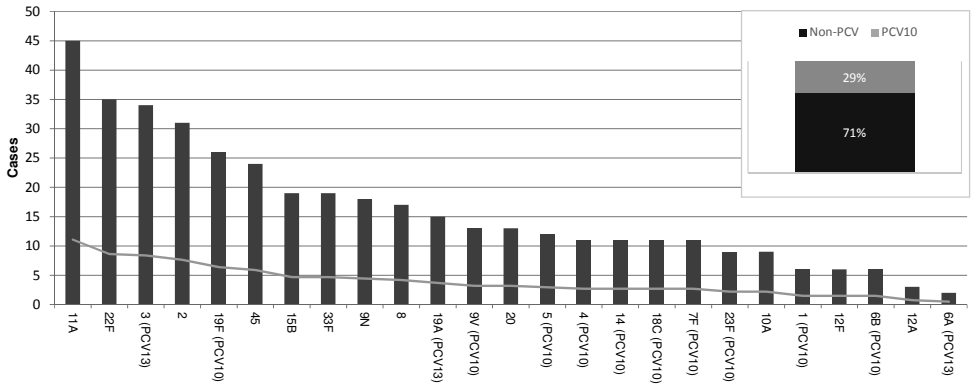

Figure 1. Distribution of serotypes causing pneumococcal CAP based on serotype-specific antibody responses. Bars indicate the number of cases, the line shows the proportion. Proportion of PCV10 coverage is shown in the right upper corner.

The serotype distribution stratified by age group is shown in Figure 2. The potential PCV10-coverage in 1-17 months was 29% vs. 28% in 18-60 months (p=0.85). Notable differences were the higher proportion of serotype 2 and 3 in cases 1-17 months old (20/193 (10%) vs. 11/213 (5.2%); p=0.05 and 21/193 (11%) vs. 13/213 (6.1%), p=0.08, respectively), whereas the proportions of serotype 9N and 33F were higher in cases 18-60 months of age (13/213 (6.1%) vs. 5/193 (2.6)%, p=0.09, and 14/213 (6.6%) vs. 5/193 (2.6%), p=0.06, respectively).

Pneumococcal antibody dynamics

Serotype-specific pneumococcal antibody response

A total of 406/1380 (31%) cases had a positive immune response for a specific pneumococcal serotype thereby representing the proportion of pneumococcal CAP. It was not possible to draw conclusions regarding the immune responses in 103 cases (7.3%), e.g. due to a response against multiple serotypes or indistinct variations in antibody dynamics. No specific immune response was detected in 871 (62%) cases. Supplementary Figure 1 shows examples of antibody dynamics of each of the three aforementioned scenarios.

Diagnostic value of 95th percentile serotype-specific antibody concentrations

The distribution of serotype-specific antibody concentrations (of all 1502 cases), including their 95th percentile concentration, are shown in Table 2A. At the time of pneumococcal CAP diagnosis when the first serum sample was drawn, <2% (n=7) of the cases had a serotype-specific antibody concentration above the 95th percentile concentration of the infecting serotype (as identified using the baseline-convalescent sample approach). In the convalescent serum, however, 165 (41%) cases with pneumococcal CAP did have a concentration above the 95th percentile for the infecting serotype. There was an inverse

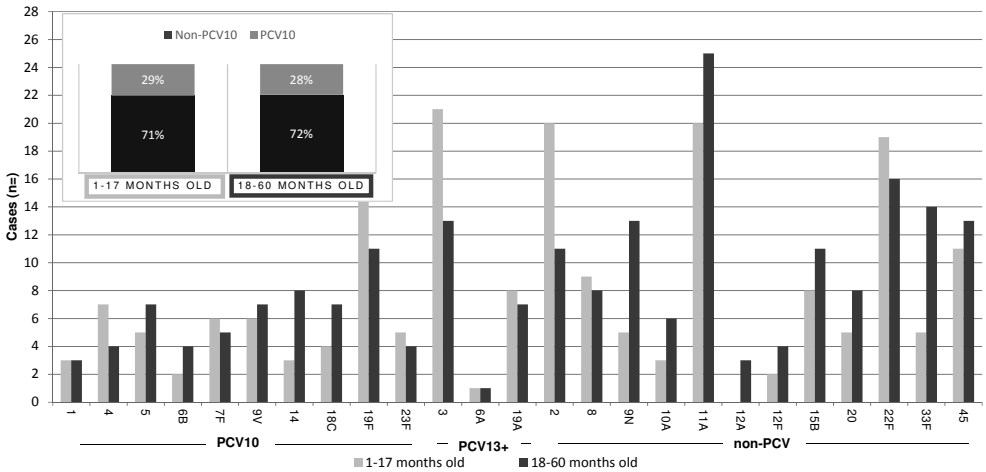

Figure 2. The overall serotype distribution based on serotype-specific antibody responses stratified by age group. Bars indicate the number of cases. Proportion of PCV10 coverage is shown in the left upper corner. PCV13+ indicates the three serotypes covered by PCV13, in addition to the serotypes covered by PCV10 (i.e. 3, 6A and 19A).

relation between the prevalence of the serotypes (as identified using the baseline-convalescent approach) and those confirmed by the 95th percentile approach on the late sample. For the top 10 prevalent serotypes the average confirmed was 30% (81/286) of the cases (max 51% for serotype 22F) vs. 60% (70/115) for the serotypes ranking 11-20 ($p<0.001$). (Table 2B).

Intra-assay correlations of early and late serotype-specific antibody concentrations

Comparing serotype antibody concentrations from the first and second serum samples, serotypes from the same serogroup generally showed a better correlation compared to unrelated serotypes. Scatterplots are shown in Supplementary Figure 2 and the full correlation matrix is shown in Supplementary Table 1. Especially serotype 12A and 12F (PCC=0.70) and 6A and 6B (PCC=0.66) correlated well. However, for 19A and 19F, the correlation was low (PCC=0.54). Noteworthy, the PCC was 0.63 for the biologically unrelated serotype 8 vs. serotype 12A and 12F. In-depth analyses considering cross-reactivity and specificity can be found in the Supplementary Text.

Discussion

By assessing serotype-specific pneumococcal antibody dynamics in serum, we were able to establish the contribution of *S. pneumoniae* in almost 1400 childhood cases with CAP in Dhaka, Bangladesh. The pneumococcal proportion was 31% (n=406). The most striking finding, based on these unique data from under-fives with CAP, was that only 29% of pneumococcal CAP was caused by a PCV10-covered serotype, in the years before

Table 2. 95th percentile of serotype-specific antibody concentrations with 95% CI (mean of first and second sample).

Part A	All cases (n=1502)				Part B	Pneumococcal pneumonia (n=406)	
	Early sample concentration (*µg/ml*)						
Serotype	All ages Median [IQR]	1-17 months Median [IQR]	18-60 months Median [IQR]	All ages 95th percentile	Serotype	Cases n= (% of total)	Late sample n= (% of serotype-specific cases) >95th percentile
1 (PCV10)	0.04 [0.02-0.06]	0.03 [0.02-0.06]	0.04 [0.02-0.07]	0.25	**1** (PCV10)	6 (1.5)	6 (100)
2	0.08 [0.02-0.32]	0.04 [0.01-0.14]	0.15 [0.05-0.59]	2.39	**2**	31 (7.6)	6 (19.4)
3 (PCV13)	0.26 [0.13-0.68]	0.23 [0.13-0.5]	0.3 [0.14-1.16]	7.68	**3** (PCV13)	34 (8.4)	10 (29.4)
4 (PCV10)	0.01 [0-0.02]	0.01 [0-0.02]	0.01 [0.01-0.04]	0.28	**4** (PCV10)	11 (2.7)	11 (100)
5 (PCV10)	0.05 [0.02-0.13]	0.04 [0.02-0.09]	0.07 [0.04-0.16]	0.57	**5** (PCV10)	12 (3)	4 (33.3)
6A (PCV13)	0.01 [0.01-0.03]	0.01 [0.01-0.02]	0.01 [0.01-0.05]	0.38	**6A** (PCV13)	2 (0.5)	2 (100)
6B (PCV10)	0.01 [0-0.02]	0.01 [0-0.01]	0.01 [0-0.05]	0.30	**6B** (PCV10)	6 (1.5)	6 (100)
7F (PCV10)	0.05 [0.03-0.13]	0.05 [0.02-0.1]	0.07 [0.03-0.18]	0.78	**7F** (PCV10)	11 (2.7)	9 (81.8)
8	0.11 [0.05-0.27]	0.07 [0.03-0.15]	0.17 [0.09-0.45]	2.48	**8**	17 (4.2)	6 (35.3)
9N	0.05 [0.02-0.18]	0.03 [0.02-0.09]	0.09 [0.04-0.31]	1.51	**9N**	18 (4.4)	4 (22.2)
9V (PCV10)	0.02 [0.01-0.06]	0.01 [0.01-0.03]	0.03 [0.01-0.12]	0.60	**9V** (PCV10)	13 (3.2)	8 (61.5)
10A	0.03 [0.01-0.08]	0.03 [0.01-0.06]	0.04 [0.02-0.11]	0.44	**10A**	9 (2.2)	7 (77.8)
11A	0.09 [0.03-0.46]	0.07 [0.03-0.28]	0.14 [0.04-0.81]	5.00	**11A**	45 (11.1)	10 (22.2)
12A	0.01 [0.01-0.03]	0.01 [0.01-0.02]	0.02 [0.01-0.05]	0.51	**12A**	3 (0.7)	0 (0)
12F	0.06 [0.03-0.14]	0.04 [0.02-0.08]	0.09 [0.05-0.21]	1.52	**12F**	6 (1.5)	1 (16.7)
14 (PCV10)	0.01 [0.01-0.1]	0.02 [0.01-0.09]	0.01 [0.01-0.1]	1.19	**14** (PCV10)	11 (2.7)	7 (63.6)
15B	0.04 [0.02-0.14]	0.04 [0.02-0.11]	0.05 [0.02-0.17]	1.31	**15B**	19 (4.7)	4 (21.1)
18C (PCV10)	0.03 [0.01-0.09]	0.02 [0.01-0.07]	0.03 [0.01-0.13]	0.80	**18C** (PCV10)	11 (2.7)	6 (54.5)
19A (PCV13)	0.07 [0.02-0.29]	0.05 [0.02-0.18]	0.1 [0.03-0.41]	1.90	**19A** (PCV13)	15 (3.7)	4 (26.7)
19F (PCV10)	0.13 [0.04-0.8]	0.08 [0.03-0.44]	0.25 [0.06-1.16]	6.55	**19F** (PCV10)	26 (6.4)	11 (42.3)
20	0.02 [0.01-0.06]	0.02 [0.01-0.05]	0.03 [0.02-0.09]	0.48	**20**	13 (3.2)	6 (46.2)
22F (PCV10)	0.02 [0.01-0.1]	0.02 [0.01-0.06]	0.03 [0.01-0.19]	1.62	**22F**	35 (8.6)	18 (51.4)

Table 2. (continued)

Part A	All cases (n=1502)				Part B	Pneumococcal pneumonia (n=406)	
	Early sample concentration (*µg/ml*)						
Serotype	All ages Median [IQR]	1-17 months Median [IQR]	18-60 months Median [IQR]	All ages 95th percentile	Serotype	Cases n= (% of total)	Late sample n= (% of serotype-specific cases) >95th percentile
23F	0.02 [0.01-0.05]	0.02 [0.01-0.05]	0.02 [0.01-0.07]	0.51	**23F (PCV10)**	9 (2.2)	8 (88.9)
33F	0.03 [0.02-0.09]	0.03 [0.01-0.07]	0.04 [0.02-0.13]	0.68	**33F**	19 (4.7)	6 (31.6)
45	0.03 [0.02-0.1]	0.02 [0.01-0.06]	0.05 [0.02-0.16]	0.74	**45**	24 (5.9)	6 (25)

Bangladesh introduced PCV10. By our best knowledge, this is the first study to report on the serotype-specific pneumococcal contribution in childhood, predominantly non-bacteremic, pneumonia cases.

PCVs have been developed based on epidemiological serotype data from Western countries and have had a proven positive impact on occurrence of IPD.[25] Few studies have reported on data regarding serotype distribution in children suffering from IPD in the region. Active IPD surveillance started in Bangladesh with support from GAVI's PneumoADIP to assess the burden of IPD (not limited to pneumonia) among children <5 years old.[26] Studies were conducted in rural and urban areas and enrolled hospitalized and/or non-hospitalized children.[9–11] The top 3 most prevalent serotypes from these three studies consisted of serotype 1, 2, 5, 6B, 14 and 19A. All were included in our panel, however, only serotype 2 was in the top 10 of most prevalent serotypes in our study limited to childhood cases with CAP (predominantly non-bacteremic).

The wide variation in serotypes likely reflects the geographical diversity between studies and difference in type of disease.[11] Bacteremic pneumonia and meningitis may be mediated by different pneumococcal serotypes than non-bacteremic disease due to a higher propensity of some serotypes to invade the bloodstream.[9] This could explain the low prevalence of serotype 1 (e.g.) in our study compared to the Bangladeshi PneumoADIP centers focusing on children with IPD.[27–29].

Our study was conducted in an urban low-income section of Dhaka that is provided health services by the icddr,b, before PCV10 was introduced in Bangladesh. PCV10 coverage was 29% of the identified pneumococcal CAP cases. Even though the PCV10-coverage for Bangladeshi children with IPD remains unclear, given the high burden of CAP among children in this region, Bangladesh could highly benefit from a vaccine that also covers serotypes causing non-bacteremic CAP. The high burden was reflected by the fact that many children participated ≥2 times in little over two years. Noteworthy, based on the identified serotypes in our study, the novel 20-valent conjugate vaccine (in phase 3 clinical research) covers 78% of the pneumococcal CAP (vs. 41% by PCV13).[30] However, given the high serotype diversity observed in Bangladesh and neighboring countries[31,32], next to ongoing replacement disease in countries with years of experience with PCVs, a recombinant protein vaccine aimed at offering serotype-independent protection seems optimal[33], and may render the risk for serotype replacement redundant.[34]

The size of the cohort allowed us to determine normal distributions of anti-polysaccharide antibodies. Yet, the diagnostic value of the 95th percentile concentrations as a threshold value in identifying a pneumococcal infection at the time of diagnosing CAP was very low, even in the convalescent serum. This indicates that it may require considerable time and, perhaps, several infections to generate high serum concentrations against capsular polysaccharides. The inverse relation between the prevalence of the serotypes as identified by the baseline-convalescent analysis and those confirmed by the 95th percentile approach using the convalescent sample does implicate early and heavy colonization by the most frequently identified serotypes (i.e. 11A, 22F and 3). Furthermore,

though two-point serology testing may be diagnostically impractical, these findings indicate its usefulness in providing a good level of evidence of a pneumococcal infection and its serotype distribution, also in the absence of a true gold standard for identification of pneumococcal pneumonia.

It has been shown that many adults with blood culture proven pneumococcal pneumonia do not show a serological antibody response, and even compared to extensive microbiological testing the same assay had a sensitivity of 42%-45% to detect pneumococcal pneumonia in Dutch adults hospitalized with CAP.[17,18] If this sensitivity holds true for children in our study, the true pneumococcal proportion might be more than twice as high. On the other hand, with the lack of a gold standard, it is not possible to be sure that each CAP case with a serotype-specific antibody response in the current study is indeed due to *S. pneumoniae.* Many children are colonized with pneumococci which may cause an immune response in parallel to another respiratory pathogen. Moreover, many children did have high antibody concentrations against multiple serotypes, which likely is a reflection of living in close contact with relatives leading to higher pneumococcal carriage rate and density. A follow-up study of similar design in this setting post vaccine exposure, including conventional microbiological testing, could shed light on the question of carriage rate, density and infection.[35]

The main strength of this study is that we obtained data from an experienced surveillance system, which has yielded a unique cohort of 1502 pneumonia cases <5 years of age. Using MIA, we were able to map the serotype distribution in childhood cases of non-bacteremic pneumococcal CAP in Bangladesh before the introduction of PCV10. The MIA technique allows identification of the proportion and serotype distribution of pneumococcal pneumonia and has proven to be valuable in detecting pneumococcal pneumonia in adults.[17,18] However, the assay was limited to identifying pneumococcal CAP cases due to the 25 serotypes included in our panel. Besides blood cultures, the detection of *S. pneumoniae* was not confirmed by conventional methods. Due to challenging technical conditions (i.e. temperature, logistics), the <3% bacteremic cases with pneumococcal CAP might well be an underestimation. The lack of a healthy control group was overcome by using the antibody measurements in cases with CAP with unknown etiology (and using measurements of the non-infecting serotypes within cases with pneumococcal CAP) as internal controls.

Conclusions

The proportion of *S. pneumoniae* in childhood cases <5 years with CAP was high in Bangladesh before introduction of PCV10. Based on serotype-specific pneumococcal antibodies measurement, the potential PCV10-coverage was only 29%. The serotype distribution differed largely compared to IPD studies from the region and from Western countries. Future research should focus on mapping the serotype-specific burden of disease in the region, aiming towards the development of vaccines directed at protecting children living in this low-income region.

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Supplementary material

Supplementary Methods

Study area & data collection

The icddr,b Kamalapur urban surveillance site comprises seven communities in four municipal wards with 350,000 residents, divided into 734 geographical clusters, each consisting of ~100 households in a 4.2 km^2 area with a median household income <US $80.00/month, a mean education of 6.6 years for men and 5.1 for women. In brief, the pneumonia surveillance system follows 5,500 children <5 years longitudinally. All children <5 years old residing in selected clusters were kept under active morbidity surveillance, after parents provided informed written consent. All study staff were trained (both clinic and field staff) to achieve standardization of case assessments, making clinical diagnosis, case management and specimen collection. Each week, trained research field assistants visited each household within the surveillance area and, using standardized calendar questionnaires, inquired about signs of pneumonia.

Signs of illness (home visit)

Clinical signs were divided into major and minor signs. Major signs included fever (axillary temperature ≥38°C), age-specific tachypnea using WHO criteria, danger signs (chest indrawing, lethargy, cyanosis, inability to drink, convulsions), difficult breathing, noisy breathing, and ear pain/discharge. Minor signs included cough, rhinorrhea, sore throat, myalgia/arthralgia, chills, headache, irritability/decreased activity, and vomiting. Children needed one major or, if absent, two minor signs for clinic referral. Thus, the basis of referrals was identification of standardized key signs and not a pneumonia diagnosis.

Definition of CAP (onsite study clinic)

All pneumonia definitions for children <5 years are based on the World Health Organisation's (WHO) program, Integrated Management of Childhood Illness (IMCI) guidelines Table 5 (p33).[s1] We have modified these by adding the definition of pneumonia, severe pneumonia and very severe pneumonia. The IMCI definition does not include auscultation findings. By incorporating auscultation findings, we will clinically detect fluid in the lungs and improve specificity. All other definitions of pneumonia are modifications of the WHO guidelines adapted for patient age.

CAP

Two criteria: 1) Age-specific tachypnoea (≥60/min if <2 months; ≥50/min if 2 – 11 months; ≥40/min if 12 – 59 months; >24/min if 5 – 9 years; >22/min if 10 – 14 years; >20/min if ≥15 years); 2) Crepitations on auscultation. Patients with concomitant wheezing would fit the definition, however based on our previous studies, would probably not have bacterial pneumonia, seem to respond differently to zinc than do non-wheezing children, and therefore

will not be be recorded as pneumonia. Wheezing without crepitations would be defined as reactive airways disease or bronchiolitis (afebrile or febrile presentation, respectively).

Severe CAP

Pneumonia plus chest indrawing.[s1]

Very severe CAP

Pneumonia plus 2) at least one of the danger signs. Danger signs include: central cyanosis, severe respiratory distress, convulsions, altered mental status, and in children < 5 years, head nodding, nasal flaring, grunting, inability to drink, and lethargy, and vomiting.[s1]

Definition of Outcome of Illness

Recovered: If child meets illness resolution criteria without any persisting or ongoing problem. Illness resolution criteria: seven disease free days (temperature <38^{0}C and respiratory rate <40/min) and no danger sign.

Recovered with Disability: If child meets illness resolution criteria with any persisting or ongoing problem. Example for recovered with disability: temperature <38^{0}C and respiratory rate <40/min and no danger sign. Lungs clear but has persisting cough/night time cough.

Continues to be ill: If illness continue up to the date of convalescent clinic visit/exit interview.

Deceased: If child died during the specific illness episode.

Lost to follow up**:** If child left the Kamalapur surveillance area after reporting the illness and the field research assistant could not communicate with the family for follow up visit of the child throughout the illness period. And the exit interview for the particular illness could not be conducted.

Serotype-specific pneumococcal antibody measurement by multiplex immune assay

Two consecutive serum samples were obtained from 1530 cases. In 28/1530 cases the late serum sample was registered under a duplicate date. Thus, two unique serum samples were available from 1502 cases, from day 1 and 9-442 days after CAP was diagnosed [median 25, IQR 19-41].

Multiplex immunoassay

The standard used for performing the MIA was calibrated against the 007sp reference serum. Three assay controls 007sp (NIBSC) in 3 dilutions were taken along in duplicate with the test samples in each assay as internal control.

The paired samples were diluted 100x in sample buffer composed of phosphate-buffered saline (PBS), pH 7.3, 5% antibody depleted human serum (ADHS) pneumococcal cell wall polysaccharide (CWPS multi), to inhibit nonspecific binding of anti-cell wall polysaccharides I and II. Diluted sera were incubated with a mixture of 25 differently color-coded microspheres r, with each region being coated with an unique polysaccharide representing the serotype. Following 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 100 (IS 2.3)(for details, see ref [s2]).

Cross-reactivity and specificity. To assess analytical specificity, a procedure described by Lal *et al.* [s3] was applied as described before, with modifications. Briefly, WHO reference serum 007sp [s4] was diluted 3200x in sample buffer containing either one of the 25 polysaccharides (2 µg/ml)(inhibitor polysaccharides). One replicate served as control. The samples were preadsorbed at RT for 1,5 hour, and subsequently processed according to the general protocol described above. Specificity results for each sample and serotype–microsphere using homologous and heterologous inhibition were determined by calculating the percent inhibition in median fluorescent intensity (MFI) signal according to the method described by Schlottmann et al. [s5]:

Percent inhibition = (MFI of serum in preadsorbent – MFI in serum in preadsorbent + inhibitor serotype /MFI of serum in preadsorbent) × 100.

The experiment was repeated 3x.

To provide insight into the MIA method, we performed intra-assay correlations of early and late serotype-specific antibody concentrations.

Supplementary Results

Pneumococcal antibody measurement

Serotype-specific pneumococcal antibody response

Within the 41 children excluded for having a delta <14 days between the early and late sample date, three (7%) showed an antibody response against one single serotype (serotype 2, 19F and 22F), whereas 53 of the 81 children with a delta >100 days did have a positive immune response (65%). Within the latter group, either one of the top 10 most prevalent serotypes was identified in 32 cases (57%) (maximum ratio of 11% for serotype 15B and 19F).

Of cases the 406 cases with pneumococcal CAP, 27 children were enrolled as a case 2 times, two children 3 times and one child 4 times. None of these 30 children were diagnosed with the same serotype more than once. The minimum and maximum time between diagnosing two pneumococcal CAP episode in these cases was 32 and 636 days, respectively.

Cross-reactivity and specificity

Considering the good correlation between concentration of antibodies against serotype 8 and serotype 12 family polysaccharides, validation data of the 25-plex were retrieved. In this validation experiment, the dilution chosen of the reference serum 007sp had a signal >500 MFI for most serotypes. Inhibition >95% and <15% are considered good and no inhibition. A background level of heterologous cross-inhibition of ~15% was generally observed between experiments. This was not related to any specific polysaccharide, but rather dependent on order of acquisition. Heterologous inhibition >15% proved reproducible. Homologues inhibition >95% was observed for all serotypes, and good cross-inhibition was found for serotype family members 6A/6B (yet A>B then B>A), and 12A/F (yet F>A then A>F). Modest cross-inhibition of ~30% was observed for both members of serotype 19. Several unexpected cross-inhibitions were observed: 1→19F, 4→14, 8→9V/11A, 9N→6A/B and 20→1. No cross-inhibition was found between serotypes 8 and 12A/F (see Supplementary Table 2 and Supplementary Figure 3).

Supplementary Discussion

Cross-reactivity and specificity

We found serologic support for antibody cross-reactivity within serotype families, e.g. serogroup 6, by correlating serotype-specific antibody concentrations. Noteworthy was the good correlation between serotype 8 and serogroup 12 members, despite their distinct composition.[s6] After having ruled out cross-contamination of polysaccharide batches (see Supplementary Figure 3), the most likely explanation for this remarkable correlation is a high degree of exposure and probably combined nasopharyngeal carriage of these serotypes.[s7] Even though, these results could be indicative for cross protection, the functionality of cross-reactive antibodies has not been established.

Currently, experiments are ongoing to determine what factors are contributing to the "so-called" unexpected cross-inhibitions. It may be related to the polysaccharide batches used, modifications introduced by the conjugation of polysaccharides to the bead allowing binding of moderately cross-reactive antibodies, or related to the artificial experimental conditions involving an excess of polysaccharide molecules during the pre-absorption phase.

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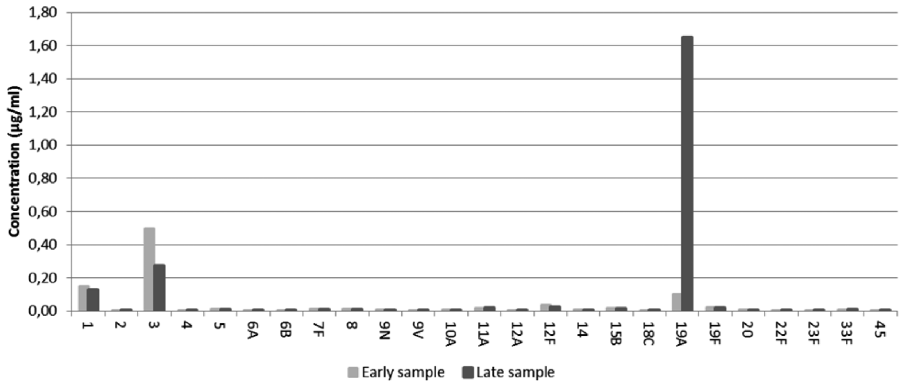
Supplementary Table 1. Correlation matrix showing Pearson correlation coefficients of correlations between serotype-specific antibody concentrations.

