# Drug effects on the risk and prognosis of community-acquired pneumonia

The work presented in this thesis was performed at the Division of Pharmacoepidemiology and Pharmacotherapy of the Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, and the departments of Clinical Pharmacy, and Pulmonary Medicine of the St. Antonius Hospital, Nieuwegein, The Netherlands.

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# Drug effects on the risk and prognosis of community-acquired pneumonia

Effecten van geneesmiddelen op het risico op en de prognose van de community-acquired pneumonie

(met een samenvatting in het Nederlands)

**Proefschrift**

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 21 november 2007 des middags te 4.15 uur

door

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geboren op 6 april 1978 te Breda

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Voor mijn ouders

# Table of contents

# 1 | General introduction

## The burden and aetiology of community-acquired pneumonia

Community-acquired pneumonia and other lower respiratory tract infections are among the most common infections world-wide. Conservative estimates for Western Europe report incidences between 1 to 10 events per 1,000 persons per year [1, 2]. For the Netherlands, the most recent estimate is that 30,000 patients are admitted to hospital for community-acquired pneumonia each year and that an additional 70,000 patients are treated for pneumonia in primary care [3-5]. This involves primarily elderly people as can been seen in Figure 1, which shows the incidence of hospital admissions for community-acquired pneumonia in different age categories in the Netherlands in 2005.

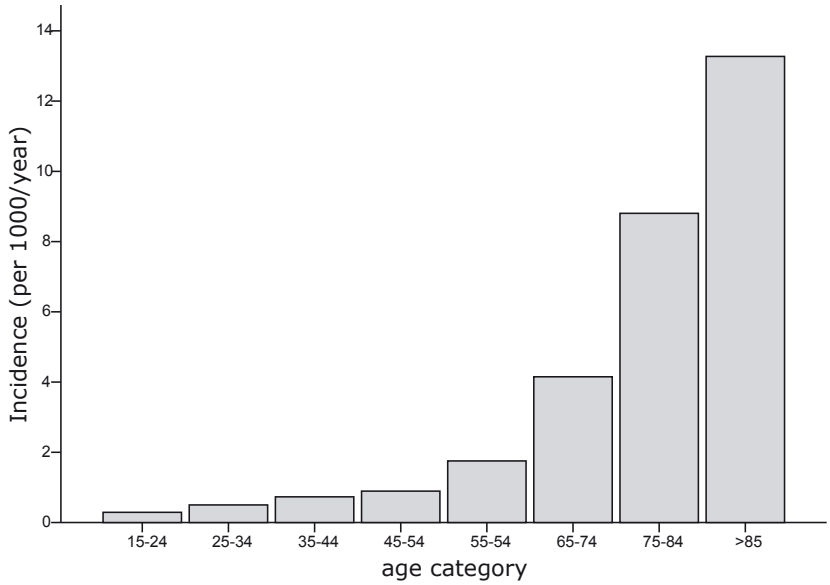


**Figure 1** *Incidence of hospitalisations for community-acquired pneumonia in the Netherlands in 2005 (source: PRISMANT, LMR diagnoses [3]).*

Many factors can contribute to the vulnerability of the older individual to pneumonia. Firstly, immune status alters with age. A major alteration of the aged immune system is an impaired T lymphocyte response [6]. Secondly, an impaired cough reflex could cause pharyngeal flora to gain access to the lower respiratory tract [7]. The typical pathogenesis of bacterial pneumonia, such as pneumococcal pneumonia, is that bacteria colonised in the nasopharynx are aspirated into the lower airways. And thirdly, the presence of multiple chronic debilitating diseases

predisposes one for pneumonia [8, 9]. Chronic diseases identified as important risk factors for pneumonia are a recent history of stroke, diabetes, asthma, and chronic obstructive pulmonary diseases (COPD) [10-13].

Although considerable progress has been made over recent years in antimicrobial treatment and supportive care for patients with pneumonia, the issue of prevention remains important especially given the disproportionate growth of the elderly population, most vulnerable to this deadly disease. All aetiological factors described above provide potential targets to reduce the burden of pneumonia. This thesis explores whether currently available medication drugs could be of help in reducing the risk of acquiring pneumonia.

### Antimicrobial treatment of pneumonia

In addition to rising incidences of pneumonia, challenges in the treatment of pneumonia also increase with age. The elderly are especially more likely to develop complications from pneumonia compared with younger patients. In the elderly, pneumonia is often a terminal event (mortality rates from 5 to 20%) [14, 15]. Particularly, site of care is an important decision regarding the outcome of pneumonia in the elderly [16]. Nowadays, many guidelines for the management of patients with suspected pneumonia differentiate between low-risk patients who are suitable for home treatment, and high-risk patients who should be monitored more closely in a hospital setting [17]. Despite these guidelines, however, there is still a group of patients which is initially treated as outpatient but admitted later [18]. Failure of initial outpatient antibacterial therapy could have consequences. For example, it could have delayed hospital treatment which is essential for good outcome [19, 20]. To study this, one prospective study (chapter 2.1) aimed to investigate the predictive value of prior outpatient antibiotic treatment for microbial aetiology of pneumonia and a second study (chapter 2.2) addresses the association between prior outpatient antibacterial treatment and in-hospital mortality of pneumonia.

### Drug-induced pneumonia

The high prevalence of comorbid chronic diseases in elderly patients introduces a considerable amount of medication involved in the management of these diseases. Drug therapy could indirectly or directly affect the risk of acquiring pneumonia by stabilising or improving chronic morbidity or a direct effect of the drugs, respectively. For example, patients with chronic obstructive pulmonary

disease (COPD) often take corticosteroid-like drugs. Corticosteroids both suppress inflammation and the primary host response to infection. The latter could increase the risk for respiratory tract infections [21]. In the recent study from Ernst et al. the use of inhaled corticosteroids was associated with an excess increased risk of pneumonia among elderly patients with COPD [22]. Another frequently prescribed class of drugs recently associated with the risk of pneumonia are gastric acid suppressing drugs. Proton pump inhibitors are associated with an almost two-fold increased risk of pneumonia [23, 24]. Gastric acid is an important barrier against pathogen invasion through the gastrointestinal tract. Raised stomach pH through proton pump inhibition could increase bacterial and viral colonisation of the oral space via the stomach. The colonised secretions may then gain access to the lower airways and cause pneumonia.
In chapter 3 and 4 of this thesis, two other frequently prescribed drugs are evaluated in light of the risk of pneumonia: (i) angiotensin-converting enzyme (ACE) inhibitors and (ii) hydroxymethylglutaryl CoA reductase inhibitors (statins). ACE-inhibitors may be important because they enhance the cough reflex and in turn could prevent aspiration causing pneumonia. Statins may be influential because of emerging evidence that statins positively affect life-threatening infections associated with cytokine dysregulation, such as bacteraemia [25-27]. Pneumonia is often complicated by the occurrence of bacteraemia [28].

### ACE-inhibitors and interference with pneumonia

Aspiration of pharyngeal flora into the lower airways has an important role in the pathogenesis of community-acquired pneumonia [7]. Patients at high risk for aspiration, such as patients with a recent history of stroke, have a more than two-fold increased risk of pneumonia [11]. A possible way to counteract the risk of aspiration is by the administration of ACE-inhibitors. Along with its effect that cleaves angiotensin I to angiotensin II, ACE also metabolises the protussive peptides substance P and bradykinin (Figure 2). The decreased metabolism of these peptides through ACE-inhibition has been shown to enhance the cough and swallowing reflex, which could prevent aspiration [29, 30].

More than 20 years after market approval of the first ACE-inhibitor, the first indications have appeared in the literature that an ACE-inhibitor enhanced cough reflex indeed has beneficial effects. In 1998, Arai et al. were the first to report that ACE-inhibitors might counteract dysphagia in patients with either cerebral

infarction or cerebral haemorrhage [31]. A few months later, Kaplan extrapolated this finding by hypothesising that ACE-inhibitors could prevent nosocomial pneumonia [32]. This hypothesis was confirmed soon after by Okaishi et al. who reported a significant protection against pneumonia among users of ACE-inhibitors in relation to patients without anti-hypertensive medication [33]. The first study to really add to the body of evidence that ACE-inhibitors may be beneficial in protecting against pneumonia was the study from Ohkubo et al [34]. In this post hoc analysis of the randomised placebo-controlled protection against recurrent stroke study (PROGRESS), perindopril protected against pneumonia compared with placebo (relative risk of 0.53). The study population comprised patients at high risk for aspiration pneumonia due to dysphagia [11]. So far, however, the protective effect of ACE-inhibitors against pneumonia has only been established in Asian populations. Two studies presented in this thesis (chapters 3.1 and 3.2) aim to assess whether this protective effect could be extended to the white population.

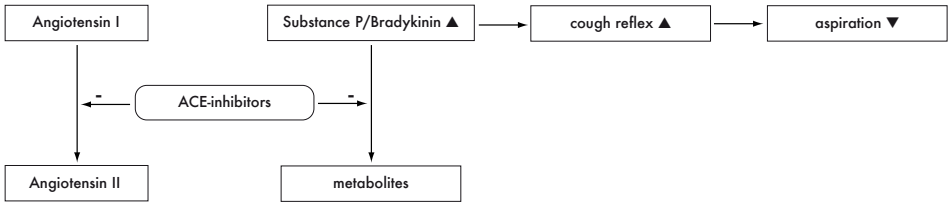


**Figure 2** *Proposed mechanism of action of ACE-inhibitors on the risk of pneumonia.*

*Genetic interaction*

As previously mentioned, until now the protective effect of ACE-inhibitors against pneumonia could only be established in Asian populations. Ohkubo et al. did include both Asian and non-Asian participants but they reported that the protective effect was present only among the Asian participants [34]. Considering the randomised nature of that study, such an ethnical difference is remarkable and could suggest genetic involvement. The ACE insertion/deletion (I/D) polymorphism provides one possible explanation. The ACE I/D polymorphism correlates with the cough threshold [35, 36] and its distribution is known to differ between Asian and non-Asian populations [37]. People with the ACE II genotype are more susceptible to cough and the prevalence of the ACE II genotype in white and African populations is low compared to Asian populations. This difference could modify the effects of ACE-inhibitors on the risk of pneumonia. First, the ACE I/D poly-

morphism could act as a confounder when the polymorphism both influences the risk of acquiring pneumonia as well as the probability of being prescribed an ACE-inhibitor [38]. Second, the polymorphism could act as an effect modifier. Effect modification implies that the protective effect of ACE-inhibitors on the risk of pneumonia is different between genotypes. To further reveal the impact of the ACE I/D polymorphism on the pathogenesis of pneumonia, the association between the ACE I/D polymorphism and the risk of acquiring pneumonia is studied in chapter 3.3.

*Clinical development of pneumonia*

Recently, one study has suggested that ACE-inhibitors also influence the outcome of pneumonia [39]. One possible explanation for this could be an increased clearance of secretions. However, there is also a possibility that the beneficial effect may be due to blunting the cytokine response so that patients have lower rates of sepsis and/or ARDS [40, 41]. To further unravel the role of ACE in the pathogenesis of pneumonia, a study is presented in chapter 3.4 that aims to assess serum ACE activity during the acute phase of pneumonia and at recovery. Concomitantly, severity of pneumonia and outcomes are captured and studied in relation to serum ACE activity. Knowledge about the course of ACE activity during an episode of pneumonia could provide insight into the mechanisms behind the protective effect of ACE-inhibitors for pneumonia.

**Hydroxymethylglutaryl CoA reductase inhibitors and pneumonia**

Another class of drugs possibly related to the risk of acquiring pneumonia are statins. The rate-limiting enzyme in cholesterol synthesis is HMG-CoA reductase which catalyses the conversion of HMG-CoA to mevalonic acid. Several fungal metabolites appeared to be potent inhibitors of this enzyme. In 1986, this knowledge resulted in market approval of the first exogenous HMG-CoA reductase inhibitor simvastatin [42]. Oral administration of statins appeared to decrease hepatic cholesterol synthesis and subsequently lead to increased synthesis of low density lipoprotein (LDL) receptors and thus increasing clearance of LDL [42]. This marked reduction in LDL-cholesterol in turn greatly reduced the risk of coronary artery disease [43]. Today, there is accumulating evidence that the cardiovascular risk reduction by statins is not solely based on the reduction in blood lipid levels [44, 45]. Evidence suggests that statins, in addition to lipid reduction, may provide other positive pleiotropic effects [46, 47]. From cardiovascular clinical trials, it has become

evident that the additional effects of statin treatment predominantly involve a reduction of inflammation. Circulating markers of systemic inflammation, such as CRP and soluble CD40L, decreased after statin treatment [48]. Another recent study demonstrated a decrease in IL-6 concentration, albeit in combination with a slight increase in CD68 positive macrophages [49]. It was suggested that not the presence but rather the activation status of macrophages was attenuated during statin treatment.

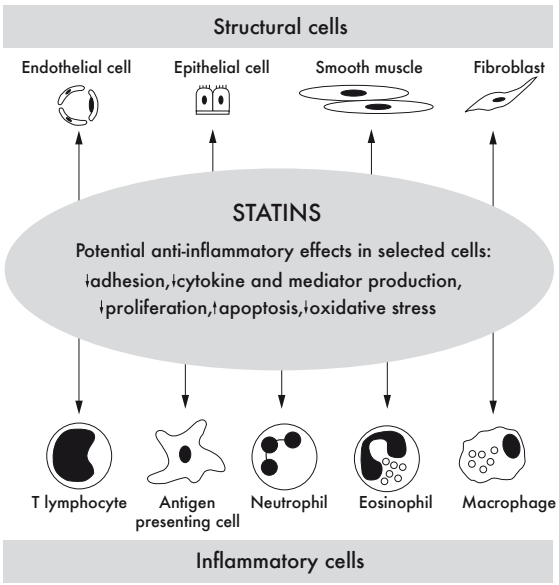


**Figure 3** *Potential anti-inflammatory effects of statins on different structural and inflammatory cells within the lungs (reproduced with kind permission from the BMJ Publishing Group Ltd.).*

Also, statins showed different anti-inflammatory effects on a pulmonary level [50]. Figure 3 shows potential effects of statins on different structural and inflammatory cells within the lungs. To further reveal the non-lipid lowering effects of statins in this thesis the association between the use of statins and risk of pneumonia is studied (chapter 4.1). Different studies have recently shown that use of statins is associated with the outcome of numerous infectious diseases, including pneumonia [51-54].

### Validation of the outcome measure

In this thesis, two large administrative databases are used to study the effects of drugs on the risk and outcome of community-acquired pneumonia. The first database is the Dutch PHARMO Record Linkage System [55]. In this database, hospital discharge records are linked to prescription drug dispensing data originating from community pharmacies. The second database used is the United Kingdom General Practice Research Database (GPRD). In this database, patient demographics, characteristics (i.e. height, weight), symptoms and diagnoses, referrals to specialist care, hospital admissions and their major outcomes, and all drug prescriptions are registered. Although the validity of both the databases has been assessed in different settings in the past [56, 57], this does not exclude potential biases when studying community-acquired pneumonia. For example, an underestimated effect could be observed when not all patients with pneumonia are assigned the corresponding codes in the database. Or, as a result of disease misclassification, bias could occur when not all observed cases are true cases. In chapter 5 of this thesis, two studies are presented addressing the appropriateness of ICD codes for studying community-acquired pneumonia. The first study aims to evaluate the accuracy of ICD code assignment in a population of patients with confirmed pneumonia. The second study aimed to calculate the positive predictive value of hospital discharge diagnoses of pneumonia, classified according to ICD-9-CM codes, as a marker for community-acquired pneumonia.

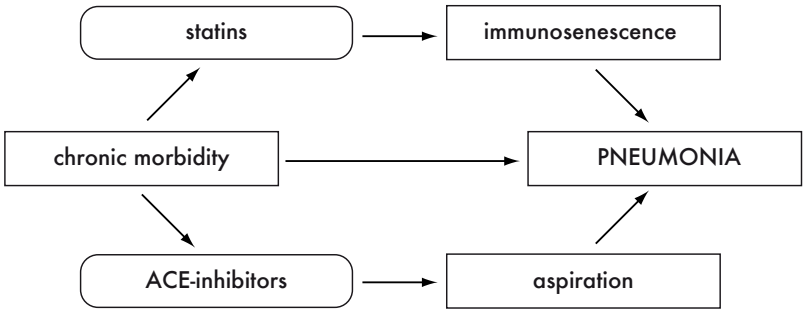


**Figure 4** *Schematic overview where ACE-inhibitors and statins could interact with the risk of acquiring pneumonia.*

## Aims and scope of this thesis

The main objectives of this thesis are to explore the impact of prior outpatient antimicrobial treatment on the prognosis of pneumonia in patients admitted to hospital, and to test the hypothesis that both ACE-inhibitors and statins affect the risk of acquiring pneumonia. Figure 4 illustrates the different sites where these drugs could interact with the risk of pneumonia.

In chapter 2.1, a study is presented on the predictive value of prior outpatient antimicrobial treatment for microbial aetiology of pneumonia. This study is conducted with data from a prospective cohort of patients admitted with community-acquired pneumonia to the St. Antonius Hospital, Nieuwegein, The Netherlands. The study in chapter 2.2 assesses whether prior outpatient antimicrobial treatment is prognostic for pneumonia-related in-hospital mortality.

The third chapter of this thesis consists of four studies focusing on the relation between angiotensin-converting enzyme and community-acquired pneumonia. Chapter 3.1 presents the association between ACE-inhibitor use and pneumonia risk in the general population, whereas in chapter 3.2 this association is studied in a population of patients with diabetes. Chapter 3.3 emphasises a possible association between the ACE insertion/deletion polymorphism and the risk and prognosis of hospitalisations for community-acquired pneumonia, and chapter 3.4 describes the relation between serum ACE activity and the severity of pneumonia. The latter two studies use data from the St. Antonius Hospital cohort.

Chapter 4.1 describes whether or not the use of statins is associated with the risk of acquiring pneumonia. This study uses data from the same population as in chapter 3.2. Special effort is made in evaluating adjustment for confounding through co-morbidities and healthy behaviour.

Two methodological aspects are addressed chapter 5. The first study assesses the accuracy of ICD-9-CM code assignment in a population with confirmed pneumonia; the second study focuses on the positive predictive value of ICD-9-CM codes for identifying pneumonia.

Finally, in chapter 6 the main findings and conclusions are discussed and put into a general perspective of efforts to prevent pneumonia.

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# 2 | Antibacterial treatment and aetiology and outcome of pneumonia

# 2.1 | Prior outpatient antibiotic use as predictor for microbial aetiology of community-acquired pneumonia: hospital-based study

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Submitted for publication

## Abstract

Different studies examined age and co-morbidities as predictors for microbial aetiology of pneumonia. We aimed to assess whether receipt of prior outpatient antimicrobial treatment is predictive for microbial aetiology of community-acquired pneumonia.

This was a hospital-based prospective observational study including all patients admitted with community-acquired pneumonia between 1 October 2004 and 1 August 2006. Microbial investigation included sputum, blood culture, sputum PCR, antigen testing, and serology. Exposure to antimicrobial drugs prior to hospital admission was ascertained through community pharmacy dispensing records. Multivariate logistic regression analysis was conducted to assess whether prior outpatient antimicrobial treatment is predictive for microbial aetiology. Patient demographics, comorbidities and pneumonia severity were considered other potential predictors.

Overall, 201 patients were included in the study. The microbial aetiology was determined in 64% of the patients. The five most prevalent pathogens were *Streptococcus pneumoniae*, *Heamophilus influenzae*, *Legionella spp.*, *Mycoplasma pneumoniae*, and Influenza virus A+B. Forty-seven of the patients (23%) had received initial outpatient antimicrobial treatment. In multivariate analyses, initial outpatient treatment was associated with a three-fold increased chance of finding atypical pathogens and a three-fold decreased probability of pneumococcal infection. The corresponding odds ratios were 3.11 (95% CI 1.16-8.33) and 0.39 (95% CI 0.17-0.91) respectively.

Prior outpatient antimicrobial therapy is very relevant information in the diagnostic workup aiming to identify the causative pathogen and planning corresponding treatment in patients hospitalised for pneumonia.

## Introduction

Community-acquired pneumonia remains a major reason for hospital admission and a common cause of death in developed countries [1, 2]. The initial management of patients hospitalised with pneumonia consists mostly of empirical antimicrobial treatment [3, 4]. Appropriate antimicrobial treatment is essential as inadequate antimicrobial treatment, generally defined as microbial ineffective therapy against the causative pathogen, can influence patient outcome [5]. Because the causative pathogen is not always identified, especially not during the first days after hospitalisation, many studies have focussed on other parameters suggestive for the causative pathogen. Most frequently studied for this purpose are patient characteristics (age and co-morbidities) and severity of pneumonia [6, 7]. Besides this, nonresponsiveness to prior outpatient antimicrobial treatment could also act as a predictor for aetiology of pneumonia. The latter, however, has not been extensively studied before. The aim of the present study was to assess whether prior outpatient antimicrobial treatment is predictive for microbial aetiology in patients admitted to hospital for community-acquired pneumonia.

## Patients and methods

The study was conducted in the St. Antonius Hospital, a 600-bed teaching hospital (Nieuwegein, The Netherlands).