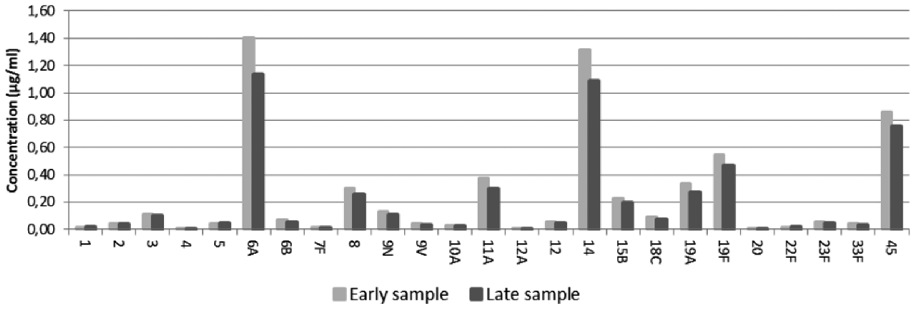
	1	2	3	4	5	6A	6B	7F	8	9N	9V	10A	11A	12A	12F	14	15B	18C	19A	19F	20	22F	23F	33F	45	
1	1,00	0,24	0,22	0,19	0,14	0,17	0,17	0,28	0,26	0,24	0,24	0,22	0,16	0,25	0,21	0,20	0,19	0,27	0,23	0,18	0,19	0,14	0,24	0,22	0,22	1
2		1,00	0,16	0,19	0,26	0,17	0,22	0,29	0,50	0,46	0,31	0,27	0,24	0,49	0,51	0,07	0,13	0,22	0,21	0,20	0,30	0,22	0,20	0,22	0,39	2
3			1,00	0,14	0,18	0,11	0,14	0,22	0,24	0,22	0,20	0,13	0,13	0,17	0,21	0,05	0,05	0,20	0,21	0,11	0,19	0,14	0,21	0,13	0,17	3
4				1,00	0,20	0,14	0,17	0,19	0,18	0,19	0,18	0,15	0,09	0,19	0,17	0,08	0,16	0,20	0,16	0,15	0,18	0,17	0,10	0,16	0,17	4
5					1,00	0,15	0,14	0,25	0,32	0,28	0,23	0,27	0,19	0,25	0,30	0,08	0,20	0,21	0,16	0,10	0,27	0,17	0,10	0,23	0,24	5
6A						1,00	0,66	0,15	0,16	0,28	0,27	0,27	0,25	0,17	0,15	0,28	0,32	0,24	0,28	0,27	0,30	0,23	0,22	0,32	0,19	6A
6B							1,00	0,18	0,16	0,29	0,30	0,20	0,21	0,20	0,16	0,26	0,27	0,29	0,27	0,24	0,24	0,22	0,25	0,26	0,18	6B
7F								1,00	0,31	0,32	0,28	0,34	0,20	0,26	0,25	0,12	0,22	0,17	0,24	0,20	0,28	0,16	0,22	0,28	0,24	7F
8									1,00	0,54	0,30	0,31	0,24	0,63	0,63	0,02	0,14	0,20	0,18	0,21	0,34	0,22	0,19	0,23	0,48	8
9N										1,00	0,48	0,39	0,22	0,52	0,50	0,10	0,22	0,22	0,27	0,25	0,36	0,27	0,28	0,30	0,47	9N
9V											1,00	0,28	0,27	0,27	0,28	0,19	0,24	0,30	0,33	0,23	0,31	0,22	0,22	0,28	0,24	9V
10A												1,00	0,25	0,29	0,27	0,15	0,38	0,14	0,24	0,22	0,38	0,28	0,16	0,42	0,32	10A
11A													1,00	0,16	0,22	0,16	0,23	0,16	0,15	0,17	0,24	0,23	0,21	0,30	0,17	11A
12A														1,00	0,70	0,11	0,21	0,22	0,19	0,21	0,31	0,25	0,22	0,22	0,52	12A
12F															1,00	0,05	0,14	0,23	0,18	0,18	0,33	0,21	0,15	0,25	0,56	12F
14																1,00	0,36	0,19	0,19	0,16	0,18	0,18	0,22	0,22	0,04	14
15B																	1,00	0,19	0,23	0,21	0,32	0,19	0,20	0,38	0,22	15B
18C																		1,00	0,28	0,17	0,25	0,21	0,22	0,16	0,20	18C
19A																			1,00	0,54	0,25	0,12	0,17	0,21	0,19	19A
19F																				1,00	0,19	0,10	0,21	0,20	0,20	19F
20																					1,00	0,28	0,22	0,36	0,33	20
22F																						1,00	0,16	0,26	0,19	22F
23F																							1,00	0,14	0,19	23F
33F																								1,00	0,26	33F
45																									1,00	45
	1	2	3	4	5	6A	6B	7F	8	9N	9V	10A	11A	12A	12F	14	15B	18C	19A	19F	20	22F	23F	33F	45	

Supplementary Table 2. Cross-reactivity and specificity.

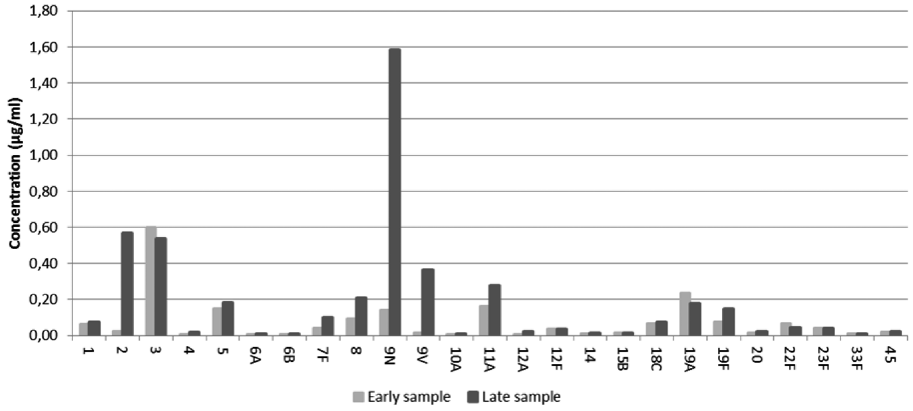
	Interrogated bead																								
	%	**1**	**2**	**3**	**4**	**5**	**6A**	**6B**	**7F**	**8**	**9N**	**9V**	**10A**	**11A**	**12A**	**12F**	**14**	**15B**	**18C**	**19A**	**19F**	**20**	**22F**	**23F**	**33F**	**45**
Inhibitor	1	99	10	10	9	13	5	12	11	11	11	13	10	13	4	16	10	10	11	14	32	15	9	15	8	10
PPS	2	14	99	16	8	17	14	16	13	14	10	13	10	12	17	24	13	11	14	13	14	18	12	15	8	15
	3	16	12	97	13	17	13	14	14	11	11	12	11	11	8	22	14	12	13	22	14	17	12	15	10	17
	4	14	13	13	98	14	7	12	12	12	11	13	11	15	3	16	53	13	10	11	11	16	9	13	10	13
	5	7	6	6	5	96	6	7	6	8	8	6	6	7	7	8	7	7	7	8	5	11	6	12	5	6
	6A	9	6	7	6	9	96	34	6	6	7	7	7	8	9	9	7	6	6	8	3	10	6	10	3	7
	6B	10	11	14	6	13	83	96	13	9	14	12	9	12	10	12	9	11	12	11	9	14	13	18	9	9
	7F	7	6	5	1	6	2	7	96	2	3	7	7	7	11	11	6	7	7	7	5	9	6	8	3	7
	8	2	5	11	-3	21	0	3	3	98	8	43	6	38	14	9	1	3	2	4	4	6	4	7	0	3
	9N	9	8	15	10	13	35	45	10	7	99	18	10	10	18	14	10	6	9	11	10	14	8	12	9	10
	9V	9	11	24	7	16	9	15	11	8	33	96	11	10	1	14	9	10	8	22	9	13	7	15	6	9
	10A	8	6	6	5	13	9	13	10	7	11	10	96	12	11	14	10	11	10	10	11	13	10	13	6	11
	11A	6	7	11	6	8	7	9	7	8	5	9	10	100	5	10	7	4	5	13	5	9	7	8	5	8
	12A	0	-3	-2	-4	-1	20	4	-3	-2	-4	0	-5	-1	99	24	-2	-1	-5	-3	-2	1	19	-1	-5	-7
	12F	2	6	11	3	8	3	6	4	5	2	6	2	11	83	95	6	6	5	3	1	10	4	8	-1	0
	14	-5	-4	-5	-6	0	-2	-4	-3	-4	-5	4	-3	-1	-11	-1	98	6	-4	-1	-5	-2	-3	-1	-6	-1
	15B	5	3	5	0	5	3	6	4	1	2	3	4	3	1	5	9	98	4	4	2	7	4	7	0	7
	18C	2	3	16	0	5	4	3	6	1	2	4	5	2	3	0	4	2	99	9	4	6	-1	5	2	8
	19A	7	6	11	1	7	3	9	4	3	5	14	5	7	4	7	4	3	8	98	32	10	5	9	2	7
	19F	5	2	14	0	9	2	6	1	2	1	4	1	3	4	3	4	3	3	23	97	6	1	6	-3	5
	20	42	1	-5	-3	3	0	1	-2	-6	0	2	-2	0	-8	2	-1	-1	-2	0	-1	98	-1	4	-3	-3
	22F	-5	-5	-15	-9	-2	-3	-1	-6	-6	-6	-3	-7	-7	-13	-5	-5	-5	-7	-7	-5	-1	100	1	-8	-6
	23F	-4	-7	-8	-9	-2	-7	-4	-5	-10	-9	-3	-7	-4	-13	-3	-4	-5	-4	-6	-7	1	-4	97	-8	-5
	33F	10	-5	0	-5	-1	-3	-4	-4	-1	6	-2	-2	-2	-10	-1	-3	-2	-2	1	-1	0	-6	2	98	-3
	45	-4	-6	5	-7	-3	-7	-3	-4	-7	-6	-3	-5	-4	-14	-1	-5	-7	-6	-4	-4	1	-5	-4	-6	97



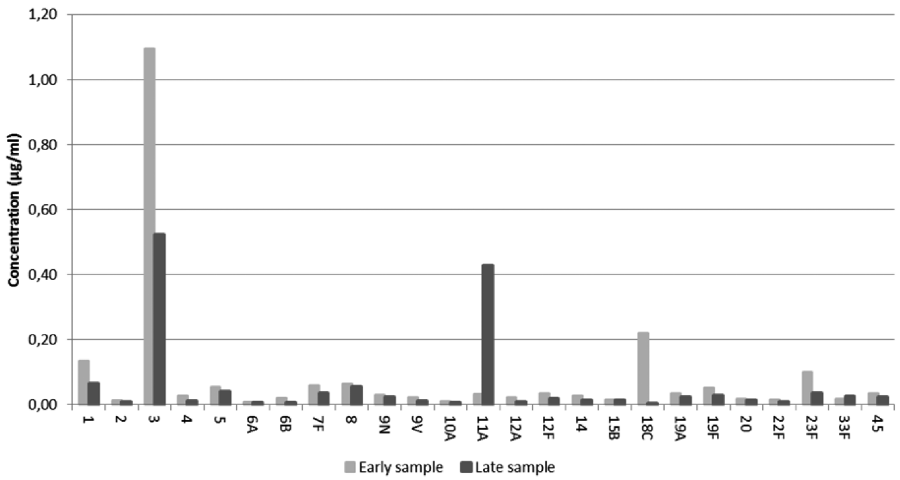
Pattern A. Single serotype-specific immune response (19A pneumococcal CAP).



Pattern B. No positive immune response against any serotype (fold-increases all below two).

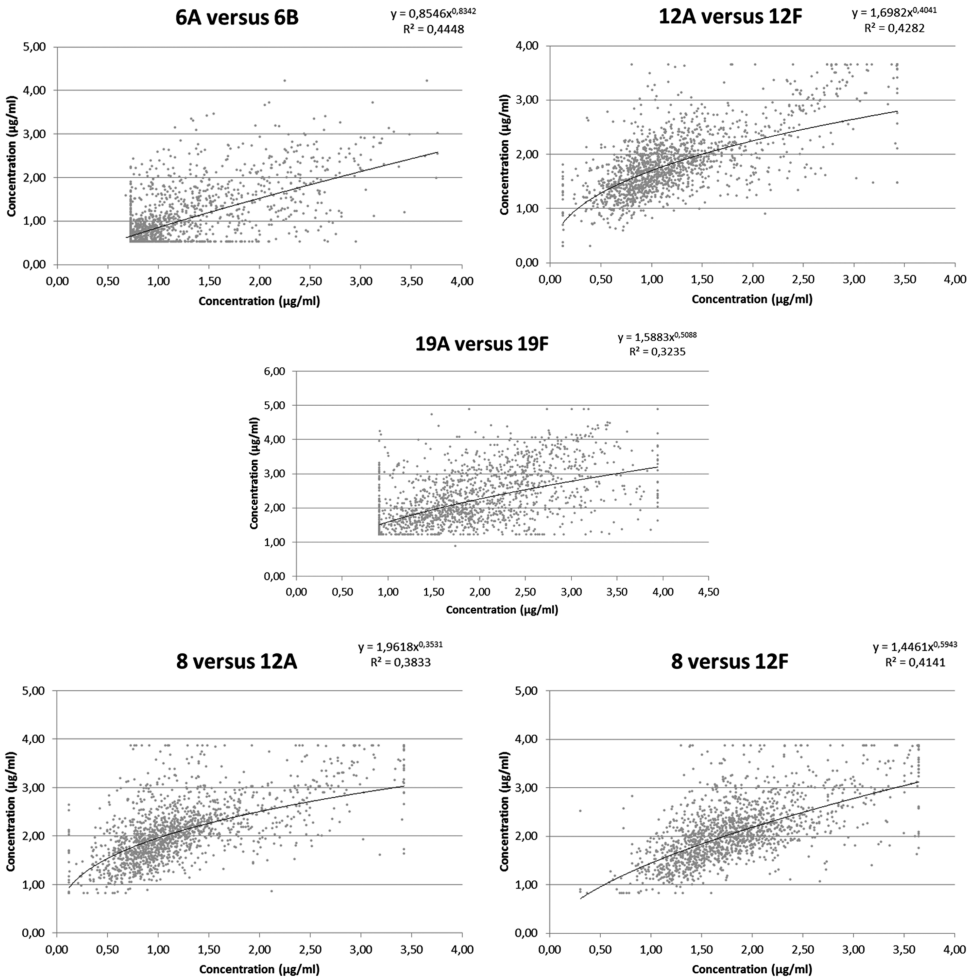


Pattern C. No single serotype-specific immune response (antibody concentration has increased for 3 serotypes: 2, 9N and 9V).

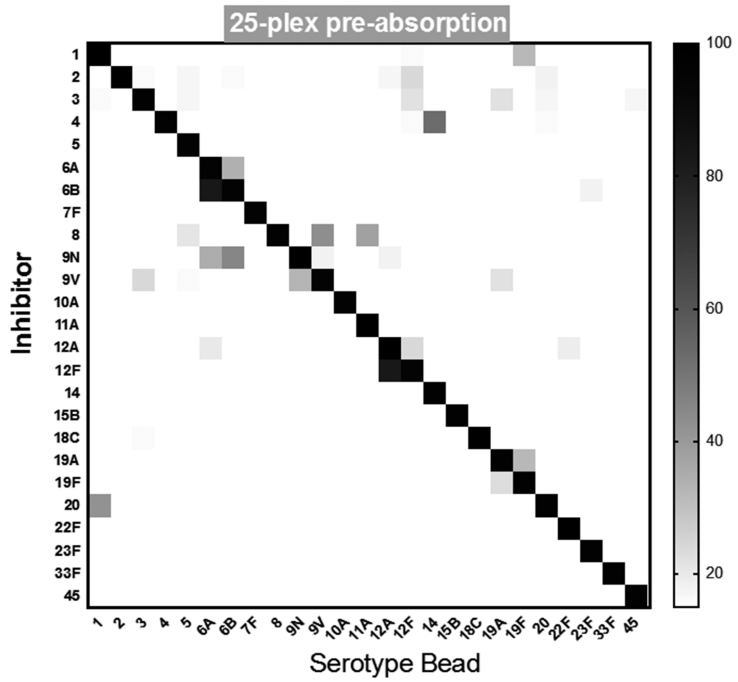


Pattern D. Indistinct antibody dynamics (increase in concentration of antibodies specific for 11A, decrease in antibodies specific for 3 and 18C).

Supplementary Figure 1. Serotype-specific antibody concentration (µg/ml) dynamics - examples of response patterns in individual patients.



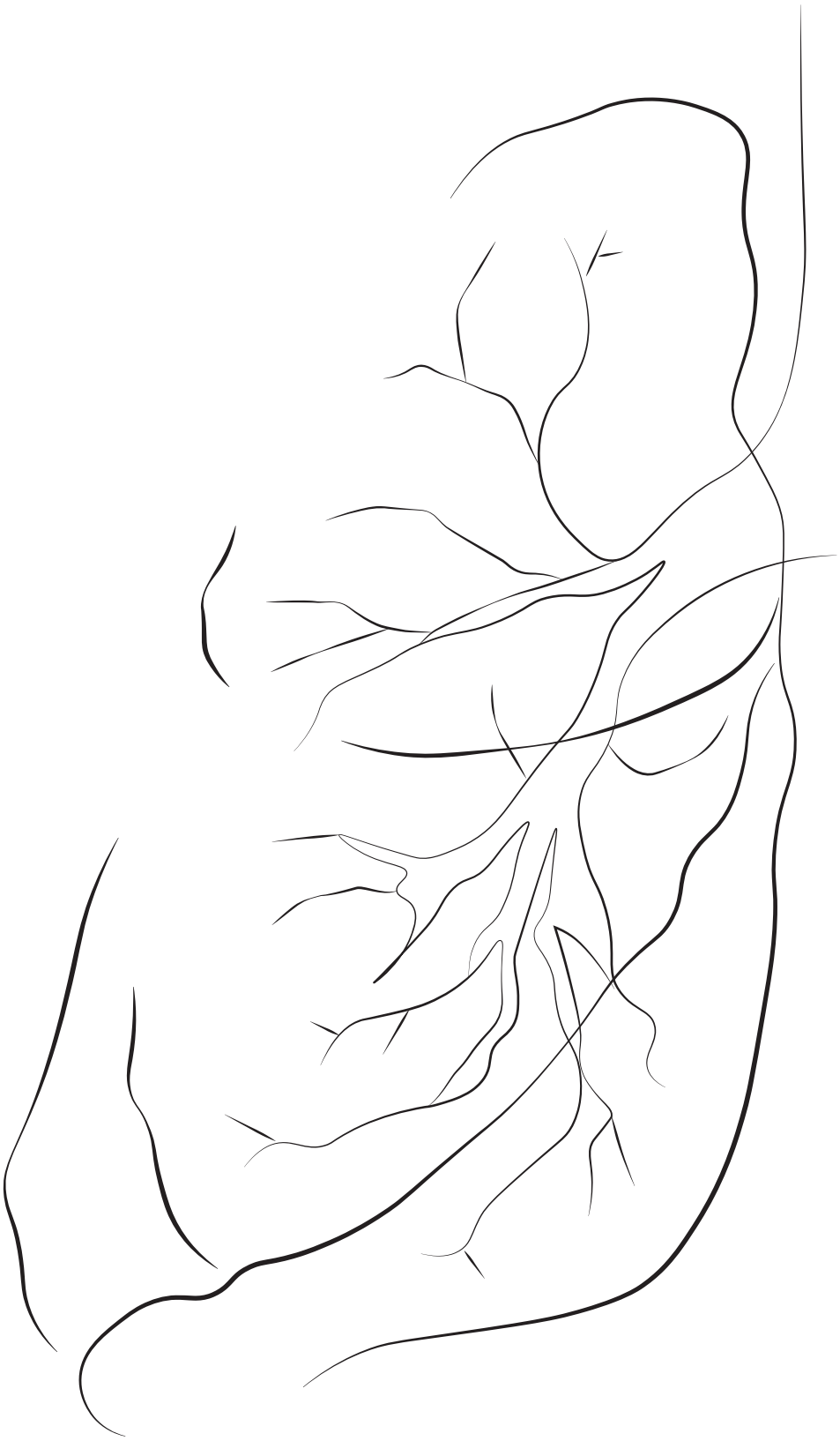
Supplementary Figure 2. Scatterplots of log-transformed serotype-specific antibody concentrations (ng/ml).



Supplementary Figure 3. Cross-reactivity and specificity.

PART III

Adjunctive corticosteroid therapy and prognostic biomarkers



CHAPTER 7

Adjunctive treatment with oral dexamethasone in adults hospitalized with community-acquired pneumonia: a randomized clinical trial

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Submitted

Abstract

Introduction

Adjunctive treatment with intravenous dexamethasone has shown to reduce length of stay in adults hospitalized with community-acquired pneumonia. Whether this beneficial effect extends to oral dexamethasone is not known. Furthermore, it remains unclear what patients will benefit most from adjunctive corticosteroid treatment. We aimed to assess the effect of oral dexamethasone on length of hospital stay and whether this effect is modified by pneumonia severity.

Methods

This multicenter, stratified randomized, double-blind, placebo-controlled trial was performed from December 2012 through November 2018 in four large teaching hospitals in the Netherlands. Immunocompetent adults hospitalized with community-acquired pneumonia were eligible for study participation. Participants were randomly assigned to receive dexamethasone (6 mg once daily p.o.) or placebo for 4 days. The primary outcome was length of hospital stay. Secondary outcomes were secondary intensive care unit admission and 30-day mortality.

Results

Among 1092 patients screened, 990 patients met inclusion criteria of whom 412 were randomized and 11 were excluded post randomization. Median length of stay was shorter in the dexamethasone group (n=203; 4.5 days (95% CI 4.0-5.0)) than in the placebo group (n=198; 5.0 days (95% CI 4.6-5.4); p=0.03). Within both pneumonia severity subgroups (Pneumonia Severity Index class I-III vs. IV-V), differences in length of stay between treatment groups were not statistically significant. Secondary intensive care unit admission rate was lower with dexamethasone (5 (3%) vs 14 (7%), p=0.03), 30-day mortality did not differ between groups. In the dexamethasone group, rate of hospital readmission tended to be higher (19 (10%) vs 9 (5%); p=0.07) and hyperglycemia (14 (7%) vs 1 (1%); p=0.001) was more prevalent.

Conclusions

A short course of oral dexamethasone reduced length of stay and secondary intensive care unit admission rate in immunocompetent adults hospitalized with community-acquired pneumonia. It remains unclear for which patients the risk-benefit ratio is optimal.

Introduction

Despite advances in antibiotic treatment and the availability of preventative measures such as vaccines, the burden of community-acquired pneumonia (CAP) remains high.[1] Therefore, non-antibiotic adjunctive therapies that modify the host response to micro-organisms remain of interest.[2]

Excessive release of cytokines in response to invading pathogens is thought to contribute to high mortality and morbidity in patients with CAP.[3] Corticosteroids have the ability to inhibit inflammation by downregulating this cytokine response.[4] Through this mechanism, adjunctive treatment with corticosteroids might result in better clinical outcomes.

Several randomized trials have shown that adjunctive corticosteroid treatment reduces length of hospital stay (LOS) and time to clinical stability in patients with CAP.[5] Most trials have studied the effects of intravenous corticosteroids such as prednisone, hydrocortisone or dexamethasone.[6–10] Intravenous dexamethasone administered during the first four days of hospitalization has shown to reduce LOS with one day.[6] Oral administration of dexamethasone has several advantages over intravenous administration as it does not hamper an early iv-to-oral switch of antibiotics, causes the patient less discomfort and carries no risk of phlebitis. Furthermore, a bioequivalence study showed that oral dexamethasone is feasible from a pharmacokinetic perspective.[11]

In literature, it is still debated which patients benefit most from corticosteroid treatment.[12] A recent individual patient data meta-analysis suggested a greater effect of corticosteroids in patients with severe CAP, as defined by a high pneumonia severity index (PSI) score.[5] So far, no randomized trial has prospectively assessed the effects of corticosteroids in pre-specified subgroups based on CAP severity.

The primary objective of this study is to investigate the effect of a short course of oral dexamethasone compared to placebo on LOS and to assess whether this effect depends on disease severity.

Methods

Study design and patients

This multicenter, stratified randomized, double-blind, placebo-controlled trial, was conducted in four non-academic teaching hospitals in the Netherlands (St. Antonius Hospital, Nieuwegein; Catharina Hospital, Eindhoven; OLVG Amsterdam; and Canisius Wilhelmina Hospital; Nijmegen). Patients presenting with CAP were screened and enrolled within 24 hours of emergency department (ED) presentation. Inclusion criteria were ≥18 years of age, presence of new opacities on chest radiograph, and two of the following signs and symptoms: cough, production of sputum, temperature >38.0 °C or <36.0 °C, abnormalities at auscultation consistent with pneumonia, C-reactive protein (CRP) >15 mg/l, white blood cell count >$10x10^9$ cells per liter or <$4x10^9$ cells per liter, or >10% of rods in leukocyte differentiation. Patients with congenital or acquired immunodeficiencies, patients receiving chemotherapy less than 6 weeks before ED presentation, receiving

corticosteroids or other immunosuppressive medication 6 weeks prior to ED presentation, requiring direct admission to the intensive care unit (ICU) at presentation, patients who had a known tropical worm infection, who were pregnant or breastfeeding, and patients with a known dexamethasone intolerance were excluded from trial participation. Written informed consent was provided by all patients. This study was approved by the Medical Ethics Committee at the St. Antonius Hospital and is registered with ClinicalTrials.gov number NCT01743755.