*Patient population*

This was a prospective observational study of patients with confirmed pneumonia admitted between October 1, 2004 and August 1, 2006. Pneumonia was defined as a new or progressive infiltrate on a chest X-ray plus at least two of the following criteria: cough, sputum production, temperature >38°C or <35°C, ausculatory findings consistent with pneumonia, leucocytosis or -penia (>10 G/L, <4 G/L, or >10% rods in leucocyte differentiation), C-reactive protein >3 times the upper reference value for normal. Patients, who were immune-compromised (systemic steroid use at admission (prednison equivalent >20 mg/daily for more than 3 days), haematological malignancies and other immunosuppressive therapy) were excluded. The study was approved by the local Medical Ethics Committee and informed consent was obtained from each patient.

*Microbial aetiology workup*

At least two blood cultures were performed and sputum was taken for Gram-stain and culture and analysed by Taqman real-time PCR for *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophyla psittaci* [8]. Pharyngeal samples were taken for viral culture. Urine was sampled for antigen testing on *Streptococcus pneumoniae* and *L.pneumophila* (Binax NOW®) [9, 10]. In addition serum samples of the day of admission and day 10 were analysed in pairs for detection of a fourfold rise of antibodies to respiratory viruses, *Coxiella burnetii*, *M.pneumoniae*, and *C.psittaci* by complement fixation assay [11]. For each patient, the total workup was completed and the microbiology department was blinded for data on outpatient antibacterial drug use. When both viruses and bacteria were identified in a patient, bacteria prevailed for definite aetiology.

*Exposure to antimicrobial therapy*

Data on outpatient antimicrobial drug use was acquired through community pharmacy dispensing records capturing all drug exposures the year before hospital admission. A patient was considered exposed to an antimicrobial drug when a prescription was filled within 14 days prior to hospitalisation. The name, dosage and amount of antimicrobial drug dispensed were also ascertained. Based on the reigning Dutch guidelines on the initial treatment of patients with suspected pneumonia, the prescribed antimicrobial drug was classified as appropriate or inappropriate [12, 13].

*Co-morbidity assessment*

Besides outpatient antimicrobial drug use, co-morbidities and other relevant patient characteristics were identified to address factors related with aetiology of community-acquired pneumonia. Co-morbidities were defined based on the presence of conditions for which the patient was under active medical supervision or was receiving treatment at the time of hospital admission. Co-morbidities evaluated were pulmonary diseases (chronic obstructive pulmonary disease, or treated asthma), congestive heart failure, diabetes (both type I and type II), history of stroke, and end-stage renal disease (serum creatinine >150 μmol/L). Furthermore, patients were classified according to the Pneumonia Severity Index (PSI) developed by Fine et al [14]. The outpatient use of oral corticosteroids and gastric acid suppressing drugs was also ascertained.

*Statistical analysis*

The SPSS statistical package (version 12.0.1 for Windows; SPSS, Chicago, IL) was used for the statistical analyses. Continuous data were expressed as mean ±SD or median (interquartile range) where appropriate. To study the association between prior outpatient antimicrobial treatment and aetiology of pneumonia multivariate logistic regression analyses were applied. Analyses were conducted for overall aetiology and relevant pathogens separately. All baseline characteristics were considered potential predictors. Predictors were included in the multivariate model when they were retained after backward stepwise elimination. Significance was set at a p-value <0.05. The model's performance (goodness-of-fit and discriminative ability) was tested by performing the Hosmer and Lemeshow test and calculating the area under the receiver operator characteristic (ROC) curve.

**Table 1** *Demographics, co-morbidities and clinical severity of 201 patients with community-acquired pneumonia*

| **Characteristic** | **n (%)** |
|---|---|
| *Age (Years)* | |
| <60 | 74 (37) |
| 60 - 69 | 39 (19) |
| 70 - 79 | 50 (25) |
| ≥80 | 38 (19) |
| *Gender* | |
| Male | 124 (62) |
| Female | 77 (38) |
| *Co-morbidities* | |
| Pulmonary diseases | 71 (35) |
| Heart failure | 18 (9) |
| Diabetes | 35 (17) |
| History of stroke | 17 (9) |
| End-stage renal disease | 10 (5) |
| Nursing home resident | 3 (1) |
| *Co-medication* | |
| Oral corticosteroids | 58 (29) |
| Gastric acid suppressing drugs | 61 (30) |
| *Fine score at admission** | |
| I | 30 (15) |
| II | 34 (17) |
| III | 53 (26) |
| IV | 56 (28) |
| V | 28 (14) |

* *Fine et al.* [14]

**Table 2** *Outpatient antibiotics utilization profile prior to hospitalisation for community-acquired pneumonia*

| Type of antimicrobial drug | n (%)* | Appropriate** |
|---|---|---|
| Amoxycillin / clavulanic acid | 18 (38) | Yes |
| Amoxycillin | 12 (26) | Yes |
| Doxycycline | 7 (15) | Yes |
| Clarithromycin | 5 (11) | Yes |
| Co-trimoxazole | 4 (8) | No |
| Ciprofloxacin | 2 (4) | No |
| Norfloxacin | 1 (2) | No |
| Azithromycin | 1 (2) | Yes |

** Total percentage exceeds 100% because some patients (n=3) had two precriptions*

*** Based on reigning Dutch guidelines NVALT and SWAB* [12, 13]

**Table 3** *Yield of different techniques for the aetiology of community-acquired pneumonia*

| | Sputum culture | Sputum PCR | Antigen testing | Blood culture | Sero-logy | Viral culture |
|---|---|---|---|---|---|---|
| Number of samples | 148 | 78 | 183 | 182 | 130 | 88 |
| Number positive | 78 | 14 | 36 | 19 | 38 | 14 |
| Percentage positive | 53 | 18 | 20 | 10 | 29 | 16 |
| ***S.pneumoniae*** | 33 | - | 30 | 17 | - | - |
| **Gram negative strain** | | | | | | |
| *H.influenzae* | 19 | - | - | 0 | - | - |
| Other | 10 | - | - | 1 | - | - |
| **Atypical** | | | | | | |
| *M.pneumoniae* | - | 7 | - | - | 8 | - |
| *Legionella spp.* | 1 | 5 | 6 | - | 7 | - |
| Other | - | 2 | - | - | 1 | - |
| **Viral** | - | - | - | - | 22 | 14 |
| **Other** | | | | | | |
| *S.aureus* | 6 | - | - | 1 | - | - |
| Gram pos. other | 2 | - | - | 0 | - | - |

## Results

In total 201 patients with pneumonia were included in the study. The mean age of the patients was 63 (±17) years and 124 were male. Three patients (1%) were admitted from a nursing home. Clinical symptoms as well as co-morbid illnesses are summarized in Table 1. The overall median duration of hospital stay was 10 days (7-14) and 21 patients were admitted to the intensive care ward. During hospital stay, 10 patients died, all due to pneumonia. The overall 28-days mortality rate was 5%. Forty-seven patients (23%) had received antimicrobial treatment in the 14 days time-window prior to hospital admission. The antimicrobial drugs dispensed to these patients are summarized in Table 2.

The majority of the patients (79%) had their prescription filled within 4 days prior to hospital admission and 85% of the prescribed antimicrobial drugs complied with the reigning Dutch guidelines [12, 13]. A microbial aetiology could be determined in 128 (64%) of the patients. Table 3 shows the yield of different techniques for the aetiological diagnosis of community-acquired pneumonia.

In the population which was hospitalised after prior outpatient antimicrobial treatment, fewer causative pathogens were found compared to patients without prior antimicrobial treatment (57% *vs.* 66%) (crude OR 0.71, 95% CI 0.36-1.38). In patients with prior treatment, an aetiology in the group comprising atypical bacterial pathogens was more probable (10 of 47 cases (21%)) (crude OR 3.51, 95% CI 1.39-8.90), and especially pneumonia due to *M.pneumoniae* (5 of 47 cases (11%)) (crude OR 4.46, 95% CI 1.15-17.37). Aetiology of *S.pneumoniae* was less prevalent in patients with prior antimicrobial treatment (8 of 47 cases (17%) (crude OR 0.40, 95% CI 0.18-0.92). In multivariate analyses, these associations remained significant. The associations (both univariate and multivariate) are listed in Table 4. The goodness-of-fit of both multivariate models was excellent with a p-value of 0.966 (Hosmer and Lemeshow test) for the model predicting pneumococcal pneumonia, and a p-value of 0.863 for the model predicting pneumonia of atypical aetiology. The corresponding areas under the ROC curve were 0.64 and 0.79 respectively. In patients aged <60 years without co-morbidities, aetiology of atypical bacterial pathogens was more prevalent (OR 4.64, 95% CI 1.72-12.56). Pulmonary co-morbidity was associated with the finding of *S.pneumoniae* and *H.influenzae* as causative pathogens (OR 1.87, 95% CI 1.00-3.47 and OR 3.72, 95% CI 1.20-11.57 respectively).

**Table 4** *Odds ratios (OR) for aetiology and prior outpatient antimicrobial treatment in patients admitted to hospital for community-acquired pneumonia*

| | Prior outpatient antimicrobial treatment | | |
|---|---|---|---|
| **Aetiology** | **Yes** | **No** | **OR (95% CI)** |
| Total no. of samples | 47 (100) | 154 (100) | |
| *Univariate* | | | |
| Pneumococcal | 8 (17) | 52 (34) | 0.40 (0.18 – 0.92) |
| Atypical | 10 (21) | 11 (7) | 3.51 (1.39 – 8.90) |
| Viral | 4 (9) | 12 (8) | 1.10 (0.34 – 3.59) |
| Gram negative strains | 3 (6) | 20 (13) | 0.46 (0.13 – 1.61) |
| Other | 2 (4) | 6 (4) | 1.10 (0.21 – 5.62) |
| Unidentified | 20 (43) | 53 (34) | 1.41 (0.73 – 2.75) |
| *Multivariate* | | | |
| Pneumococcal | - | - | 0.39 (0.17 – 0.91) * |
| Atypical | - | - | 3.11 (1.16 – 8.33) ** |

*OR: Odds Ratio; CI: Confidence Interval*

** Adjusted for pulmonary diseases and use of oral corticosteroids*

*** Adjusted for age and pulmonary diseases*

## Discussion

Our study shows that in patients admitted for community-acquired pneumonia, whether the patient received initial outpatient treatment is associated with a three-fold decreased chance of having an infection with *S.pneumoniae* and a three-fold increased probability of having pneumonia of atypical aetiology. These findings indicate that information about prior outpatient antimicrobial therapy is very relevant in the diagnostic workup aiming to identify the causative pathogen and planning corresponding treatment in patients with pneumonia.

The initial management of patients hospitalised with pneumonia has been under constant study in different settings during the past decades. Choice of antimicrobial treatment, time to first antimicrobial drug administration, and route of administration all have appeared to be relevant factors in relation to the outcome of pneumonia [15-17]. In light of choice of antimicrobial treatment, knowledge of predominant microbial patterns in CAP represents an essential basis for initial decisions about empirical antimicrobial treatment. In literature, the most frequently found pathogens in community-acquired pneumonia are *S.pneumoniae*, *H.influenzae*, Influenza virus A and B, *Legionella spp.* and *C.pneumoniae* [3]. The aetiology distribution found in the present study is in accordance with this litera-

ture. Because *S.pneumoniae* is the most frequently found pathogen, beta-lactam antibiotics are preferred as initial empirical antimicrobial treatment in treatment guidelines on CAP [3, 13, 18]. Beta-lactam antibiotics, however, do not cover *Legionella spp*., *C.pneumoniae*, and *M.pneumoniae*, the so-called atypical pathogens. Therefore, patients with pneumonia of atypical aetiology treated as outpatient with beta-lactam antibiotics will probably not respond to treatment which could cause deterioration of the situation and lead to subsequent hospital admission. Our finding of an increased prevalence of atypical pathogens in patients with prior outpatient antimicrobial treatment supports such an explanation, but also confirms what has already been suggested in the current British Thoracic Society guideline for the management of community-acquired pneumonia in adults [3, 19]. In this guideline is stated that after failure of initial empirical antibiotic treatment, the microbiological examination should be reassessed with a view to excluding less common pathogens such as atypical pathogens. To our knowledge, the present study is the first study to specifically document failure of initial outpatient antibiotic treatment as predictor for microbial aetiology of community-acquired pneumonia.

Another explanation for the observed reduced likelihood of pneumococcal pneumonia in patients who received prior outpatient antimicrobial treatment could be a growth suppression of *S.pneumoniae* in blood and sputum cultures through the presence of antibiotics. This could mask *S.pneumoniae* as the causative pathogen. We think, however, that such an explanation is less plausible, especially because we also used antigen testing for identification of the causative pathogen [20]. In addition, such a mechanism can not explain the finding of an increased probability of pneumonia caused by atypical pathogens.

The present study was conducted in a single teaching hospital in the Netherlands, what could pose questions about extrapolation to other hospitals and clinical settings. We think, however, that the external validity of the present study is sufficiently high. First, because the percentage of identified aetiology (64% in the present study) complies with other studies using a similar extent and nature of microbiologic techniques [21-23]. Second, because our patient characteristics very much comply with a previous nationwide study on prior outpatient antibacterial therapy as prognostic factor for mortality in patients hospitalised for pneumonia [24]. In that large database study, the percentage of patients hospitalised after initial outpatient antimicrobial treatment was almost identical to the

percentage observed in the present study (27% *vs.* 23%). In addition, also age distribution, co-morbidities and outpatient antibiotics utilization profile were very similar as were the median duration of hospital stay and in-hospital mortality. Unfortunately, due to limited numbers, we were unable to study an association between prior outpatient antimicrobial treatment and mortality in the present study.

Besides prior antimicrobial treatment, our study also showed an association between aetiology and age, and pulmonary co-morbidity. Patients aged <60 years without co-morbidities were more likely to have an aetiology comprising viral or atypical bacterial pathogens, and pulmonary co-morbidity was independently associated with *S.pneumoniae* and *H.influenzae* as causative pathogens. These findings confirm previous studies on the impact of age, and co-morbidity on microbial aetiology of community-acquired pneumonia [6]. A limitation of the present study, however, is that we were not able to adjust for confounding by smoking habits and alcohol intake of the patients. Previous studies on determinants for pneumonia aetiology also showed that these factors are significant predictors for pneumococcal infection [6, 7]. On the other hand, we do not expect prior antimicrobial therapy and smoking and alcohol intake to coincide in such a way that this would result in finding a null effect when this information was available.

In conclusion, in patients admitted for pneumonia, whether or not a patient has received prior outpatient antimicrobial therapy is very relevant information in the diagnostic workup aiming to identify the causative pathogen and planning initial treatment at the time of hospital admission. This finding supports further strengthening of continuity of care at the interface between the extramural and hospitalised settings.

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# 2.2 | Prior outpatient antibacterial therapy as prognostic factor for mortality in hospitalized pneumonia patients

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## Abstract

The aim of the present study was to assess whether prior outpatient antibacterial treatment is associated with outcome in patients hospitalized for community-acquired pneumonia (CAP).

Data were obtained from the Dutch PHARMO Record Linkage System. All patients with a first hospital admission for CAP between 1995 and 2000 were selected. Patients were divided into two groups, one of patients with use of antibacterial agents prior to hospitalization and one of patients treated as inpatient directly. The main outcome measures were duration of hospital stay and in-hospital mortality.

The two patient groups comprised 296 and 794 patients, respectively. The median duration of hospital stay was 10 days and was similar for both groups. In patients with respiratory diseases or heart failure, the median duration of hospital stay was 12 and 14 days, respectively. The overall in-hospital mortality was 7.2% and did not largely differ between both groups. In patients with congestive heart failure, the mortality was 9.8% for controls and 23.3% for patients hospitalized after initial outpatient treatment (adjusted odds ratio (OR) 2.78, 95% confidence interval (CI) 1.01-7.81).

In conclusion, prior outpatient antibacterial therapy is not associated with outcome in hospitalized pneumonia patients. In patients with underlying chronic heart failure, prior outpatient antibiotic is associated with a significant increased mortality.

## Introduction

Community-acquired pneumonia (CAP) is a major direct cause of death in the elderly with mortality rates for inpatients varying from 5% to 20% [1, 2]. Approximately 20% of all patients with pneumonia require inpatient treatment [3, 4].

Whether to treat a patient with CAP as an outpatient or as an inpatient is an extremely important decision. Nowadays many guidelines give directions for making this decision [5-9]. Despite these guidelines still patients fail initial outpatient treatment and get hospitalized subsequently. To our knowledge hardly any studies about factors influencing outcome in hospitalized pneumonia patients include failure of initial outpatient antibacterial drug treatment as prognostic factor. Considering that the clinical course of patients with CAP within the first 2-3 days is crucial in relation to disease outcome [10], it seems logical to suspect that patients who fail initial outpatient antibacterial therapy and get subsequently hospitalized could be at increased risk for worse outcome compared with patients treated as an inpatient directly. The objective of this study was to assess whether prior outpatient antibacterial therapy is associated with outcome in patients hospitalized for CAP.

## Patients and Methods

*Data setting*

The setting of the study was the PHARMO record linkage system (www.pharmo.nl). PHARMO includes pharmacy dispensing records from community pharmacies linked to hospital discharge records of more than 600,000 community-dwelling residents of more than eight population-defined areas in the Netherlands from 1985 onwards [11]. Since virtually all patients in the Netherlands are registered with a single community pharmacy independently of prescriber, pharmacy records are virtually complete with regard to prescription drugs.

The computerized drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. The duration of use of each dispensed drug is estimated by dividing the number of dispensed units by the prescribed number of units to be used per day. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification. The hospital discharge records are obtained from PRISMANT (www.prismant.nl), previously known as the Dutch Center for Healthcare Information (LMR database), an institute that collates nationwide all hospital discharge records in the Netherlands since the 1960s into

a standardized format. These records include detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical and treatment procedures, type and frequency of consultations with medical specialists and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9).

*Study base*

The study base comprised all patients with a first hospital admission for community-acquired pneumonia between January 1, 1995 and December 31, 2000. All patients were selected according to the following criteria: adult (>18 years old) and hospitalized with a primary discharge diagnosis of CAP: pneumococcal pneumonia (ICD-9 code 481), other bacterial pneumonia (ICD-9 code 482), bronchopneumonia, organism unspecified (ICD-9 code 485) or pneumonia, organism unspecified (ICD-9 code 486). Besides this, patients with a signature of bacterial pneumonia (secondary discharge diagnosis) coupled with a primary pulmonary diagnosis (ICD-9 codes 491-493 or 496) were selected. For all subjects pharmacy dispensing data were collected. Patients were eligible if they had at least 365 days of exposure information prior to hospital admission available in the PHARMO record linkage system.

*Definition of groups*

For each patient, we identified all prescriptions for antibacterial agents (ATC code J01A to J01X) within the year prior to the date of hospital admission. In the Netherlands the standard duration of antibacterial drug use for pneumonia varies from 7 to 10 days [12-14]. Patients were divided into two groups. Patients hospitalized without a prescription for an antibacterial drug within 2 weeks prior to the hospital admission date were considered inpatient treatment only. All patients who received one or more antibacterial drugs within the 2 weeks prior to hospitalization were considered as hospitalized after prior outpatient treatment. For these patients the duration of outpatient antibacterial drug use was calculated as the number of days between dispensing and hospital admission. Based on duration of use, patients with prior antibiotic use were further subdivided in early failure (duration of use ≤3 days) and late failure (duration of use >3 days). Antibiotics prescribed were classified as appropriate when mentioned in the reigning Dutch guidelines for initial treatment of pneumonia [12-14].

*Outcome measures*

The duration of hospital stay was defined as the difference between the admission and discharge date. If the date of death in the PHARMO record linkage system was similar to the hospital discharge date, the patient was considered to have died in the hospital.

*Co-morbidity assessment*

Besides antibacterial agents also cardiovascular medication, respiratory drugs, immunosuppressive agents, cancer medication and flu vaccination were identified to address co-morbidities related with outcome in CAP [1, 2, 15, 16]. When a patient received bronchodilators and or inhaled corticosteroids, the patient was considered as suffering from respiratory diseases. The combination of a diuretic with a cardiac glycoside was considered as signature for congestive heart failure (CHF). For these groups of drugs one was considered exposed if more than one dispensing occurred in the 12-month period prior to hospitalization, except for flu vaccination, which required just one dispensing. To evaluate the overall health status of the patients, for each patient the Chronic Disease Score (CDS) was calculated based on patterns of use of selected prescription medications during a 1-year period identified by a consensus judgment process [17].

*Data Analysis*

Mann-Whitney U-tests were used to compare duration of hospital stay between groups. Logistic regression analysis was used to calculated crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) (SPSS for Windows, version 11.0; SPSS Inc., Chicago, Illinois, USA).

## Results

The study population comprised 1,090 patients with a first hospital admission for community-acquired pneumonia. We identified 296 patients with prior antibiotic use (27% of all CAP admissions) and 794 patients hospitalized with no prior antibiotic use. Table 1 shows the characteristics of both groups. The overall mean age was 67 years (±0.7 years) and was not different between both groups. Also, the CDSs were comparable, indicating that the overall health status did not differ between both groups. In our study population almost 97% of all patients had a primary diagnosis of CAP, and most of them had a pneumonia with unspecified organism (about 72%) recorded in the hospital abstract.