Randomization and masking

Eligible patients were randomly allocated (1:1 ratio) to receive either 6 mg oral dexamethasone or placebo once a day for 4 days. A previous pharmacokinetic study showed that 6 mg dexamethasone orally equals the exposure of 5 mg dexamethasone phosphate (=4 mg dexamethasone) intravenously, as studied in the trial by Meijvis et al.[6,11] Randomization was performed in blocks of 4 using PASW Statistics software version 18.0.03. Patients were stratified by enrolling center and CAP severity. To that end two subgroups were defined: non-severe CAP and severe CAP. Non-severe CAP was defined as PSI class I-III and severe CAP was defined as PSI class IV-V.[13] Randomization was set up to ensure that in each CAP severity subgroup 50% of patients received dexamethasone and 50% of patients received placebo. After randomization patients were assigned a medication kit number using a central computer assisted allocation system. Corresponding coded medication kits containing four tablets of 6 mg dexamethasone or placebo were available at the ED of each of the participating hospitals. Patients, treating physicians, and investigators were masked to treatment allocation.

Procedures

Eligible patients were enrolled by a research physician or research nurse. Patients in the dexamethasone group received 6 mg of oral dexamethasone (TioFarma BV, Oud-Beijerland, the Netherlands) once a day for four days and patients in the placebo group received one placebo tablet (TioFarma BV) once a day for four days. Study treatment was initiated within 24 hours of ED presentation. Baseline blood samples for blood chemistry testing and hematology were obtained before initiation of study treatment in the ED as part of standard care. Measurements included CRP, electrolytes, glucose, renal function, and a complete blood count. All patients were treated with antibiotics prior to receiving the first dose of study medication. Decisions regarding antibiotic type, route of administration, and treatment duration were made by the treating physician and were based on Dutch national guidelines.[14,15] Microbiological testing included sputum cultures, blood cultures, polymerase chain reaction (PCR) assays for respiratory viruses and atypical pathogens, and urinary antigen tests for the detection of *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae*. The decision to transfer a patient to the ICU or to discharge a patient was made by the treating physician. The general rule for discharge in all four hospitals was that

patients were clinically stable (improvement of shortness of breath, consistent decrease in CRP concentrations, absence of hyperthermia or hypothermia, adequate oral intake, and adequate gastro-intestinal absorption) and in well enough condition to leave the hospital. Baseline characteristics included comorbidities, medical history and variables necessary to calculate the PSI score.[13]

Outcomes

The primary outcome was LOS measured in 0.5 days and was calculated from time of ED presentation to the day of discharge, day of ICU admission, or day of death. If the patient was admitted to the ED before 12:00 h, day of presentation was counted as 1 day. If the patient was admitted to the ED after 12:00 h the day of ED presentation was counted as 0.5 days. The discharge date was defined as the date that a patient was medically ready for discharge (hereby excluding waiting time for admission to a nursing home). Time of discharge was set at 12:00 h for all patients as patients are generally discharged late morning or early afternoon depending on ward logistics. Secondary outcomes were admission to the ICU after initial admission to the general ward and all-cause mortality within 30 days of hospital admission.

Statistical analyses

Sample size estimation was based on our hypothesis that dexamethasone could reduce median LOS in all patients with CAP by 1 day and reduce median LOS in patients with severe CAP by 2 days. With sample data pseudo-randomly generated from available data from our previous trial[6], and assuming that 50% of patients have severe CAP, it was simulated that 300 patients were needed in each arm to provide >80 percent power maintaining a type 1 error rate of 0.05 (two-sided).

The Kaplan-Meier method was used to calculate median LOS for each treatment group and to assess the difference in LOS between treatment groups by analyzing time to discharge. An unadjusted hazard ratio with 95% confidence interval (CI) was calculated using Cox proportional hazards regression. Patients who died, were admitted to the ICU, or were transferred to a different hospital were censored seeing as these patients would have a short LOS which could lead to an incorrect representation of the median LOS. For the Kaplan-Meier method, a Gehan-Breslow-Wilcoxon test was used as this test emphasizes early differences.[16] Differences in secondary outcomes between the two treatment groups were analyzed with a chi-squared test and risk ratios were calculated, a two-tailed p value <0.05 was deemed significant. Statistical analyses were performed in IBM SPSS version 24.0. The primary analysis was performed according to the intention-to-treat principle after which the analysis was repeated in the per-protocol population. Patients who missed one or more doses of study medication while admitted the general ward, whose diagnosis was altered, with exclusion criteria unknown at time of study entry, or who were discharged on the day of study entry were excluded from the per-protocol analysis. The following

predefined subgroup analyses were performed: (1) CAP severity (non-severe CAP vs severe CAP) (2) Initial CRP level at ED presentation (above median vs below median) and (3) *S. pneumoniae* urinary antigen test result.

Categorical variables are shown as number (%). Continuous variables are presented as median [IQR] or mean (SD) for variables with a non-parametrical or parametrical distribution, respectively.

Interim analyses to monitor the frequency of serious side-effects related to either dexamethasone or placebo were pre-planned at 200, 400 and 500 patients. The analyses and the review of the results were performed by an external independent data safety and monitoring board.

Results

From December 23rd 2012 to November 28th 2018, 1092 patients were screened for eligibility. Screening logs were not available for one hospital. 412 patients were randomly allocated to receive either dexamethasone or placebo of which 11 were excluded post-randomization (Figure 1). The study was prematurely terminated after the second interim analysis due to a slower inclusion rate than anticipated combined with a lower 30-day mortality and shorter LOS than used in our sample size calculation. Because there was no difference in 30-day mortality between treatment groups we did not expect different outcomes at 600 patients. The independent data safety and monitoring board found no ground for early termination based on safety concerns.

There was no difference in baseline characteristics between the intervention and the placebo group (Table 1). The mean PSI score calculated for all patients was 81 (±29 SD). The severe CAP subgroup consisted of 156 (39%) patients. In patients with non-severe CAP the dexamethasone subgroup had a higher median CRP. In the severe CAP subgroup diabetes mellitus was more frequent in the placebo arm compared to the dexamethasone arm (Table 1). A causative pathogen was identified in 176 (44%) patients. *S. pneumoniae* was the most frequently identified pathogen (n=75, 19%) followed by *Legionella* species (n=27, 7%). Microbiological etiology and antibiotic treatment are presented in Supplementary Table 1 and Supplementary Table 2. There was no difference in distribution of causative organisms and initial antibiotic treatment between treatment groups (Supplementary Table 1, Supplementary Table 2).

In the intention-to-treat population, median LOS was 0.5 days shorter in the dexamethasone group (4.5 days (95% CI 4.0 to 5.0)) than in the placebo group (5.0 days (95% CI 4.6 to 5.4)) (Table 2). Kaplan Meier analysis of LOS showed a significant difference between treatment groups (p=0.03, Figure 2). Although non-statistically significant, in the non-severe CAP subgroup LOS was 1.0 days shorter in the dexamethasone group compared to the placebo group (Table 2, Figure 3). There was no difference in LOS between treatment groups in the severe CAP subgroup (Table 2, Figure 3). Results were similar in the per-protocol population (Supplementary Table 3).

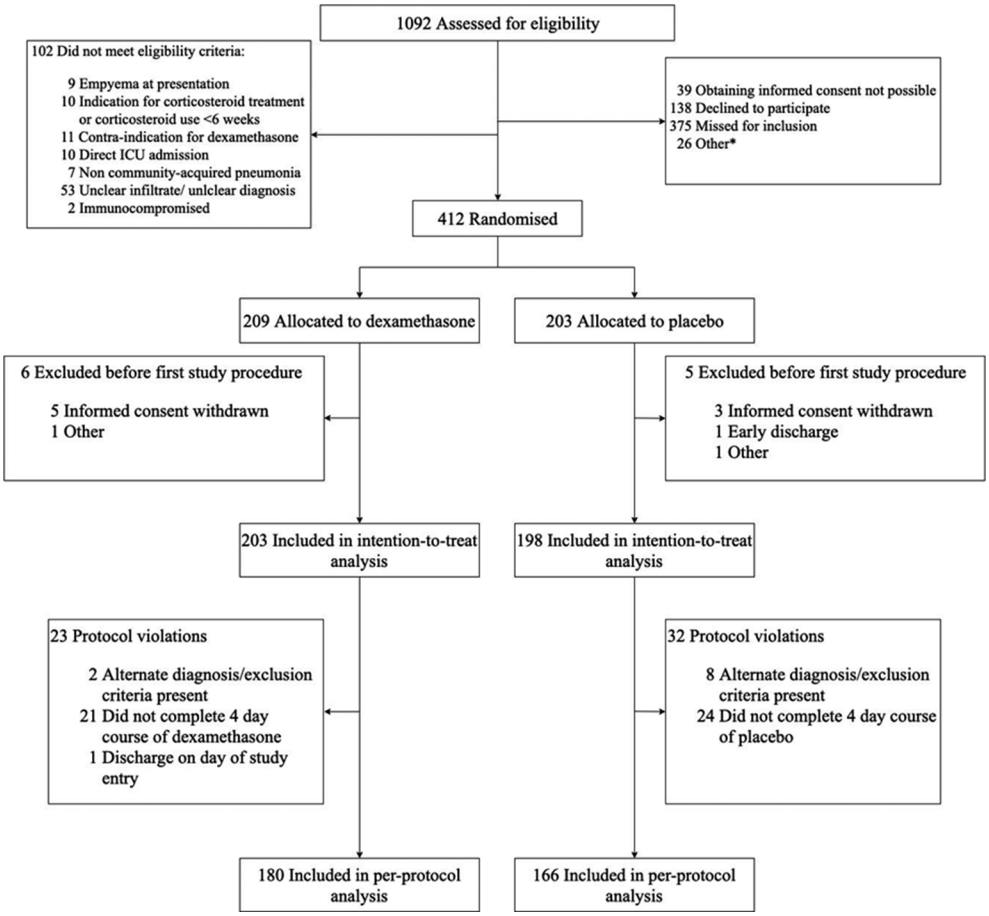


Figure 1. Study profile. No patient was lost to follow-up before reaching the primary endpoint. * e.g. Transferred to another hospital, or patient opting for palliative care.

For secondary outcomes, the secondary ICU admission rate was lower in the dexamethasone group (n=5, 3%) than in the placebo group (n=14, 7%; p=0.03). Respiratory failure was the most common reason for ICU admission (Supplementary Table 5). The 30-day mortality rate did not differ between both treatment groups (Table 2). Causes of death are shown in Supplementary Table 6. In the non-severe CAP subgroup no patients in the dexamethasone arm were admitted to the ICU, compared to 6 (5%) patients in the placebo arm (p=0.011). There were no differences in secondary endpoints between treatment groups in the severe CAP subgroup (Table 2). All the above reported results for the intention-to-treat population were similar in the per-protocol population (Supplementary Table 3).

Results of predefined subgroup analyses are presented in Supplementary Table 4. Patients with an initial CRP above median (≥ 210 mmol/l) receiving dexamethasone had

Table 1. Baseline characteristics of enrolled patients.

	All patients		PSI I-III		PSI IV-V	
	Placebo (n=198)	**Dexamethasone (n =203)**	**Placebo (n=119)**	**Dexamethasone (n = 126)**	**Placebo (n=79)**	**Dexamethasone (n =77)**
Men	120 (61)	116 (57)	58 (49)	63 (50)	62 (79)	53 (69)
Age (years)	67 [54-76]	68 [57-76]	61 [44-69]	61 [50-70]	77 [68-83]	76 [69-83]
Ethnicity						
Caucasian	186 (94)	197 (97)	111 (93)	122 (97)	75 (95)	75 (97)
Other	11 (6)	6 (3)	7 (6)	4 (3)	4 (5)	2 (3)
Elderly home resident	1 (1)	6 (3)	0 (0)	2 (2)	1 (1)	4 (5)
Current smoker	45 (23)	53 (26)	26 (22)	39 (31)	19 (24)	14 (18)
Antibiotic treatment prior to admission	57 (29)	56 (28)	40 (34)	35 (28)	17 (22)	21 (27)
Comorbidities						
Neoplastic disease	6 (3)	8 (4)	1 (1)	0 (0)	5 (6)	8 (10)
Liver disease	2 (1)	2 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Congestive heart failure	17 (9)	20 (10)	4 (3)	4 (3)	13 (17)	16 (21)
Renal disease	27 (14)	32 (16)	6 (5)	7 (6)	21 (27)	25 (33)
Diabetes Mellitus	47 (24)	41 (20)	14 (12)	22 (18)	33 (42)	19 (25)
Chronic obstructive pulmonary disease	35 (18)	40 (20)	20 (17)	22 (18)	15 (19)	18 (23)
Physical examination findings						
Temperature (^{0}C)	38.3 (1.2)	38.4 (1.1)	38.3 (1.1)	38.4 (0.9)	38.3 (1.3)	38.4 (1.3)
Systolic blood pressure (mmHg)	128 (22)	130 (22)	127 (20)	131 (18)	121 [112-147]	130 [104-148]
Heart rate (beats per minute)	98 [87-110]	99 [87-111]	98 [90-110]	100 [90-111]	98 (20)	98 (23)
Respiratory rate (breaths per minute)	20 [18-25]	20 [16-25]	21 (5)	20 (5)	23 (7)	23 (7)
Altered mental status	14 (7)	13 (6)	0 (0)	1 (1)	14 (18)	12 (16)

Table 1. (continued)

	All patients		PSI I-III		PSI IV-V	
	Placebo (n=198)	**Dexamethasone (n =203)**	**Placebo (n=119)**	**Dexamethasone (n = 126)**	**Placebo (n=79)**	**Dexamethasone (n =77)**
Inflammatory parameters						
C-reactive protein (mg/L)	198 [82-309]	211 [86-330]	190 [8-291]	249 [131-336]	203 [61-323]	153 [41-314]
White-blood-cell count (10^9 cells per L)	12.6 [9.8-17.6]	13.2 [10.1-17.8]	12.5 [9.6-17.6]	13.6 [10.2-18.3]	12.9 [10.0-17.1]	12.8 [10.0-16.8]
Pneumonia severity index score	82 (29)	81 (29)	69 [52-76]	65 [52-76]	106 [97-115]	106 [97-120]
Pneumonia severity index risk class						
Class 1	25 (13)	27 (13)	25 (21)	27 (21)	-	-
Class 2	40 (20)	55 (27)	40 (34)	55 (44)	-	-
Class 3	54 (27)	44 (22)	54 (45)	44 (35)	-	-
Class 4	70 (35)	64 (31)	-	-	70 (90)	64 (82)
Class 5	9 (5)	13 (6)	-	-	9 (11)	13 (17)

Table 2. Overview of primary and secondary endpoints for the intention-to-treat population.

Endpoint	Dexamethasone (n=203)	Placebo (n=198)	Hazard ratio or risk ratio (95% CI)	p-value
Length of stay (days)				
All patients	4.5 (4.0 to 5.0)	5.0 (4.6 to 5.4)	HR 1.14 (0.93 to 1.39)	0.03*
PSI class I-III	4.0 (3.6 to 4.4)	5.0 (4.5 to 5.5)	HR 1.19 (0.92 to1.54)	0.07*
PSI class IV-V	5.5 (4.6 to 6.4)	6.0 (5.1 to 6.9)	HR 1.06 (0.76 to 1.48)	0.27*
Secondary ICU admission				
All patients	5 (3)	14 (7)	RR 0.35 (0.13 to 0.95)	0.03‡
PSI class I-III	0 (0)	6 (5)	-	0.01‡
PSI class IV-V	5 (7)	8 (10)	RR 0.64 (0.22 to1.87)	0.41‡
30-day mortality				
All patients	4 (2)	7(4)	RR 0.56 (0.17 to 1.87)	0.34‡
PSI class I-III	1(1)	2 (2)	RR 0.47 (0.04 to 5.14)	0.53‡
PSI class IV-V	3 (4)	5 (6)	RR 0.62 (0.15 to 2.49)	0.49‡

Data are median (95% CI) or number (%). ICU = Intensive care unit. PSI = Pneumonia Severity Index. HR = Hazard ratio. RR = risk-ratio. *Gehan-Breslow-Wilcoxen test. ‡Chi-squared test.

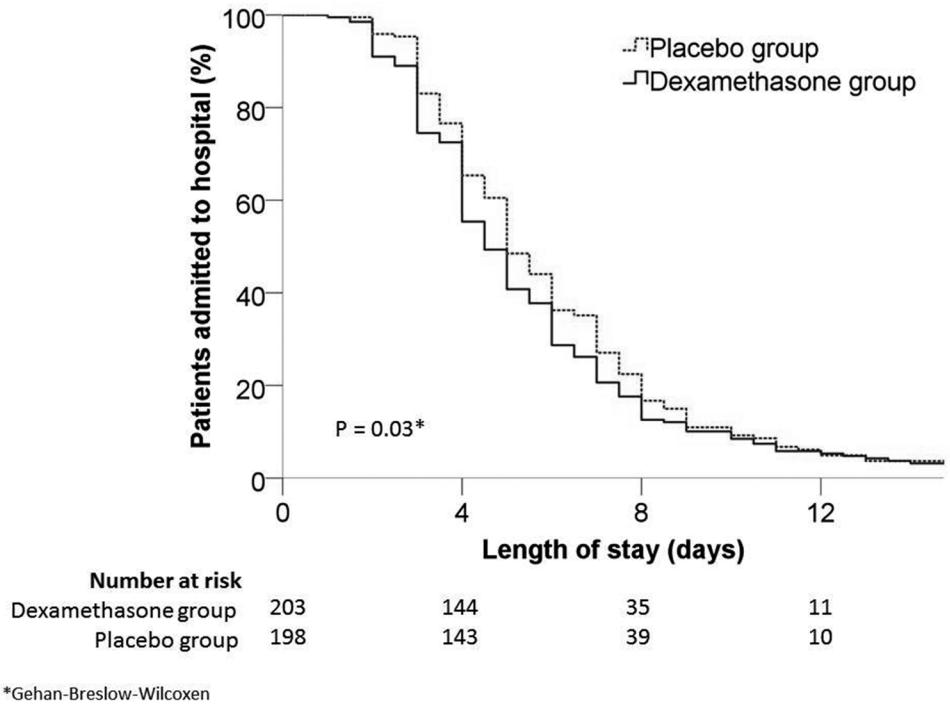
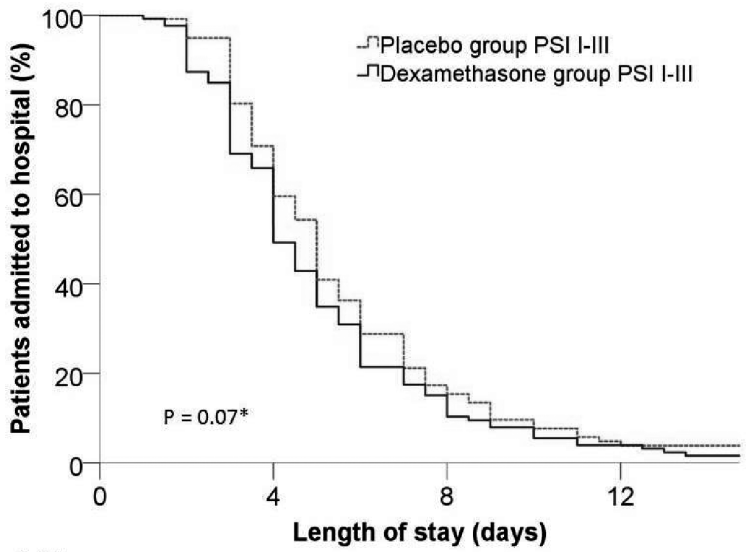
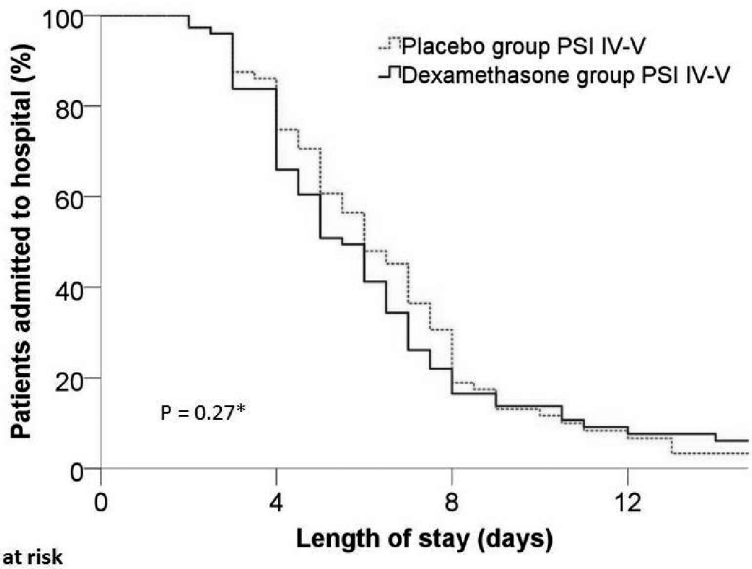


Figure 2. Kaplan-Meier analysis of the effect of dexamethasone on length of hospital stay in all enrolled patients. Patients who were admitted to the intensive care unit and/or died in hospital (n=21), and patients who were transferred to another hospital (n=2) were censored on the day of admission to the intensive care unit, day of death or the day of transfer to another hospital.



Number at risk				
Dexamethasone group	126	83	19	5
Placebo group	119	82	18	5



Number at risk				
Dexamethasone group	77	61	16	6
Placebo group	79	61	21	5

*Gehan-Breslow-Wilcoxen

Figure 3. Kaplan-Meier analysis of the effect of dexamethasone on length of hospital stay stratified according to CAP severity. Patients who died, were admitted to the intensive-care unit, or were transferred to a different hospital were censored on the day of death, the day of admission to the intensive-care unit, or the day of transfer.

a shorter LOS compared to placebo (5.0 (95% CI 4.4 to 5.6) vs 5.5 (95% CI 4.9 to 6.1), p=0.046), there was no difference in LOS between treatment groups in patients with an initial CRP below median. The rate of secondary ICU admission was also lower in the dexamethasone group compared to the placebo group in patients with an initial CRP above median (2 (2%) vs 8 (9%), p=0.03). Furthermore, length of stay was shorter in patients with a negative *S. pneumoniae* urinary antigen test receiving dexamethasone compared to those receiving placebo (4.5 (95% CI 4.1 to 4.9) vs 5.0 (95% CI 4.5 to 5.5), p=0.03).

An overview of adverse events is shown in Supplementary Table 8. Readmission rate within 30 days of study entry was higher in the dexamethasone group compared to the placebo group (19 (10%) vs 9 (5%), p=0.07). Reasons for readmission are shown in Supplementary Table 7. Hyperglycemia was reported by physicians in 14 (7%) patients in the dexamethasone group and one (1%) patient in the placebo group (p=0.001). The frequency of empyema, neuropsychiatric complaints and cardiac events did not differ between both treatment groups (Supplementary Table 8). In the placebo group, one patient required surgery for a newly diagnosed myxoma and one patient was diagnosed with HIV. Both were transferred to an academic hospital for further treatment. In the dexamethasone group, one patient had a perforated jejunal diverticulitis which required surgical intervention. This patient already had abdominal complaints before study entry. Furthermore, in the dexamethasone group three patients had an ischemic cerebrovascular accident and one patient developed deep venous thrombosis of the right leg.

Discussion

In this trial we observed a reduction in median LOS of 0.5 days in patients with CAP treated with oral dexamethasone for four days compared to controls. Furthermore, patients receiving dexamethasone had 2.8 times less the risk of being admitted to the ICU than controls.

This finding supports our hypothesis that dexamethasone reduces LOS in patients with CAP. However, a 0.5 days reduction is lower than the hypothesized 1 day reduction and lower than reported by Briel and colleagues who also found a 1 day reduction of LOS in their individual patient data meta-analysis (IPDMA) of six trials.[5] Median LOS in our study was shorter compared to all trials included in the IPDMA by Briel et al. which may explain the difference in absolute reduction in LOS. Still, the relative reduction in LOS was 10% in our trial compared to 12.5% found by Briel et al. Thus the relative effect of dexamethasone on LOS in our study was similar. The difference in overall LOS could be explained by the fact that most studies in the IPDMA used intravenous study medication which may have hampered an early iv-to-oral antibiotic switch and consequently a swiftly discharge. Furthermore, there were less patients with severe CAP in our trial compared to the two trials in the IPDMA with similar inclusion criteria (39% vs 47% and 49%, respectively).[6,17]

This is the first study to show a reduction in frequency of secondary ICU admissions in patients with CAP receiving corticosteroids. However, as respiratory failure was the main reason for ICU admission (n=14, 74%), this finding is in line with the meta-analysis by Stern et

al. who showed a lower risk of new respiratory failure in patients receiving corticosteroids.[18] In line with the IPDMA by Briel et al, we did not observe a beneficial effect of corticosteroids on 30-day mortality. Stern et al. did show corticosteroids to reduce mortality. However in that meta-analysis, small studies with an unclear allocation concealment were mainly responsible for that finding.[19–21]

Contrary to our hypothesis, we did not observe a beneficial effect of dexamethasone in patients with severe CAP. In fact, the beneficial effects of dexamethasone were more outspoken in the non-severe CAP subgroup. In the latter group, no patients receiving dexamethasone were admitted to the ICU and median LOS was 1.0 day shorter in patients receiving dexamethasone compared to those receiving placebo (although not statistically significant). This counterintuitive finding could be related to the fact that we used the PSI score to define severe CAP. PSI score is good predictor of mortality, yet PSI score does not necessarily correspond with level of inflammation. The PSI score is mainly determined by age and the presence of comorbidities. We therefore performed an additional explorative analysis using the CURB65 score. This score is based on clinical parameters, does not include comorbidities and is less influenced by age. Indeed, we found the largest reduction in LOS in patients under the age of 65 with high CURB65 scores (≥2 points) (Supplementary Figure 1). Furthermore, in a predefined subgroup analysis we found that dexamethasone reduced LOS and the rate of secondary ICU admission in patients with a CRP above median. We did not find this effect in patients with a CRP below median. Two post hoc analyses of randomized controlled trials investigating corticosteroids in CAP have also noted that patients with a high level of inflammation benefitted most from corticosteroids.[22,23] Remmelts et al. previously observed that dexamethasone was most effective in patients with a high level of pro-inflammatory cytokines combined with discrepantly low cortisol.[22] Urwyler et al. found that only a high level of pro-inflammatory cytokines predicted a positive response to steroids.[23] Consequently a prediction score based solely on the level of inflammation is of interest as it might aid identifying the subgroup of patients that would benefit most from dexamethasone.

Regarding safety, the rate of patients readmitted was twice as high as in the placebo group (5% vs 10%, number needed to harm 20). However, this difference did not reach statistical significance. Furthermore, the rate of hyperglycemia was higher in the dexamethasone group, which is in line with the pharmacology of corticosteroids and with an earlier trial.[6]

Our study has several strengths. First, it is the second largest multicenter trial assessing the effects of corticosteroids in patients with CAP and the first trial to use a stratified randomization to assess the effects of corticosteroids within subgroups based on CAP severity. Second, we studied a short course of oral dexamethasone which has several advantages over longer courses of intravenously administered corticosteroids.

There were several limitations to this study. First, the results cannot be generalized to all patients with CAP. Patients directly admitted to an ICU and thus the most critically ill patients were excluded. Second, the trial was prematurely terminated due to slower inclusion rates than anticipated. The results of the interim review of the study's data at 400

patients showed a lower 30-day mortality and shorter LOS than used in our sample size calculation. However, as there was no difference in 30-day mortality between treatment groups we did not expect different findings when the planned 600 patients would have been included. Last, the number of patients reported to have hyperglycemia is substantially lower than described by Briel et al. We cannot exclude the possibility that this is due to underreporting as the presence of hyperglycemia was based on voluntarily reporting by local research physicians instead of a structured assessment. Glucose was only measured on day 4, a time when many patients were already discharged. In hindsight, this might limit an all-inclusive benefit-risk assessment. On the other hand, the relative risk was similar to other studies.

The benefits of dexamethasone should be weighed against the risks. A 10% reduction in LOS and reduction in ICU admissions is of considerable benefit for patients and contributes to optimal deployment of health care resources. The possible rise in readmissions and a higher risk of hyperglycemia should not outweigh these benefits. Therefore, it is important to identify subgroups of patients who benefit most and/or suffer least from corticosteroid treatment. High levels of inflammatory biomarkers such as cytokines, procalcitonin, pro-adrenomedullin and a high neutrophil-lymphocyte ratio have been associated with unfavorable outcomes in CAP.[23–25] In other studies only measurement of the inflammation based on cytokine levels has shown to predict response to corticosteroids. In the present study we found that in patients with a high CRP dexamethasone had a greater effect. Future research is necessary to determine how CRP and other inflammatory biomarkers can predict response to corticosteroids, preferably using biochemical tests which are readily available and provide fast results.

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Supplementary material

Supplementary Table 1. Etiological diagnosis for all enrolled patients.

	Placebo Group (n=198)	Dexamethasone group (n=203)
Streptococcus pneumoniae	35 (18)[1]	40 (20)[2]
Legionella spp.	15 (8)[3]	12 (6)[4]
Haemophilus influenzae	8 (4)[5]	7 (3)[6]
Mycoplasma pneumoniae	6 (3)	6 (3)
Chlamydia psittaci	4 (2)	2 (1)
Staphylococcus aureus	4 (2)[7]	1 (0)[8]
Influenza A/B virus	9 (5)[9]	8 (4)
Other pathogen*	3 (2)[10]	5 (2)[11]
Other viruses‡	5 (3)	6 (3)
Unidentified	109 (55)	116 (57)

*Other pathogens: *Coxiella burnetti*, *Pneumocystis jiroveci*, *Escheria coli*, group A streptococci, *Haemophilus haemolyticus*, *chlamydia pneumoniae*, *Neisseria meningitidis*

‡Other virusses: Parainfluenza virus, Rhinovirus, Respiratory synctiel virus, human metapneumovirus (hMPV).

[1]Mixed infection with: influenza A virus (n=1), *Moraxella catarrhalis* (n= 1), hMPV (n=1), Rhinovirus (n=2), *H. influenzae* (n = 1), *H. influenza* and Rhinovirus *(n=1)*.

[2]Mixed infection with: *S. aureus* (n=1), Influenza type A (n=2), *H. influenza* (n=1), *E. coli* (n=1)

[3]Mixed infection with: hMPV (n=1), Influenza type B (n=1)

[4]Mixed infection with: *S. pneumoniae* (n=1)

[5]Mixed infection with: *S. aureus* (n=2), Influenza type A (n=1)

[6]Mixed infection with: *Klebsiella pneumoniae* and *E. coli* (n=1), Influenza type A virus (n=2)

[7]Mixed infection with: *Pseudomonas aeruginosa* and Rhinovirus (n=1)

[8]Mixed infection with: Rhinovirus (n=1)

[9]Mixed infection with: *Candida albicans* (n=1)

[10]Mixed infection with: Rhinovirus (n=1), *M. pneumoniae* (n=1)

[11]Mixed infection with: Rhinovirus (n=1)

Supplementary Table 2. Initial antibiotic regimen at time of hospital admission.

	Dexamethasone group (n= 203)	Placebo group (n= 198)
Penicillin monotherapy*	81 (40)	80 (40)
Cephalosporin monotherapy	31 (15)	28 (14)
Fluoroquinolone, macrolide or doxycycline monotherapy	5 (3)	10 (5)
Penicillin combined with a fluoroquinolone, macrolide or doxycycline	38 (19)	37 (19)
Cephalosporin combined with a fluoroquinolone, macrolide or doxycycline	36 (18)	32 (16)
Other	10 (5)	10 (5)
Unknown	2 (1)	1 (1)

Data are number (%). *Penicillin, amoxicillin or amoxicilline/clavuanicacid.

Supplementary Table 3. Overview of primary and secondary endpoints for the per-protocol population.

Endpoint	Dexamethasone (n=180)	Placebo (n=166)	Hazard ratio or risk ratio (95% CI)	p-value
Length of stay (days)				
All patients	4.5 (4.2 to 4.8)	5.0 (4.6 to 5.4)	HR 1.18 (0.95 to 1.47)	0.02*
PSI class I-III	4.0 (3.6 to 4.4)	5.0 (4.5 to 5.5)	HR 1.21 (0.92 to1.59)	0.054*
PSI class IV-V	5.5 (4.4 to 6.6)	6.5 (5.5 to 7.5)	HR 1.15 (0.80 to 1.66)	0.16*
Secondary ICU admission				
All patients	4 (2)	12 (7)	RR 0.31 (0.10 to 0.93)	0.03‡
PSI class I-III	0 (0)	6 (6)	-	0.009‡
PSI class IV-V	4 (6)	6 (9)	RR 0.65 (0.19 to 2.18)	0.48‡
30-day mortality				
All patients	3 (2)	7(4)	RR 0.40 (0.10 to 1.50)	0.16‡
PSI class I-III	0(0)	2 (2)	-	0.13‡
PSI class IV-V	3 (5)	5 (8)	RR 0.58 (0.14 to 2.33)	0.44‡

Data are median (95% CI) or number (%). ICU = Intensive care unit. PSI = Pneumonia Severity Index. HR = Hazard ratio. RR = Risk ratio. *Gehan-Breslow-Wilcoxen test. ‡Chi-squared test. Numbers analysed: PSI I-III placebo (n= 102) and dexamethasone (n=114). PSI IV-V: placebo (n= 64) and dexamethasone (n=66).

Supplementary Table 4. Overview primary and secondary endpoints for subgroup analyses.

Endpoint	Dexamethasone	Placebo	Hazard ratio or risk ratio (95% CI)	p-value
Length of stay (days)				
Initial CRP at admission				
CRP < 210 mg/l	4.5 (4.0 to 5.0)	5.0 (4.6 to 5.4)	HR 1.09 (0.82 to 1.46)	0.28*
CRP ≥ 210 mg/l	5.0 (4.4 to 5.6)	5.5 (4.9 to 6.1)	HR 1.17 (0.88 to 1.56)	0.046*
Pneumococcal urinary antigen test result				
Positive	5.0 (3.9 to 6.1)	6.0 (5.2 to 6.8)	HR 0.94 (0.55 to 1.60)	0.45*
Negative	4.5 (4.1 to 4.9)	5.0 (4.5 to 5.5)	HR 1.18 (0.94 to 1.48)	0.03*
Secondary ICU admission				
Initial CRP at admission				
CRP < 210 mg/l	3 (3)	6 (6)	RR 0.54 (0.14 to 2.11)	0.37‡
CRP ≥ 210 mg/l	2 (2)	8 (9)	RR 0.22 (0.05 to 1.01)	0.03‡
Pneumococcal urinary antigen test result				
Positive	0 (0)	0 (0)	-	-
Negative	4 (3)	11 (7)	RR 0.38 (0.12 to 1.17)	0.08‡
30-day mortality				
Initial CRP at admission				
CRP < 210 mg/l	3 (3)	5 (5)	RR 0.65 (0.16 to 2.65)	0.54‡
CRP ≥ 210 mg/l	1 (1)	2 (2)	RR 0.44 (0.04 to 4.77)	0.49‡
Pneumococcal urinary antigen test result				
Positive	0 (0)	1 (4)	-	0.26‡
Negative	4 (3)	5 (3)	RR 0.84 (0.23 to 3.06)	0.79‡

Data are median (95% CI) or number (%). ICU = Intensive care unit. HR = Hazard ratio. RR = Risk ratio. CRP = C-reactive protein. Numbers analysed (dexamethasone/placebo): CRP < 210 mg/l (96/104), CRP ≥ 210 mg/l (107/94), Positive pneumococcal urinary antigen test result (32/26), negative pneumococcal urinary antigen test result (154/161). *Grehan-Breslow-Wilcoxen test. ‡Chi-squared test.

Supplementary Table 5. Reasons for ICU admission.

Patients	Age	PSI class	Reason for ICU admission
Placebo			
1	42	3	Respiratory failure
2	82	4	Respiratory failure
3	75	3	Respiratory failure
4	81	4	Respiratory failure
5	67	3	Observation after VATS[1] for empyema
6	85	3	Respiratory failure
7	69	2	Observation after VATS for empyema
8	66	3	Respiratory failure
9	59	4	Respiratory failure
10	58	4	Respiratory failure
11	85	4	Sepsis;Hypotension
12	65	4	Respiratory failure
13	56	4	Respiratory failure
14	80	5	Sepsis; Hypotension
Dexamethasone			
1	76	4	Respiratory failure
2	52	4	Respiratory failure
3	85	5	Arrhythmia with hypotension
4	85	4	Respiratory failure
5	80	4	Respiratory failure and pulmonary hemorrhage

[1]Video assisted thoracic surgery.

Supplementary Table 6. Cause of death.

Patients	Age	PSI risk class	Cause of death
Placebo			
1	82	4	Respiratory failure; Severe legionella pneumonia
2	75	3	Respiratory failure; post-obstruction pneumonia newly diagnosed lung tumor
3*	67	3	Died after VATS# for empyema
4	58	4	Sepsis; Respiratory failure
5	85	4	Sepsis
6	77	4	Respiratory failure due to influenza pneumonia and congestive heart failure
7	84	4	Respiratory failure after opting for palliative care
8	81	4	Died 3 days after discharge; unknown cause of death
Dexamethasone			
1*	76	4	Died after ICU discharge due to multiple complications
2	80	4	Respiratory failure; Pulmonary hemorrhage
3	79	3	Strangulated femoral hernia after readmission
4	82	4	Respiratory failure; pulmonary infection and congestive heart failure
5	94	5	Died 10 days after discharge; unknown cause of death

*Died in hospital after 30 days of hospital admission. #Video assisted thoracic surgery.

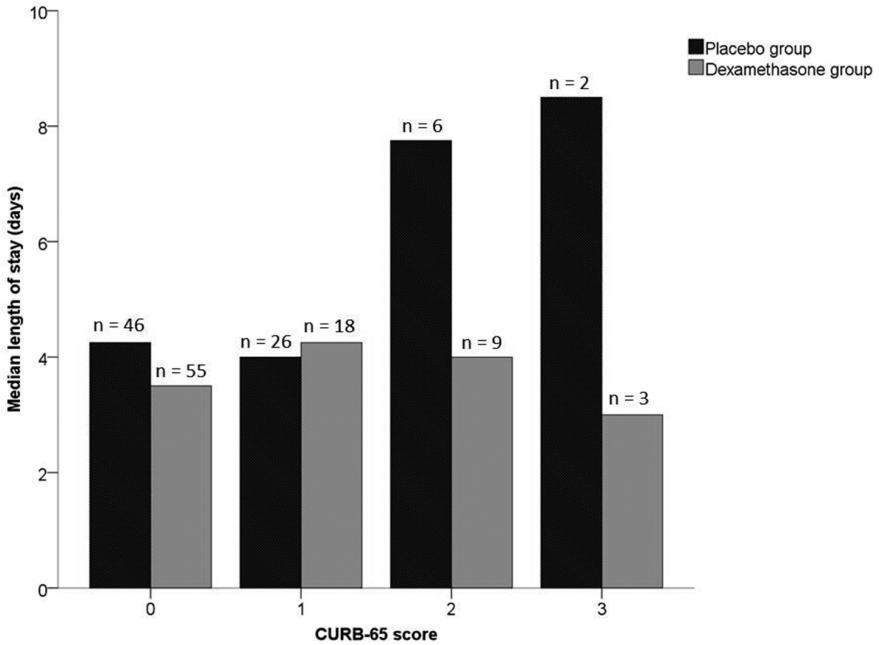
Supplementary Table 7. Reasons for readmission <30 days of admission.

Patients	Age	PSI risk class	Reason for readmission
Placebo			
1	52	3	Antrum gastritis
2	70	3	Mediastinitis
3	44	1	Hospital-acquired pneumonia; urticarial reaction to amoxicillin/clavulanic acid
4	90	4	Urosepsis
5	54	3	Relapse of pulmonary infection
6	71	4	Psychiatric complaints
7	40	1	Bronchiolitis
8	67	3	Relapse of pulmonary infection
9	71	5	Relapse of pulmonary infection
Dexamethasone			
1	79	3	Strangulated femoral hernia
2	82	4	Relapse of pulmonary infection and congestive heart failure
3	69	5	Congestive heart failure
4	74	3	Relapse of pulmonary infection
5	56	2	Altered mental status
6	84	4	Hospital-acquired pneumonia
7	61	2	Angina Pectoris
8	46	1	Relapse of pulmonary infection
9	76	4	Relapse of pulmonary infection
10	61	2	Elective cardioversion for atrial fibrillation
11	85	5	Fever of unknown origin
12	54	2	Urine retention
13	56	2	Relapse of pulmonary infection
14	61	3	Chest pain caused by pleurisy
15	61	5	Ischemic cerebrovascular accident
16	84	4	Fatigue
17	27	1	Relapse of pulmonary infection
18	71	4	Dehydration and altered mental status
19	64	4	Relapse of pulmonary infection

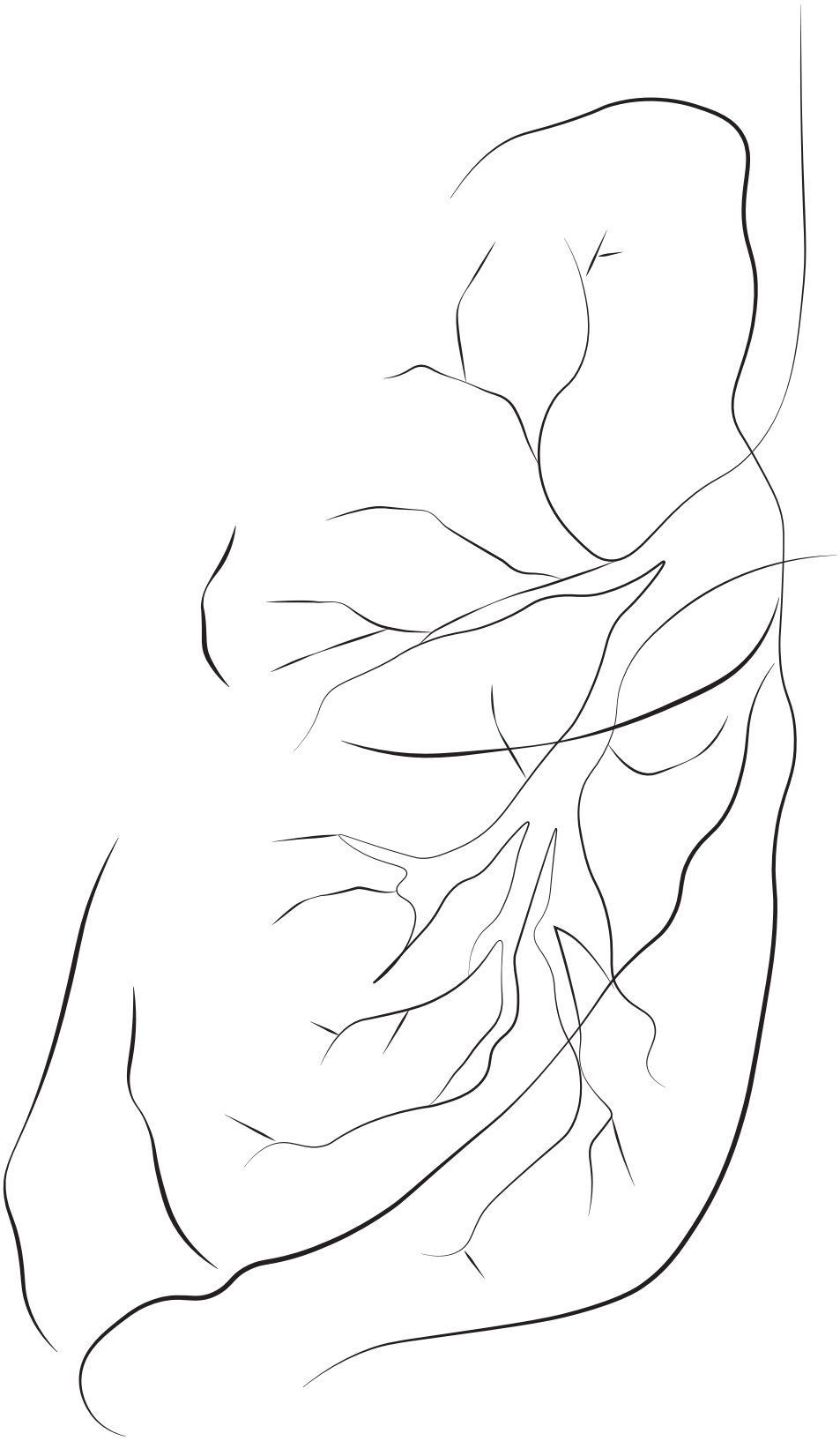
Supplementary Table 8. Overview of Adverse events.

Adverse event	Dexamethasone (n = 203)	Placebo (n = 198)	Risk ratio (95% CI)	P value*
Readmission‡	19 (10)	9 (5)	1.99 (0.92 to 4.28)	0.07
Empyema	3 (2)	5 (3)	0.59 (0.14 to 2.42)	0.45
Hyperglycaemia	14 (7)	1 (1)	13.7 (1.81 to 103)	0.001
Neuropsychiatric complaints (e.g. delirium, agitation)	10 (5)	7 (4)	1.39 (0.54 to 3.59)	0.49
Cardiac events (e.g. arrhythmia, congestive heart failure, myocardial infarction)	9 (4)†	4 (2)	2.19 (0.69 to 7.01)	0.17

Data are number of patients (%). *Chi-squared test. ‡ 201 patients analysed in the dexamethasone group and 189 patients analysed in the placebo group (Excluding missings (n=2) and patients who died in hospital (n=9)). † One patient suffered myocardial infarction and was admitted to the cardiac ward, one patient was admitted to the cardiac ward after discharge due to ongoing angina pectoris and fatigue.



Supplementary Figure 1. Length stay according to CURB-65 score and treatment group in patients under 65 years of age.



CHAPTER 8

Antipyretic effect of dexamethasone in community-acquired pneumonia

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Abstract

Adjunctive dexamethasone therapy in patients hospitalized with community-acquired pneumonia (CAP) can reduce the length of hospital stay. We questioned whether the antipyretic properties of dexamethasone contributed to this finding. Therefore we collected body temperature registrations of patients that participated in the original randomised placebo-controlled trial conducted by Meijvis et al. We found significantly lower body temperatures in patients during treatment with dexamethasone. This did not seem to affect the length of hospital stay in the dexamethasone group, because differences in length of stay between groups occurred after temperature differences had already disappeared. Our findings support the hypothesis that adjunctive dexamethasone treatment in patients hospitalized with CAP causes a faster cure of disease.

Introduction

The cornerstones of the treatment of community-acquired pneumonia (CAP) are early diagnosis and initiation of appropriate antibiotic therapy.[1] Despite prevention with vaccination, and optimal antibiotic treatment, CAP is associated with high mortality and morbidity and significant healthcare costs.[2,3] Adjunctive therapy for CAP could help to reduce disease severity, and indeed, addition of dexamethasone to antibiotic treatment in patients hospitalized with CAP has shown to reduce the length of hospital stay by one day.[4] Recent trials showed similar results.[5 6] One of the comments raised to our previous study was that the antipyretic effect of dexamethasone might be the major underlying explanation for this result.[7] In patients hospitalized with CAP, measurement of body temperature is part of standard care. Stable defervescence is one of the criteria used to define clinical stability.[8] Other clinical markers used in the decision to discharge a patient are respiratory and haemodynamic stability, the ability to maintain oral intake and a normal mental status.[9-11] Besides white cell count, C-reactive protein can be a useful a marker of treatment response.[10,11]

In a post hoc analysis, we assessed the effect of dexamethasone on body temperature in our previously conducted trial[4] and studied whether its antipyretic properties may have influenced the length of hospital stay.

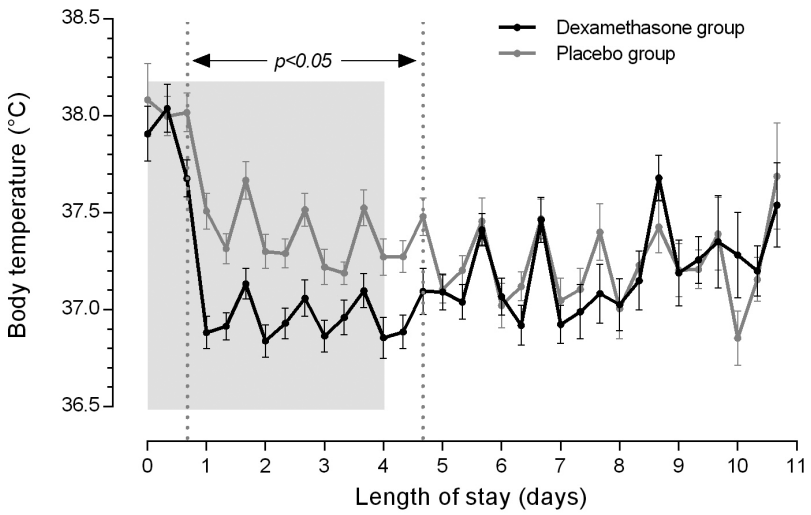
Methods

In the original clinical trial, patients hospitalized with CAP were randomised to a 4-day course of 5 mg intravenous dexamethasone or placebo, both in addition to standard care. More details of the study protocol are described elsewhere.[4] We retrospectively collected body temperature registrations from the medical charts of the study participants. Our aim was to collect three temperature measurements during each day of admission per patient. Differences in body temperature were assessed using the Students' *t* test. We analysed the time to persistent defervescence, defined as a body temperature <37.8°C for 8 consecutive hours, with the chi-square test and with the Kaplan-Meier method and a log-rank test.

Results

Body temperature registrations could be retrieved from 143 patients (94.7%) in the dexamethasone group and 137 patients (89.5%) in the placebo group. Baseline patient characteristics are evenly distributed over both treatment arms and reflect the characteristics in the original trial. The amount of patients that received antibiotic therapy prior to hospital admission did not differ between groups. Neither did the duration.

In the dexamethasone group, body temperature already started to decrease on the day of admission, whereas the decrease in the placebo group commenced one day later (Figure 1). At the end of the day of admission, mean temperature was 37.68°C (SD 1.04) in the dexamethasone group and 38.02°C (SD 1.07) in the placebo group (p=0.015,



Number at risk

Placebo group	137	134	125	112	96	86	78	60	44	40	31
Dexamethasone group	143	141	130	121	96	79	61	45	31	25	22

Figure 1. Mean morning, afternoon and evening body temperature from admission to day 10. Dotted lines show the period during which differences in body temperature between groups are significant ($p<0.05$). Gray box indicates the period of dexamethasone or placebo administration. Error bars show standard error.

95%CI 0.07-0.61). Mean time to persistent defervescence was significantly shorter in patients treated with dexamethasone (mean 0.97 vs. 1.77 days, $p<0.001$). The temperature difference between the dexamethasone and placebo group persisted until the end of day 4 of hospital stay (=24 hours after the last administration of study medication). Average body temperature from day 1 to day 4 of hospital stay was 37.3°C in the placebo group and 36.9°C in the dexamethasone group. On every day from day 0 until day 4, the cumulative proportion of patients that reached defervescence was significantly higher in the dexamethasone group. By day 4, all patients in the dexamethasone group had reached stable defervescence, while 10.3% in the placebo group still had fever ($p<0.05$, Table 1). The number of patients discharged did not diverge during the period that dexamethasone and placebo were given (Figure 1). Discharge rates did not start diverging until day 7 and 8 ($p<0.05$, Table 1).

Discussion

We found that body temperature was significantly suppressed in patients treated with dexamethasone. This resulted in a shorter time to persistent defervescence, one of the criteria used to define clinical stability.[10] There was no difference in discharge rates between the study groups during the period that a temperature difference was present. The difference in discharge rates between study groups started to occur only from day 5 onwards. At that time, body temperature did no longer differ between the two study groups.