**Table 1** *Characteristics of both groups*

| Characteristic | Prior outpatient treatment (n=296) | Inpatient treatment only (n=794) | Crude OR (95% CI) |
|---|---|---|---|
| *Age (years)* | | | |
| <40 | 33 (11.1) | 64 (8.1) | |
| 40-49 | 22 (7.4) | 68 (8.6) | |
| 50-59 | 23 (7.8) | 92 (11.6) | |
| 60-69 | 55 (18.6) | 133 (16.8) | |
| 70-79 | 92 (31.1) | 260 (32.7) | |
| 80-89 | 62 (20.9) | 152 (19.1) | |
| ≥ 90 | 9 (3.0) | 25 (3.1) | |
| *Gender* | | | |
| Male | 169 (57.1) | 466 (58.7) | 1.00 (reference) |
| Female | 127 (42.9) | 328 (41.3) | 1.07 (0.82-1.40) |
| *Primary discharge diagnosis* | | | |
| Pneumococcal pneumonia | 12 (4.1) | 69 (8.7) | |
| Other bacterial pneumonia | 21 (7.1) | 62 (7.9) | |
| Bronchopneumonia, organism unspecified | 37 (12.5) | 100 (12.6) | |
| Pneumonia, organism unspecified | 219 (74.0) | 536 (67.5) | |
| *Chronic Disease Score* | | | |
| 0 | 58 (19.6) | 162 (20.4) | 1.00 (reference) |
| 1-3 | 55 (18.6) | 152 (19.1) | 1.01 (0.66-1.55) |
| 4-6 | 67 (22.6) | 205 (25.8) | 0.91 (0.61-1.37) |
| 7-9 | 54 (18.2) | 142 (17.9) | 1.06 (0.69-1.64) |
| ≥10 | 62 (20.9) | 133 (16.8) | 1.30 (0.85-1.99) |
| *Co-medication* | | | |
| Respiratory drugs | 128 (43.2) | 322 (40.6) | 1.12 (0.85-1.46) |
| Cardiovascular drugs | 162 (54.7) | 466 (58.7) | 0.85 (0.65-1.11) |
| Flu vaccination | 58 (19.6) | 180 (22.7) | 0.83 (0.60-1.16) |
| In-hospital mortality | 24 (8.1) | 55 (6.9) | 1.19 (0.72-1.95) |

*OR: Odds Ratio; CI: Confidence Interval*

Table 2 lists all the antibiotics prescribed for the prior antibiotic group within the 2 weeks prior to hospitalization. Figure 1 shows the duration of outpatient antibacterial drug use before hospitalization. Based on this, 126 patients were classified as "early failure" and 170 patients as "late failure". More than 92% of the antibiotics prescribed was appropriate according to the reigning Dutch guidelines for the initial treatment of CAP [12-14]. Almost 90% of all prescriptions originated from a general practitioner. The overall median duration of hospital stay was 10 days and similar for both groups (10 *vs.* 10 days, respectively) and did not show a significant difference (p=0.787). For "late failure" patients, the median duration of hospital stay was 11 days. In patients with respiratory diseases and CHF, the median duration of hospital stay was significantly higher (12 and 14 days, respectively; p<0.01).

**Table 2** *Outpatient antibiotics utilization profile prior to hospitalization for community acquired pneumonia*

| Type of antibacterial drug | ATC-code | No. of users (%) | | Appropriate* |
|---|---|---|---|---|
| Amoxicillin/clavulic acid | J01CR02 | 74 | (22.0) | yes |
| Amoxicillin | J01CA04 | 73 | (21.7) | yes |
| Doxycyclin | J01AA02 | 53 | (15.7) | yes |
| Clarithromycin | J01FA01 | 35 | (10.4) | yes |
| Ofloxacin | J01MA01 | 18 | (5.3) | no |
| Co-trimoxazol | J01EE01 | 17 | (5.0) | yes |
| Azythromycin | J01FA10 | 16 | (4.7) | yes |
| Roxithromycin | J01FA06 | 11 | (3.3) | yes |
| Ciprofloxacin | J01MA02 | 10 | (3.0) | no |
| Erythromycin | J01FA01 | 9 | (2.7) | yes |
| Cephalosporin | J01DA | 3 | (0.9) | yes |
| Norfloxacin | J01MA06 | 3 | (0.9) | no |
| Trimethoprim | J01EA01 | 3 | (0.9) | no |
| Flucloxacilin | J01CF05 | 2 | (0.6) | no |
| Aminoglycosides | J01G | 1 | (0.3) | no |
| Other antibacterials | J01X | 9 | (2.7) | unknown |

** Based on reigning Dutch guidelines NVALT, SWAB and NHG [12-14]*

From all patients, 79 deceased in hospital. This gives an overall in-hospital mortality of 7.2%. All patients who died during their hospital admission were compared with patients that left the hospital alive with respect to prior antibiotic use, age, respiratory diseases, immunosuppressive therapy, congestive heart failure and flu vaccination (Table 3). Age was significantly associated with an increased risk of in-hospital mortality (adjusted OR 1.37, 95% CI 1.14-1.66). Overall, prior antibiotic use was not associated with the in-hospital mortality (adjusted OR 1.09, 95% CI 0.65-1.83). Furthermore, no differences were observed between "early

failure" and "late failure". Considering different co-morbidities, patients with CHF showed a higher in-hospital mortality (adjusted OR 1.83, 95% CI 1.01-3.32). Patients with heart failure and failure of prior outpatient treatment had a significant higher mortality rate (23.3%) compared with heart failure patients treated as inpatient directly (mortality rate 9.8%; adjusted OR 2.78, 95% CI 1.01-7.81). Respiratory disease was not associated with increased mortality in CAP (adjusted OR 0.92, 95% CI 0.54-1.55).

**Table 3** *Odds ratios (OR) for in-hospital mortality and the use of outpatient antibacterial therapy, co-morbidity and other drug treatment*

| | **Dead (n=79)** | **Alive (n=1,011)** | **Crude OR (95% CI)** | **Adjusted OR* (95% CI)** |
|---|---|---|---|---|
| Outpatient therapy | 24 (30.4) | 272 (26.9) | 1.19 (0.72-1.95) | 1.09 (0.65-1.83) |
| -without CHF | 14 (17.7) | 239 (23.6) | 0.85 (0.44-1.62) | 0.87 (0.45-1.68) |
| -with CHF | 10 (12.7) | 33 (3.3) | 2.96 (1.07-8.23) | 2.78 (1.01-7.81) |
| -early failure | 6 (7.6) | 120 (11.9) | 0.60 (0.26-1.43) | 0.61 (0.26-1.45) |
| -late failure | 18 (22.8) | 152 (15.0) | 1.92 (1.06-3.48) | 1.53 (0.87-2.71) |
| Respiratory diseases | 37 (46.8) | 413 (40.9) | 1.28 (0.81-2.02) | 0.92 (0.54-1.55) |
| CHF | 59 (74.7) | 569 (56.3) | 2.49 (1.42-4.38) | 1.83 (1.01-3.32) |
| Flu vaccination | 19 (24.1) | 219 (21.7) | 1.15 (0.67-1.96) | 0.93 (0.53-1.62) |
| Cancer medication | 4 (5.7) | 47 (4.6) | 1.09 (0.38-3.12) | 1.13 (0.38-3.33) |
| Immunosuppressive | 18 (22.8) | 155 (15.3) | 1.63 (0.94-2.83) | 1.40 (0.75-2.58) |

** Adjusted for age, gender, respiratory diseases, flu-vaccination, immunosuppressive drugs, cancer medication; OR: Odds Ratio; CI: Confidence Interval; CHF: congestive heart failure*

## Discussion

In this study, prior outpatient antibiotic treatment was not associated with outcome in patients hospitalized for pneumonia. The duration of hospital stay and in-hospital mortality rates found in this study were comparable with other studies concerning hospitalized pneumonia patients [1, 2]. For patients with heart failure who were hospitalized after initial outpatient therapy, the in-hospital mortality was two-fold increased compared with heart failure patients treated as inpatient directly. Literature indicates that in approximately 20% of all pneumonia cases inpatient treatment is required because the clinical situation does not allow outpatient treatment [2-4]. The decision between outpatient and inpatient treatment will in most cases be made by the general practitioner. This notion has been confirmed by the present study with almost 90% of the prescriptions for antibacterial drugs originating from a general practitioner. Still many patients, however, fail the initial outpatient antibacterial treatment and get subsequently hospitalized.

In the present study almost 30% of the patients was hospitalized after initial outpatient treatment. Although mortality seemed a little higher for these patients compared with patients treated as inpatient directly (8.1 % *vs.* 6.9 % respectively), no significant association could be observed between initial outpatient treatment and mortality in patients hospitalized for pneumonia. Our study had sufficient power to detect a two-fold difference in mortality rates between the two groups ($\alpha$=0.05; (1-$\beta$)=0.80). When considering literature on this subject, there is the recent study of Marrie et al. which showed an indicative increased in-hospital mortality of 11.3% for patients with former outpatient antibacterial therapy compared with patients hospitalized without, i.e. 8.2% [15]. In contrast, however, Johnson et al. associated antibiotic utilization prior to hospitalization for pneumonia with a decreased in-hospital mortality [18] , something which could not be confirmed by the present study.

When considering different types of patients, we found that heart failure was associated with an increased in-hospital mortality (adjusted OR 1.83, 95% CI 1.01-3.32). This finding complies with literature addressing CHF as risk factor for worse outcome in CAP [1, 2, 15, 16, 19]. Most interestingly, our study indicated that heart failure patients hospitalized after prior outpatient antibacterial therapy had an even higher mortality compared with heart failure patients treated as inpatient directly (23.3% *vs.* 9.8%). One possible explanation for the observed higher mortality in these patients could be that they received inappropriate outpatient antibacterial therapy [18]. However, more than 90% of the antibiotics prescribed were appropriate according to the reigning Dutch guidelines for initial antibacterial therapy in pneumonia. Additionally, the percentage of appropriate antibacterial therapy did not differ between patients who died and patients who left the hospital alive. A more obvious explanation for the observed higher mortality for the cases with heart failure might be related to the fact that patients with a combination of pneumonia and heart failure are more susceptible for deterioration of the clinical situation and with this that initial outpatient treatment would delay the hospital treatment which is essential to rescue this type of severe patients. This may actually indicate that general practitioners underestimate the severity and impact of pneumonia in these patients, leading to an increased mortality in hospital when outpatient therapy fails. Although the Pneumonia Severity of Illness score and AMBU-65 scores [20, 21] use heart failure to predict outcome it may be suggested to directly admit all pneumonia patients with underlying chronic heart failure to the hospital in order to decrease mortality.

Strength of our database study in comparison to other studies about factors influencing in-hospital mortality in CAP is that we had the possibility to study a large number of patients. But our study also had limitations. One limitation is that we included pneumonia based on ICD-9 codes [22]. In our study, over 70% of the patients had a diagnosis of pneumonia of unspecified organism recorded. It is known from literature that the etiology of the infectious agent in many pneumonia cases remains unknown [23]. This could imply that, for example, some non-pneumonic exacerbations of COPD could be incorrectly recorded as pneumonia. But other studies using advanced analytical techniques report percentages with known etiology up to 87% [24, 25], indicating that the fact no organism was specified using conventional techniques does not imply no pathogen was involved. An additional limitation is that we had no information about the exact clinical situation at the time of hospitalization, so both groups may not be entirely comparable. The CDS scores, however, showed no differences between both groups. Another limitation is the definition of heart failure used in this study. We realize that the use of a diuretic in combination with a cardiac glycoside as marker for heart failure can be debated and that it is difficult to assess the exact severity of CHF based on drug prescriptions and we can therefore not further distinguish between different classes of these patients. However, previous studies have shown that the use of this marker is in general adequate [26-28].

In summary, our study confirms that many pneumonia patients are hospitalized after prior outpatient treatment. Although the overall duration of hospital stay and in-hospital mortality are not significantly increased after failure of outpatient therapy, we conclude that patients with CHF are at increased risk for worse outcome when outpatient therapy of CAP fails and they get hospitalized subsequently. Based on these findings, we suggest that patients with CHF should be carefully monitored when treated as outpatient or treated as inpatient directly when presenting with symptoms of pneumonia.

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# 3 | Angiotensin-converting enzyme and community-acquired pneumonia

# 3.1 | Angiotensin-converting enzyme inhibitor use and pneumonia risk in a general population

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**Abstract**

The aim of the present study was to assess whether the use of angiotensin-converting enzyme (ACE) inhibitors is associated with a decreased risk of hospitalisation for community-acquired pneumonia (CAP) in a general, essentially white population.

Data were obtained from the Dutch PHARMO Record Linkage System. Cases were defined as patients with a first hospital admission for CAP. For each case, up to four population controls were matched by age and sex.

The study population comprised 1,108 patients with a first hospital admission for CAP and 3,817 matched controls. After adjusting for several confounders, ACE-inhibitor use was not associated with a decreased incidence of pneumonia (adjusted odds ratio (OR) 1.12; 95% confidence interval (CI) 0.88-1.43). Additionally, no significant association was observed in patients with diabetes, respiratory diseases, heart failure, or patients with both of the last two conditions. Furthermore, adjustment of treatment effects on pneumonia risk using stratification on balancing score also showed no significant association between ACE-inhibitor use and pneumonia risk within the different strata (overall adjusted OR 1.09; 95% CI 0.87-1.36).

In contrast with previous findings in Asian populations, the current authors were not able to confirm the beneficial effect of ACE-inhibitors on pneumonia risk in a general, essentially white population.

## Introduction

Community-acquired pneumonia (CAP) is a major direct cause of death in the elderly, with mortality rates ranging 5-20% [1, 2]. The increased incidence of CAP in the elderly is thought to be caused first by silent or manifest aspiration of oropharyngeal flora into the lungs and, secondly, by decreased function of the immune system [3, 4]. Angiotensin-converting enzyme (ACE) has a number of functions in the inflammatory/immune system. Along with its effect that cleaves angiotensin I to angiotensin II, ACE also metabolises the protussive peptides, substance P and bradykinin [5]. The decreased metabolism of these peptides by ACE inhibition could enhance the cough reflex and prevent aspiration. Besides this, ACE inhibition prevents the angiotensin II-induced transcription of the pro-inflammatory nuclear factor-kß [6, 7].

Recent studies have demonstrated that ACE-inhibitor use is associated with a reduced incidence of pneumonia, particularly in the elderly [8-12]. These studies were performed in subjects of Asian ethnicity with a history of stroke. Although these studies add to the body of evidence about the possible effects of these drugs on pneumonia risk, little is known about the extent of the protective effect and whether or not it is present in the general population. The aim of the present study was to assess whether use of ACE-inhibitors is associated with a decreased risk of hospitalisation for CAP in a general, essentially white population.

## Patients and Methods

*Data source*

The PHARMO record linkage system (www.pharmo.nl) was used to provide data for the study. PHARMO includes pharmacy dispensing records from community pharmacies, and is linked to the hospital discharge records of >2,000,000 community-dwelling residents of >40 population-defined areas in the Netherlands from 1985 onwards [13]. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to receipt of prescription drugs.

The computerised drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. The duration of use of each dispensed drug is estimated by dividing the number of dispensed units by the prescribed number of units to be used per day. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification. The hospital discharge

records are obtained from PRISMANT, previously known as the Dutch Centre for Healthcare Information (LMR database), an institute that collates nationwide all hospital discharge records in the Netherlands since the 1960s into a standardised format. These records include detailed information concerning the primary and secondary discharge diagnoses, as well as diagnostic, surgical and treatment procedures, type and frequency of consultations with medical specialists and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).

*Patients*

The study period consisted of a 6-yr period between January 1, 1995 and December 31, 2000. Cases were selected according to the following criteria: adult (aged >18 yrs) hospitalised with a primary discharge diagnosis of CAP and pneumococcal pneumonia (ICD-9-CM code 481), other bacterial pneumonia (ICD-9-CM code 482), bronchopneumonia, organism unspecified (ICD-9-CM code 485) or pneumonia, organism unspecified (ICD-9-CM code 486). Besides this, patients with a signature of bacterial pneumonia (secondary discharge diagnosis) coupled with a primary pulmonary diagnosis (ICD-9-M codes 491-493 or 496) were selected. Deaths that were recorded during hospitalisation were also selected as cases. For each case, up to four population controls were matched by age and sex. Controls were patients who were present in the PHARMO database without a hospitalisation for CAP. The date of admission was the index date. For all subjects, pharmacy dispensing data were collected. Patients with no pharmacy dispensing history for the period minus 365 days until the index date were excluded.

*Study design*

The current study was a population-based case-control study.

*Exposure assessment*

For each patient, all prescriptions for ACE-inhibitors and angiotensin-II antagonists (ATC code C09A to C09D) were identified between entry into the cohort and the index date. The exposure time of ACE-inhibitor and angiotensin-II antagonist use was determined by calculation of the legend duration of treatment episodes. Treatment episodes were defined as a series of subsequent prescriptions refills for these drugs. A new episode was assumed if an interval of ≥ 30 occurred between the theoretical end date of a prescription and the dispensing date of the

next prescription for the same patient. Patients were classified as current users if the index date was between the start date and end date of a treatment episode. Patients were classified as past users if they were not using at the index date, but had a history of use in the year before the index date. Patients were classified as new users if they received a first dispensing <14 days prior to the index date.

*Assessment of potential confounders*

Potential confounders in this study were drugs and 'medical signatures' as measured by the receipt of prescriptions drugs that have been associated with an increased or decreased risk of CAP [14-17]. Cardiovascular drugs (cardiac glycosides and diuretics) were used as a marker for heart failure. Respiratory morbidity was measured by assessing the use of inhaled glucocorticoids, β-agonists or anticholinergic drugs. Furthermore, diabetes was assessed through the use of oral antidiabetic agents and/or insulin [18]. Identified confounding drugs were systemic glucocorticoids [19] and gastric acid-suppressing drugs [20]. For all these drugs, a patient was considered exposed if more than one dispensing took place within a 1-yr period prior to the index date.

*Analysis*

The data analysis was approached in two ways. First, as measure of the association between ACE-inhibitor use and the occurrence of hospitalisation for CAP, estimations of the relative risk through odds ratios (OR) were used. These were calculated using multivariable conditional logistic regression. Statistical interaction terms were used to determine potential modification of the association by comorbidities (heart failure, respiratory diseases or diabetes). Secondly, a balancing score method was applied to further determine potential modification of the effect of ACE inhibition by overall pneumonia risk [21, 22]. This technique enabled assessment of the association of ACE-inhibitor use with pneumonia occurrence in patients who had an equal probability of being a case. Logistic regression analysis was used to model for being a case. Variables considered for inclusion in this model included all prescription drugs (except ACE-inhibitors and angiotensin II antagonists) evaluated as potential confounders in the current study. The model was used to calculate the probability of being a case for each individual patient in the full data set (1,108 cases; 3,817 controls). Patients were stratified by quintiles of the distribution of their balancing score. The goodness of fit of the balancing score was evaluated using its ability to balance the covariates within each quintile

group, using the logistic regression. Subsequently, the effect of ACE-inhibitor use on pneumonia risk was assessed within each quintile group. An overall effect was calculated using the Mantel-Haenszel statistic.

## Results

A total of 1,108 patients with a primary diagnosis of CAP were identified and matched to 3,817 controls. Baseline characteristics are shown in table 1. Both cases and controls had a mean ±SEM age of 67 ±0.51 yrs and ~ 55% of the patients were male.

**Table 1** *Patient characteristics of both cases and controls*

| Characteristic | Cases (n=1,108) | | Controls (n=3,817) | |
|---|---|---|---|---|
| *Age (years)* | | | | |
| <40 | 99 | (8.9) | 396 | (10.3) |
| 40-49 | 91 | (8.2) | 352 | (9.2) |
| 50-59 | 116 | (10.5) | 461 | (12.1) |
| 60-69 | 190 | (17.1) | 637 | (16.7) |
| 70-79 | 353 | (31.9) | 1,048 | (27.5) |
| 80-89 | 218 | (19.7) | 798 | (20.9) |
| ≥ 90 | 41 | (3.7) | 125 | (3.3) |
| *Gender* | | | | |
| Male | 650 | (58.7) | 1,998 | (52.3) |
| Female | 458 | (41.3) | 1,819 | (47.7) |
| *CAP discharge diagnosis* | | | | |
| Primary diagnosis of CAP | 1,100 | (99.3) | | |
| Pneumococcal pneumonia | 84 | (7.6) | | |
| Other bacterial pneumonia | 90 | (8.1) | | |
| Bronchopneumonia, organism unspecified | 140 | (12.6) | | |
| Pneumonia, organism unspecified | 794 | (71.7) | | |
| *Co-morbidities* | | | | |
| Heart failure | 126 | (11.4) | 108 | (2.8) |
| Respiratory diseases | 454 | (41.0) | 333 | (8.7) |
| Diabetes | 134 | (12.1) | 259 | (6.8) |

The use of prescription drugs for both cases and controls is shown in table 2. The prevalence of using ACE-inhibitors (current use) was higher among the cases (15.2%) than among controls (9.7%), yielding a crude OR of 1.65 (95% confidence interval (CI) 1.36-1.99). As only a very small number of patients (2.1%) were using angiotensin-II antagonists, this was not analysed further. None of the patients could be classified as new users of ACE-inhibitors or angiotensin-II

antagonists. After adjusting for respiratory diseases, heart failure, diabetes, the use of systemic corticosteroids and gastric acid-suppressing drugs, the OR decreased to 1.12 (95% CI 0.88-1.43). Considering different co-morbidities, as shown in table 3, no significant association was observed in patients with diabetes (adjusted OR 1.02, 95% CI 0.59-1.77), patients with respiratory diseases (adjusted OR 0.97, 95% CI 0.59-1.60), patients with heart failure (adjusted OR 0.69, 95% CI 0.30-1.60) or patients with both of the last two conditions (adjusted OR 1.06, 95% CI 0.40-2.87).