Table 1. Cumulative percentages of patients free of fever per day of admission# and patients discharged on each day of admission.

	Dexamethasone n=143		**Placebo** n=137	
Days	**Free of fever**	**Patients discharged**	**Free of fever**	**Patients discharged**
0	15.9	0	6.4**	0
1	89.8	0	67.9**	0.7
2	98.9	0.7	79.5**	1.5
3	98.9	4.2	88.5**	4.4
4	100	12.6	89.7**	13.1
5	100	25.9	96.2¶	23.4
6	100	42	97.4	32.1¶
7	100	56.6	97.4	42.3**
8	100	64.3	100	52.6**
9	100	74.8	100	67.2
10	100	76.9	100	70.1

#: based on two consecutive readings. *: $p<0.05$; ¶: $p<0.1$, dexamethasone versus placebo.

The most likely mechanism by which body temperature is suppressed in patients treated with dexamethasone is the suppression of pro-inflammatory and –pyretic cytokines.[12,13] The courses of C-reactive protein, IL-6 and IL-10 from patients included in this study have been shown elsewhere.[4] In accordance with our findings, Snijders et al. found that defervescence (and C-reactive protein decrease) was reached faster in hospitalized patients with CAP that were treated with 40mg of prednisolone for a total of 7 days, compared to placebo (median 2 ± 1 days vs 3 ± 2, $p<0.01$).[14]

In a comment on our trial it was suggested that the "defervescence caused by the corticosteroids" might be the explanation for the reduced length of hospital stay in patients who received dexamethasone.[4,7] The fact that discharge rates started to diverge at day 7 and that by that time temperature differences had disappeared for days (Table 1), makes such an explanation unlikely. A delayed effect on discharge decision making cannot be ruled out however. Absence of hypothermia or hyperthermia was only one of the criteria used to define clinical stability in the original trial. The other criteria that were used as a rule for hospital discharge were: improvement of shortness of breath, a consistent decrease of C-reactive protein concentrations, and adequate oral intake and gastrointestinal absorption.[4] Except for C-reactive protein concentration, daily registration of these criteria was not part of the original study protocol. It was thereby commented that daily measurement of C-reactive protein may have influenced the length of stay.[15] Together with a concurrent body temperature suppression, this might have favoured dexamethasone.

The main limitation of our study is that body temperature measurements during hospital stay were not part of the original study protocol. Consequently, body temperature values could not be retrieved for all patients at every time point. However, the occurrence of missing data can be considered fully non-differential because of the randomised nature

of the trial. A point of critique to our concept of determining defervescence might be that temperature can increase again after 2 consecutive measures below 37.8°C. Only 2 patients in the dexamethasone group and 11 patients in the placebo group developed fever after being labeled having stable defervescence. Furthermore, the prescription of paracetamol, often prescribed because of its anti-pyretic effect, was not registered. We can therefore only speculate on the paracetamol effect. If any difference did occur, the prescription rate of paracetamol might be expected to be highest in the placebo group, since defervescence was more rapidly attained in patients treated with dexamethasone. It is highly unlikely that unevenly distributed use of paracetamol would account for the faster defervescence in the dexamethasone treated patients.

In summary, our findings support the hypothesis that the reduction in length of hospital stay was the result of a faster overall clinical recovery of pneumonia caused by dexamethasone, rather than a 'cosmetic' suppression of fever.

Acknowledgements

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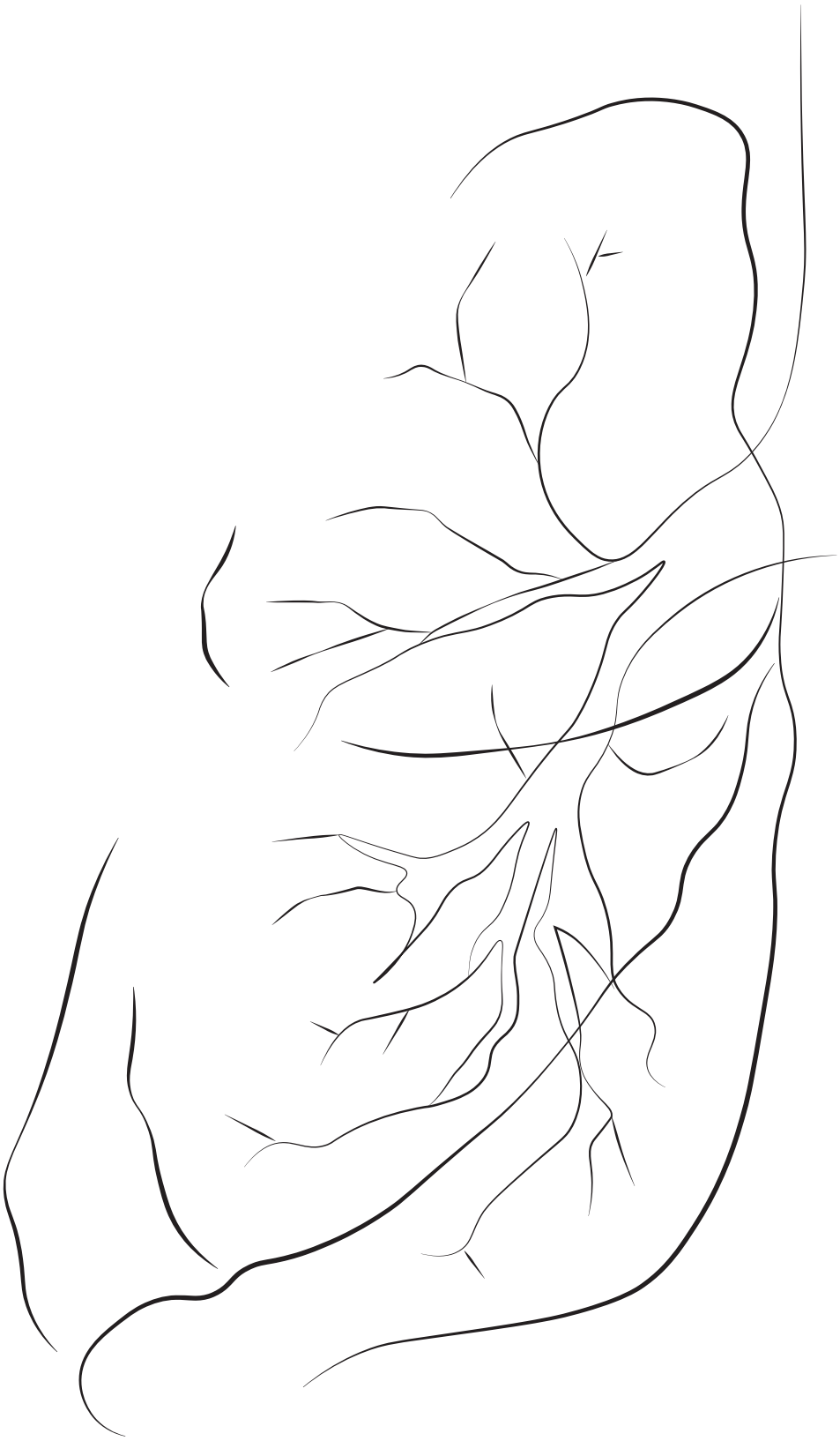

CHAPTER 9A

High-sensitivity cardiac troponin T predicts mortality after hospitalisation for community-acquired pneumonia

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Respirology, 2017

Abstract

Background and objective

Mortality after hospitalisation with community-acquired pneumonia (CAP) is high, compared to age-matched controls. Available evidence suggests a strong link with cardiovascular disease. Our aim was to explore the prognostic value of high-sensitive cardiac troponin T (cTnT) for mortality in patients hospitalised with CAP.

Methods

CTnT level on admission was measured (assay conducted in 2015) in 295 patients hospitalised with CAP that participated in a randomised placebo-controlled double-blind trial on adjunctive dexamethasone treatment. Outcome measures were short- (30-day) and long-term (4.1-year) mortality.

Results

CTnT levels were elevated (≥14 ng/L) in 132 patients (45%). Pneumonia severity index (PSI) class was 4-5 in 137 patients (46%). Short- and long-term mortality was significantly higher in patients with elevated cTnT levels. Admission cTnT level combined with PSI classification was significantly better in predicting short-term mortality (area under curve (AUC) 0.903; 95%CI 0.847 – 0.960), compared to PSI classification alone (AUC 0.818; 95%CI 0.717 – 0.919). An optimal cTnT cut-off level of 28 ng/L was independently associated with both short- and long-term mortality (odds ratios 21.9; 95%CI 4.7-101.4 and 10.7; 95%CI 5.0-22.8, respectively).

Conclusion

Elevated cardiac troponin T level on admission is a strong predictor of short- and long-term mortality in patients hospitalised with community-acquired pneumonia.

Introduction

It has been shown that both short- and long-term mortality are high after hospitalisation with CAP, compared to age-matched controls.[1-3] Exact mechanisms explaining the high mortality in CAP survivors have not yet been elucidated. A strong association with cardiovascular disease has, however, been suggested.[4,5] This theory is supported by an increased rate of cardiac complications after an episode of CAP.[6] Inflammation induced platelet activation may play an important role in the acute phase of pneumonia, linking inflammation and cardiovascular disease.[7,8]

Pneumonia severity index (PSI) and CURB-65 are clinical scoring systems which predict mortality in patients with CAP.[9,10] To further increase the predictive value of these models, biomarkers in CAP have been subject of study, including cardiac biomarkers.[11-13] For example, a recent prospective study assessed the relationship between cardiac troponin T (cTnT) elevation and in vivo markers of platelet activation in acute CAP. In this study, cTnT was elevated in 144 out of 278 (52%) of patients, of which 31 (22%) had at least one additional criteria of myocardial infarction. In 78% of cases, cTnT elevation was isolated. Markers of platelet activation were significantly higher in patients with myocardial infarction.[14] In sepsis it also has been shown that elevated cardiac troponin levels, measured shortly after admission, are associated with mortality.[15,16] It is unclear whether cTnT elevation during CAP is a reflection of myocyte damage, possibly due a temporary oxygen demand/supply mismatch or of underlying coronary artery disease.[17]

The aim of this post-hoc analysis was to explore the prognostic value of high-sensitive cTnT for mortality in hospitalised patients with CAP. We hypothesised that the cTnT level at the time of hospital admission predicts mortality in patients admitted with CAP.

Methods

Participants

Adult patients hospitalised with CAP that participated in a prior randomised double-blinded, placebo-controlled trial (NCT00471640) were included. Only patients of whom serum from time of admission was available were enrolled in this study.

The trial was conducted between November 2007 and September 2010. It was primarily designed to assess the effect of adjunctive dexamethasone treatment on length of hospital stay in adult patients with CAP. Secondary endpoints included in-hospital and 30-day mortality, intensive care unit (ICU) admission and hospital readmission. Patients were enrolled in the St. Antonius Hospital in Nieuwegein or the Gelderse Vallei Hospital in Ede, <24 hours after initial presentation at the emergency department. Inclusion and exclusion criteria from the original study are described in detail elsewhere.[18] Important exclusion criteria were an immunocompromised state, administration of chemotherapy or corticosteroids within the last 6 weeks or an indication for systemic corticosteroid therapy.

On admission, comorbidities were registered and baseline PSI score was calculated. Long-term survival status was collected by contacting patients' general practitioners and

information was verified by checking hospital records using business intelligence software. Maximum follow-up duration ranged from 4.1 – 6.9 years. We used 4.1 years for our long-term analyses. Antiplatelet therapy usage was collected from hospital pharmacist's files and patients' medical records. It was defined as current use of a platelet aggregation inhibitor on admission and prescription in the last six consecutive months prior to admission. Proof of symptoms indicating cardiac complication (chest pain, novel arrhythmia, signs of heart failure or novel ECG abnormalities) were collected from patients' medical charts. All patients gave informed consent and ethical approvals were obtained from the Medical Ethical Committee of the St. Antonius Hospital.

High-sensitive cardiac troponin T measurement

Blood samples were obtained and deep frozen (-80ºC / - 112ºF) directly after initial collection on day of admission. Serum levels were determined in all blood samples using fifth generation high-sensitive cardiac troponin T (Roche Diagnostics, Cobas 6000) assays, in accordance with the manufacturer's instructions. The assay was conducted in 2015. The 99th percentile upper reference limit of this high-sensitive assay was determined at 14 ng/L, based on manufacturers guidelines.[19] Values ≥14 ng/L were considered to be elevated.

Statistical analyses

Descriptives were stated as number (%) and continuous data were presented as mean (SD) or median [IQR]. Area under receiver-operating-characteristics curve (AUC) analysis was used to determine the cTnT cut-off level on admission with the highest combined sensitivity and specificity, using 30-day mortality as state variable. Values above this cut-off level will hereafter be named 'high'. Baseline differences between patients, based on admission cTnT level, were tested with an independent sample T-test, chi-square test or Mann-Whitney U test, where appropriate. Patients were categorised into three baseline groups based on the cTnT upper reference level and the determined optimal cut-off level for mortality prediction. Because multiple comparisons were made to evaluate baseline differences, a p-value <0.001 was applied in Table 1. Crude survival of patients with different cTnT cut-off levels on admission was calculated using Kaplan-Meier analysis with the log-rank test.

Multivariable logistic regression analyses were applied to study the association between cTnT level on admission and 30-day and long-term mortality. Potential confounding variables were selected for inclusion in the model based on rational judgment. For statistical reasons (no deaths occurred in patients with PSI class I), PSI was categorised as 1-3 and 4-5 for regression analyses. Possible outcome predictors included in the PSI were not simultaneously included in the multivariable model. An interaction between current antiplatelet therapy and high cTnT on admission could be expected and was tested statistically.

By comparing areas under the operating curve (AUC), the potential of cTnT and PSI prognostication (both separately and combined) was compared. To assess the discriminative ability of the final model, we performed receiver operating curve (ROC) analysis.

Data analysis was performed using IBM SPSS statistics, version 22, for Windows. A two-tailed p-value of <0.05 or <0.001 (where appropriate) was considered significant. Figures were created using Prism® software (GraphPad Corp., San Diego, CA).

Table 1. Baseline characteristics and outcomes of the 295 patients hospitalised with community-acquired pneumonia, based on cardiac troponin T (cTnT) level on admission.

Baseline	All patients (n=295)	1) cTnT <14 ng/L (n=163)	2) cTnT 14-28 ng/L (n=64)	3) cTnT >28 ng/L (n=68)
Male sex	167 (56.6)	83 (50.9) #	45 (70.3)	39 (57.4)
Age in years	63.6 (18.3)	54.8 (17.1) ##	72.8 (11.7)	76.1 (14.0) **
Caucasian ethnicity	286 (96.9)	160 (98.2)	63 (98.4)	63 (98.4)
Nursing home	16 (5.4)	4 (2.5)	3 (4.7)	9 (14.3) *
Smoking §	80 (27.1)	65 (43.3) #	9 (15.5)	6 (9.7) **
Comorbidities				
Chronic renal failure	27 (9.2)	2 (1.2) #	6 (9.4) ¶	19 (27.9) **
Diabetes mellitus	42 (14.2)	17 (10.4)	9 (14.1)	16 (23.5) *
Liver disease	2 (0.7)	0	1 (1.6)	1 (1.5)
Neoplastic disease	19 (6.4)	4 (2.5) #	9 (14.1)	6 (9.4) *
Chronic heart failure	49 (16.6)	10 (6.1) #	14 (21.9)	25 (15.2) *
PSI class				
Classes 1-3	158 (53.6)	124 (76.1) ##	16 (25.0)	18 (26.5) **
Classes 4-5	137 (46.4)	39 (23.9) ##	48 (75.0)	50 (73.5) **
Blood pressure				
Systolic in mm Hg	132 (22)	130 (19)	134 (25)	133 (24)
Diastolic in mm Hg	74 (12)	76 (11)	73 (13)	72 (13) *
Temperature in °C	38.2 (1.1)	38.2 (1.1)	38.1 (1.2)	38.0 (1.0)
Laboratory parameters				
CRP in mg/L	217 (140)	240 [112-342]	194 [63-280]	138 [70-296] *
WBC x10^9/L	14.2 (6.5)	14.5 (6.3)	13.6 (7.2)	13.7 (6.1)
Thrombocytes x10^9/L	260 (103)	260 (100)	261 (127)	256 (82)
Days ill before admission	5 [3-7]	5 [3-7]	5 [1-7]	4 [2-7] *
Pretreated with antibiotics at home	81 (27.5)	55 (33.7) #	13 (20.3)	13 (20.3) *
Antiplatelet therapy	76 (26.0)	28 (17.3) #	23 (35.9)	25 (37.9) *
Dexamethasone ‖	146 (49.5)	81 (49.7)	29 (45.3)	36 (52.9)

Data are presented as number (%), mean (SD) or median [IQR].
Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; PSI, pneumonia severity index; WBC, white blood count; § 24 missings; ‖ Dexamethasone was given as part of a clinical trial. Difference between group 1 and 2 are indicated by symbol #;
difference between group 1 and 3 are indicated by symbol *;
difference between group 2 and 3 are indicated by symbol ¶.
One symbol indicates a *p*-value <0.05, two indicate *p*<0.001.

Results

Patients

Serum samples for cTnT measurement on admission were available in 295 out of 304 (97.0%) patients hospitalised with CAP that participated in the original trial.[18] Median age was 67 years, 56.6% of patients were male and 27.1% were active smoker. Heart failure (16.6%) was the most common co-morbidity, 9.2% had chronic renal failure. Twenty-six percent used antiplatelet therapy on admission. In 46.4% of patients PSI class was 4-5. Median cTnT level was 12 ng/L [IQR 6-26]. In 132 patients (44.7%) admission cTnT was elevated (≥14 ng/L).

Based on our data, the calculated optimal admission cTnT cut-off level for 30-day mortality prognostication was 28 ng/L (sensitivity 0.88; specificity 0.81). The prognostic value of this 'high' cut-off level will be described in a later paragraph. Baseline characteristics of patients, categorised in three groups based on cTnT level on admission (<14 vs. 14-28 vs. >28 ng/L) are shown in Table 1.

Patients with elevated or high cTnT levels were older and generally had more comorbidities. Thereby, diabetes mellitus, chronic renal failure and heart failure rates were higher in patients with cTnT elevation. Compared to patients with elevated cTnT, significantly more patients with high cTnT level had chronic renal failure. In contrast, active smoking was more common in patients without elevated cTnT. Of patients with PSI 4-5, 36.5% had a high cTnT level on admission, compared to 11.4% of patients with a PSI 1-3 (p<0.001).

Survival

Sixteen patients (5.4%) died during admission; five of whom had cardiac symptoms in retrospect. Fifteen of 16 patients had elevated cTnT levels on admission. Nineteen patients (6.4%) died before the planned outpatient clinic visit at day 30. Forty-nine patients (16.4%) did not survive more than 1 year after hospital admission. The long-term (4.1 years) mortality rate for all-cause mortality was 32%. Differences in ICU admission and mortality rates are shown in Table 2.

Crude survival based on cTnT cut-off level is shown in Figure 1. In the current study, crude survival did not differ between the dexamethasone and placebo group. In patients with cTnT <14 ng/L, long-term mortality was significantly higher in current antiplatelet therapy users, compared to those not on antiplatelet therapy (Supplementary Figure 1). Similar trends (but no statistical significant differences) were found when comparing survival, based on antiplatelet therapy use, within the two other baseline groups.

Prognostic value of cTnT on admission

Logistic regression analysis of our data shows that high cTnT on admission is a significant predictor of both 30-day and long-term mortality (Table 3).

Besides cTnT, no variable was significantly associated with 30-day mortality. Active smoking was associated with a reduced chance of long-term mortality. No significant

Table 2. Outcomes for enrolled patients based on cardiac troponin T (cTnT) group on admission.

	All patients (n=295)	cTnT <14 ng/L (n=163)	cTnT 14-28 ng/L (n=64)	cTnT >28 ng/L (n=68)
ICU admission	16 (5.4)	5 (3.1)	3 (4.7)	8 (11.8) ¶¶
In-hospital mortality	16 (5.4)	1 (0.6)	1 (1.6) **	14 (20.6) ¶¶
30-day mortality	17 (5.8)	1 (0.6)	1 (1.6) **	15 (22.1) ¶¶
One-year mortality	49 (16.6)	5 (3.1) ##	8 (12.5) **	36 (52.9) ¶
Long-term mortality	80 (27.1)	10 (6.1) ##	21 (13.7) **	49 (72.1) ¶¶

Data are presented as number (%). Abbreviations: ICU, intensive care unit. # difference between group 1 and 2; ¶ difference between group 1 and 3; * difference between group 2 and 3 (one symbol indicates a *p*-value <0.05, two indicate $p<0.01$).

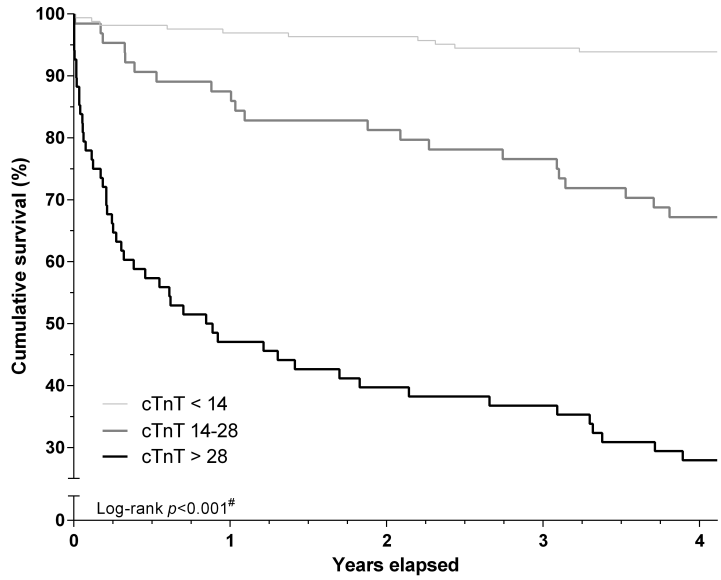

Figure 1. Crude survival based on cardiac troponin T (cTnT) level on admission. CTnT is categorised into three groups based on admission level (as in Table 1). # for difference between every group.

interaction between antiplatelet therapy and cTnT for predicting long-term mortality could be identified (β -0.95, p=0.2 for the interaction term high cTnT*antiplatelet therapy).

ROCs of cTnT level on admission and PSI class with corresponding AUCs are shown in Figure 2 and Figure 3. The combined model using cTnT and PSI, was significantly better in predicting 30-day mortality, compared to using PSI alone (Figure 2). For long-term mortality prognostication (Figure 3), the combined model (AUC 0.904; CI95% 0.868-0.940) was

Table 3. Association between high cardiac troponin T (cTnT) level on admission and mortality.

	30-day mortality		Long-term mortality	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
cTnT ng/L (≤ / > 28)	21.9 (4.7-101.4)	<0.001	10.7 (5.0-22.8)	<0.001
PSI class (1-3 / 4-5)	4.2 (0.9-20.2)	0.075	7.3 (3.3-16.0)	<0.001
Antiplatelet therapy (no / yes)	1.1 (0.4-3.3)	0.865	1.9 (0.9-3.9)	0.102
Smoking (no / yes)			0.4 (0.2-0.99)	0.49

Abbreviations: PSI, Pneumonia Severity Index.

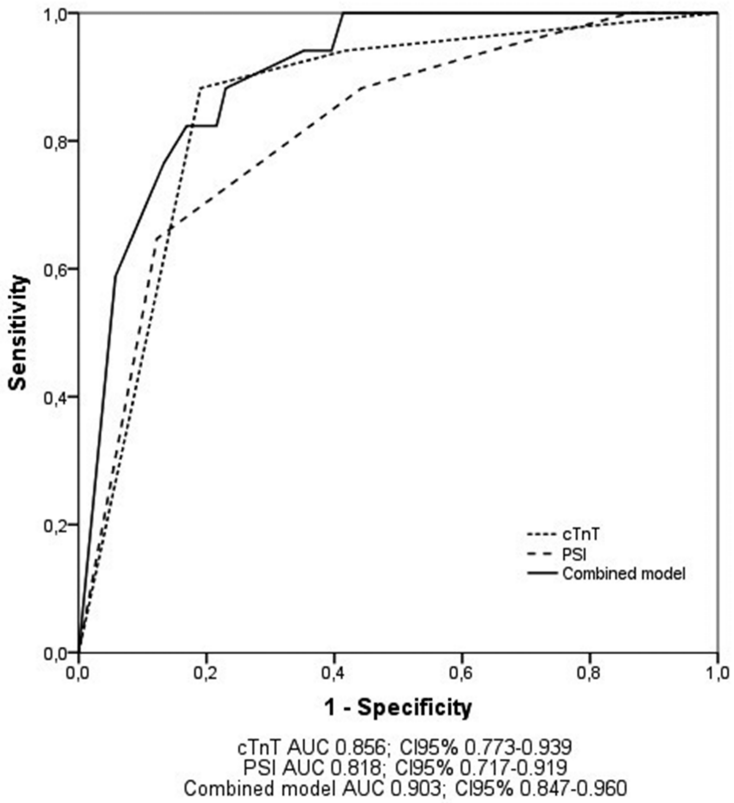

Figure 2. Operating Characteristics curve of cardiac troponin T (cTnT) on admission and the Pneumonia Severity Index (PSI) class for predicting 30-day mortality. Data on the day of admission are shown, cTnT is categorised into three groups based on admission level (as in Table 1), PSI is categorised into five classes, the final model combines the predictive value of cTnT and PSI. Abbreviations: AUC, area under the curve; CI, confidence interval; PSI, Pneumonia Severity Index.

significantly better than both PSI classification (AUC 0.829; CI95% 0.781-0.877) or cTnT alone (AUC 0.843; CI95% 0.790-0.896).

Discussion

CTnT elevation on admission with CAP is common and is a strong independent predictor of short- and long-term mortality. The prevalence of elevated cTnT (45% of enrolled patients) is in accordance with prior results in CAP that showed 52% cTnT elevation within 48 hours after diagnosing CAP.[14] Similar to our findings, Chang et al. found an association between cTnT level on admission and 30-day mortality in patients hospitalised with CAP, although these results did not remain significant after adjusting for potential confounders.[13] To the best of our knowledge, no other studies have assessed the predictive value of admission cTnT on long-term mortality in patients with CAP. In a recent study in patients with CAP it has been shown that cardiac troponin I on admission was independently associated with in-ICU

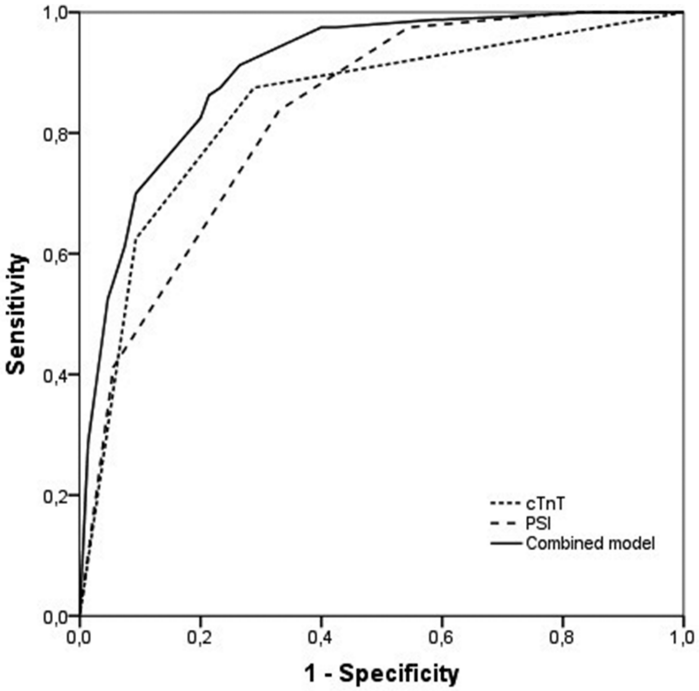

Figure 3. Operating Characteristics curve of cardiac troponin T (cTnT) on admission and the Pneumonia Severity Index (PSI) for predicting long-term mortality. Data on the day of admission are shown, cTnT is categorised into three groups based on admission level (as in Table 1), PSI is categorised into five classes, the final model combines the predictive value of cTnT and PSI. Abbreviations: AUC, area under the curve; CI, confidence interval; PSI, Pneumonia Severity Index.

mortality (adjusted hazard ratio; 1.398; 95%CI 1.005-1.945). All patients were primarily admitted to the ICU with severe pneumonia in the absence of acute coronary syndrome.[20]

In our data, cTnT was a stronger predictor of 30-day mortality than PSI, which is a validated 30-day mortality prediction score in patients with CAP.[21] Using AUC analyses, we demonstrated that cTnT on admission adds significant predictive value to the PSI for both 30-day and long-term mortality prognostication (Figure 2).

The crude analysis of our data showed that the mortality rate is significantly higher in patients with elevated cTnT levels on admission, compared to patients without cTnT elevation. The mortality rate of patients with elevated cTnT levels on admission is high, compared to a similar and well described Dutch cohort with mild-severe CAP and is especially high compared to the population control group.[3] Even though age and comorbidity rates were significantly higher in patients with elevated and high cTnT level in our cohort, cTnT remained a strong predictor of mortality in the multivariable analyses. Besides DM, all comorbidities shown in Table 1 are part of the PSI, and were therefore not used separately in the multivariable analyses. Furthermore, neither pretreatment with antibiotics, nor nursing home residency significantly influenced the model. Remarkably, cTnT elevation on admission was more common in non-smokers. This is in contrast to findings in patients with acute myocardial infarction in whom cTnT was higher in smokers, compared to non-smokers.[22] We showed that active smoking was associated with a lower long-term mortality rate in the multivariable analyses. This intriguing finding was probably the result of the exclusion of patients in need for corticosteroid therapy from the original trial. Thus, smokers who are more likely to suffer from COPD were frequently excluded from inclusion, while smokers without COPD (younger patients, less pack-years and less comorbidity) were included. This explanation is supported by the younger age of smokers compared to non-smokers (mean 55.2 (SD 15.9) *vs.* 67.2 (SD 17.9), respectively).

CTnT is used widely as a clinical predictor of myocardial damage.[23,24] Possible mechanisms causing acute cardiac injury in patients admitted with CAP (and other infections) are oxygen supply-demand mismatch or damage due to the systemic inflammatory response which may directly influence atherosclerotic plaques and the coronary arteries.[25,26] Furthermore, in vivo platelet activation markers on admission with CAP have recently been associated with myocardial infarction during hospital stay.[14] Inflammation induced platelet activation may link CAP to cardiovascular disease. Platelet activation potentially causes deterioration of pre-existing coronary artery disease, in turn leading to ischemia.[7,8,27] This hypothesis could explain our finding that elevated cTnT levels on admission with CAP are associated with short- and long-term mortality. Thus, cTnT level elevation during CAP might unveil clinically unrecognised coronary artery disease and from this perspective pneumonia may be seen as a cardiac stress test.

For this reason, further studies are needed to evaluate whether diagnostic testing for cardiac injury is warranted in patients admitted with CAP with concurrent cTnT elevation. A cardiac work up, including electrocardiographic assessment and cTnT monitoring, may be

warranted.[28] Second, treatment options to prevent mortality in patients admitted with CAP with high cTnT should be investigated as well.[29]

In our study, a trend for higher long-term mortality rates was seen in current antiplatelet users, compared to dose not on antiplatelet therapy. This finding is probably the result of more prior recognised, and thus more severe, vascular disease in current antiplatelet therapy users on admission. Cangemi et al. showed that the myocardial infarction rate was not different in current aspirin (ASA) users vs. non-ASA-users with CAP.[14] However, initiating ASA treatment in patients hospitalised with CAP with more than one risk factor for cardiovascular disease has shown to be effective in preventing acute coronary syndrome (ACS) within one month after admission. ACS occurred in 1.1% of ASA-users and in 10.6% of non-ASA-users (p=0.015). After one month mortality was 3.3% in ASA-users vs. 9.6% in non-ASA-users (p=0.151). Thereby, a significant reduction on the risk of cardiovascular death was found.[30] Studies are needed to investigate whether initiating antiplatelet therapy in patients admitted with CAP is especially effective in case of cTnT level elevation on admission.

The most important limitation of this study is that cause of death was not reported. Therefore, we could not explore a possible relationship between cause of death and cTnT. Also, patients directly admitted to the ICU were excluded. If of any influence, exclusion of these patients most likely resulted in underestimating the predictive value of cTnT for both short- and long-term mortality. It is unclear if performing the assay 5-8 years after collection of the blood samples has influenced cTnT levels. However, in 15 patients cTnT was measured for clinical reasons on admission. These levels were similar to those determined in the stored samples and reference levels have not changed since the time of initiation of the original.

In conclusion, our findings support the hypothesis that cardiac troponin T level on admission is a predictor of 30-day and long-term mortality in patients hospitalised with community-acquired pneumonia.

Acknowledgements

We acknowledge the substantial contribution of Sabine CA Meijvis, Douwe H Biesma, Hans Hardeman, Rik Heijligenberg, Hilde HF Remmelts, Heleen van Velzen-Blad and G Paul Voorn to the original trial. Furthermore, we thank Gertjan Wagenvoort (Department of Medical Microbiology and Immunology, St Antonius Hospital) for providing the data on long-term mortality.

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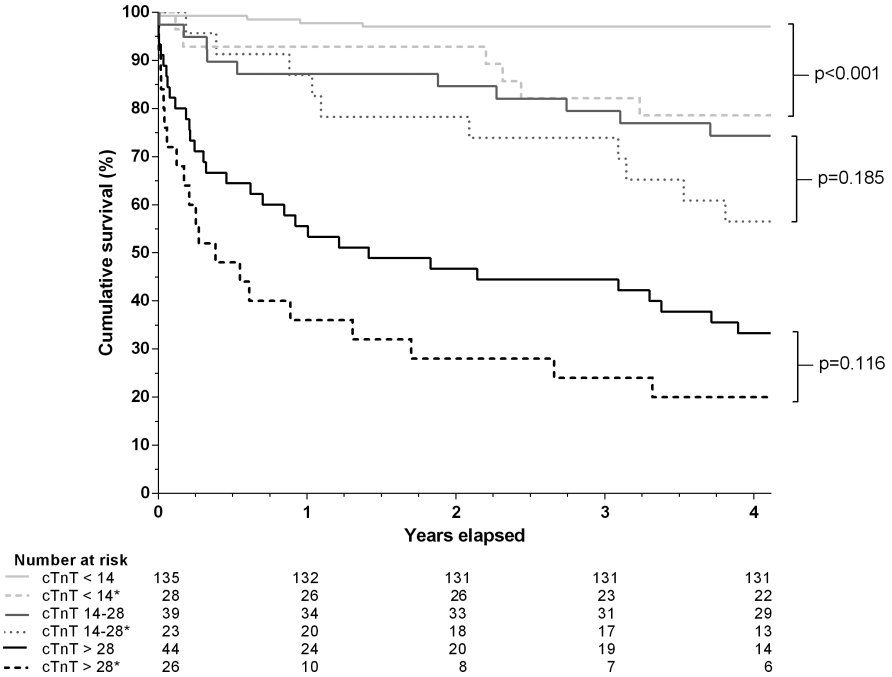
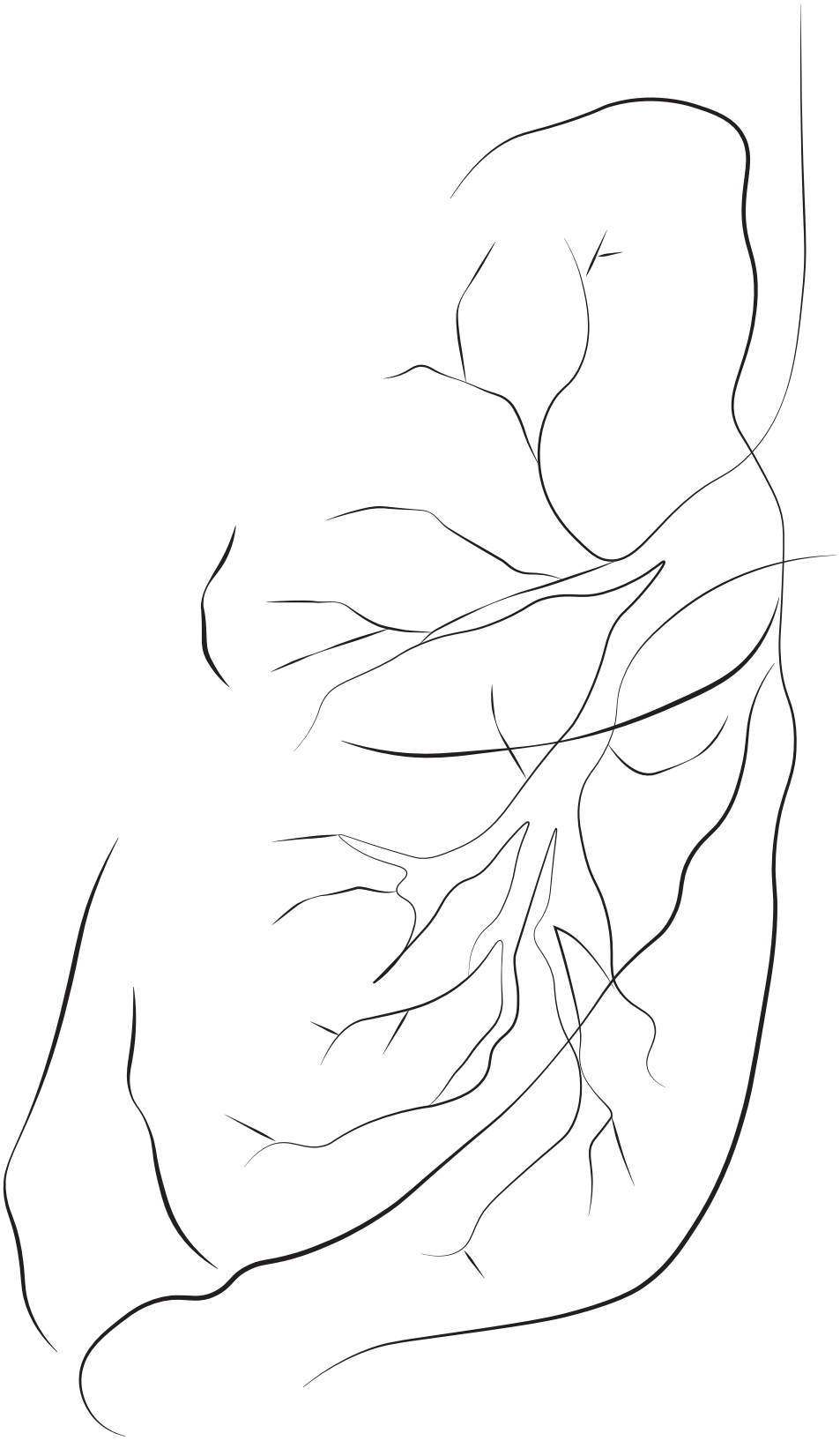
Supplementary material



Supplementary Figure 1. Crude survival based on cardiac troponin T (cTnT) level on admission and antiplatelet therapy use. CTnT is categorised into three groups based on admission level (as in Table 1). * indicates antiplatelet therapy users. Differences between groups were tested using the log-rank test.



CHAPTER 9B

Inclusion of sepsis and hypoxaemia in mortality prediction of hospitalized patients with community-acquired pneumonia – Authors' reply

Stefan M.T. Vestjens, Simone M.C. Spoorenberg,
Ewoudt M.W. van de Garde, Willem Jan W. Bos

Respirology, 2017

This letter was written as a reply to 'Inclusion of sepsis and hypoxaemia in mortality prediction of hospitalized patients with community-acquired pneumonia' by Berk Takir H et al. (*Respirology, 2018*)

We showed that elevated high-sensitivity cardiac troponin T (hs-cTnT) at the time of hospital admission predicts mortality in patients admitted with community-acquired pneumonia (CAP).[1]

Dr Berk Takir et al. argue that presence of sepsis or septic shock instead of (or due to) CAP may have influenced our findings by referring to evidence showing that the degree of sepsis-induced cardiac dysfunction is an important prognostic factor in critically ill patients.[2]

Our study was conducted in a subset (n=295) of a cohort of (n=304) patients hospitalized with CAP in a non-academic teaching hospital.[1] Direct intensive care unit (ICU) admission was an exclusion criterion. Sixteen patients (5%) were admitted to the ICU later during admission. Forty-five patients (15%) presented with a pneumonia severity index[3] (PSI) class V, of which only four patients were later admitted to the ICU. We acknowledge the assumption that secondary ICU admission might act as a surrogate of septic shock in patients with secondary ICU admission. However, given the low rate of these events it seems unlikely that this has influenced our results. We have therefore conducted a sensitivity analysis in which we excluded patients admitted to the ICU later during admission. Both Kaplan-Meier and logistic regression analyses showed very similar findings compared to the original analyses. Thus secondary ICU admission (as a surrogate of sepsis) was not an important confounder.

In addition, Dr Berk Takir et al. suggest that hs-cTnT might have functioned as a surrogate marker of hypoxemia (oxygen supply–demand mismatch), rather than an indication of myocardial ischaemia in our study. This hypothesis was mentioned in our manuscript.[1] As stated, we believe that cTnT level elevation during CAP admission might unveil clinically unrecognized coronary artery disease, which can be one explanation of the high long-term mortality rates we described. Other studies have shown evidence supporting this theory.[4,5] Furthermore, we do acknowledge that hs-cTnT levels were influenced by comorbid disease (i.e. renal failure and heart disease). In our cohort, of all patients with elevated hs-cTnT (≥14ng/L) 18.9% had chronic renal failure, 29.5% had chronic heart failure and 6.8% had both. Other proposed mechanisms potentially playing a role are inflammation-induced platelet activation[6] and indirect or direct damage caused by the *Streptococcus pneumonia* bacterium. The latter was shown in a recent study in non-human primates, demonstrating that *Streptococcus pneumoniae* has the ability to invade the myocardium, resulting in cardiomyocyte death.[7]

More detailed studies would be needed to differentiate between possible underlying mechanisms of hs-cTnT elevation in patients admitted with CAP.

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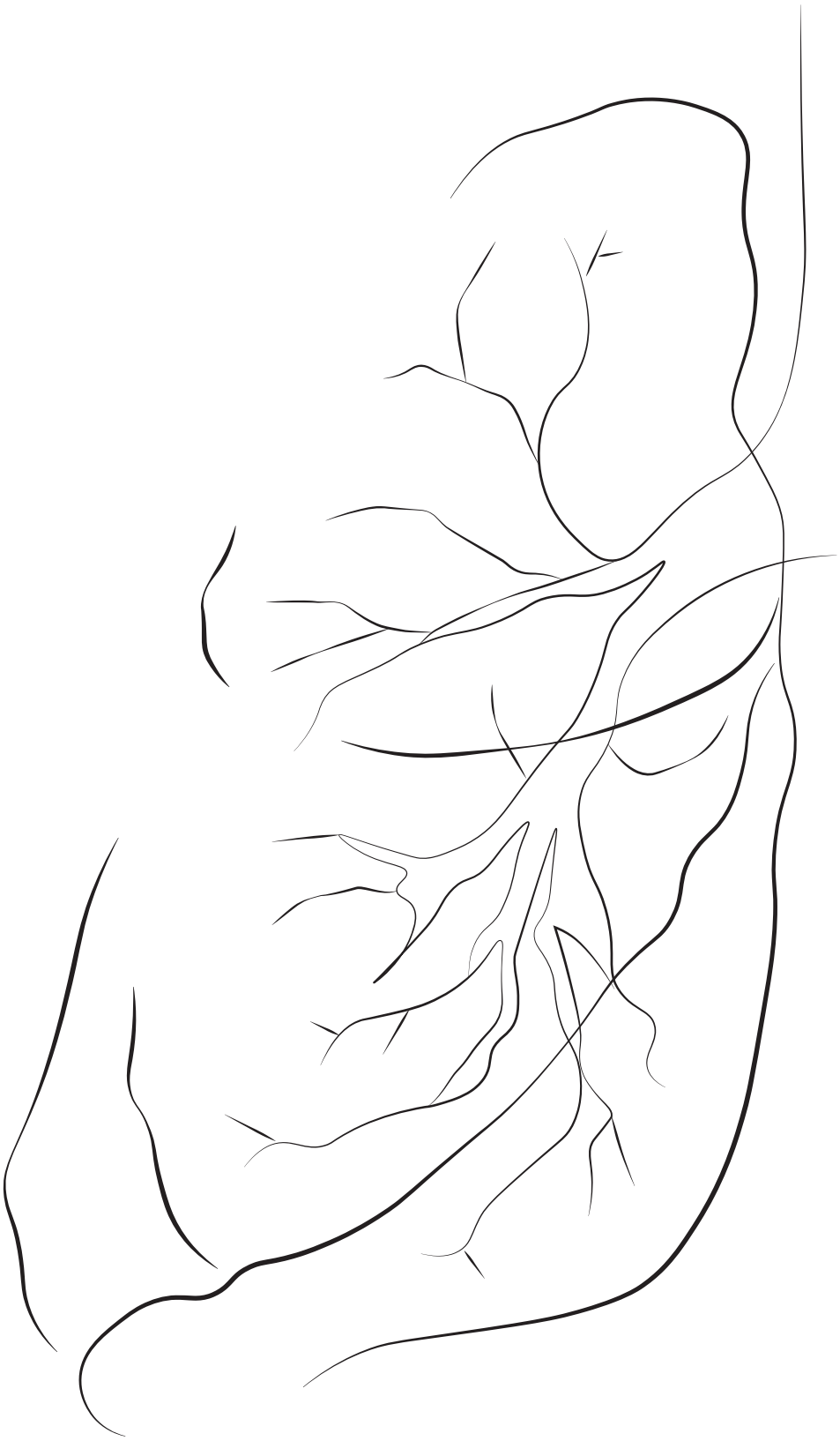

CHAPTER 10

Summary & general discussion

Summary

This thesis provides insight into the care of patients with community-acquired pneumonia (CAP), the pneumococcal serotype distribution in patients with pneumococcal CAP and the burden of invasive pneumococcal disease (IPD) in the Netherlands. The development of antimicrobial therapies and pneumococcal vaccines were important landmarks that have strongly improved the prognosis of both CAP and IPD. Despite these progresses, the mortality and morbidity of both conditions remains high.[1,2] To further improved patient outcomes, the use of adjunctive corticosteroids in hospitalized adults with CAP has been proposed. However, their role in the management of CAP is still uncertain, since the balance between their beneficial and adverse effects is delicate. Meanwhile, the relationship between CAP and cardiovascular events is becoming increasingly evident. Biomarkers for cardiovascular disease might help to identify those at risk for cardiovascular complications during or after hospitalization with CAP. With regard to IPD, the introduction of pneumococcal conjugate vaccines (PCV) in the Dutch immunization program reduced the incidence of IPD and also its case fatality rate (CFR). In less developed countries such as Bangladesh, however, the impact of the recently introduced 10-valent PCV (PCV10) remains unclear.

Part I – Proportionality of diagnostic testing

Chapter 2 indicates that large inter-hospital variation exists with regard to the extent of diagnostic testing in the management of adults with CAP. A counterintuitive inverse association between the costs for microbiological testing and antibiotic alteration was found. An interactive electronic trigger tool designed to optimize the intravenous to oral switch of antibiotics in one of the hospitals likely caused the inverse association.

Chapter 3 shows that the extent of microbiological testing is positively associated with antibiotic alteration in adults hospitalized with CAP. A PCR assay for atypical pathogens was most strongly associated with early antibiotic treatment alteration.

Part II – Pneumococci, pneumococcal vaccination and serotype-specific antibodies

Chapter 4 shows that there has been a sustained reduction of IPD incidence in children and younger adults twelve years after PCV introduction in the Netherlands. The switch from the 7-valent to the 10-valent pneumococcal conjugate vaccine (PCV7 to PCV10) did not have additional impact on the IPD incidence in older adults or on the overall case-fatality rate due to the emergence of and replacement by non-vaccine serotype IPD.

Chapter 5 shows that the proportion of *Streptococcus pneumoniae* in hospitalized patients with CAP decreased from 37% to 26% comparing the pre-PCV7 period with the PCV10 period. For other pathogens, no sustained shifts were observed. The proportion of PCV7-type disease decreased both in bacteremic and non-bacteremic patients, without shifts in CAP caused by one of the three serotypes additionally covered by PCV10.

Chapter 6 demonstrates that, before the introduction of PCV10, in Bangladeshi under-fives with CAP, 31% of cases had a serotype-specific antibody response against a single pneumococcal serotype, suggesting *S. pneumoniae* is the causative pathogen in ≥30% of cases. Within this group, only 29% was caused by a PCV10 serotype, indicating that PCV10-coverage will be low.

Part III – Adjunctive corticosteroid therapy and prognostic biomarkers

Chapter 7 describes the findings of a multicenter randomized placebo-controlled trial with a 4-day course of oral adjunctive dexamethasone in 401 immunocompetent adults hospitalized with CAP. The median length of hospital stay (LOS) was 0.5 days shorter in patients treated with adjunctive oral dexamethasone therapy. Also, the rate of secondary ICU admission was lower in patients treated with dexamethasone. In subgroup analyses in patients with a PSI class I-III and IV-V, no significant differences in LOS were present, but the rate of secondary ICU admission was significantly lower in PSI class I-III dexamethasone treated patients. There were no differences in mortality rates, but there was a clear trend towards more readmissions in the dexamethasone group. These results confirm that corticosteroids reduce the LOS in adults with CAP, but it remains unclear which patients have an optimal risk-benefit ratio.

Chapter 8 illustrates that the reduction in LOS in patients with CAP participating in an earlier placebo-controlled trial evaluating the effects of adjunctive dexamethasone therapy, was likely the result of a faster clinical recovery, rather than from "cosmetic" suppression of fever by dexamethasone.

Chapter 9A shows that elevated cardiac troponin T (cTnT) levels at the time of hospital admission with CAP are common and associated with both 30-day and long-term mortality. The mortality rate was significantly higher in patients with high cTnT levels on admission, compared with patients with normal cTnT levels. CTnT remained a strong predictor of mortality after adjustment for potential confounders. As shown in **chapter 9B**, this was still the case after excluding patients that were admitted to the ICU.

General discussion

The studies presented in this thesis give insight in the proportionality of diagnostic testing in the clinical management of CAP, whether adjunctive dexamethasone in patients hospitalized with CAP has a differential effect in certain subgroups, how body temperature is influenced by dexamethasone, and in cTnT as prognostic biomarker in CAP. In addition, this thesis addresses the impact of PCV on IPD and non-bacteremic CAP in the Netherlands, but also provides insight into the potential PCV10-coverage in young children from Bangladesh with CAP. In this general discussion, the implications of the results of the studies presented in this thesis and the opportunities for future research are discussed. This general discussion offers perspectives on the main findings in this thesis.

Part I – Proportionality of diagnostic testing

Is the extent of microbiological testing associated with antibiotic therapy alteration in CAP?

Chapter 2 shows that large inter-hospital variation exists in resource utilization in the management of adults hospitalized with CAP. The differences were particularly pronounced in the microbiological diagnostics. Furthermore, an inverse association between costs of testing and antibiotic alteration by day 3 of hospital admission was observed between hospitals. Surprisingly, the hospital with the lowest costs for (microbiological) diagnostic testing per patient had the highest rate of antibiotic alteration.