**Table 2** *Univariate odds ratios (OR) of hospitalisation for community acquired pneumonia and use of medication in the year before the index date*

| Characteristic | Cases (n=1,108) | | Controls (n=3,817) | | Crude OR (95% CI) |
|---|---|---|---|---|---|
| Cardiac glycosides | 124 | (11.2) | 156 | (4.1) | 2.92 (2.26-3.77) |
| ACE-inhibitors | 168 | (15.2) | 370 | (9.7) | 1.65 (1.36-1.99) |
| Ca-channel blockers | 179 | (16.2) | 346 | (9.1) | 1.82 (1.49-2.24) |
| Beta-blockers | 155 | (14.0) | 575 | (15.1) | 0.87 (0.72-1.06) |
| Diuretics | 392 | (35.4) | 638 | (16.7) | 3.16 (2.66-3.75) |
| Oral corticosteroids | 176 | (15.9) | 58 | (1.5) | 11.41 (8.27-15.76) |
| Anticholinergic inhalation | 258 | (23.3) | 134 | (3.5) | 8.43 (6.58-10.80) |
| Beta-agonists inhalation | 324 | (29.2) | 183 | (4.8) | 7.69 (6.21-9.53) |
| Inhaled corticosteroids | 347 | (31.3) | 196 | (5.1) | 8.07 (6.52-9.98) |
| H2-antagonists | 93 | (8.4) | 172 | (4.5) | 1.95 (1.49-2.55) |
| Proton pump inhibitor | 144 | (13.0) | 226 | (5.9) | 2.33 (1.85-2.92) |
| Flu vaccination | 244 | (22.0) | 560 | (14.7) | 2.52 (1.94-3.28) |

*OR: odds ratio; CI: confidence interval*

The balancing score derivation model, which included the variables use of cardiac glycosides, diuretics, calcium channel blockers, oral corticosteroids, anticholinergic inhalation, β-agonists, inhaled corticosteroids, gastric acid-suppressing drugs, cancer medication and influenza vaccination, was reliable since the OR for component variables were all between 0.90 and 1.10. None of the variables reached a significance level of 0.05 in any of the strata. In none of the balancing score strata was ACE inhibitor use significantly associated with pneumonia risk (table 4; overall adjusted OR 1.09, 95% CI 0.87-1.36).

**Table 3** *Associations between hospitalisation for CAP and the use of ACE-inhibitors in all patients and different subgroups*

| Characteristic | Crude OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|
| *All patients* | | |
| current use | 1.65 (1.36-1.99) | 1.12 (0.88-1.43)* |
| past use | 2.09 (1.39-3.13) | 1.43 (0.89-2.31)* |
| *Patients with diabetes* | | |
| current use | 1.10 (0.69-1.75) | 1.02 (0.59-1.77)** |
| *Patients with respiratory diseases* | | |
| current use | 0.98 (0.61-1.52) | 0.97 (0.59-1.60)** |
| *Patients with heart failure* | | |
| current use | 0.81 (0.39-1.80) | 0.69 (0.30-1.60)** |
| *Patients with both respiratory diseases and heart failure* | | |
| current use | 1.05 (0.40-2.75) | 1.06 (0.40-2.87)** |

*OR: odds ratio; CI:confidence interval*

** Adjusted for diabetes, respiratory diseases, heart failure, use of systemic corticosteroids and gastric acid suppressing drugs*

*** Adjusted for the use of systemic corticosteroids and gastric acid suppressing drugs*

**Table 4** *Adjusted treatment effects on pneumonia risk using stratification on balancing score*

| | n | OR | (95% CI) | |
|---|---|---|---|---|
| Total sample | 4,925 | 1.09# | 0.87 | 1.36 |
| 1st quintile group | 982 | 1.48 | 0.68 | 3.22 |
| 2nd quintile group | 986 | 1.22 | 0.63 | 2.39 |
| 3rd quintile group | 694 | 1.66 | 0.82 | 3.37 |
| 4th quintile group | 1,278 | 0.90 | 0.56 | 1.45 |
| 5th quintile group | 985 | 1.03 | 0.75 | 1.42 |

*OR: odds ratio; CI:confidence interval*

*# Calculated using the Mantel-Haenszel statistic. Strata vary in size because a large group of patients with similar balancing score near the third quintile cut-off were entirely allocated to the fourth quintile group*

## Discussion

In the present study, no significant association between the use of ACE-inhibitors and reduced risk of hospitalisation for CAP could be observed in a general, essentially white population. The initially observed overall higher use of ACE-inhibitors for the cases compared with controls could, in the current authors' opinion, be explained by cardiovascular morbidity as a risk factor and indication for in-patient treatment of CAP [1, 16]. The fact that many of the patients used an ACE-inhibitor in combination with a diuretic and cardiac glycosides could indicate that they were suffering from congestive heart failure as well. Additionally, the use of respiratory drugs was also higher for the cases, confirming chronic pulmonary diseases as risk factors for CAP [17]. The striking lower use of ß-blockers for the cases could, in the current authors' opinion, be explained as confounding by contraindication. In the observed study period, the use of ß-blockers was contraindicated for both heart failure and patients with asthma or chronic obstructive pulmonary disease (COPD; non-selective ß-blockers) [23].

When considering the reduction of CAP risk using ACE-inhibitors, there are several reasons to explain the difference between the present findings and those of the previously mentioned Asian studies [8, 10, 11]. One major difference with the study of Okaishi et al. is that the current study included patients with CAP admitted to a hospital, instead of nosocomial pneumonia in an in-patient ward. In addition, Okaishi et al. only included 55 cases and almost 80% of all cases (47% for controls) in this study were suffering from dementia and were in bedridden state. It is unclear in what way pneumonia risk could be affected by these underlying chronic conditions. Previous studies have shown an increased pneumonia risk among patients in bedridden state. It is presumed that ACE-inhibitors can be protective in this specified patient group, but that their protective effect is absent in a larger general population. In contrast with the present study, Ohkubo et al. only included patients with a history of stroke or transient ischemic attack. In this randomised trial, a subject was considered as a case if pneumonia was reported by the patient during a routine follow-up with the researcher. The high percentage of fatal pneumonia in the study by Ohkubo et al. (115 fatal and 155 nonfatal) is remarkable. In the current study, only 7.4% of all pneumonias was fatal, which corresponds well with literature reporting mortality rates ranging 5-20% [1, 2]. Given this information, this could mean an underestimation of nonfatal pneumonias in their study population. Another difference that was previously mentioned

is the ethnicity. Okaishi et al. used Asian subjects exclusively and Ohkubo et al. has a high percentage (39%) of Asian subjects as well. Although Ohkubo et al. studied a more ambulatory population of patients with a history of stroke, they were not able to associate ACE-inhibitor use with reduced pneumonia risk in their non-Asian participants, which is something that seems to be confirmed in the present study. One possible explanation for this is the higher prevalence of the I allele and II genotype in Asian participants. The ACE insertion/deletion polymorphism accounts for 47% of the total variance of serum ACE, with lowest ACE levels in the II genotype [24].

As mentioned previously, induction of the cough reflex is one possible explanation for the protective effect of ACE-inhibitors on pneumonia risk. The mechanisms by which ACE-inhibitors induce cough are thought to be inhibition of ACE and, with this, the metabolism of bradykinin and substance P, both inflammatory peptides that sensitise airway sensory nerves and enhance the cough reflex [5]. Considering that patients with the II genotype already have the lowest ACE activity, administration of an ACE-inhibitor in these patients could increase bradykinin levels possibly above a cough threshold. This is something that seems to be confirmed by the study of Ye et al., which showed that the cough induced by ACE-inhibitors was related to I allele and II genotype and that ACE levels were significantly lower in patients with ACE-inhibitor-induced cough [25]. In addition, ACE-inhibitor-related cough has been reported to be more prevalent in individuals of Asian ethnicity [26]. As the prevalence of the II genotype in the white and African populations is low compared with the Asian population (18% *versus* 39%) [24], this provides a possible explanation why the current authors were not able to associate ACE-inhibitor use with a reduced pneumonia risk in a general, essentially white population. However, in the recent study by Ohkubo et al. [10] this hypothesis could not be confirmed, possibly indicating that other unknown factors are involved.

The strength of the present study is that a large number of patients were included, it was population based and there was good data quality regarding exposure assessment. Furthermore, different methods were used to adjust for potential confounding. In both models no association between ACE-inhibitor use and pneumonia risk was observed. The application of the balancing score method to stratify patients in the present case-control study provided additional information that ACE-inhibitor use was not associated with hospitalisation for CAP in both low- and high-risk patients.

A limitation of the current study is that only cases of hospitalised CAP were selected. Considering that not all CAP is treated as in-patient and that only the cases at risk of a worse outcome are hospitalised means it cannot be concluded whether or not ACE-inhibitors are protective in low-risk patients. Another limitation is that pneumonia based on ICD-9 codes was included [27]. In the present study, >70% of the patients had a diagnosis of pneumonia of unspecified organism recorded. It has been found that the aetiology of the infectious agent in many pneumonia cases remains unknown [28]. This could imply that, for example, some nonpneumonic exacerbations of COPD could be incorrectly recorded as pneumonia. However, other studies using advanced analytical techniques report percentages with known aetiology of up to 87% [29, 30], indicating that the fact no organism was specified using conventional techniques does not imply any pathogen was involved. Concerning adjusting for confounding in the current study, as many confounders as possible were corrected for. However, the current authors realise that there are additional risk factor that could not be controlled for [15-17]. In the present study, "medical signatures" by the receipt of prescription drugs were used to adjust for confounding through comorbidities. For this reason, the present authors were not able to adjust for other additional risk factors (*e.g.* current smoking, low body mass index, excessive use of alcohol, hypertension or stroke) as these medical conditions do not share a specific pharmacological treatment that could be uniquely measured. An additional limitation is that patients with no pharmacy dispensing history for the period minus 365 days until the index date were excluded. This could imply selection bias through exclusion of CAP patients without antecedent pharmacological treatment. However, due to the high age of patients with pneumonia and the fact that many patients at least receive antibiotics for their pneumonia, the number of patients hospitalised for CAP without ever using prescription drugs is expected to be negligible.

In conclusion, angiotensin-converting enzyme inhibitor use does not reduce risk of hospitalisation for community-acquired pneumonia in a general, essentially white population. Further steps to elucidate the possible effects of angiotensin-converting enzyme inhibitors on community-acquired pneumonia may include more detailed information of angiotensin-converting enzyme genotype, angiotensin-converting enzyme gene expression profiles and (inflammatory) biomarkers to ascertain community-acquired pneumonia disease severity.

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# 3.2 | ACE-inhibitor use and protection against pneumonia in patients with diabetes

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## Abstract

Because of the high risk of pneumonia in patients with diabetes, we aimed to assess the effect of angiotensin-converting enzyme (ACE) inhibitor use on the occurrence of pneumonia in a general population of patients with diabetes.

The study population comprised all patients in the UK General Practice Research Database who had a diagnosis of diabetes (both type 1 and type 2) between 1987 and 2001. Cases were defined as patients with a first diagnosis of pneumonia. For each case, up to four controls were matched by age, gender, practice, and index date. Patients were classified as current ACE-inhibitor user when the index date was between the start and end date of ACE-inhibitor therapy. Conditional logistic regression analysis was used to estimate the strength of the association between ACE-inhibitor use and pneumonia risk.

ACE-inhibitors were used in 12.7% of 4,719 cases and in 13.7% of 15,322 matched controls (crude odds ratio [OR]: 0.92, 95% confidence interval [CI] 0.82-1.01). After adjusting for confounding, ACE-inhibitor therapy was associated with a significant reduction in pneumonia risk (adjusted OR: 0.72, 95% CI 0.64-0.80). The protective association was consistent across different relevant subgroups with the strongest association in patients with a history of stroke. There was a significant dose-effect relationship (p for trend <0.001).

In conclusion, the use of ACE-inhibitors is associated with a significant reduction in pneumonia risk and, apart from blood pressure-lowering properties, may be useful in the prevention of pneumonia in patients with diabetes.

## Introduction

Community-acquired pneumonia is a major direct cause of death in the elderly with overall mortality rates varying from 5%-20% [1, 2]. Elderly patients and patients with co-morbidities, such as diabetes, are at increased risk for pneumonia [1, 3]. In the literature, there is evidence that angiotensin-converting enzyme (ACE) inhibitors, apart from their blood pressure-lowering properties, could protect against pneumonia [4-6]. To date, these studies were all conducted in patients with a history of stroke because induction of the cough reflex and prevention of aspiration was noted as the mechanism behind this protective effect [7, 8]. Currently, there is accumulating evidence that ACE-inhibitors also have different anti-inflammatory and immunomodulatory effects [9-11], providing an additional explanation for a protective effect, which could also be present in patients without a history of stroke. Therefore, we assessed the effects of ACE-inhibitor use on the risk of acquiring pneumonia in an unselected high-risk population of diabetic patients.

## Patients and Methods

*Data setting*

Data for this study were obtained from the General Practice Research Database (GPRD), which contains the computerized medical records of approximately 650 general practices. The GPRD is owned by the UK Department of Health and managed by the Medicines Control Agency. Approximately 6.5% of the total population of England and Wales is represented in the database. The computer records contain patient demographics, characteristics (i.e. height, weight), symptoms and diagnosis (using Oxford Medical Information System (OXMIS) and Read codes, which are mapped onto International Classification of Disease codes), referrals to specialist care, hospital admissions and their major outcomes, and all drug prescriptions in chronological order. The computerized recording of patient information was started by many general practitioners in the late 1980s, and replaced the handwritten records used previously. Several independent validation studies have shown that the GPRD database has a high level of completeness and validity [12, 13]. The study was approved by the Scientific and Ethical Advisory Group of the GPRD.

*Study design and population*

A population-based retrospective case-control study among diabetic patients (both type 1 and type 2) was conducted using data from 1 June 1987 to 21 January 2001.

Cases were defined as patients aged 18 and older who had a first diagnosis of pneumonia (for selected OXMIS codes see Appendix 1). The date of the pneumonia diagnosis recorded was the index date. For each case, up to 4 controls were matched on sex, age (±2 years), general practice and index date. Controls were selected from patients also present in the GPRD database without a record for pneumonia. Both cases and controls were eligible when they had a medical history in the database for at least 365 days before the index date.

*Drug exposure*

For each patient, we identified all prescriptions for ACE-inhibitors prior to the index date (cilazapril, captopril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril and trandolapril). The timing of ACE-inhibitor use was determined by calculation of the legend duration of treatment episodes. Treatment episodes were defined as series of subsequent prescriptions for these drugs. A new episode was assumed if an interval of 14 days or more occurred between the theoretical end date of a prescription and the date of the next prescription for the same patient. Patients were classified as current users when the index date was between the start and the end date of a treatment episode. Patients were classified as past users when they were not a current user, but had a history of use in the year before the index date. Prescribed daily doses were expressed as the number of defined daily doses (DDDs) per day. This unit corresponds to the average daily dose of a drug for its main indication in adults and is recommended by the World Health Organization [14].

*Potential confounders*

For each patient, we identified co-morbidities and the receipt of prescriptions for drugs that have been associated with an increased or decreased risk of pneumonia. We evaluated the presence or absence of the following frequently occurring co-morbidities as potential confounders: congestive heart failure, history of stroke, evident alcohol abuse and pulmonary diseases (asthma and chronic obstructive pulmonary disease) [15-18]. Potential confounding drugs were pneumococcal vaccination, statins, oral glucocorticoids, gastric acid suppressing drugs and flu-vaccination [19-23]. A patient was considered to have been exposed to a prescription drug if more than one prescription was issued within a 1-year period prior to the index date, except for vaccination drugs that required only one prescription. In addition, diuretics (thiazides and loop diuretics) were examined as control drug

because diuretics share the same indication as ACE-inhibitors but are not expected to protect against pneumonia. Furthermore, smoking status was evaluated as a potential confounder. We calculated the number of general practitioner (GP) visits in the year before the index date as a proxy for general health status and health consumption.

*Statistical analysis*

Univariate analysis by the chi-squared test and Student's *t*-test was applied to test for statistically significant differences in baseline characteristics between cases and controls. Conditional logistic regression analysis was used to estimate the strength of the association between ACE-inhibitor use and pneumonia and was expressed as odds ratios (OR) and 95% confidence intervals (CI). Potential confounders were included in the multivariate model when they were retained after backward stepwise elimination ($p<0.10$). To assess a potential dose-response relationship ACE-inhibitor exposure was categorized into three categories of less than 1 DDD per day, 1 - 1.5 DDD per day and more than 1.5 DDD per day, respectively.

## Results

The study population comprised 4,719 patients with a diagnosis of pneumonia and 15,322 controls. Among the cases, 271 had a diagnosis of pneumococcal pneumonia, 46 had other bacterial pneumonia, 2,050 had a diagnosis of bronchopneumonia with unspecified organism and 2,291 had pneumonia with unspecified organism. Approximately 28% of all cases were referred to a hospital. Approximately 48% of the patients were male and both cases and controls had a mean ±SD age of 73 ±11 years but, in cases, the proportion of very old was higher than in controls (Table 1). The prevalence of stroke, congestive heart failure, pulmonary diseases, alcohol abuse, and smoking was higher in cases than controls, as was the use of diuretics, gastric acid suppressing drugs and oral glucocorticoids. Pneumococcal vaccination was more frequent among controls. Cases visited the GP more often than controls (15 versus nine visits per year, respectively, $p<0.01$).

Among the 4,719 cases, 600 patients (12.7%) were current users of ACE-inhibitors compared to 13.7% for the control group (crude OR 0.92, 95% CI 0.89-1.01). After adjusting for confounders, current use of ACE-inhibitors (OR 0.72, 95% CI 0.64-0.80) was significantly associated with a decreased risk of pneumonia.

Covariates used for adjustment were age, congestive heart failure, history of stroke, pulmonary diseases, smoking, number of GP visits/year, oral glucocorticoid use, statin use, pneumococcal vaccination, and use of gastric acid suppressing drugs. The decrease of the odds ratio was primarily caused by adding congestive heart failure to the model. The association was similar for all type of ACE-inhibitors and consistent across all seasons (data not shown).

**Table 1** *Characteristics of both cases and controls*

| Characteristic | Cases (n=4,719) | | Controls (n=15,322) | | Crude OR (95% CI) |
|---|---|---|---|---|---|
| *Age (years)* | | | | | |
| <60 | 525 | (11.1) | 1,776 | (11.6) | N/A |
| 60-69 | 785 | (16.6) | 3,066 | (28.9) | N/A |
| 70-79 | 1,533 | (32.5) | 5,665 | (37.0) | N/A |
| 80-89 | 1,622 | (34.4) | 4,431 | (28.9) | N/A |
| ≥90 | 254 | (5.4) | 384 | (2.5) | N/A |
| *Gender* | | | | | |
| Male | 2,275 | (48.2) | 7,336 | (47.9) | N/A |
| Female | 2,444 | (51.8) | 7,986 | (52.1) | N/A |
| *Comorbid conditions* | | | | | |
| History of stroke | 1,186 | (25.1) | 1,901 | (12.4) | 2.37 (2.19-2.57) |
| Congestive heart failure | 1,421 | (30.1) | 2,158 | (14.1) | 2.63 (2.43-2.84) |
| Pulmonary diseases | 949 | (20.1) | 1,592 | (10.4) | 2.17 (1.99-2.37) |
| Alcohol abuse | 17 | (0.4) | 34 | (0.2) | 1.62 (0.91-2.91) |
| *Prescription drugs* | | | | | |
| Diuretics | 831 | (17.6) | 1,990 | (13.0) | 1.43 (1.31-1.56) |
| Pneumococcal vaccination | 144 | (3.1) | 569 | (3.7) | 0.82 (0.68-0.98) |
| Influenza vaccination | 1,955 | (41.4) | 6,114 | (39.9) | 1.07 (1.00-1.14) |
| Gastric acid suppressing drugs | 967 | (20.5) | 1,988 | (13.0) | 1.73 (1.59-1.88) |
| Oral glucocorticoids | 311 | (6.6) | 329 | (2.1) | 3.22 (2.74-3.77) |
| Statins | 63 | (1.3) | 365 | (2.4) | 0.55 (0.42-0.73) |
| *Behavioral habbits* | | | | | |
| Current smoking* | 407 | (12.7) | 1,169 | (9.2) | 1.48 (1.31-1.67) |

*CI: confidence interval; OR: Odds Ratio.*

** Percentage for current smoking calculated in subjects with information on smoking history recorded in GPRD*

Furthermore, a protective effect was present in all co-morbidity related subgroups with the strongest association in patients with a history of stroke (adjusted OR 0.54, 95% CI 0.43-0.69; no history of stroke: adjusted OR 0.77, 95% CI 0.68-0.88). For patients with heart failure and or pulmonary diseases the adjusted OR was 0.72 (95% CI 0.63-0.80). The protective effect of current ACE-inhibitor use could only be established for pneumonia treated in primary care (adjusted OR

0.61, 95% CI 0.53-0.70). We could not demonstrate such an association between ACE-inhibitor use and risk of pneumonia requiring hospitalization (adjusted OR 1.00, 95% CI 0.82-1.24). In the analyses regarding a potential dose-response, dosages of ACE-inhibitors of more than 1.5 DDD per day showed a stronger inverse association with pneumonia risk (OR 0.54, 95% CI 0.44-0.67) than lower daily dosages of ACE-inhibitors with a significant dose-response relationship (Figure 1, p for trend <0.001). Past use of ACE-inhibitors was not associated with a decreased pneumonia risk (OR 1.83, 95% CI 1.50-2.23). Furthermore, current use of diuretics was not associated with a decreased pneumonia risk (OR 0.96, 95% CI 0.86-1.07) when adjusted for age, congestive heart failure, history of stroke, pulmonary diseases, smoking, number of GP visits/year, oral glucocorticoid use, statin use, pneumococcal vaccination and use of gastric acid suppressing drugs.