A possible explanation for this inverse association is earlier recognition of clinical improvement in the hospital utilizing the least diagnostics because of an automated advisory message, alerting a physician as to when parenteral antibiotics have been administered for >48 hours. It has been shown that interventions that promote a timely iv to oral switch of antibiotic therapy can reduced the length of iv therapy, without comprising clinical recovery.[3,4] There were no differences in secondary ICU admission between the three hospitals in **chapter 2** and patient characteristics were similar, indicating that the higher rate of early antibiotic alteration did not lead to a higher therapy failure rate. These findings support the Dutch guideline on the management of CAP stating that empiric antibiotic therapy should be de-escalated when a definitive microbiological diagnosis is made, but also when a patients has clinically improved.[5] The latter likely explains the inverse association between hospitals found in **chapter 2**. The single hospital evaluation from **chapter 3** shows that microbiological testing does facilitate an early alteration of antibiotic therapy in patients with CAP. Moreover, for each additional test performed, a stepwise increase in percentage of patients with an altered antibiotic regimen within the first 2 days of admission was found. Performing a PCR assay for atypical pathogens was most strongly associated with alteration of antibiotic treatment, likely because both positive and negative results often result in antibiotic alteration (either adding atypical pathogen coverage in case of a positive result, or removing atypical coverage in case of a negative result).

Considering the findings from both **chapter 2** and **3**, combining extensive microbiological testing with actively alerting physicians as to when parenteral antibiotics have been

administered longer than a certain amount of time seems optimal. These findings underline the value of inter-hospital comparisons. A PCR assay for atypical pathogens should be performed for at least every patient receiving 'atypical antibiotic coverage'. With the aim to facilitate early antibiotic de-escalation or directed therapy, both positive and negative atypical PCR results should be actively communicated with the clinician. For this purpose, an interactive electronic trigger tool should be implemented in current state of the art electronic healthcare records. These should also be used for sending automated messages when parenteral antibiotics have been administered >48 hours in patients admitted with CAP. Such interventions, combined with correct microbiological testing can help to reduce healthcare costs and may also reduce workload of hospital staff, complications related to intravenous (iv) access, and antibiotic resistance. Guidelines should acknowledge the importance and possibilities of ways of communicating test results to the physician.

Part II – Pneumococci, pneumococcal vaccination and serotype-specific antibodies

Invasive pneumococcal disease in the Netherlands before and after pneumococcal vaccination

Chapter 4 demonstrates that the incidence of IPD decreased in The Netherlands after the 7-valent PCV (PCV7) was introduced in the national immunization program in 2006. After the switch from PCV7 to PCV10 in 2011, a further reduction in IPD incidence was observed in children <5 years and in 18-49 years, but not in those aged 50-64 years and ≥65 years due to replacement disease by non-vaccine serotypes. Overall, the remaining proportion of PCV10-type IPD was only 11% in 2016-2018, compared to 65% in 2004-2006 before PCV7 was introduced, suggesting that the maximum impact of PCV10 has almost been reached.

The case fatality rate (CFR) decrease that was observed after PCV7 introduction (from 16% to 12%) did not continue after PCV10 was introduced. The increasing incidence of serotypes associated with higher fatality rates such as 19A, 9N and 3, and a reduction of PCV10 serotypes with a lower CFR (mainly 1 and 7F) can explain this finding. In addition, an increase in empyema was observed in the post-PCV7 period, but its incidence stabilized after the switch to PCV10 due to a reduction of PCV10-type empyema.

In 2016-2018, the three serotypes causing most IPD were in descending order 8, 19A and 3 (together 46.9%). Whereas serotype 8 is not covered by any of the currently used PCVs, serotype 19A and 3 are both covered by PCV13. At present, the available evidence indicates that both PCV10 and PCV13 are effective in reducing IPD caused by serotypes included in the particular vaccine, in both vaccinated and unvaccinated individuals (indirectly in the latter via herd immunity). However, it was shown in England and Wales, after switching from PCV7 to PCV13 in 2010, that both the direct and indirect vaccine effectiveness of PCV13 for serotype 19A was low and even non-significant for serotype 3.[6] Still, a continuous reduction in IPD incidence across all ages was observed in the first three years after the switch from PCV7 to PCV13.[6] However, from 2015 onward, replacement disease by non-PCV13 serotypes, especially occurring in adults, eroded the previous PCV13 benefit and only a small net benefit was retained in 2016-2017.

Differential impact of PCV10, and PCV13 on serotype 19A was shown recently with data from Belgium, where an increase of serotype 19A IPD was observed in children under 2 years of age two years after switching from PCV13 to PCV10 in 2015-2016.[7] Also based on data from a Swedish study comparing different counties who switched from PCV7 either to PCV10 or PCV13, a decrease of the serotype 19A incidence might be expected after PCV13 introduction.[8] There currently is insufficient evidence to determine whether there is a difference between the impact of the two vaccines on the overall IPD burden over time. Given the continuous replacement disease with non-vaccine pneumococcal serotypes, the effects of serotype replacement should be meticulously followed.

Routine PPV23 vaccination every five year of adults 60-75 years has recently been advised by the Dutch Health Council. Start of vaccination is planned for the end of 2020. The potential additional benefit of PPV23 in older adults has increased after PCV10 introduction in children, because of the expanded PPV23-coverage of emerging serotypes like 8, 9N and 12F. PPV23 is not effective in young children, but the observation that the maximum impact of PCV10 has almost been reached does raise the question whether replacement of PCV10 will be desirable in the near future. Since the added value of switching from PCV10 to PCV13 in unclear, childhood pneumococcal vaccine developers should focus on the development of non-encapsulated inactivated whole-cell vaccines or multi-component purified protein vaccines. Both could offer serotype-independent protection, but might also largely reduce carriage rates and thereby impair herd immunity formation.[9] Though, these vaccines are still years from clinical implementation. Novel higher-valent vaccines might offer a solution for ongoing replacement disease in the nearer future, also retaining indirect protection.[10]

Changes in pathogens and pneumococcal serotypes in adults with CAP

Chapter 5 shows that the proportion of *S. pneumoniae* decreased from 37% pre-PCV to 27% post-PCV10 in hospitalized adults with CAP. Although increased colonization with *Staphylococcus aureus* and *Haemophilus influenzae* has been observed in parents of vaccinated children[11,12], the ecological effects of PCVs on the microbiome did not result in a higher proportion of these pathogens in the etiology of adults hospitalized with CAP. Moreover, the distribution of the other common CAP pathogens remained relatively stable over time in these three comparable CAP cohorts.

The vast majority of pneumococcal pneumonia is non-invasive, non-bacteremic pneumonia (figure 1).[13] As vaccine policymaking is guided by impact studies focusing on IPD, potential clinically relevant changes in serotype distribution for non-invasive disease might be missed. **Chapter 5** shows that, based on the serological data from hospitalized adults with CAP, trends and shifts in the proportion of PCV-covered serotypes causing invasive and non-invasive CAP are similar. This is in line with what was found earlier.[14] The method used in **chapter 5** to detect a pneumococcal infection based on the polysaccharide antibody response cannot capture all non-bacteremic pneumococcal CAP cases, since it is limited to the serotypes included in the assay and the premise that all patients are immunocompetent.

The sensitivity of the panel to detect pneumococcal CAP was 42% compared to conventional methods, which is in line with a prior study applying this technique in adults with CAP.[15] However, this method did also detect 51 (non-invasive) cases which were not detected by conventional methods. In only 1/27 cases (3.7%) with a serotype identified by both Quelling and serology, there was a discordant serotyping result.

Within pneumococcal CAP patients, we observed a continuing decrease in the proportion of cases with CAP caused by PCV7-covered serotypes and an increase in non-PCV10 serotypes. Like in an earlier study, no significant herd-protection in non-bacteremic pneumococcal CAP was found for the three additional serotypes covered by PCV10 (serotype 1, 5, and 7F).[14] The sampling up to 5 years after introduction of PCV10 should have been long enough to observe potential herd effects on non-bacteremic pneumococcal pneumonia.[16]

Overall, the findings in **chapter 5** confirm that PCV introduction in infants does impact the microbial etiology of adults hospitalized with CAP. The most likely explanation for the significant reduction in proportion of pneumococcal CAP are herd protection of childhood PCV, resulting in a decline in vaccine-serotypes in adults and replacement by non-vaccine-serotypes with an overall lower disease potential, leading to less pneumococcal pneumonia. However, the decrease of the proportion of *S. pneumoniae* as a causative pathogen was accompanied by an increase in the proportion of patients with CAP with no pathogen identified, subsequent to a decrease in proportion of atypical pathogens, using similar diagnostic testing. However, during the same period serology was replaced

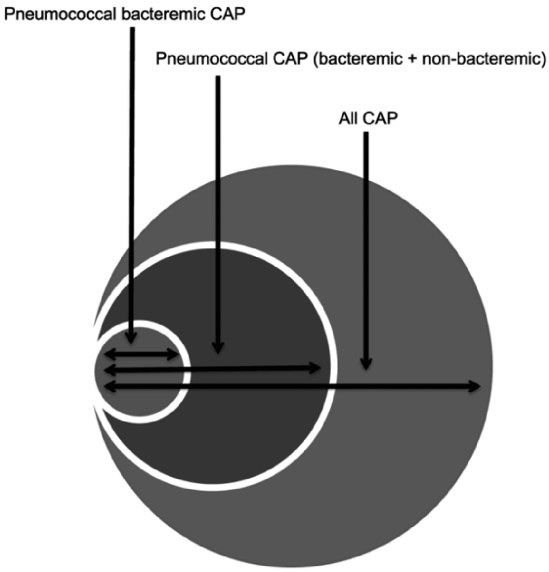

Figure 1. The relative proportion of CAP, non-bacteremic pneumococcal pneumonia, and bacteremic pneumococcal pneumonia. (From: Said MA et al. PLoS One. 2013).

by molecular testing with throat swabs for the identification of atypical pathogens. This might have led to an underestimation of the proportion of atypical pathogens in the PCV10 cohort, but PCRs generally have a higher sensitivity, making it unlikely that our comparisons were biased by decreased detection chances over time.[17] In contrast to the 2007–2009 PCV7 period (Q-fever outbreak), in the 2012-2016 PCV10 period there were no national outbreaks or regional signals of or increases of the atypical pathogens, which explains why less atypical pathogens were identified from 2012-2016.[18–20] However, this still does not explain the increase in patients with no pathogen identified in the latter period.

New methods are needed to further increase the yield of microbiological testing, facilitating directed antibiotic therapy and thereby preventing the development of antimicrobial resistance. In the near future, next-generation sequencing of microbial DNA directly from patient samples may further improve diagnostic accuracy and will reduce the proportion of unidentified pathogens.[21] Still, we must remain vigilant for over-detection (of pathogens of doubtful relevance). Extended serotype assays could contribute in identifying more non-invasive pneumococcal CAP. Also, molecular methods for serotyping of *S. pneumoniae,* without the need for phenotypic confirmation, would greatly enhance our understanding of pneumococcal epidemiology.[22]

Proportion and serotype distribution of S. pneumoniae in Bangladeshi children with CAP

In **chapter 4** the impact of PCV7 and PCV10 introduction in Netherlands on all ages is described. The IPD incidence decreased dramatically in young children due to the decrease of vaccine-type IPD. Since PCV introduction, the vast majority of the remaining new IPD cases in young children are due to non-vaccine-type IPD, which has a lower CFR. Similar but less pronounced serotype shifts occurred in adults and elderly. In **chapter 5**, we have shown that serotype shifts also occurred in adult patients hospitalized with non-invasive CAP.

Chapter 6 shows that 31% of Bangladeshi children <5 years of age with CAP have a serotype-specific antibody response against one single pneumococcal serotype included in our assay, indicating that the true proportion of *S. pneumoniae* is ≥31%. Within these cases with pneumococcal CAP, only 29% was due to a PCV10-covered serotype. Considering that these data have been collected before PCV10 introduction in Bangladesh in 2015, this may indicate that the potential effectiveness of PCV10 will be low.

The pneumococcal proportion results in **chapter 6** were obtained using a 25-plex serotype-specific multiplex immunoassay, with which serotype-specific antibodies against 25 different serotypes were measured. We were interested in the sensitivity of this serological assay to detect invasive pneumococcal disease in particular, and *S. pneumoniae* mediated CAP in general. This assay can only detect serotypes included in the panel. Blood culturing is considered the gold standard technique to identify invasive pneumococcal disease, and is included in this study. In contrast to **chapter 5**, no results were available of other conventional microbiological tests. This limited the ability to establish sensitivity of the serological assay in CAP in **chapter 6**.

It was found that <3% of cases had bacteremic pneumococcal CAP. Due to challenging technical conditions, it is very likely that this percentage is an underestimation. Therefore the results obtained from blood cultures were insufficient to test the sensitivity of the serological assay for detecting invasive pneumococcal CAP.

In **chapter 5** it has been shown that the assay has a sensitivity of 42%-45% when compared with conventional methods to detect pneumococcal pneumonia in Dutch adults hospitalized with CAP. If this sensitivity also holds true for children from Bangladesh, the true pneumococcal proportion in **chapter 6** might be >2 as high.

It is reasonable to assume that the serological antibody response in children is easier to interpret than in adults. Indeed, "background" levels of antibody concentrations in children are generally lower since children have had less exposure to different serotypes in their short life. Consequently, humoral immunity cannot interfere with the infecting serotype yet, and the primo humoral response will result in a strong fold-increase of serotype-specific antibodies, well detectable at convalescence. This may lead to a higher sensitivity of the assay. On the other hand, many children in this study proved to have high antibody concentrations against multiple serotypes. This possibly is a reflection of the living conditions of these children, living in close contact with relatives under poor conditions, thereby possibly increasing the pneumococcal carriage rate and density.

In **chapter 6**, we also evaluated whether serological analysis of a single serum sample would suffice to identify *S. pneumoniae* as pathogen responsible for the CAP. The hypothesis was that pneumococcal CAP could be serologically identified in these young children using only one serum sample. For this purpose, the serotype-specific antibody concentrations from the early (admission) serum sample were compared with the 95th percentile concentrations of the pooled data (from all early and late sample concentrations combined, per serotype). However, within the children that were identified as having pneumococcal CAP based on a specific antibody response, <2% (n=8) had a serotype-specific antibody concentration above the 95the percentile of the infecting serotype. This indicates that it may require a considerable amount of time and, probably, several infections to accumulate high serum concentrations against capsular polysaccharides. The relatively high 95th percentile concentrations can also be explained by the many close contacts these children have due to their living conditions in a densely populated area, leading to high and frequent exposure to various serotypes. To conclude, our study shows that diagnosing pneumococcal CAP based on concentration of anti-capsular serotype specific antibodies will not be possible, at least not in young children living in these conditions.

The most surprising finding with the highest impact was the low estimated potential PCV10-coverage of only 29% within the serologically determined pneumococcal CAP cases. To put this into perspective: in the Netherlands the proportion of PCV7-serotypes causing IPD (not limited to pneumonia) in under-fives *before* PCV7-introduction was 70%. It is unclear whether the 29% potential PCV10-coverage also holds true for Bangladeshi children with IPD. In the limited data from literature on the serotype distribution in children with IPD from

Bangladesh, serotype 1, 2, 3, 5, 11A, 12A, 14, 22F, 19A and 45 were among the most found serotypes (data from different studies in different settings and from different regions).[23–26] Of these serotypes, serotype 2, 3, 11A, 19A, 22F and 45 were all in the top 10 most identified serotypes in our study and all were included in the panel. Noteworthy, in our top 10 only serotype 19F is covered by PCV10, where the novel 20-valent conjugate vaccine (in phase 3 clinical research) would cover seven top 10 serotypes.[10]

Further research is required to confirm the potentially limited PCV10-coverage in young children from Bangladesh with CAP, using conventional diagnostics. Ideally, the multiplex method used in this study will be applied simultaneously to determine its sensitivity in children with CAP. The multiplex immunoassay has proven to be a useful serological method in assessing the pneumococcal serotype distribution in a low-income region, possibly also in determining the pneumococcal proportion in CAP.

Part III – Adjunctive corticosteroid therapy and prognostic biomarkers

Corticosteroids in CAP: weighing the pros and cons

In the last decade, adjunctive corticosteroid therapy in hospitalized patients with CAP has been subject of many studies, all based on the hypothesis that corticosteroids dampen the excessive inflammatory response in patients hospitalized with CAP. The latter would result in improved patient outcomes.[27] At present, multiple trials[27–35] and meta-analyses[36–45] have evaluated the effects of different corticosteroid regimes on various endpoints. All studies found that patients treated with corticosteroids have a significantly shorter length of hospital stay (LOS) compared to a placebo. However, side effects of corticosteroids are not uncommon, especially hyperglycemia. Because of these adverse effects, the exact role of corticosteroids in the treatment of CAP is still a matter of debate. Earlier studies have suggested that patients with severe CAP would benefit most from corticosteroids.[35,46]

Dexamethasone in patients hospitalized with CAP with a high versus low PSI class

Chapter 7 describes the results of the first placebo-controlled trial specifically designed to assess whether the effect of adjunctive corticosteroids differs between immunocompetent adults hospitalized with severe CAP (PSI IV-V) compared to patients with mild-to-moderate severe CAP (PSI I-III).

The a priori hypothesis was that patients with severe CAP would benefit most from adjunctive dexamethasone. This hypothesis was based on an earlier finding that adjuvant dexamethasone treatment is associated with a significant decrease in mortality/ICU admission in patients presenting with a high pro-inflammatory cytokine response, but a discrepantly low cortisol.[46]

In the trial described in **chapter 7**, patients were randomized to receive either a 4-day oral regime of dexamethasone 6 milligram or placebo. Reasons to choose an oral regimen were safety considerations, ease of use, but also to avoid possible bias by unintended extension of the length of stay due to need for intravenous administration.[27] It has been shown before

that the bioavailability of oral dexamethasone in patients hospitalized with pneumonia is adequate.[47] Primary study outcome was LOS. Secondary outcome measures were secondary intensive care unit admission and 30-day mortality. The study was prematurely terminated after the second interim analysis, when two third of the originally planned sample size was enrolled. The reasons for the early termination were a slower than expected enrolment rate and a lower than expected overall mortality rate causing an important reduction in statistical power to detect the assumed 50% reduced mortality in patients with severe CAP.

The positive effects of corticosteroids in patients hospitalized with CAP

Besides the 0.5 days reduction in LOS, the study in **chapter 7** is the first study showing a reduction in secondary ICU admissions in patients treated with adjunctive corticosteroids versus placebo. Although non-statistically significant, in the non-severe CAP subgroup LOS was 1.0 days shorter in the dexamethasone group compared to the placebo group. There was no difference in LOS between treatment groups in the severe CAP subgroup. Even though a number of meta-analyses found that corticosteroids reduce mortality in severe CAP but not in non-severe CAP[38,40], there was no difference in 30-day mortality rate within both pneumonia severity subgroups (or within the whole cohort). However, the studies included in the meta-analyses that did find clear survival benefit had high risk of bias because of differences in baseline characteristics between study groups.[30,33]

Even without a mortality benefit, the reduction in LOS and secondary ICU admissions can be of considerable benefit to patients and does contribute to optimal use of health care resources and cost reductions.[48] The shorter LOS in the dexamethasone group described in **chapter 7** is line with what the most similar trials found.[27,34] Main reasons to choose a medically-fit based LOS as primary outcome measure was its patient-centered character. Even though the 0.5 day (10%) reduction in LOS might sound minor, from a patient's perspective this might be major.

One might discuss that the reduction in LOS observed in all the studies in patients treated with corticosteroids is the result of their antipyretic properties, merely masking symptoms rather than promoting faster recovery from disease.[27] **Chapter 8** does clearly show the antipyretic properties of dexamethasone in patients with CAP. However, because discharge rates started to diverge between treatment groups by the time that temperature differences had disappeared for days, makes such an explanation unlikely. Also, the rate of readmissions was similar between study arms in the original study, suggesting the recovery was persistent.[27] This supports the hypothesis that the reduction of LOS was the result of a more rapid and persistent clinical recovery caused by dexamethasone, rather than a "cosmetic" suppression of fever.

The adverse effects of corticosteroids

In addition to their antipyretic and anti-inflammatory effects, corticosteroids stimulate gluconeogenesis, which can lead to hyperglycemia. Similar to other studies, **chapter 7**

shows that hyperglycemia was more frequently present in the corticosteroids group compared to the placebo group (7% vs. 1%; p=0.001).[27,34] Also, and maybe even more importantly, the rate of readmissions was also higher in the dexamethasone group (10%, n=19 vs. 5%, n=9; p=0.07). No other individual trial has found a significant increase in re-admissions before. However, in an individual patient data meta-analysis a higher rate of CAP-related readmissions was found the intervention group compared to placebo (5.0% vs. 2.8%; number needed to harm of 45). Yet, in general, few adverse events other than hyperglycemia have been reported in corticosteroid groups in the variety of trials so far.

Which patients hospitalized with CAP benefit most from adjunctive corticosteroids?

Given the risk of adverse effects on the one hand, but on the other hand the reduction 0.5-1 day reduction in LOS and potentially a lower risk of secondary ICU admission, it is worthwhile to search for a subgroup of patients who will benefit from the faster recovery and not suffer from side effects. This group likely consists of patients with severe CAP, based on a combination of parameters related to inflammation, rather than on PSI. The PSI score was designed to predict mortality and is mainly driven by age (and the presence of comorbidities).[49] For this reason, PSI does not necessarily correspond with the degree of inflammation. This is also demonstrated in an explorative analysis in **chapter 7**, where the largest reduction in LOS was found in patients <65 years with a CURB-65 score of ≥2 points. In contrast to the PSI, the CURB-65 (acronym for: confusion, urea, blood pressure and [an age of] ≥65 years) does not include comorbidities and is less influenced by age.

Besides clinical symptoms, another option for stratification could be an inflammatory biomarker such as CRP or PCT. This is also demonstrated in **chapter 7**, were we found that patients with a CRP above median (≥210 mmol/l) receiving dexamethasone had a shorter LOS and a lower rate of secondary ICU admissions compared to placebo. Moreover, in a previous placebo-controlled trial in which only patients admitted to the ICU with a CRP above 150 mg/dL were enrolled, less treatment failure was found in the group who received methylprednisolone for five consecutive days.[35] In another post-hoc analyses of the trial by Meijvis et al, patients presenting with a high pro-inflammatory cytokine response and a discrepantly low cortisol showed a significantly lower mortality/ICU admission rate.[46] However, this finding could not be confirmed in another cohort.[50] In addition, no significant effect modification by degree of inflammation (based on number of SIRS criteria), CRP, or CAP severity (based on PSI) on 30-day mortality was found in the earlier individual patient data meta-analysis.[45] The above illustrates that conventional parameters are likely to be insufficient for predicting adjunctive corticosteroid effectiveness in CAP. In other words, additional studies with modern techniques, for example metabolomics in exhaled breath, is needed to hopefully find relevant parameters to identify patients with the optimal risk-benefit balance for adjunctive corticosteroid treatment.[51–53]

Progress should also be made in exploring outcomes other than LOS. Since recovery from CAP continues after discharge, assessment of quality of life during and after hospitalization

might change how to judge the beneficial effects of corticosteroids. Results from such a study in American veterans with severe CAP are currently expected (defined as being of sufficient severity to require ICU admission), and might help getting closer to identifying the subgroup of patients hospitalized with CAP with the optimal adjunctive corticosteroid benefit-risk ratio.[54]

CAP, cardiac events and the added value of cardiac troponin T as biomarker in CAP?

In **chapter 7** and **chapter 8**, mainly short-term outcomes in patients with CAP have been addressed. **Chapter 9** focuses on longer-term outcomes after hospitalization with CAP. In recent years, the association between hospitalization with pneumonia and the subsequent risk of both short-term and long-term cardiovascular disease has been well established.[55,56] Therefore, multiple cardiac biomarkers have been subject of study in patients hospitalized with CAP.[57] In **chapter 9** the prognostic value of cardiac troponin T (cTnT), which is used widely as predictor of myocardial damage, is studied towards predicting mortality in patients admitted with CAP. An elevated or high cTnT concentration at hospital admission with CAP was associated with more severe CAP and appeared to be a strong predictor for both short-term and long-term mortality, with odds ratios as high as 21.9 and 10.7 respectively. These findings suggest that cardiac injury is common in patients admitted with CAP.

Earlier studies have shown that cardiovascular complications in patients hospitalized with CAP consist of cardiac arrhythmias such as atrial fibrillation, but also of acute coronary syndrome, heart failure and strokes. It has been shown that 12% to 18% of patients have a cardiovascular event during hospitalization with CAP.[58,59] Especially arrhythmias occur frequently, an event in which cTnT can also be elevated. The increased risk for cardiovascular complications in CAP is likely to be multifactorial, but whether a hospitalization (or less severe episode) of CAP contributes to the pathogenesis of cardiac complications or if it aggravates pre-existent problems remains to be elucidated.

A first hypothesis is that that CAP acts as a cardiac stressor, in which systemic inflammation with catecholamine release cause tachycardia, and peripheral vasodilation in combination with low blood oxygenation cause an increased cardiac oxygen demand in combination with a decreased cardiac oxygen supply resulting in acute cardiac stress. A second hypothesis is that the disease process of CAP or its causative pathogen leads to plaque instability in patients with pre-existing plaques (atherosclerosis), which in turn might lead to an acute coronary syndrome.[60] Other contributors in the process might be an oxygen supply-demand mismatch and endothelial dysfunction. The latter promotes platelet activation and can eventually even lead to acute coronary syndrome.[61] Lastly, infection by *S. pneumoniae* might also play a role in cardiac disease. Animal studies show a direct effect of *S. pneumoniae* on the myocardium, forming micro-lesions that results in myocardial fibrosis.[62]

The highest risk to develop cardiovascular complications with an increased risk of death is observed during the first 30 days of hospitalization.[63] This risk has been shown to persist

up to 1.5 fold, even 10 years after the original CAP episode.[56,59] Why both cardiovascular and all-cause long-term mortality are elevated after a CAP episode remains to be elucidated. Possibly hospitalization with CAP can be seen is an indicator for underlying cardiac disease. Mortality due to cardiovascular events during an episode of CAP might also be caused by a persistent or imbalanced systemic inflammatory activity that leads to a continuous pro-coagulant state and subsequent atherosclerosis.