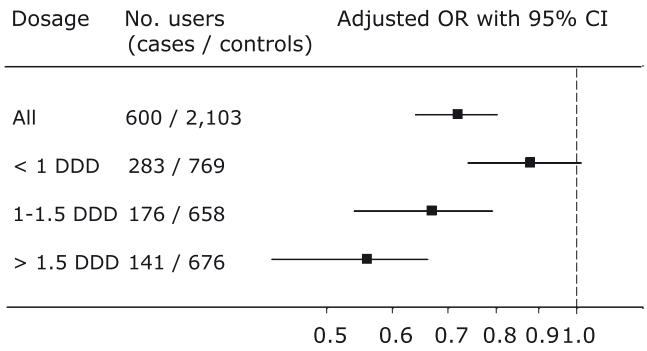


**Figure 1** *Current use of different dosages of ACE-inhibitors and association with pneumonia.*

** Adjusted for age, congestive heart failure, history of stroke, pulmonary diseases, smoking, number of GP visits/year, oral glucocorticoid use, statin use, pneumococcal vaccination, and use of gastric acid suppressing drugs; OR: Odds Ratio; CI: Confidence Interval; DDD: Defined Daily Dose*

## Discussion

The present study demonstrates that use of ACE-inhibitors is associated with a reduction in the risk of pneumonia in diabetic patients and that the effect is consistent across several relevant subgroups with the strongest effect in patients with a history of stroke. Furthermore, a significant dose-response relation is present. The study has several strengths, including the use of a large representative and valid database, the inclusion of a high-risk population of diabetic patients and the possibility to adjust for important potential confounders. Experimental studies have suggested two potential mechanisms along which ACE-inhibitors

may reduce the occurrence of pneumonia: (i) decreased metabolism of the protussive peptides bradykinin and substance P by ACE inhibition could enhance the cough reflex and prevent aspiration [7] and (ii) ACE inhibition could influence the inflammatory response to pathogens in the lung. For example, ACE-inhibitors have been linked to lower tumor necrosis factor-k and interleukin-6 levels in humans and reduced lipopolysaccharide-induced pulmonary neutrophil influx [11]. Furthermore, angiotensin II is believed to play an important role in tissue repair and remodeling [24] and inhibition of ACE has revealed an important role in acute lung injury-related endothelial cell damage and acute respiratory distress syndrome [10]. One possible mechanism behind this is the prevention of angiotensin II-induced transcription of the proinflammatory nuclear factor-kB [25]. Nuclear factor-kB regulates the transcription of several genes that encode proteins (such as cytokines, chemokines, adhesion molecules and enzymes) involved in mediator synthesis and the further amplification and perpetuation of the inflammatory response [26]. The findings from the present study that the protective effect was also present for patients without a history of stroke and that there was an evident dose-effect relationship suggest that the protection against pneumonia could, in addition, be explained by the proposed immunomodulatory effect of ACE-inhibitors. Further research is necessary to explore the anti-inflammatory properties of ACE-inhibitors in community-acquired pneumonia.

In addition, to our knowledge, the present study is the first study to associate the use of ACE-inhibitors with reduced pneumonia risk in a predominantly white population. Previous studies with similar results were solely conducted in elderly Asian populations [4-6], except for the study of Ohkubo et al. who included non-Asian participants as well but was not able to confirm a beneficial effect in their non-Asian participants [5]. The finding of a null effect was explained by potential genetic differences in the ACE gene between Asian en non-Asian populations [27, 28]. In addition, this is also the first study indicating a possible role for ACE inhibition in preventing pneumonia in outpatients. Approximately 80% of all pneumonia is treated in an outpatient setting [29, 30]. In the present study, the protective effect of ACE inhibition was primarily present for outpatient-treated pneumonia. The fact that no association was observed for hospitalized pneumonia could possibly be explained by selection bias through co-morbidities treated by ACE-inhibitors serving as indication for inpatient treatment [31]. In particular, heart failure is a strong reason for inpatient treatment in different prediction rule models and,

subsequently, this could lead to an overestimation of ACE-inhibitor use in identified admitted pneumonia cases versus population controls. This might explain why, in two previous case-control studies, no protective association between ACE-inhibitor use and pneumonia was observed [32, 33].

Our observational study also has some potential limitations. First, our study was based on computerized databases and subject to the limitations of such studies. Second, the identification of cases with pneumonia has a potential for misclassification due to inaccurate diagnostic assessment or incorrect coding. For example, acute exacerbations of chronic obstructive pulmonary diseases could be inaccurately classified as pneumonia. However, most variables that were considered to be risk factors for pneumonia appeared to be associated with pneumonia in our study [15, 16, 19-21], reducing the plausibility of misclassification, unless the misclassified diseases share comparable risk factors. Also, the incidence of pneumonia during the year showed an expected pattern, with most cases occurring during the winter season. Finally, if anything, misclassification of pneumonia would have been random, which will always lead to the finding of a null effect.

Another concern could be that people on ACE-inhibitors take more comfort out of good health support, which can also lower pneumonia risk. However, we believe that this healthy user effect, if present, is small in the UK because ACE-inhibitors are prescribed for numerous indications associated with an increased pneumonia risk (e.g. hypertension, heart failure). The fact that use of ACE-inhibitors in the present study is still associated with a decreased risk of pneumonia provides good evidence for ACE-inhibitors being beneficial, as well as because diuretics did not show a protective effect (adjusted OR 0.96, 95% CI 0.86-1.08). Another limitation of this study is the lack of information on certain prognostic factors, such as socio-economic status (SES) which has been proposed as a potential confounder [34]. We tried to minimize such confounding through matching subjects on practice, and thereby on geographical region and city area. However, the use of postcodes as proxy for SES is debated in literature [35, 36]. We cannot rule out confounding due to unknown or unmeasured factors in this observational study.

Furthermore, misclassification of exposure to ACE-inhibitor therapy is a concern because we used prescription data. Patients using ACE-inhibitors could have been non-compliant with their therapy and therefore could have used less ACE-inhibitors than prescribed. However, this would have led to an underestimation of the association between ACE-inhibitor use and pneumonia occurrence.

To our knowledge, the mechanism of ACE inhibition is not different between patients with and without diabetes, indicating that the association found in the present study may be applicable for the general population at large. However, the association in the general population could be less pronounced due to the lower probability of pneumonia compared to diabetic patients [3]. Further studies are required to elucidate a possible protective effect in non-diabetic patients.

In conclusion, the use of ACE-inhibitors is associated with a significant dose-dependent reduction in risk of pneumonia in diabetic patients. These findings could provide additional support for using ACE-inhibitors in the treatment of hypertension and heart failure in patients with diabetes.

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**Appendix 1** *Description of OXMIS and Read codes used with case selection*

| Codes | Description |
|---|---|
| H28..00 | Atypical pneumonia |
| H261.0 | Basal pneumonia due to unspecified organism |
| 485 | Bronchopneumonia |
| H25..00 | Bronchopneumonia due to unspecified organism |
| H21..00 | Lobar (pneumococcal) pneumonia |
| 481 B | Lobar pneumonia |
| H260.0 | Lobar pneumonia due to unspecified organism |
| H22..00 | Other bacterial pneumonia |
| 486 | Pneumonia |
| H2…0 | Pneumonia and influenza |
| 486 AP | Pneumonia aspiration |
| 483 AT | Pneumonia atypical |
| 481 BA | Pneumonia basal |
| H26..00 | Pneumonia due to unspecified organism |
| 483 M | Pneumonia mycoplasal |
| H2z..00 | Pneumonia or influenza nos |
| 481 A | Pneumonia pneumococcal |
| 4823 | Pneumonia staphycoccal |
| 486 T | Pneumonitis |

# 3.3 | Angiotensin-converting enzyme insertion/deletion polymorphism and risk and outcome of pneumonia

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## Abstract

Recent studies have suggested involvement of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism in the susceptibility to and severity of pneumonia in Asian populations. We have explored the hypothesis that the ACE I/D polymorphism affects the risk and outcome of community-acquired pneumonia in a Dutch white Caucasian population.

This is a hospital-based prospective observational study including patients with community-acquired pneumonia admitted between October 2004 and August 2006. All patients were genotyped and pneumonia severity and clinical outcome were compared between patients with the II, ID, and DD genotype of the ACE gene. Pneumonia severity was assessed on day of admission and consecutively on day 2,3,5, and 10 of hospital stay using APS scoring. Outcomes evaluated were: duration of hospital stay, ICU admittance, in-hospital and 28-days mortality. To study the association between ACE genotype and risk of pneumonia, the distribution of the ACE I/D polymorphism was compared with healthy control subjects from the same geographical region.

In total, 200 patients with pneumonia and 200 control subjects were included in the study. The mean age of the patients was 63 years. APS scores were not different between the genotype groups on any of the days and all clinical outcomes (duration of hospital stay, ICU admittance, in-hospital and 28-days mortality) were comparable between the three genotype groups. The ACE I/D genotype distribution was identical for patients and control subjects (p=0.973).

The ACE I/D polymorphism is not associated with risk and outcome of community-acquired pneumonia in the Dutch white Caucasian population.

## Introduction

Community-acquired pneumonia (CAP) ranks in the top-10 leading causes of death with estimated mortality rates varying between 5 and 20% [1, 2]. Despite substantial progress in standards of care and the availability of prediction rule models to identify patients at high risk [3], the mortality rate and impact of pneumonia on health remain high [4, 5]. Therefore, it is considered that, besides demographics and co-morbidities, also genetic factors play an important role in the susceptibility to and severity of pneumonia.

Recently, the involvement of the renin-angiotensin system in the pathogenesis and evolution of pneumonia has gained substantial interest. The use of angiotensin-converting enzyme (ACE)-inhibitors has been associated with lower risk of pneumonia, particularly in elderly patients, and patients using ACE-inhibitors are less likely to die from pneumonia [6-8]. ACE-inhibitors may act on the pathogenesis of pneumonia in two different ways: first, they induce the cough reflex through inhibition of the degradation of the protussive peptides bradykinin and substance P [9, 10], and second, they have an immunomodulatory effect through lowering angiotensin II levels [11-16]. Serum ACE levels are also determined genetically through the identified insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene. The I/D polymorphism has been reported to account for 47% of the variance in serum ACE level, whereas the DD genotype is associated with the highest levels of serum ACE [17].

The ACE I/D polymorphism can also been linked to pneumonia as persons with the DD genotype have a lower cough reflex compared with II and ID [18, 19], and the DD genotype carriers have higher serum levels of the pro-inflammatory angiotensin II [20]. Morimoto et al. already showed that the ACE D allele is an independent risk factor for (fatal) pneumonia in an Asian population [21]. We have explored the hypothesis that the ACE I/D polymorphism affects the risk and clinical outcome of community-acquired pneumonia in a Dutch white Caucasian population.

## Patients and Methods

*Study design and subjects*

The study was conducted in St. Antonius Hospital, a 600-bed teaching hospital (Nieuwegein, The Netherlands), and approved by the local Medical Ethics Committee. Informed consent was obtained from each subject. The ethnicity of the population in and around the city of Nieuwegein is primarily (>94%) white Caucasian [22].

This was a prospective observational study of patients with confirmed pneumonia admitted between October 1, 2004 and August 1, 2006. Pneumonia was defined as a new or progressive infiltrate on a chest X-ray plus at least two of the following criteria: cough, sputum production, temperature >38°C or <35°C, ausculatory findings consistent with pneumonia, leucocytosis or -penia (>10 G/L, <4 G/L, or >10% rods in leucocyte differentiation), C-reactive protein >3 times the upper limit of normal. Patients, who were immune-compromised (systemic steroid use at admission (prednison equivalent >20 mg/daily for more than 3 days), hematological malignancies and other immunosuppressive therapy) were excluded.
Microbiological confirmation was sought using sputum for Gram-stain and sputum and blood for culture. Sputum was analysed by PCR for atypical pathogens (*Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophyla psittaci*). Urine was sampled for antigen testing on *Streptococcus pneumoniae* and *L.pneumophila*. In addition, serum samples of the day of admission and day 10 were analysed in pairs for detection of a fourfold rise of antibodies to respiratory viruses, *Coxiella burnetii*, *M.pneumoniae*, and *C.psittaci* by complement fixation assay. Pharyngeal samples were taken for viral culture.

As control group, ACE I/D genotype data were used from a population of healthy employees of the St. Antonius Hospital who volunteered for venapunction. All control subjects were Dutch white Caucasians. Other characteristics of this population have been described elsewhere [23]. The control subjects did not have a history of pneumonia.

*Sample size calculation*
In the recent study of Morimoto et al., the relative risk (DD vs. II+ID) was 2.9 for pneumonia and 4.4 for fatal pneumonia [21]. To detect a clinical significant effect of ACE I/D polymorphism on pneumonia outcome we hypothesized that carriers with the DD genotype of the ACE gene would have a 3-fold increased mortality risk compared with carriers of the II and ID genotype. Considering a baseline mortality risk of 10% combined with 25% DD genotype carriers, this resulted in a estimated sample size of 196 patients ($\alpha$=0.05, power=0.80). For the effect of the ACE polymorphism on the susceptibility for pneumonia, considering a relative risk for pneumonia of 2 for carriers of the DD genotype compared with II+ID [21], the required sample size to detect a significant effect of genotype on pneumonia risk was estimated at 153 patients and 153 control subjects ($\alpha$=0.05,

power=0.80). Beforehand, the aim of the present study was set at the inclusion of 200 patients and 200 control subjects.

*Outcome measures and illness severity assessment*

The following outcome measures were identified for all patients: duration of hospital stay, the need for intensive care admittance, survival to hospital discharge, and 28-days mortality. To quantify illness severity the APS (Acute Physiology Score) score was calculated for each patient on admission and consecutively on day 2,3,5 and 10 of hospital stay [24]. In addition, for each patient the highest APS score during hospital stay and the occurrence of ARDS (Acute Respiratory Distress Syndrome) was identified.

*Genotyping*

Genomic DNA of patients was isolated from EDTA blood using the MagNA Pure LC DNA Isolation kit 1 (MagNA Pure; Roche Diagnostics). ACE I/D polymorphisms were determined by real-time PCR using fluorescent hybridization probes and a LightCycler (Roche Diagnostics) as described earlier with some slight modifications [17, 25, 26]. Briefly, the reaction volume was 20 μl, containing 1 μl of DNA (40-80 ng), 0.2 μM forward primer and 0.8 μM reversed primer reported by Rigat et al. [17], 2 μl of 10x reaction buffer (LightCycler DNA master hybridization probes, Roche Diagnostics), 1.6 μl of 25 mM $MgCl_2$ stock solution and 0.1 μM of each probes. The detection probes were the same as described by Somogyvari et al. [26]. The PCR conditions were as follows: denaturation at 95°C for 60s, followed by 50 cycles denaturation (95°C for 10s), annealing (first 10 cycles: 67°C for 20s, followed by 0.5°C stepwise decrease per cycle to 61°C) and extension (72°C for 30s). Melting curve analysis consisted of heating to 95°C for 5s, 45°C for 60s, followed by an increase of the temperature to 75°C at 0.2°C/s. To exclude mistyping of I/D heterozygotes as D/D homozygotes, a second PCR reaction was conducted under the same conditions except for using the primer pair as described earlier [25, 26]. Verification of the real-time PCR results with those of electrophoresis and using SSP-PCR revealed no mistyping. ACE I/D polymorphisms were determined after follow-up of the patients, excluding confounding by indication.

*Co-morbidity assessment*

Besides ACE genotyping, co-morbidities were identified to address factors related with outcome in community-acquired pneumonia. Co-morbidities were defined

based on the presence of conditions for which the patient was under active medical supervision or was receiving treatment at the time of hospital admission. Co-morbidities evaluated were lung diseases (chronic obstructive pulmonary disease, or treated asthma), congestive heart failure, diabetes (both type I and type II), and end-stage renal disease (serum creatinine >150 μmol/L). Furthermore, patients were classified according to the Pneumonia Severity Index (PSI) developed by Fine et al [3]. The use of ACE-inhibitors and angiotensin-II receptor blockers was also assessed.

*Statistical analysis*

The SPSS statistical package (version 12.0.1 for Windows; SPSS, Chicago, IL) was used for the statistical analyses. Continuous data were expressed as mean ±SD or median (range) where appropriate. Categorical data were analysed by chi-square and continuous data by Student's t tests, rank tests, and one-way analysis of variance where appropriate. Multivariate logistic regression analyses were applied to study the association between ACE genotype and need for ICU admittance, in-hospital mortality, and 28-days mortality. All baseline characteristics were considered potential prognostic factors for clinical outcome. Non-significant variables ($p>0.05$) were removed stepwise from the model. The chi-square tables were used to compare the observed number of each genotype with those expected for a population in Hardy-Weinberg equilibrium and to compare genotype frequencies between the patients with pneumonia and the control subjects. For all tests, a p-value of $<0.05$ was considered significant.

## Results

In total 201 patients with pneumonia and 200 control subjects were included in the study. For one patient a DNA sample was missing, leaving 200 patients and 200 control subjects eligible for further analysis.

There were no major differences in demographics and clinical characteristics of the patients by ACE genotype (Table 1). Based on microbiological data the patients were categorized as pneumococcal pneumonia, atypical pneumonia, pneumonia with gram negative strain, viral pneumonia, or etiology unknown. In total, etiology was available for 127 patients (64%). Etiology was not different for the three ACE genotype groups (Table 1).

**Table 1** *Baseline characteristics of 200 patients with community-acquired pneumonia by ACE I/D polymorphism*

| | all<br>n=200 | II<br>n=42 | ID<br>n=106 | DD<br>n=52 | p-value |
|---|---|---|---|---|---|
| *Demographics* | | | | | |
| Age (SD) | 63 (17) | 61 (17) | 65 (17) | 60 (18) | 0.224 |
| Male sex | 124 (62) | 20 (48) | 70 (66) | 34 (65) | 0.097 |
| *Co-morbidity* | | | | | |
| Renal disease | 10 (5) | 2 (5) | 4 (4) | 4 (8) | 0.567 |
| CHF | 18 (9) | 3 (7) | 9 (9) | 6 (12) | 0.734 |
| Diabetes | 34 (17) | 7 (17) | 20 (19) | 7 (14) | 0.695 |
| Lung diseases | 70 (35) | 18 (43) | 38 (36) | 14 (27) | 0.264 |
| ACE/ATII use | 43 (22) | 7 (17) | 28 (26) | 8 (15) | 0.197 |
| *Etiology* | | | | | |
| Pneumococcal | 60 (30) | 17 (41) | 30 (28) | 13 (25) | 0.402 |
| Atypical | 21 (11) | 5 (12) | 11 (10) | 5 (10) | |
| Viral | 16 (8) | 3 (7) | 11 (10) | 2 (4) | |
| Gram negative strain | 22 (11) | 6 (14) | 9 (9) | 7 (14) | |
| Other | 8 (4) | 0 (0) | 4 (4) | 4 (8) | |
| Unknown | 73 (37) | 11 (26) | 41 (39) | 21 (40) | |
| *Risk class** | | | | | |
| Low I | 30 (15) | 7 (17) | 15 (14) | 8 (15) | 0.252 |
| Low II | 34 (17) | 8 (19) | 16 (15) | 10 (19) | |
| Low III | 53 (27) | 4 (10) | 35 (33) | 14 (27) | |
| Moderate IV | 56 (28) | 17 (40) | 25 (24) | 14 (27) | |
| High V | 27 (13) | 6 (14) | 15 (14) | 6 (12) | |

* *Pneumonia Severity Index based on Fine et al.* [3]

The overall median duration of hospital stay was 9.5 days and 21 patients were admitted to the intensive care ward (Table 2). During hospital stay, 10 patients died, all due to pneumonia. The overall 28-days mortality rate was 5.0% and not statistically different between the three ACE genotypes (7.1%, 3.8%, and 5.8% for II, ID and DD respectively; p=0.668). The mean highest APS score during hospital stay was 23.9 and not statistically different between the genotype groups. There was no trend towards an association between ACE genotype and risk of ARDS.