So far, two trials have evaluated the effects of aspirin (both a platelet aggregation inhibitor and an anti-inflammatory drug) in the reduction of acute coronary syndromes and cardiovascular mortality in patients with pneumonia. The first enrolled 91 patients with pneumonia who had more than one risk factor for cardiovascular disease and found that aspirin 300 mg/day for 1 month was effective in preventing acute coronary syndromes (rate of 1.1% (n=1) in the aspirin group vs. 10.6% (n=10) in controls; p=0.015).[64] The second study enrolled 278 patients both with and without elevated cTnT (>0.014 µg/l) at admission. These patients were followed up until discharge. Among 144 patients with elevated cTnT, 31 had signs of myocardial infarction and 113 did not. No differences in myocardial infarction rate was observed between the 123 patients taking aspirin (100 mg/day) and those who were not (12% vs. 10%; p=0.649).[65]

CTnT seems a good candidate for the early identification of patients with CAP that are at high risk of cardiac injury during (or after) hospitalization. However, the clinical meaning and implications of elevated cTnT at admission with CAP need further research.

Conclusions

The purpose of this thesis was to gain more insight into the care of patients with CAP, into on *S. pneumoniae* as pathogen of CAP and the pneumococcal serotype distribution in CAP and IPD. Even though other pathogens can cause CAP, the pneumococcus remains the most important bacterial pathogen, doing justice to its name. Modern diagnostic techniques have made rapid identification of *S. pneumonia* possible. This thesis shows that utilization of more microbiological test results is associated with earlier alteration of antibiotic therapy in CAP. Antibiotic therapy might even be further streamlined by extensive testing in combination with alerting physicians as to when parenteral antibiotics have been administered for longer than a certain amount of time, and by notifying them of both positive and negative test results of a PCR for atypical pathogens.

The Netherlands has a very efficient IPD sentinel surveillance system to monitor the effects of PCVs, which have shown to be effective in reducing IPD incidence, especially in young children and young adults. Herd-immunity has protected the non-vaccinated, but the maximum effects of the current PCV10 are almost reached due to continuous emergence of non-vaccine serotypes. Serotype-specific pneumococcal antibody measurement is a useful screening method to assess the serotype distribution in adults and children with pneumococcal pneumonia. It is detected by this method that the potential PCV10 effectiveness in under-fives from Bangladesh with CAP is low.

Adjunctive dexamethasone therapy in CAP reduces the LOS in patients hospitalized with CAP, but can also lead to more readmissions. Though there were less secondary ICU admissions in patients treated with dexamethasone (also in the subgroup PSI I-III), the adverse effects of corticosteroids makes them unsuitable for all patients. There was no difference in LOS and 30-day mortality between treatment arms in the subgroups PSI I-III vs. PSI IV-V. The search for markers associated with an optimal benefit-risk ratio for corticosteroids, also pursuing minimum side effects, continues.

The association between CAP and cardiovascular events has become increasingly clear. CTnT concentration at admission with CAP is a good predictor of both short-term and long-term mortality after hospitalization with CAP.

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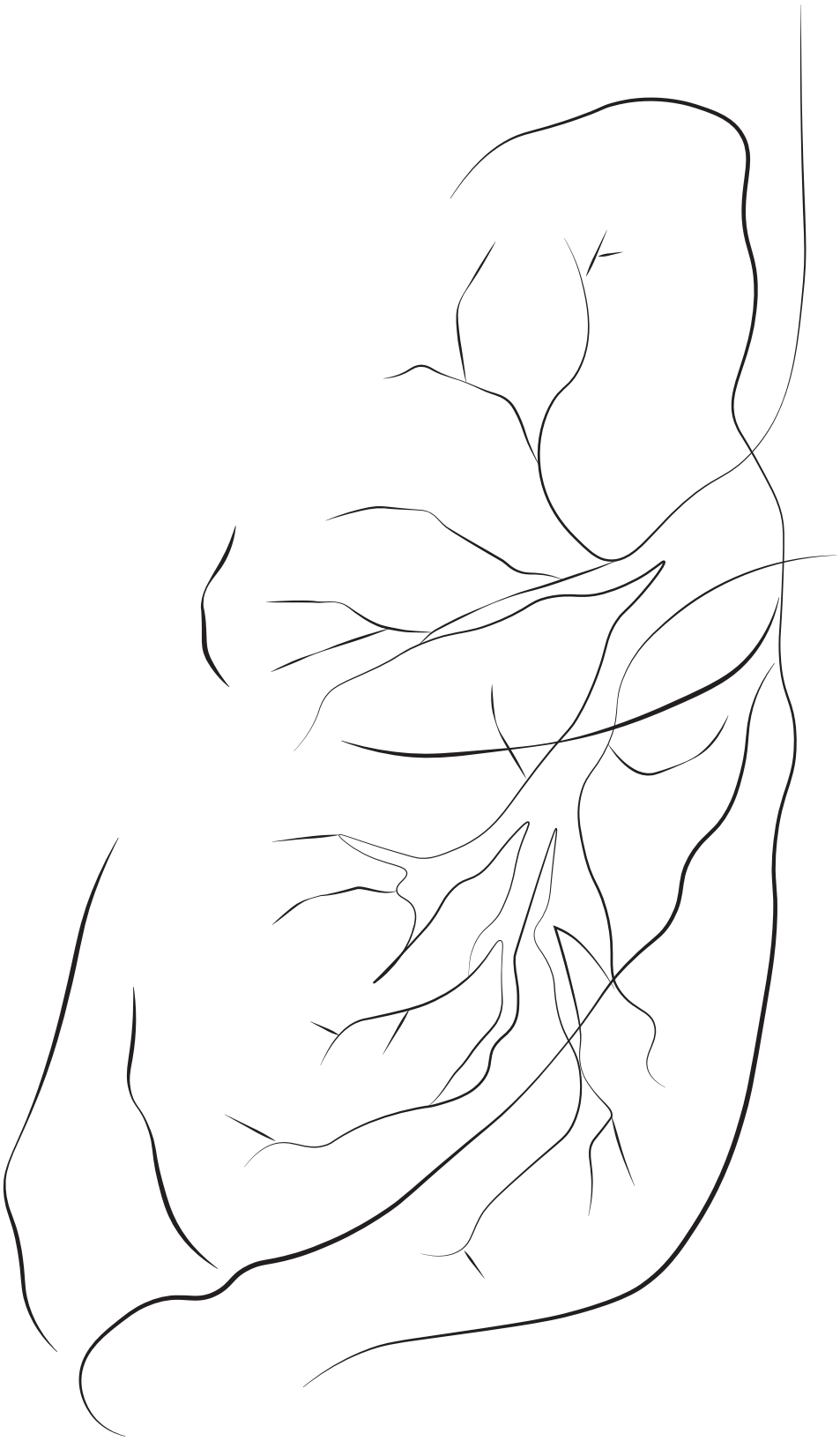

CHAPTER 11

Nederlandse samenvatting
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Nederlandse samenvatting

Longontsteking is een veelvoorkomende aandoening die vaak lijdt tot ziekenhuisopname en niet zelden tot overlijden. *Streptococcus pneumoniae* is de meest voorkomende bacteriële verwekker van een thuis-opgelopen longontsteking (in de Angelsaksische literatuur 'community-acquired pneumonia' genoemd, CAP). Voor de leesbaarheid van deze samenvatting noemen we de CAP longontsteking en de bacterie pneumokok. De ontwikkeling van antibiotica heeft ervoor gezorgd dat het sterftepercentage ten gevolge van longontstekingen en infectieziekten sterk is verminderd. Desondanks zorgen longontstekingen en ziekten veroorzaakt door pneumokokken nog altijd voor een grote ziektelast. In **hoofdstuk 1** worden beide onderwerpen uitgebreid geïntroduceerd.

Deel I van dit proefschrift staat in het teken van doelmatige diagnostiek bij volwassenen opgenomen met een longontsteking. Vanwege stijgende zorgkosten is doelmatigheid van zorg een belangrijk thema. In **hoofdstuk 2** en **3** leggen we het verband tussen de hoeveelheid uitgevoerde diagnostiek (en kosten) en aanpassing van antibiotica (binnen de eerste twee opnamedagen) bij patiënten opgenomen met een longontsteking. Antibiotische therapie kan worden gewijzigd wanneer het veroorzakend micro-organisme gevonden is (bijvoorbeeld gerichte therapie voor pneumokokken). Anders gebeurt dit vaak pas wanneer de patiënt voldoende is opgeknapt, bijvoorbeeld de overstap van antibiotica via het infuus naar orale therapie.

In **hoofdstuk 2** werd dit verband in drie ziekenhuizen onderzocht. We vonden, onverwacht, dat de kosten van (met name microbiologische) diagnostiek omgekeerd evenredig waren met de frequentie van aanpassen van antibiotica. Met andere woorden: minder diagnostiek was geassocieerd met meer aanpassing van antibiotica. Bij navraag in het ziekenhuis met de laagste kosten én de meeste antibiotica aanpassingen bleek dat het ziekenhuis een systeem had om behandelend artsen erop te attenderen wanneer een patiënt met antibiotica twee dagen opgenomen is. Dit met het idee dat een arts hierdoor eerder kritisch het antibiotische beleid zal beoordelen. Hoogstwaarschijnlijk heeft deze 'antibiotic-stewardship' interventie ervoor gezorgd dat artsen simpelweg eerder naar reeds bekende uitslagen van microbiologische diagnostiek (ook de negatieve testen) hebben gekeken, waardoor er eerder aanpassing van antibiotica plaatsvond.

In **hoofdstuk 3** onderzochten we in één ziekenhuis het verband tussen de hoeveelheid uitgevoerde microbiologische diagnostiek en aanpassing van antibiotisch beleid binnen twee dagen na opname met een longontsteking. Deze associatie was wel positief: met iedere extra uitgevoerde test steeg het percentage aanpassingen in antibiotisch beleid. Deze resultaten tonen dat het uitvoeren van uitgebreidere microbiologische diagnostiek kan leiden tot snellere aanpassing van antibiotica. Dit is gunstig omdat "bredere" antibiotische behandeling leidt tot meer antibioticaresistentie ontwikkeling.

De resultaten van **hoofdstuk 2** en **3** tonen aan dat microbiologische diagnostiek belangrijk is om antibiotica aan te kunnen passen, maar dat het wel nodig kan zijn om behandelende artsen actief te attenderen op testuitslagen.

Deel II van proefschrift gaat over de bacterie *Streptococcus pneumoniae* (de pneumokok). De pneumokok ontleent zijn naam aan het feit dat het een beruchte en veel voorkomende veroorzaker is van longontstekingen. Waarschijnlijk dragen alle mensen de pneumokok ergens gedurende het leven bij zich in de keel, zonder dat we er ziek van worden (dat heet kolonisatie). Soms kunnen de pneumokokken die we bij ons dragen echter wel tot ziekte leiden. Denk bijvoorbeeld aan long- maar ook aan oorontstekingen. Wanneer een bacterie door de buitenste barrières van het lichaam heen weet te dringen en een normaliter steriele lichaamsvloeistof infecteert, zoals hersenvloeistof (meningitis) of bloed (bacteriëmie), dan wordt dit een invasieve infectie genoemd. De verzamelterm van aandoeningen waarbij de pneumokok een bacteriëmie en/of een hersenvliesontsteking veroorzaakt is 'invasieve pneumokokkenziekte' (IPZ) genoemd. IPZ is een ernstige aandoening die vaak resulteert in overlijden; in het bijzonder bij jonge kinderen, maar ook bij ouderen. Om deze reden en met als doel de ziektelast van IPZ te verminderen, zijn er pneumokokkenvaccins ontwikkeld. Deze dienen bescherming te bieden tegen de meest voorkomende en ziekmakende pneumokokken typen (serotypen). De pneumokok wordt namelijk getypeerd op basis van het "kapsel" dat de pneumokok omgeeft. Dit kapsel zit aan de buitenkant van de bacterie en bestaat uit suikerketens (polysachariden). Er zijn al meer dan 90 verschillende pneumokokkenserotypen beschreven, elk met een eigen polysacharidenkapsel. Het kapsel vormt een beschermende laag rondom de bacterie, maar het menselijk immuunsysteem is in staat om er antistoffen tegen te vormen. Deze zijn kapsel-specifiek (of serotype-specifiek) en dus gericht tegen één van de 90 serotypen. De huidige pneumokokkenvaccins maken gebruik van dit principe. Ze bevatten namelijk kleine hoeveelheden pneumokkenkapsel van een aantal van de meest voorkomende serotypen. De momenteel gebruikte pneumokokkenvaccins in Rijksvaccinatieprogramma's wereldwijd zijn gericht op het voorkomen van IPZ en bieden, afhankelijk van het vaccin, bescherming tegen 10 of 13 serotypen. De polysachariden aanwezig in deze vaccins zijn gekoppeld (geconjugeerd) aan een eiwit. Conjugatie aan eiwitten stelt het menselijke immuunsysteem in staat om geheugen te vormen, waardoor vaccinatie in theorie levenslang bescherming biedt. Deze vaccins worden pneumokokken conjugaatvaccins (PCV) genoemd. In Nederland werd de introductie van het 7-valente vaccin (PCV7, wat bescherming biedt tegen de top 7 serotypen) in 2006 gevolgd door een daling van incidentie van IPZ in kinderen <2 jaar, maar ook in volwassenen (en met name in 65+'ers). Deze afname in niet gevaccineerde volwassenen wordt kudde (of groeps-)immuniteit genoemd en valt te verklaren doordat gevaccineerde kinderen geen vaccin-type pneumokokken meer bij zich dragen, waardoor ze er zelf niet ziek van worden maar op hun beurt ook geen volwassenen meer kunnen besmetten (of drager maken). Daarnaast werd er na de invoering van PCV7 een daling van het percentage sterfgevallen gezien in patiënten die nog wel IPZ infectie opliepen, waarschijnlijk doordat deze andere serotypen (er zijn er immers nog 90-7 = 83 over!) minder ziekmakend zijn.

In **hoofdstuk 4** onderzochten we het effect van de invoering van PCVs in het Nederlandse Rijksvaccinatieprogramma. In 2011 werd PCV7 vervangen door het 10-valente

vaccin (PCV10). We vergeleken de periodes vóór invoering van PCV7 (2004-2006), na invoering van PCV7 en na invoering van PCV10 (2016-2018). De eerste en laatste periode vergelijkend, vonden dat er een daling in aantal IPZ heeft plaatsgevonden in kinderen <5 jaar, volwassenen van 18-49 jaar en in 65+'ers. Kijkend naar de hele populatie was deze afname niet zo duidelijk. De verklaring hiervoor is dat er in alle leeftijdscategorieën wel een afname was van de 7 serotypen gedekt door PCV7 (en tussen 2016-2018 ook de drie extra gedekt door PCV10), maar dat er gelijktijdig een toename heeft plaatsgevonden van niet-vaccin gedekte serotypen. Daarnaast bleef het percentage sterfgevallen wat daalde na de invoering van PCV7, gelijk na de overstap naar PCV10. Onze bevindingen wijzen erop dat het maximale effect van PCV10 bereikt lijkt te zijn. Gezien de continue vervanging van vaccin-type pneumokokken door niet-vaccin-serotypen is het maar de vraag of vervanging door een hoger-valent vaccin (bijv. PCV13, wat meer serotypen zou dekken) effectiever zou zijn. Een pneumokokken vaccin dat alle 90 serotypen omvat zou hiervoor een oplossing kunnen bieden.

In **hoofdstuk 5** hebben we het effect van PCVs in Nederland bestudeerd binnen volwassenen opgenomen met een longontsteking. In deze groep patiënten is er meestal geen infectie van de bloedbaan en is er in het geval van een pneumokken longontsteking dus geen sprake van IPZ. Een daling van de pneumokok als verwekker van longontstekingen zou ertoe kunnen leiden dat een andere, potentieel meer ziekmakende bacterie zijn plek zou innemen. Ondanks dat we wel vonden dat het aandeel pneumokokken longontstekingen afgenomen was in de periodes na PCV invoering t.o.v. ervoor, steeg het aandeel van andere verwekkers niet. Wel was er een toename in het aandeel patiënten waarbij geen verwekker gevonden werd. De reden hiervoor is onduidelijk. Daarnaast toonden we aan dat het aandeel PCV7-serotypen ook binnen de niet-IPZ pneumokokken longontstekingen duidelijk afnam in de PCV10 periode in vergelijking met de pre-PCV7 periode. Dit toont aan dat er ook binnen deze groep vaccin-effecten lijken te hebben plaatsgevonden.

In **Hoofdstuk 6** hebben we onderzocht wat het pneumokokkenaandeel is in een grote groep kinderen <5 jaar uit Bangladesh met een longontsteking. Deze kinderen leven onder slechte leefomstandigheden en in armoede in een zeer dichtbevolkt gebied in de hoofdstad Dhaka. In 2015 heeft Bangladesh PCV10 in het Rijksvaccinatieprogramma ingevoerd. Door specifieke pneumokokkenantistoffen te meten in bloedmonsters van alle deelnemende kinderen waren we in staat te bepalen dat 406 (31%) van hen geïnfecteerd moet zijn geweest door een pneumokok. Doordat de gemeten antistoffen serotype-specifiek zijn, waren we instaat aan te tonen dat slechts 29% van de 406 kinderen met een pneumokokken longontsteking geïnfecteerd was door een serotype gedekt door PCV10 (en 61% dus niet). Het aandeel IPZ longontsteking was erg laag in deze groep en daarom weten we niet of de PCV10-dekking van kinderen met IPZ uit de regio vergelijkbaar is.

Deel III, het laatste deel van het proefschrift, staat geheel in het teken van volwassenen met een longontsteking. In **hoofdstuk 7** beschrijven we de resultaten van een onderzoek in vier Nederlandse ziekenhuizen waarin het effect van behandeling met dexamethason werd bestudeerd (de Santeon-CAP studie). Nadat 2/3 van de 600 beoogde patiënten had

deelgenomen werd er een tussen-analyse uitgevoerd. Vanwege de lange duur van het onderzoek en onvermogen om de onderzoeksvraag nog te kunnen gaan beantwoorden bij continueren, werd besloten de studie vroegtijdig te stoppen. In deze studie werd door middel van loting bepaald wie er gedurende vier dagen aanvullend behandeld zou worden met dexamethason (een ontstekingsremmer en het te onderzoeken medicijn) of met een placebo (een nepmedicijn). Zowel patiënten als onderzoekers waren niet op de hoogte van de groep waarin de patiënt zat. Op basis van een gevalideerde score werden de behandelgroepen verder onderverdeeld naar ernst van de longontsteking (niet ernstig of ernstig). Alle deelnemers werden intensief gevolgd. Een uitkomst van dit onderzoek was dat patiënten behandeld met dexamethason gemiddeld 0,5 dagen korter waren opgenomen dan de patiënten die de placebo kregen (4,5 en 5 dagen). Binnen de subgroepen van patiënten met een niet-ernstige en een ernstige longontsteking was er geen verschil te zien. Verder werden er in de dexamethasongroep 5 patiënten (3%) overgeplaatst naar een intensive care, waar dit er 14 (7%) waren in de placebogroep. Een belangrijke bevinding was daarnaast dat 19 (10%) van de patiënten behandeld met dexamethason heropgenomen werden na eerder ontslag uit het ziekenhuis, tegen 9 (5%) in de placebogroep. Onze bevindingen en ook eerder onderzoek laat zien dat behandeling van longontstekingen met dexamethason positieve effecten heeft, maar zeker ook negatieve effecten, zonder dat deze eenduidig meer uitgesproken waren binnen patiënten met een niet-ernstige of een ernstige longontsteking. Dexamethason heeft daarom nog geen plek in de standaardbehandeling van volwassenen opgenomen met een longontsteking.

Hoofdstuk 8 betreft een analyse van een eerdere studie in 304 patiënten opgenomen met een longontsteking waarin tevens geloot werd voor dexamethason of een placebo. Ons doel was te bepalen of onderdrukking van koorts door dexamethason een belangrijke verklaring is voor kortere opnameduur die ook in deze studie gevonden werd. Het koortsonderdrukkende effect van dexamethason t.o.v. de placebo was overduidelijk. Echter, het verschil in opnameduur tussen de behandelgroepen trad pas op vanaf dag 6, twee dagen na de laatste toediening van studiemedicatie en wanneer het percentage koortsvrije patiënten alweer gelijk verdeeld was over de twee groepen. Het is daarom onwaarschijnlijk dat het koortsonderdrukkende effect van dexamethason van invloed was op de studieresultaten.

In **hoofdstuk 9A** hebben we onderzocht wat de relatie was tussen troponine T in bloed (een indicator van hartschade) en korte- en langtermijn overlijden bij volwassen opgenomen met een longontsteking. Beide waren sterk geassocieerd met een verhoogde troponine T bij opname, ook na correctie voor mogelijk beïnvloedende factoren (zoals leeftijd en onderliggende aandoeningen). Mogelijke verklaringen voor onze bevindingen zijn bijvoorbeeld zuurstoftekort t.g.v. longontsteking of de overmatige ontsteking welke direct of indirect zouden kunnen leiden tot hartschade.

In **hoofdstuk 9B**, een reactie op een commentaar op hoofdstuk 9A, onderzochten we of de gevonden relatie het gevolg zou kunnen zijn geweest van zogenaamde sepsis-geïnduceerde hartschade. Door patiënten die mogelijk leidden aan deze aandoening uit

te sluiten van de eerdere analyse en deze vervolgens te herhalen, toonden we aan dat dit de relatie niet beïnvloed heeft.

Samenvattend laat dit proefschrift zien dat het uitvoeren van microbiologische diagnostiek in de zorg voor volwassenen opgenomen met een longontsteking bijdragend is aan vroege aanpassing van antibiotica, en dat middelen om een behandelaar alert te maken op uitslagen verder kunnen bijdragen aan vroegtijdige aanpassing. Ook laat het zien dat er serotype-verschuivingen hebben plaatsgevonden na de invoering van pneumokokken conjugaatvaccins binnen patiënten met invasieve pneumokokkenziekte in Nederland en dat waarschijnlijk hierdoor het IPZ sterftepercentage gedaald is. Verder demonstreert het dat er na invoering van PCVs ook serotype-verschuivingen hebben plaatsgevonden binnen niet-IPZ patiënten opgenomen met een longontsteking en dat het aandeel van de pneumokok als verwekker lijkt te zijn afgenomen. Ook illustreert dit proefschrift dat pneumokokken longontsteking veel voorkomt bij jonge kinderen uit Bangladesh en dat slechts een klein deel van de pneumokokken gedekt lijkt te worden door het in Bangladesh gebruikte vaccin. In volwassenen opgenomen met een longontsteking resulteerde additionele dexamethason behandeling in een verkorting van opnameduur (en snel verdwijnen van koorts), maar verhoogde wel de kans op heropname. Ten slotte vonden we dat een verhoogd troponine T in patiënten opgenomen met een longontsteking onafhankelijk geassocieerd was met overlijden op zowel de korte- als lange-termijn na ziekenhuisopname.

List of publications (not included in this thesis)

Lubbers R, Sutherland JS, Goletti D, de Paus RA, van Moorsel CHM, Veltkamp M, **Vestjens SMT**, Bos WJW, Petrone L, Del Nonno F, Bajema IM, Dijkman K, Verreck FAW, Walzl G, Gelderman KA, Groeneveld GH, Geluk A, Ottenhoff THM, Joosten SA, Trouw LA. Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis. Front Immunol 2018;9:2427.

Spoorenberg SMC, **Vestjens SMT**, Voorn GP, van Moorsel CHM, Meek B, Zanen P, Rijkers GT, Bos WJW, Grutters JC; Ovidius study group. Course of SP-D, YKL-40, CCL18 and CA 15-3 in adult patients hospitalised with community-acquired pneumonia and their association with disease severity and aetiology: A post-hoc analysis. PLoS One 2018;13(1):e0190575.

Spoorenberg SM, **Vestjens SM**, Rijkers GT, Meek B, van Moorsel CH, Grutters JC, Bos WJ; Ovidius Study Group. YKL-40, CCL18 and SP-D predict mortality in patients hospitalized with community-acquired pneumonia. Respirology 2017;22(3):542-550.

Spoorenberg SMC, **Vestjens SMT**, Albrich WC, Rijkers GT. Corticosteroids for all adult patients with community-acquired pneumonia? Pneumonia 2015;6:44-47.

Dankwoord

Het is zover: mijn proefschrift is af!

Dit proefschrift zou niet tot stand zijn gekomen zonder de bijdrage van vele direct en indirect betrokkenen. Hiervoor ben ik jullie allen zeer dankbaar. Ik wil graag een aantal personen in het bijzonder bedanken.

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Curriculum vitae

Stefan Vestjens was born on the 9th of September 1988 in Deurne, The Netherlands. After secondary school at the "Peellandcollege" in Deurne, from which he graduated in 2007, he studied Medicine at the Utrecht University. After obtaining his bachelor degree in 2011, he took his first steps in medical research, working as a student on mapping risk factors of child abuse at emergency departments. During his master's degree program, he combined his senior and research internship in Internal Medicine at the St. Antonius hospital, during which he worked on his first community-acquired pneumonia research project. In December 2014 he obtained his Master of Science degree in Medicine. Stefan continued his research work during the time he worked as a resident (not in training) at the department of Internal Medicine at the St. Antonius Hospital from May 2015 – September 2016, and during his job as 'Epic Research Integration Lead Analyst and Trainer' up to January 2018.

From February 2015 onwards he worked on various research projects as a PhD student at the Department of Internal Medicine at the St. Antonius Hospital, also coordinating the Santeon-CAP trial until May 2018. For his *Streptococcus pneumoniae* research work, Stefan closely collaborated with the Department of Medical Microbiology and Immunology of the St. Antonius Hospital. The research for this thesis was supervised by Prof. dr. J.C. Grutters, Prof. dr. ir. G.T. Rijkers, Prof. dr. W.J.W. Bos and Dr. E.M.W. van de Garde.

In May 2018, Stefan has started his residency in Medical Microbiology at the University Medical Center Utrecht and the St. Antonius Hospital.