**Table 2** *Clinical outcomes and illness severity by ACE I/D polymorphism*

| | II<br>n=42 | ID<br>n=106 | DD<br>n=52 | p-value |
|---|---|---|---|---|
| *Clinical outcomes* | | | | |
| Duration of stay [median(range)] | 11.5 (4-49) | 9 (2-143) | 9 (3-59) | 0.548 |
| ICU admittance [n(%)] | 7 (17) | 11 (10) | 3 (6) | 0.230 |
| Days on ICU [median(range)] | 5 (1-13) | 8 (3-64) | 4 (4-16) | 0.282 |
| In-hosp. mortality [n(%)] | 2 (5) | 6 (6) | 2 (4) | 0.883 |
| 28-days mortality [n(%)] | 3 (7) | 4 (4) | 3 (6) | 0.668 |
| *Illness severity* | | | | |
| APS [mean (SD)]* | 26 (13) | 23 (12) | 23 (13) | 0.350 |
| ARDS [n(%)] | 1 (2) | 3 (3) | 0 (0) | 0.481 |

* *Mean based on highest score for each individual*

For both patients with pneumonia and control subjects, the ACE I/D genotype distribution was compatible with the Hardy-Weinberg equilibrium. The genotype and allele frequencies did not differ between patients and control subjects (Table 3).

**Table 3** *Genotype and allele frequencies of the ACE I/D polymorphism*

| | Pneumonia (n=200) | Controls (n=200) | p- value |
|---|---|---|---|
| *Genotype* | | | |
| II | 42 (21) | 43 (22) | 0.973 |
| ID | 106 (53) | 107 (54) | |
| DD | 52 (26) | 50 (25) | |
| *Allele* | | | |
| I | 190 (48) | 193 (48) | 0.832 |
| D | 210 (52) | 207 (52) | |

Figure 1 shows the mean APS scores during the episode of pneumonia by ACE genotype. Using one way analysis of variance, the scores were not statistically different on any of the days (p=0.350). None of the patients in the low PSI risk classes (risk class I-II) died during hospital stay. For the patients with a moderate (risk class IV) or high risk (risk class V), the in-hospital mortality rates were 5.4% and 22.2% respectively. In univariate analysis, the risk class at admission was significantly associated with in-hospital mortality (p<0.01). In the multivariate analyses, no associations between ACE I/D polymorphism and need for ICU admittance, in-hospital mortality, nor 28-days mortality could be detected as ACE

genotype did not reach significance in any of the models. When ACE genotype (DD vs. II+ID) was added to the final model afterwards, this yielded odds ratios of 0.81 (95% CI 0.15-4.29), 1.53 (95% CI 0.34-6.86), and 0.47 (95% CI 0.12-1.77) for respectively in-hospital mortality, 28-days mortality, and ICU admittance. Exclusion of patients using ACE-inhibitors or angiotensin II receptor blockers from the analyses did not cause a change in the findings (data not shown).

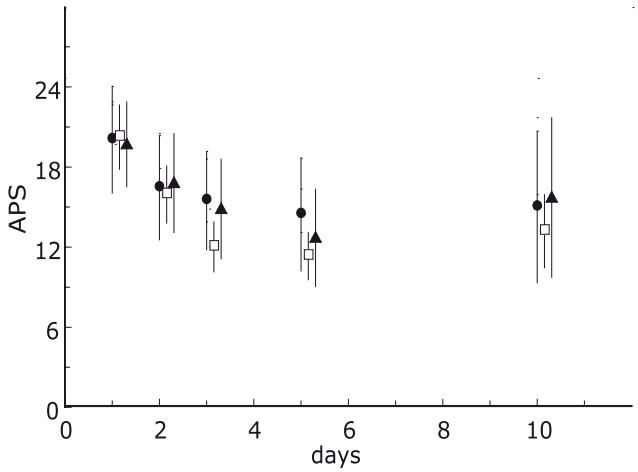


**Figure 1** *Mean APS scores with 95% confidence intervals by ACE genotype ( ● = II, □ = ID, ▲ = DD) on day of hospital admission (day 1) and during hospital stay (days 2,3,5, and 10).*

## Discussion

In this hospital-based prospective observational study, no differences in clinical development of community-acquired pneumonia were observed between patients with the DD, ID, and II genotype of the ACE gene. Furthermore, there was a similar distribution of genotypes and allele frequencies of the ACE I/D gene in patients with pneumonia and control subjects, suggesting no association between the ACE I/D polymorphism and the risk of acquiring pneumonia.

The recent study of Morimoto et al. [21] found that the ACE D allele was an independent risk factor for pneumonia in elderly patients with a relative risk of 2.9 (95% CI 1.7-4.8). Although our study had sufficient power to detect an odds ratio of 1.60 or more, we were not able to confirm this association. A major difference between the present study and the study from Morimoto et al. is the ethnicity of the population under study. Morimoto studied Japanese patients solely, whereas

we studied a Dutch white Caucasian population. Reports have shown marked ethnic differences between polymorphisms of the renin-angiotensin system components, especially of the ACE gene [27]. The prevalence of the DD genotype is small in Asian populations compared to white and African populations [28]. When we compare the ACE I/D genotype frequencies between our control group and the control group of Morimoto we can confirm this difference (frequency DD genotype: 25% *versus* 11%, p<0.001). The genotype frequency of our control group is very much in line with other white Caucasian control groups published in literature [29, 30]. The ACE I/D polymorphism also occurs in multiple haplotypes. Possibly, the ACE I/D polymorphism is not a functional polymorphism but rather a marker for a true functional polymorphism for which the linkage dysequilibrium with the true functional polymorphism is different between ethnic groups.

Another difference between the present study and the study of Morimoto is the setting of the study. Morimoto studied elderly inpatients in a long-term care hospital because of the known increased risk of pneumonia due to aspiration in this group of patients. Furthermore, they studied patients only for the non-winter months in order include mostly aspiration events. Increased risk of aspiration through decreased activity of the cough reflex via decreased local levels of the protussive peptides bradykinin and substance P is proposed as one possible mechanism responsible for the effect of ACE D allele on pneumonia risk [18]. We think, however, that this difference cannot explain the finding of a null effect in the present study. Firstly, when we limited our analysis to non-winter (April-November) events the genotype distribution of the patients remained identical to that of the control subjects (II/ID/DD: 21/50/24; p=0.929). Secondly, the genotype distribution of the patients did not differ with age.

At last, the finding of a null effect could also be explained by the inclusion of admitted pneumonia cases solely. Approximately 60% of patients with pneumonia is treated at home. Therefore, one might argue that admission itself is dependent on genotype, either because those of one genotype (i.e. DD) die before referral or because those of one genotype (i.e. II) are not sufficiently unwell to be admitted. Such admission bias, however, seems unlikely given that genotype distribution is in Hardy-Weinberg equilibrium and very similar to our control group.

Besides no association between ACE I/D polymorphism and pneumonia risk, our study also showed no association between the ACE I/D polymorphism and pneumonia outcome. The pneumonia illness severity, as quantified by APS, was not

different for the three genotype groups as was the duration of hospital stay, in-hospital mortality, and 28-days mortality. Recently, Harding et al. showed that the ACE D allele is associated with increased risk of organ dysfunction and death with meningococcal meningitis [31]. Another study from Marshall et al. suggested an important role for the ACE I/D polymorphism in the susceptibility and outcome in ARDS [32]. This was partly confirmed by Jerng et al. concluding that the ACE I/D polymorphism is a prognostic factor for the outcome of ARDS [33]. Also, Adamzik et al. showed an association between the ACE DD genotype and increased 30-day mortality in ARDS [29]. In the present study, we were not able to extend these findings to community-acquired pneumonia. There was no trend towards an association between ACE I/D polymorphism and the occurrence of ARDS in our patients (Table 2). This is in accordance with the previous findings from Jerng et al. and Adamzik et al.. Due to limited numbers we were unable to examine the association between ACE I/D polymorphism and outcome in ARDS. Regarding the association between the ACE I/D polymorphism and clinical outcome of community-acquired pneumonia, we realize that the numbers of outcomes in the present study were smaller than expected and that this could explain the finding of a null effect due to lack of power. On the other hand, the absence of any trend towards an effect of ACE DD genotype on pneumonia outcome makes the need for additional studies with larger numbers questionable. Also a subgroup analysis (data not shown) in patients with confirmed pneumococcal pneumonia showed no trend of an effect of ACE genotype on disease severity. Our study still had sufficient power to detect a 10% absolute difference in mortality between the DD and ID+II genotypes.

In conclusion, according to our findings, the ACE gene I/D polymorphism is not associated with risk and outcome of community-acquired pneumonia in the Dutch white Caucasian population.

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# 3.4 | ACE I/D corrected serum ACE activity and severity assessment of community-acquired pneumonia

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## Abstract

Different studies described decreased serum angiotensin-converting enzyme (ACE) activity in patients with pneumonia. The present study aimed to evaluate the role of ACE in pneumonia by comparing ACE insertion/deletion (I/D) genotype corrected serum ACE activity and to establish whether the severity of the disease correlates with lower ACE activity.

This was a prospective hospital-based observational study including 134 patients with pneumonia. Serum ACE activity was determined at admission, on day 2, 3, 5, and 10 of hospital stay, and at recovery. Based on ACE genotype and reference values, corresponding Z-scores were calculated. Disease severity, quantified by the Acute Physiology Score (APS), and clinical outcome were compared between tertile groups of the Z-scores.

A significant decrease in serum ACE activity during an episode of pneumonia with return to control range during recovery was observed for all 3 genotypes (II, ID and DD). The calculated Z-scores showed a negative correlation with APS scores ($p=0.050$). No significant association between decreased serum ACE activity and clinical outcome could be observed.

Serum ACE activity is significantly decreased during the acute phase of pneumonia. Despite correction for ACE I/D genotype, decreased ACE activity did not show a prognostic value. Further studies are suggested on the mechanisms behind and diagnostic value of a decreased ACE activity in community-acquired pneumonia.

## Introduction

Community-acquired pneumonia remains a major reason for hospital admission and a common cause of death in developed countries [1, 2]. Therefore, pneumonia is subject to many studies on demographic variables, co-morbidities, and biological markers in order to predict outcome and to evaluate a patients' management.

The renin-angiotensin system is a feedback regulated system. In response to a fall in blood pressure renin is secreted into the circulation. Renin cleaves angiotensinogen to generate angiotensin I. Angiotensin I has no appreciable activity, but is acted on by a second proteolytic enzyme, angiotensin-converting enzyme (ACE) (peptidyldipeptidase A, EC 3.4.15.1) to form the highly active angiotensin II. The majority of ACE is expressed on the surface of (pulmonary) endothelial cells [3] and leaked into the circulation from ACE-expressing cells by proteolytic cleavage [4]. Circulating concentrations of ACE have been extensively studied in relation to different human lung disorders [5, 6]. Today, one quite established value of serum ACE measurements is in the diagnosis and follow-up of sarcoidosis [7, 8]. Furthermore, serum ACE has also been studied in patients with adult respiratory distress syndrome (ARDS) and pneumonia where ACE activity showed a strong decrease in the acute phase of the disease with return to control range within a few days [9-13]. Although proposed, so far no relation between these alterations in serum ACE activity and clinical development of both ARDS and pneumonia could be established [14]. One possible explanation for this could be the large inter-patient variations in serum ACE activity observed in these studies. Nowadays, the identified ACE insertion/deletion (I/D) polymorphism, accounting for almost half of the variance in serum ACE activity, provides an explanation for these inter-patient variations but also a need to re-investigating the possible clinical value of serum ACE activity in pneumonia by considering ACE I/D genotype [15].

The objective of the present study was to evaluate the role of ACE in community-acquired pneumonia by comparing the genotype corrected serum ACE activity and to establish whether the severity of the disease correlates with lower ACE activity.

## Patients and Methods

The study was conducted in St. Antonius Hospital, a 600-bed teaching hospital (Nieuwegein, The Netherlands). The ethnicity of the population in and around the city of Nieuwegein is primarily (>94%) white-Caucasian [16].

*Patient population*

This was a prospective observational study of patients with confirmed pneumonia admitted between October 1, 2004 and August 1, 2006. Pneumonia was defined as a new or progressive infiltrate on a chest X-ray plus at least two of the following criteria: cough, sputum production, temperature >38°C or <35°C, ausculatory findings consistent with pneumonia, leukocytosis or -penia (>10 G/L, <4 G/L, or >10% rods in leukocyte differentiation), C-reactive protein >3 times the upper limit of the reference interval for normal values. Patients, who were immunocompromised (systemic steroid use at admission (prednisone equivalent >20 mg/daily for more than 3 days), haematological malignancies and other immunosuppressive therapy) or who were using ACE-inhibitors, angiotensin II receptor blockers, or aldosterone-antagonists were excluded. All patients were required to sign an informed consent and the study was approved by the Ethics Committee of the St. Antonius Hospital. In total, 158 patients with pneumonia were included in the study. For twenty-four of these patients, no blood sample for ACE activity measurement was collected at time of hospital admission and an appropriate DNA sample was missing for one patient. Finally, 134 patients were eligible for further analysis.

*Determination of ACE activity*

Blood samples were collected aseptically into lithium heparin tubes on admission and on day 2, 3, 5, and 10 of hospital stay. At least 30 days after the resolution of the acute infection, the patients were requested to visit the out-patient clinic to provide another blood sample. Quantification of ACE activity was measured in lithium heparin plasma using the Bühlmann ACE kinetic test, according to previously described methods (Bühlmann Laboratories AG, Switzerland) [17, 18]. The manufacturers' reference interval is 12-68 U/L.

*Genotyping*

Genomic DNA of patients was isolated from EDTA blood using the MagNA Pure LC DNA Isolation kit 1 (MagNA Pure; Roche Diagnostics). ACE I/D polymorphisms were determined by real-time polymerase chain reaction (PCR) using fluorescent hybridization probes and a LightCycler (Roche Diagnostics) as described earlier with some slight modifications [15, 19, 20]. Briefly, the reaction volume was 20 μl, containing 1 μl of DNA (40-80 ng), 0.2 μM forward primer and 0.8 μM reversed primer reported by Rigat et al. [15], 2 μl of 10x reaction buffer (LightCycler DNA master

hybridization probes, Roche Diagnostics), 1.6 μl of 25 mM $MgCl_2$ stock solution and 0.1 μM of each probes. The detection probes were the same as described by Somogyvari et al. [20]. The PCR conditions were as follows: denaturation at 95°C for 60s, followed by 50 cycles denaturation (95°C for 10s), annealing (first 10 cycles: 67°C for 20s, followed by 0.5°C stepwise decrease per cycle to 61°C) and extension (72°C for 30s). Melting curve analysis consisted of heating to 95°C for 5s, 45°C for 60s, followed by an increase of the temperature to 75°C at 0.2°C/s. To exclude mistyping of I/D heterozygotes as D/D homozygotes, a second PCR reaction was conducted under the same conditions except for using the primer pair as described earlier [19, 20]. Verification of the real-time PCR results with those of electrophoresis and using sequence-specific primer PCR revealed no mistyping. ACE I/D polymorphisms were determined after follow-up of the patients.

*Genotype corrected ACE activity*

After genotyping, all serum ACE activities were translated into Z-scores. The Z-score was calculated as ($ACE_{patient}$ – mean $ACE_{reference\ group}$) / $SD_{reference\ group}$ ,where mean $_{reference\ group}$ and $SD_{reference\ group}$ are calculated from the ACE values measured in previously described II, ID and DD reference groups originating from the same geographical region as the patients [21]. This reference group consisted of healthy employees of the St. Antonius Hospital who volunteered for venapunction.

*Pathogen identification*

At least two blood cultures were performed and sputum was taken for Gram-stain and culture and analysed by Taqman real-time PCR for *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Chlamydophyla psittaci* [22]. Pharyngeal samples were taken for viral culture. Urine was sampled for antigen testing on *Streptococcus pneumoniae* and *L.pneumophila* (Binax NOW®) [23, 24]. In addition serum samples of the day of admission and day 10 were analysed in pairs for detection of a four-fold rise of antibodies to respiratory viruses, *Coxiella burnetii*, *M.pneumoniae*, and *C.psittaci* by complement fixation assay [25]. Based on the findings, patients were classified as pneumonia with bacterial origin, viral pneumonia, or pneumonia with unknown aetiology.

*Outcome measures and illness severity assessment*

The following outcome measures were identified for all patients: duration of hospital stay, the need for intensive care unit (ICU) admittance, survival to hospital

discharge, and 28-days mortality. Illness severity was quantified by Acute Physiology Score (APS) scoring on admission and consecutively on day 2, 3, 5 and 10 of hospital stay [26].

*Co-morbidity assessment*

Besides demographic variables, co-morbidities were identified to address factors related with outcome of community-acquired pneumonia. Co-morbidities were defined based on the presence of conditions for which the patient was under active medical supervision or was receiving treatment at the time of hospital admission. Co-morbidities evaluated were lung diseases (chronic obstructive pulmonary disease (COPD), or treated asthma), congestive heart failure, diabetes (both type I and type II), and end-stage renal disease (serum creatinine >150 μmol/L). Furthermore, patients were classified according to the Pneumonia Severity Index (PSI) developed by Fine et al [27].

*Statistical analysis*

The SPSS statistical package (version 12.0.1 for Windows; SPSS, Chicago, IL) was used for the statistical analyses. Continuous data were expressed as mean ±SD or median (interquartile range) where appropriate. Categorical data were analysed by chi-square and continuous data by Student's t tests, rank tests, and one-way analysis of variance where appropriate. To study the association between serum ACE and severity of disease a correlation coefficient between Z-score and APS was calculated. Besides this, first, a multivariate linear regression analysis was conducted to identify determinants for serum ACE activity (using ACE activity at recovery), and subsequently, a linear regression analysis was conducted with APS as dependent and serum ACE activity and all parameters that appeared independent determinants for serum ACE activity included in the model. The prognostic usefulness of serum ACE activity was studied in two ways. First, clinical outcomes were compared between tertile-based groups of the distribution of the Z-scores. Secondly, a statistical analysis was focused on the ability of serum ACE activity to predict the outcome of pneumonia. For this purpose, logistic regression models adjusted by the comorbidities, ACE genotype and including serum ACE activity were constructed. The relative risk for having a certain outcome was estimated by odds ratio (OR) and 95% confidence interval (CI). For all tests, a p-value <0.05 was considered significant.

## Results

The mean age of the patients was 61 years (±19) and 79 (59%) of the patients were male (Table 1). On day of hospital admission, the serum ACE activity was significantly different for the II, ID and DD genotype groups with mean serum ACE activities of 24, 28 and 39 U/L respectively (Table 2).

**Table 1** *Demographic and general characteristics of the study population*

| Characteristic | n=134 |
|---|---|
| *Demographics* | |
| Age (SD) | 61 (19) |
| Male sex | 79 (59) |
| *Co-morbidity* | |
| Renal disease | 8 (6) |
| CHF | 10 (8) |
| Diabetes | 17 (13) |
| Lung diseases | 48 (36) |
| ACE genotype (II/ID/DD) | 32/63/39 |
| *Risk class** | |
| Low I | 25 (19) |
| Low II | 24 (18) |
| Low III | 30 (22) |
| Moderate IV | 40 (30) |
| High V | 15 (11) |

** Risk class based on Fine et al.*[27]

**Table 2** *Serum ACE activity (U/L) according to the ACE genotype on admission and at recovery*

| | II | ID | DD | p-value ° |
|---|---|---|---|---|
| *ACE acute (n=134)* | | | | |
| Mean (SD) | 24 (9) | 28 (12) | 39 (15) | <0.001 |
| *ACE recovery (n=96)** | | | | |
| Mean (SD) | 27 (7) | 37 (12) | 52 (17) | <0.001 |
| p-value # | 0.019 | <0.001 | <0.001 | |

** Number of patients in acute and recovery group vary in size because not all patients visited the out-patient clinic for a recovery sample (8 patients died and 30 lost to follow-up). # paired-sample t-test (n=96). ° one-way analysis of variance*

In total 96 patients visited the out-patient clinic for a recovery sample. The reason for not visiting was either death (n=8) or lost to follow up (n=30). The

ACE genotype distribution did not differ between both time points (II/ID/DD at time of admission was: 32/63/39; at time of recovery: 25/48/23 (p=0.733)) and the genotype distribution was similar to that of the reference group (43/107/50; p=0.356). When analysed as paired sample (n=96) the serum ACE activity differed significantly between admission and recovery in all three genotype groups (Table 2).

The decrease in serum ACE activity was most evident in carriers of the DD genotype. Figure 1 shows the serum ACE activity on day of admission, on day 2, 3, 5, and 10 of hospital stay and at recovery for the three genotype groups. The serum ACE activity of the reference group is also presented in Figure 1. The mean serum ACE activities at hospital admission were 29, 29, and 34 U/L for pneumonia with bacterial origin (n=81), viral pneumonia (n=12), and pneumonia with unknown aetiology (n=41) respectively.

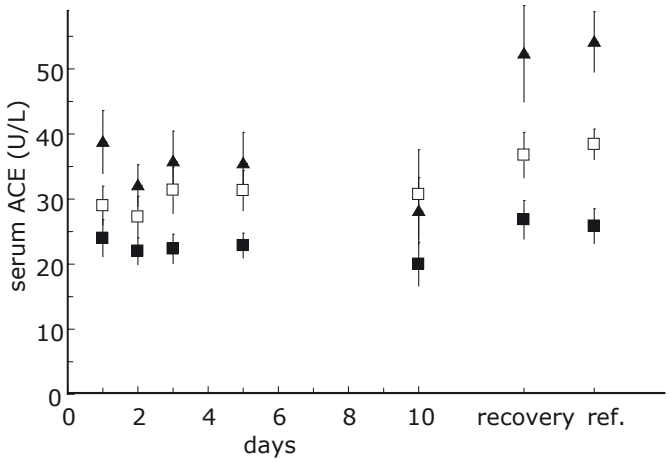


**Figure 1** *Mean serum ACE activity with 95% confidence intervals on admission (day 1) and on day 2, 3, 5, 10 of hospital stay and after recovery for different genotypes ( ■ = II, □ = ID, ▲ = DD). The last column represents the ACE activity of the reference (ref.) group.*

**Table 3** *Clinical outcomes and illness severity by Z-score tertiles (Z-score calculated based on serum ACE activity at time of hospital admission)*

| Severity and outcome | Z-score (-3.2 to -1.2) n=46 | (-1.2 to -0.4) n=43 | (-0.4 to 3.2) n=45 | p-value |
|---|---|---|---|---|
| APS [mean(SD)] | 22 (14) | 19 (10) | 17 (12) | 0.111 |
| Duration of stay [median(interquartile range)] | 10 (6-15) | 9 (7-14) | 8 (6-15) | 0.908 |
| ICU admittance [n(%)] | 5 (11) | 3 (7) | 6 (13) | 0.618 |
| In-hospital mortality [n(%)] | 3 (7) | 2 (5) | 3 (7) | 0.906 |
| 28-days mortality [n(%)] | 3 (7) | 2 (5) | 3 (7) | 0.906 |

The overall median duration of hospital stay was 9 days and 14 patients were admitted to the intensive care ward. During hospital stay, 8 (6%) patients died, all due to pneumonia. Based on the calculated Z-scores, the patients were divided into three groups of equal size (based on tertiles). This resulted in groups with Z-scores from -3.2 to -1.2 (n=46), from -1.2 to -0.4 (n=43), and from -0.4 to 3.2 (n=45). Duration of hospital stay, in-hospital and 28-days mortality as well as intensive care admittance were not statistically different between the three groups (Table 3). However, there was a trend towards increased disease severity with lower Z-scores. The mean APS score was the highest in the group of patients with lowest Z-scores and APS showed a negative correlation with the calculated Z-scores (p=0.050). In the multivariate linear regression analysis, serum ACE was not significantly associated with APS (p=0.156). Serum ACE activity at recovery was significantly determined by ACE genotype and lung diseases (COPD and asthma). In the multivariate logistic regression analyses, no associations between serum ACE activity and need for ICU admittance, in-hospital mortality nor 28-days mortality could be detected as the serum ACE activity did not reach significance in any of the models. The ACE I/D polymorphism also showed no association with need for ICU admittance and both in-hospital and 28-days mortality (data not shown).

**Discussion**

In accordance with the previous findings from Kerttula et al. and Altshuler et al. our study showed a significant decrease in serum ACE activity during an episode of community-acquired pneumonia with return to control range during recovery. The decrease was evenly pronounced in pneumonia with bacterial origin, viral pneumonia, as pneumonia with unknown aetiology. Despite correction for ACE I/D genotype and a significant correlation with the APS, we were not able to establish a prognostic value for the decreased ACE activity on the outcome of pneumonia.

The pathophysiological mechanisms behind the decrease in serum ACE activity are still unclear. Considering that ACE in the peripheral blood is identical to that produced by the pulmonary endothelial cells, one possible explanation could be an attenuated enzyme release from damaged pulmonary vascular endothelium. The observation from Altshuler et al. that ACE activity decreased more in patients with polysegmentated pneumonia is supportive to such a mechanism [28]. Another possible explanation could be an increased demand for angiotensin II leaving a depleted ACE pool. Hilgenfeldt et al. previously observed higher angiotensinogen levels combined with lower ACE levels in patients with sepsis [29]. The fact that in the present study serum ACE activity also correlated with mean arterial pressure (used in the calculation of the APS score) is supportive to such a mechanism [26]. A third explanation could be the concomitant presence of circulating endogenous inhibitors. This, however, was studied by Altshuler et al. but not observed.

In addition to the studies from Altshuler and Kertulla, we included the ACE I/D polymorphism in the association between decreased serum ACE activity and severity of pneumonia but we were still unable to show a solitaire prognostic value for decreased serum ACE activity during the active phase of pneumonia. However, although non significant, our findings show a trend towards a negative correlation between ACE activity and disease severity. Based on these findings we think that further studies with larger numbers are warranted to explore a prognostic value for decreased ACE activity in community-acquired pneumonia. We realise, however, that pneumonia severity is multifactor determined which could preclude clinical significance for the decreased ACE activity itself. The severity and clinical outcome remain strongly determined by demographic patient characteristics and comorbidities as well as the pneumonia aetiology and antibacterial treatment. To

further study the mechanism responsible for the observed decreased serum ACE activity during an episode of pneumonia, concomitant angiotensin II sampling would be helpful.

Although in the present study we were unable to establish a prognostic value for the decreased ACE activity in the acute phase of pneumonia, there might be a diagnostic applicability. To explore this possibility we compared the serum ACE activity at admission with those of the healthy control subjects used for our calculation of the Z-scores. For example, considering a cut point of 52 U/L for patients with the DD genotype for deciding about the diagnosis of pneumonia, ACE activity showed a sensitivity of 52% and a specificity of 90%. These data indicate that an ACE activity above this cut point practically excludes the diagnosis of pneumonia. We realize, however, that such a diagnostic applicability requires further study especially because this was not the primary aim in the present study. Furthermore, also serum ACE activity of initially suspected pneumonia that is not confirmed at follow-up will need to be assessed.

There are some possible limitations to our study. As it was conducted in admitted patients, less severe episodes of pneumonia attended normally in the primary care setting could not be included, although, 59% of the episodes were grouped into the low-risk classes (class I-III). Secondly, one could argue about the sample size of the present study. Beforehand, no solid power calculation was conducted as decrease in serum ACE activity was not predictable. Besides this, the mixed character (e.g. different aetiologies) of the study population could have weakened the power to detect an association between serum ACE activity and outcome of pneumonia. In a post hoc power calculation: our study had sufficient power to detect a three-fold increased in-hospital mortality for patients with a Z-score below -2 compared to patients with higher Z-scores ($\alpha$:0.05; power (1-$\beta$):0.80). Another possible limitation is the lack of information about smoking status. Smoking is associated with both an increased risk of pneumonia and increased plasma ACE levels [30]. Therefore, smoking could modify the decrease in serum ACE activity during pneumonia. Unfortunately, it was not possible to evaluate this in the present study. At last, we observed a decrease in circulating concentrations of ACE and this may not necessarily represent a decrease of ACE levels in the lung. Although ACE is mainly derived from endothelial cells and the lung represents the body's largest endothelial surface, in ARDS patients an increased ACE

activity has been reported in bronchoalveolar lavage fluid, despite a decrease in circulating concentrations [31].

In conclusion, serum ACE activity is significantly decreased during the acute phase of pneumonia with return to normal during recovery. Despite correction for ACE I/D genotype, the decrease in ACE activity did not show a prognostic value. Further studies are suggested on the mechanisms behind and diagnostic value of a decreased ACE activity in community-acquired pneumonia.

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# 4 | Statin treatment and community-acquired pneumonia

# 4.1 | Statin treatment and reduced risk of pneumonia in patients with diabetes

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## Abstract

Recent prognostic studies have shown that previous treatment with statins is associated with a better outcome in patients admitted to hospital with pneumonia. Because of an increased risk of pneumonia in patients with diabetes, we assessed the effects of statin use on the occurrence of pneumonia in adult diabetic patients.

All patients with a diagnosis of diabetes (type 1 and type 2) enlisted in the UK General Practice Research Database between 1 June 1987 and 21 January 2001 were included. A case-control study was performed with cases defined as patients with a first recorded diagnosis of pneumonia. For each case up to four controls were matched by age, sex, practice, and index date. Patients were classified as current users when the index date was between the start and end date of statin treatment. Conditional multiple logistic regression analysis was used to estimate the strength of the association between statin treatment and the occurrence of pneumonia.

Statins were used in 1.1% of 4,719 cases and in 2.1% of 15,322 matched controls (crude odds ratio (OR) 0.51, 95% CI 0.37-0.68). After adjusting for potential confounders, treatment with statins was associated with a significant reduction in the risk of pneumonia (adjusted OR: 0.49, 95% CI 0.35-0.69). The association was consistent among relevant subgroups (cardiovascular diseases, pulmonary diseases) and independent of the use of other prescription drugs.

In conclusion, use of statins is associated with a considerable reduction in the risk of pneumonia in diabetic patients. In addition to lowering the risk of cardiovascular disease, statins may be useful in preventing respiratory infections.

## Introduction

Community-acquired pneumonia (CAP) ranks in the top-10 leading causes of death in the elderly and is a significant and increasing cause of primary care consultations and hospital admissions [1-3]. Individuals with co-morbid conditions such as diabetes types 1 and 2 are at increased risk for lower respiratory tract infections and their complications [4, 5]. Furthermore, the impact of diabetes on deaths associated with pneumonia is substantial [6]. Recent in vitro studies have shown that inhibitors of hydroxy-methylglutaryl coenzyme A reductase (statins) have different immunomodulatory effects and that statins modulate both lipopolysaccharide induced pulmonary inflammation and pulmonary bacterial infection [7]. Clinical prognostic studies have confirmed a potential immune regulatory effect and showed that previous treatment with statins was associated with decreased rates of severe sepsis and mortality in patients admitted to hospital with community-acquired bacterial infections such as pneumonia [8-12]. We therefore hypothesised that, if the use of statins can improve the outcome in pneumonia, statin treatment may prevent development of the infection itself. The aim of this study was to assess whether the use of statins is associated with a decreased risk of developing pneumonia in an ambulatory population of diabetic patients.

## Patients and Methods

*Data Source*

The data were obtained from the United Kingdom General Practice Research Database (GPRD) which contains the computerised medical records of approximately 650 general practices. The GPRD is owned by the UK Department of Health and managed by the Medicines Control Agency. Approximately 6.5% of the total population of England and Wales is represented in the database. The computer records contain patient demographic characteristics (height, weight), symptoms and diagnosis (using the Oxford Medical Information System (OXMIS) and Read codes, which are mapped onto International Classification of Disease codes), referrals to specialist care, hospital admissions and their major outcomes, and all drug prescriptions in chronological order. The computerised recording of patient information was started by many general practitioners in the late 1980s and replaced the handwritten records used previously. Several independent validation studies have shown that the GPRD database has a high level of completeness and validity [13, 14]. The study was approved by the Scientific and Ethical Advisory Group of the GPRD.

*Study design and population*

This retrospective case-control study was conducted in a population of 142175 patients with diabetes (both types 1 and 2) recorded in the GPRD from 1 June 1987 to 21 January 2001 (original data extraction early 2002). This population has been used for previous studies on other topics not related to the present research study [15, 16].

Cases were defined as patients aged 18 years and older with a first medical attendance for an episode of community acquired pneumonia (for selected OXMIS and Read codes see Appendix 1). The date of pneumonia diagnosis was recorded as the index date. For each case up to four controls were matched for sex, age (±2 years), general practice, and index date of the case. Controls were randomly selected from the baseline cohort of diabetic patients without a record of pneumonia. To be able to control for potential prognostic differences between comparison groups, both cases and controls were eligible for inclusion in the study if they had a medical history in the database for at least 365 days before the index date.

*Exposure to statin therapy*

For each patient we identified all prescriptions for statins before the index date (atorvastatin, cerivastatin, fluvastatin, pravastatin, simvastatin). Episodes of statin treatment were defined as a series of subsequent prescription refills for these drugs. We assumed that a new episode of treatment started if an interval of 14 days or more occurred between the theoretical end date of a prescription and the date of the next prescription for the same patient. The theoretical end date was defined as the prescription date plus the duration of use (number of tablets prescribed divided by prescribed daily dose). Patients were classified as current statin users when the index date was between the start and the end date of a treatment episode. Patients were classified as past users when they were not a current user but had a history of use in the year before the index date.

*Potential confounders*

To be able to control for potential confounding, medical information was obtained on pneumonia risk factors that could potentially confound the association between statin treatment and outcome. For each patient we evaluated the presence or absence of the following frequently occurring co-morbidities as potential confounders: cardiovascular diseases (congestive heart failure, stroke),

pulmonary diseases, and alcoholism [17-20]. In addition, the latest data on smoking status (non-smoking, ex-smoking, current smoking, or unknown) and body mass index (<20, 20-24, 25-30, >30 kg/$m^2$, or unknown) were assessed. Possible confounding drugs examined were influenza vaccination, pneumococcal vaccination, immunosuppressive drugs (methotrexate, cyclosporin, tacrolimus, etanercept, infliximab, fludarabin, cladrimycin, rituximab, or alemtuzimab), oral glucocorticoids, and use of gastric acid suppressing drugs [21-24]. A patient was considered to have been exposed to a drug if more than one prescription was issued in the year before the index date, except for vaccination drugs which required only one prescription. The prescription drugs shown above were selected because they have been linked to decreased or increased pneumonia risk, and those receiving these drugs may be more likely to receive statins because of their likelihood of attending a physician. We calculated the number of general practitioner (GP) visits in the year before the index date as a proxy for overall health status and healthcare consumption.

*Statistical analysis*

Univariate analysis by $\chi^2$ tests and Student's t tests was used to test for statistically significant differences in baseline characteristics between cases and controls. Conditional logistic regression was used to estimate the strength of the association between statin treatment and the risk of pneumonia and expressed as odds ratios (OR) and 95% confidence intervals (CI). We included all potential confounders and age in the multivariate analysis to adjust for the baseline differences between both groups. Exposure to statins was categorised as current, past and never (reference). In addition, to elucidate a possible time trend effect, the study population was divided into quartiles based on ranked index dates and analysed separately. Stratified analyses were conducted to detect possible differences in effect between co-morbidity related subgroups.

## Results

The study population comprised 4,719 patients with a diagnosis of pneumonia and 15,322 matched controls. A diagnosis of pneumococcal pneumonia was recorded for 271 of the cases, 46 had a diagnosis of other bacterial pneumonia, 2,050 had a diagnosis of bronchopneumonia with unspecified organism recorded, and 2,291 had pneumonia with unspecified organism recorded. 28% of cases was referred to hospital because of their pneumonia diagnosis. About 48% of cases

and controls were men; the mean age of both cases and controls was 73 years but the proportion of very old was higher among the cases than in the controls (table 1). Matching on age had a median difference in age of 1.00 year with a mean difference of – 0.16 years. The median duration of observation in the GPRD was 4.0 years for cases and 4.1 years for controls.

**Table 1** *Characteristics of diabetic patients with pneumonia and controls*

| **Characteristic** | **Cases n=4,719 (%)** | | **Controls n=15,322 (%)** | | **Crude OR (95% CI)** |
|---|---|---|---|---|---|
| *Age (years)* | | | | | |
| <60 | 525 | (11.1) | 1,776 | (11.6) | * |
| 60-69 | 785 | (16.6) | 3,066 | (20.0) | * |
| 70-79 | 1,533 | (32.5) | 5,665 | (37.0) | * |
| 80-89 | 1,622 | (34.4) | 4,431 | (28.9) | * |
| ≥ 90 | 254 | (5.4) | 384 | (2.5) | * |
| *Gender* | | | | | |
| Male | 2,275 | (48.2) | 7,336 | (47.9) | * |
| Female | 2,444 | (51.8) | 7,986 | (42.1) | * |
| *Co-morbidities* | | | | | |
| Cardiovascular disease | 2,189 | (46.4) | 3,611 | (23.6) | 2.81 (2.62-3.00) |
| Pulmonary disease | 949 | (20.1) | 1,592 | (10.4) | 2.17 (1.99-2.37) |
| Alcoholism | 17 | (0.4) | 34 | (0.2) | 1.62 (0.91-2.91) |
| *Body mass index (kg/m$^2$)* | | | | | |
| 20-24 | 689 | (14.6) | 2215 | (14.5) | 1.00 (reference) |
| <20 | 251 | (5.3) | 466 | (3.0) | 1.73 (1.45-2.06) |
| 25-30 | 1083 | (22.9) | 4939 | (32.2) | 0.71 (0.63-0.79) |
| >30 | 485 | (10.3) | 2365 | (15.4) | 0.66 (0.58-0.75) |
| unknown | 2211 | (46.9) | 5337 | (34.8) | 1.33 (1.21-1.47) |
| *Prescription drugs* | | | | | |
| Influenza vaccination | 1,955 | (41.4) | 6,114 | (39.9) | 1.07 (1.00-1.14) |
| Gastric acid suppressing drugs | 967 | (20.5) | 1,988 | (13.0) | 1.73 (1.59-1.88) |
| Immunosuppressive drugs | 27 | (0.6) | 38 | (0.2) | 2.32 (1.41-3.80) |
| Oral glucocorticoids | 311 | (6.6) | 329 | (2.1) | 3.22 (2.74-3.77) |
| Pneumococcal vaccination | 144 | (3.1) | 569 | (3.7) | 0.82 (0.68-0.98) |
| Statins (current use) | 50 | (1.1) | 318 | (2.1) | 0.51 (0.37-0.68) |
| No. GP visits/year (mean, SD) | 14.8 | (11.0) | 9.1 | (7.7) | N/A |
| Current smoking # | 407 | (12.7) | 1,169 | (9.2) | 1.48 (1.31-1.67) |

** Matching variable. # Percentage for current smoking was calculated in subjects with information on smoking history recorded in the GPRD. GP: General Practitioner*

The presence of cardiovascular diseases, pulmonary diseases, smoking, and alcoholism was higher in cases than in controls, as was the use of gastric acid suppressing drugs and oral glucocorticoids. Cases visited the GP more often than

controls (15 *vs.* 9 visits per year, respectively, p<0.01).

Of the 4,719 cases, 50 patients (1.1%) were on active statin treatment compared with 2.1% for the control group. Univariate analysis showed that current statin treatment was associated with a reduced risk of pneumonia (crude OR: 0.51, 95% CI 0.37-0.68). After adjusting for age, cardiovascular diseases, pulmonary diseases, body mass index, alcoholism, smoking, influenza vaccination, pneumococcal vaccination, gastric acid suppressing drugs, immunosuppressive drugs, oral glucocorticoids, and number of GP visits in the year before the index date, the association did not alter substantially, yielding an adjusted OR of 0.49 (95% CI 0.35-0.69). The protective effect was similar for all statins and was consistent for both summer and winter seasons. The adjusted ORs for the different statins were 0.57 (95% CI 0.30-1.11) for atorvastatin (n=57), 0.48 (95% CI 0.14-1.71) for cerivastatin (n=17), 0.37 (95% CI 0.10-1.31) for fluvastatin (n=19), 0.36 (95% CI 0.17-0.75) for pravastatin (n=47), and 0.52 (95% CI 0.35-0.76) for simvastatin (n=228). Comparable effects were observed in all co-morbidity related subgroups with a strongest association in patients with a history of cardiovascular disease (table 2).

**Table 2** *Association between current statin therapy and pneumonia occurrence in all patients and in different (co-morbidity) subgroups*

| **(Sub)groups** | **Crude OR (95% CI)** | **Adjusted OR* (95% CI)** |
|---|---|---|
| All patients | 0.51 (0.37-0.68) | 0.49 (0.35-0.69) |
| Men | 0.65 (0.42-1.02) | 0.56 (0.39-0.91) |
| Women | 0.51 (0.33-0.78) | 0.40 (0.25-0.62) |
| No prior cardiovascular diseases | 0.63 (0.42-0.93) | 0.60 (0.40-0.91) |
| Prior cardiovascular diseases | 0.35 (0.21-0.57) | 0.36 (0.22-0.62) |
| No prior pulmonary diseases | 0.50 (0.35-0.72) | 0.41 (0.28-0.61) |
| Prior pulmonary diseases | 0.87 (0.47-1.58) | 0.85 (0.45-1.63) |

*OR: Odds ratio; CI: Confidence Interval.*

** Adjusted for age, cardiovascular diseases, pulmonary diseases, smoking, alcoholism, body mass index, gastric acid suppressing drug, flu vaccination, immunosuppressive drugs, pneumococcal vaccination, oral glucocorticoids, and number of GP contacts, with the exception of the grouping variable in the subgroup analysis.*

The effect did not differ substantially between younger (≤ 70 years; OR 0.56, 95% CI 0.37-0.86) and older patients (>70 years; OR 0.35, 95% CI 0.20-0.61), and the effect of current statin treatment on the risk of pneumonia was similar for episodes of community-acquired pneumonia treated in primary care (OR 0.52, 95% CI 0.36-0.77) and those requiring admission to hospital (OR 0.50, 95% CI 0.28-0.89). Furthermore, the association was equally present in the more recent years of the study period and in the first years, despite a consistent increase in statin prescriptions with time (Figure 1). Past use of statins was not associated with a decreased risk of pneumonia (OR 0.95, 95% CI 0.63-1.42).

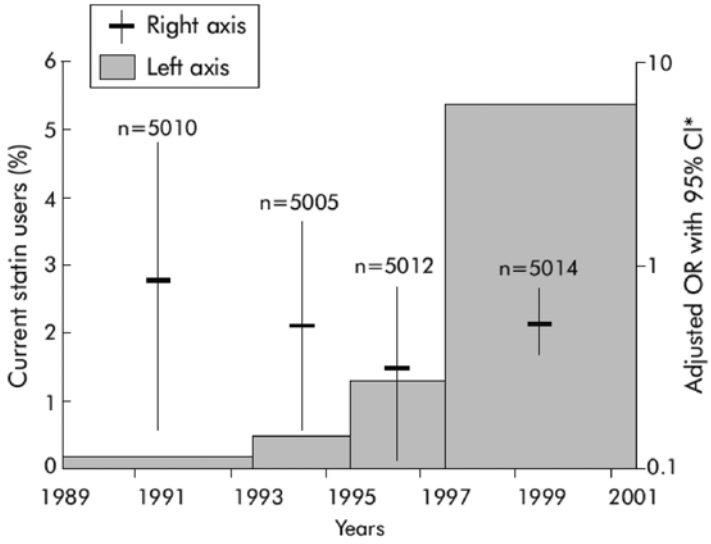


**Figure 1** *Prevalence of current statin use (left axis) and the association between statin use and pneumonia occurrence (right axis) in different time windows based on quartiles of ranked index dates of all subjects.*

## Discussion

In this study of diabetic patients the use of statins was associated with a considerably reduced risk of pneumonia. These findings add to the accumulating evidence that statins may have immunomodulatory and anti-inflammatory properties in addition to their lipid lowering effects. In vitro studies have indicated that statins have a potentially beneficial effect by normalising the inflammatory response in respiratory bacterial infections [9, 25]. This finding seems to have been confirmed by several prognostic clinical studies which found a substantial reduction in severe outcomes among statin users with pneumonia [7, 8, 10]. Our large case-control study with a long study period and a representative high-risk population is the first to extend these findings to examine the possible prophylactic effect of statins on the incidence of pneumonia.

This is important as it suggests that statins not only influence the development of pulmonary infection but could also prevent primary infection. The mechanisms behind this protective effect, however, remain speculative. To our knowledge, the only study addressing the possible effects of statins on primary host defence is the experimental study of Fessler et al. [7] which suggested impaired host defence in a murine model. However, there are several limitations in that study which preclude translation to the clinical situation. These include (1) the differences between a murine model and men; (2) the very high doses of lovastatin used (10 times that routinely used in humans); (3) observation of mice for only 48 hours after intratracheal ingestion of bacteria; and (4) lack of correlation between number of colony forming units and clinical appearance. Furthermore, it is possible that the other anti-inflammatory effects of statins (on macrophages and monocytes) may outweigh any impaired neutrophilic microbicidal capacity.

The prevalence of statin use in our study was relatively low, certainly in comparison with recent years. This is primarily caused by the period under study. We think, however, this has no impact on the validity of our study. Firstly, no obvious time dependent effect was observed (Figure 1). The association was consistently pronounced in the more recent years of the study period compared with earlier years, despite a constant increase in the use of statins with time. The large confidence interval in the first quartiles is most likely explained by small numbers. Additionally, although the power to detect differences between individual statins is somewhat limited, all the statins had odds ratios in the same range below 1. This suggests that the effect of statins results from their intrinsic properties and

is independent of trends in the prescribing of statins. Furthermore, adjustment of the associations for the presence of many important and frequently occurring co-morbidities and use of other prescription drugs did not substantially change the risk estimates. However, we cannot rule out the possibility of confounding by unknown or unmeasured factors.

This study was conducted in a high risk population of diabetic patients. Although patients with diabetes are more susceptible to infection, to our knowledge the mechanism of action of statins does not differ between diabetic and non-diabetic patients. The protective properties of statins may therefore be applicable to the general population, but further research is needed to establish a possible protective effect in non-diabetic patients.

Our study has some limitations. Firstly, identification of cases of pneumonia has the potential for misclassification due to incorrect coding. It is possible that, because of the broad definitions of setting the diagnosis of community acquired pneumonia, GPs could have applied the diagnostic criteria differently. For example, some acute exacerbations of chronic obstructive pulmonary diseases could be identified as pneumonia. We believe, however, that such misclassification is independent of statin use and would only lead to an underestimation of the true association between statins and the occurrence of pneumonia. In addition, the overall validity of the GPRD coding has previously been assessed several times and other validation studies on pneumonia coding in different medical databases have shown positive predictive values ranging from 61% to 85% [26, 27]. Assuming that the diagnosis in patients admitted to a hospital is more accurate (better accessibility to chest radiography and sputum and blood cultures), it is reassuring that the association between statin use and pneumonia in admitted hospital was identical to that in patients treated in primary care. In agreement with Laheij et al. [24], our study also showed an association between the use of gastric acid suppressing drugs and pneumonia.

A second possible limitation is misclassification of exposure to statin treatment since we used prescription data. Patients using statins could have been non-compliant with their treatment and therefore could have used fewer statins than were prescribed. However, this would have led to an underestimation of the association between statin use and the occurrence of pneumonia.

Another concern is that people on statins take more comfort out of good health support - the so-called "healthy user effect"- which can induce confounding. It is possible that statins were preferentially prescribed to patients with higher socioeconomic status and a lower risk of pneumonia. We tried to reduce such confounding by matching subjects on general practice (thereby on geographical region and city area). However, the use of postcodes as a proxy for socioeconomic status has been debated in the literature [28, 29]. On the other hand, greater co-morbidity (and therefore a greater risk of pneumonia) could decrease the likelihood that an older person will receive effective therapy for asymptomatic conditions such as raised cholesterol levels. If this was the case, a substantial difference would have been seen between younger and older patients in the protective association between statins and pneumonia and this was not seen in our study. Finally, we cannot rule out the possibility of confounding by unmeasured factors. However, the finding that the protective effect disappeared after discontinuing statin treatment strongly suggests that it is attributable to the properties of statins.

It must be emphasized that these findings on the preventive potential of statins should not be interpreted as suggesting that statins should be given to all diabetic patients to prevent pneumonia. Further confirmation is needed, particularly form large scale prospective randomized trials. In Influenza pandemics statins could possibly provide additional support in the prevention of bacterial super infections of the lung.

In conclusion, treatment with statins is associated with a considerably decreased risk of pneumonia in diabetic patients and their effects were consistent in all clinically relevant subgroups studied. Evidence is accumulating that treatment with statins may also influence bacterial respiratory infections, which may therefore broaden the indications for these drugs.

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**Appendix 1** *Medical codes used for case selection*

| Code | Description |
|---|---|
| H28..00 | Atypical pneumonia |
| H261.0 | Basal pneumonia due to unspecified organism |
| 485 | Bronchopneumonia |
| H25..00 | Bronchopneumonia due to unspecified organism |
| H21..00 | Lobar (pneumococcal) pneumonia |
| 481 B | Lobar pneumonia |
| H260.0 | Lobar pneumonia due to unspecified organism |
| H22..00 | Other bacterial pneumonia |
| 486 | Pneumonia |
| H2…0 | Pneumonia and influenza |
| 486 AP | Pneumonia aspiration |
| 483 AT | Pneumonia atypical |
| 481 BA | Pneumonia basal |
| H26..00 | Pneumonia due to unspecified organism |
| 483 M | Pneumonia mycoplasal |
| H2z..00 | Pneumonia or influenza nos |
| 481 A | Pneumonia pneumococcal |
| 4823 | Pneumonia staphylococcal |
| 486 T | Pneumonitis |

# 5 | Methodological aspects

# 5.1 | International classification of diseases codes showed modest sensitivity for detecting community-acquired pneumonia

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## Abstract

The aim of the present study was to estimate the sensitivity of International Classification of Diseases (ICD-9-CM) coding for detecting hospitalized community-acquired pneumonia and to assess possible determinants for misclassification.

Based on microbiological analysis data, 293 patients with a principal diagnosis of community-acquired pneumonia at seven hospitals in the Netherlands were assigned to three categories (pneumococcal pneumonia, pneumonia with other organism, or pneumonia with no organism specified). For these patients, the assigned principal and secondary ICD-9-CM codes in the hospital discharge record were retrieved and the corresponding sensitivity was calculated. Furthermore, pneumonia-related patient characteristics were compared between correctly and incorrectly coded subjects.

The overall sensitivity was 72.4% for the principal code and 79.5% for combined principal and secondary codes. For pneumococcal pneumonia (ICD-9-CM code 481) and pneumonia with specified organism (ICD-9-CM code 482-483) the sensitivities were 35% and 18.3% respectively. Patient characteristics were not significantly different between correctly and incorrectly coded subjects except for duration of hospital stay which correlated negatively with coding sensitivity ($p=0.01$).

In conclusion, ICD-9-CM codes showed modest sensitivity for detecting community-acquired pneumonia in hospital administrative databases, leaving at least one quarter of pneumonia cases undetected. Sensitivity decreased with longer duration of hospital stay.

## Introduction

Community-acquired pneumonia (CAP) is a common and potentially fatal infection of lung tissue and is associated with high health care costs. Therefore, CAP is subject to many epidemiological and economical studies. In about 20% of all pneumonia cases inpatient treatment is required because the clinical situation does not allow outpatient therapy [1, 2]. Many of the studies on pneumonia, therefore, identify cases of hospitalized CAP because these are most likely to result in death and resource use. A common approach to identify cases of hospitalized CAP is using hospital discharge records as coded according to the International Classification of Diseases-9$^{th}$ revision-Clinical Modification (ICD-9-CM). The common ICD-9-CM codes used for this purpose are 481, 482.x, 483.x, 485, and 486 [3-8]. Information, however, on the validity of such data is inconclusive or lacking in many cases. Several researchers have assessed the validity of hospital discharge records of various diseases, including pneumonia, by identifying cases through ICD-9-CM codes and subsequently reviewing medical charts to confirm or reject the correct diagnosis [9-12]. This approach, however, leaves cases of CAP without an ICD-9-CM code for pneumonia undetected and provides no information on the sensitivity of ICD-9-CM coding for detection of cases of CAP. The aim of this study is to estimate the sensitivity of ICD-9-CM code assignment in a population of patients admitted with a principal diagnosis of CAP. Furthermore, we aim to assess possible determinants for misclassification.

## Patients and Methods

This study used patient data from a randomized open label clinical trial (July 2000-March 2004) on efficacy of an early switch of intravenous antibacterial treatment to oral treatment of CAP [13]. All adult patients hospitalized for CAP in seven hospitals (two university medical centers and five teaching hospitals) in the Netherlands were eligible for inclusion in that study. Pneumonia was defined as a new or progressive infiltrate on a chest X-ray plus at least two of the following criteria: cough, sputum production, rectal temperature >38 °C or <36 °C, ausculatory finding consistent with pneumonia, leucocytosis (>10.000/mm$^3$, or >15% bands), C-reactive protein >3 times the upper limit of normal, positive blood culture or positive culture of pleural fluid. Patients with cystic fibrosis, a history of colonization with Gram negative bacteria due to structural damage to the respiratory tract, malfunction of the digestive tract, life expectancy of less than 1 month due to underlying diseases, infections other than pneumonia nee-

ding antibiotic treatment, severe immunosuppression (neutropenia (<0.5 x $10^9$/L) or a CD4 count <200 /$mm^3$), and needing mechanical ventilation in an intensive care unit were excluded.

*Identification of pathogen and patient categorization*

For each patient, sputum samples and blood samples were collected, cultured and evaluated following standard procedures. In addition, Binax NOW-tests were used to detect urinary antigen for *Legionella pneumophila* and *Streptococcus pneumoniae*. Acute and convalescent serology samples were collected and evaluated for *Mycoplasma pneumoniae, L. pneumophila* and *Chlamydophila pneumoniae*. Based on microbiological analyses performed, patients were categorized patients as having pneumococcal pneumonia (defined as *S. pneumoniae* isolated from a blood sample or adequate sputum sample containing >25 polymorphonuclear neutrofils and <10 epithelial cells per high power field), pneumonia with other pathogen specified, or pneumonia with no organism specified. When in a patient more than one pathogen was specified, *S. pneumoniae* was defined as the pathogenic organism.

*Coding accuracy measurement*

In the Netherlands the principal diagnosis is defined as the medical condition most responsible for admission to a hospital. The principal diagnosis should be coded in the primary position on the hospital discharge record. In addition, different secondary diagnoses and complications can be coded in the secondary positions [14]. All records are coded according to the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM).

For all patients, we retrieved the coded hospital discharge record from the medical registration department of the participating hospitals. This was done more than 2 years after closure of the previously mentioned randomized clinical trial (March 2006). The following ICD-9-CM codes were considered to be correct for each different patient category: 481 for pneumococcal pneumonia (the ICD-9-CM specifically excludes coding pneumococcal pneumonia as 482.3); 482.x, and 483.x for pneumonia with other organism specified; and 485-486 for pneumonia with no organism specified. For each category of pneumonia, we evaluated the proportion of patients for whom the correct ICD-9-CM code was listed in the primary or secondary positions on the hospital discharge record and calculated the sensitivity. Sensitivity was defined as the number of correctly coded patients

divided by total number of patients in that category. Analyses were conducted for all patients together and for the seven hospitals separately. For subjects without an ICD-9-CM code of 481-486, we identified the other codes assigned in the primary position.

*Determinants for misclassification*

To assess possible determinants for misclassification, we identified and analyzed the following characteristics between correctly and incorrectly coded subjects: age, gender, co-morbidities (heart failure, history of stroke, liver disease, malignancy, renal disease), pneumonia severity index [15], and whether the patient was a nursing home resident. The reason we identified the previously mentioned characteristics was because they have been linked to pneumonia outcome in different prediction rule models [16-18] and that, especially, differences in prevalence of these items are important to predict possible selection bias in etiological or prognostic studies using cases of CAP identified through ICD-9-CM codes. In addition, we evaluated the following pneumonia outcome measures: duration of hospital stay and in-hospital mortality.

To study determinants for misclassification, multiple variable logistic regression analysis was conducted with incorrect code assigned as dependent. All possible determinants were included in the multivariate model when they were retained after backward stepwise elimination ($p<0.10$).

## Results

Of the 293 patients hospitalized for CAP, 40 (14%) had confirmed pneumococcal pneumonia, 82 (28%) had pneumonia with another organism specified, and 171 (58%) had pneumonia with no organism specified (Table 1). In total, 212 patients had any pneumonia related ICD-9-CM code (481-486) as principal diagnosis, yielding an overall sensitivity for any pneumonia-related ICD-9-CM code of 72.4%. The overall sensitivity for six of the seven participating hospitals separately ranged from 61.5 to 82.0% (one hospital excluded for including only one patient). Expanding the criteria to a correct ICD-9-CM code as principal diagnosis or as any secondary diagnosis increased the overall sensitivity to 79.5%. The sensitivities for all three categories individually are shown in Table 1. When both ICD-9-CM codes 481 and 482.3 were considered valid for pneumococcal pneumonia, the sensitivity for that category increased from 35% to 47.5%. For cases without an ICD-9-CM code 481-486 as principal or secondary diagnosis ($n$=60),

the most frequently occurring other ICD-9-CM codes were 496 (chronic airway obstruction, not classified), 507.0 (pneumonitis due to inhalation of food or vomitus), and 162.9 (malignant neoplasm of bronchus or lung), respectively assigned to 11, 3, and 3 patients. Other codes assigned as principal diagnosis were diverse and occurred not more than once. None of the patients had an ICD-9-CM code for viral pneumonia (480.x) or influenza with pneumonia (487.x).

**Table 1** *(Overall) sensitivity for ICD-9-CM code assignment for hospitalised community-acquired pneumonia*

| | | Principal diagnosis code assigned | | | | |
|---|---|---|---|---|---|---|
| **CAP diagnosis** | **n (%)** | **481** | **482-483** | **485-486** | **Other** | **Sensitivity** |
| All diagnoses | 293 (100) | 32 | 30 | 150 | 81 | 72.4 %* |
| Pneumococcal pneumonia | 40 (14) | 14 | 7 | 13 | 6 | 35.0 %† |
| Pneumonia with other organism specified | 82 (28) | 10 | 15 | 30 | 27 | 18.3 %† |
| Pneumonia, organism unspecified | 171 (58) | 8 | 8 | 107 | 48 | 62.6 %† |

** Overall sensitivity for any pneumonia related ICD-9-CM code (481-486)*
*† Sensitivity for individual diagnoses*

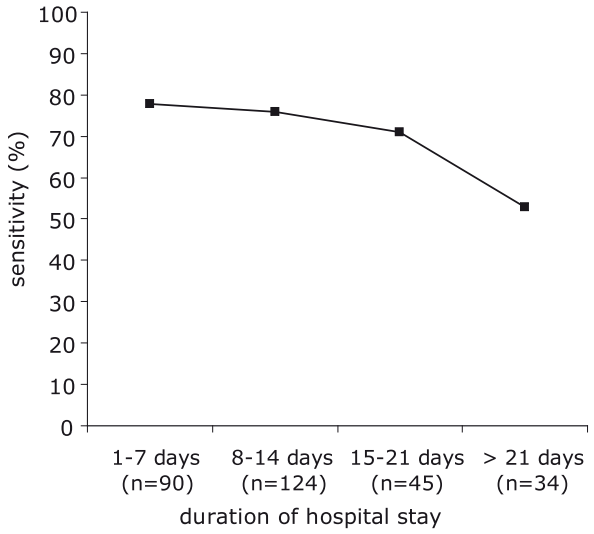


**Figure 1** *Duration of hospital stay and overall sensitivity.*

The characteristics of the correctly and incorrectly coded patients were not significantly different except duration of hospital stay, which was significantly associated with risk of misclassification in the multivariable regression analysis (Table 2; p=0.01). Although not significant, malignancies were surprisingly more prevalent in incorrectly coded subjects. Figure 1 shows the relation between duration of hospital stay and sensitivity of ICD-9-CM coding.

**Table 2** *Characteristics of correctly and incorrectly coded patients*

| | **Incorrectly coded (n=81)** | **Correctly coded (n=212)** |
|---|---|---|
| Mean age (SD) | 70.0 (13.1) | 69.5 (14.1) |
| *Sex* | | |
| Male | 54 (66.7) | 139 (65.6) |
| Female | 27 (33.3) | 73 (34.4) |
| *Co-morbidities* | | |
| Heart failure | 9 (11.1) | 27 (12.7) |
| History of stroke | 8 (9.9) | 19 (9.0) |
| Liver disease | 1 (1.2) | 2 (0.9) |
| Renal disease | 7 (8.6) | 20 (9.4) |
| Malignancy | 24 (29.6) | 41 (19.3) |
| Nursery home resident | 3 (3.7) | 8 (3.8) |
| Mean length of hospital stay, days (SD) * | 15.2 (11.6) | 11.7 (8.6) |
| In-hospital mortality | 4 (4.9) | 16 (7.5) |
| *Pneumonia severity index* | | |
| Risk class 1 | 0 | 0 |
| Risk class 2 | 1 (1.2) | 15 (7.1) |
| Risk class 3 | 6 (7.4) | 18 (8.5) |
| Risk class 4 | 57 (70.4) | 143 (67.5) |
| Risk class 5 | 17 (21.0) | 36 (17.0) |

** p=0.01*

## Discussion

Our study showed that in patients hospitalized with confirmed CAP, overall, only 72% was assigned any ICD-9-CM code for pneumonia (481-486) as the principal diagnosis on the hospital discharge record. For "pneumococcal pneumonia" and "pneumonia with other organism specified", sensitivity was as low as 35% and 18.3%, respectively.

Ideally, the ICD-9-CM code in the primary position (principal diagnosis) on the hospital discharge record always represents the medical condition that is chiefly responsible for the admission of the patient to a hospital. In the present study, however, we observed that this was the case in only 72%. Errors in classification can occur in any stage of the long chain of events leading to assignment of an ICD-9-CM code to a hospital discharge record: hospitalization, diagnosing, medical record keeping, filling the discharge abstract form by the treating physician, and interpretation by the coding clerk. In this study, all patients were hospitalized with a principal diagnosis of CAP as confirmed according to a standardized exam. Therefore, lack of information on hospitalization or diagnosing are probably less plausible explanations for the errors in classification. This makes medical record keeping, filling the discharge abstract form by the treating physician, and interpretation by the coding clerk more probable causes for the inaccurate coding observed in this study. But also complications or additional diagnoses could decrease the likelihood that the diagnosis at admission remains the evident principal diagnosis in the medical record. Our findings are supportive to such an effect since the true principal diagnosis and the assigned code were most likely to coincide in brief (and probably uncomplicated) admissions and diverged during prolonged admissions.

Concerning the pneumonia-related patient characteristics evaluated in the present study, except for duration of hospital stay, there were no significant differences between correctly and incorrectly coded subjects. This important finding implies that when cases of pneumonia are identified through ICD-9-CM codes no specific patient categories remain undetected except for an underestimation of patients with prolonged and probably complicated hospital stay. Especially when studying quality of care, outcomes, resource use, or the development of predictive instruments, inclusion of cases representing severe pneumonia is essential, as they represent the pneumonia cases that are most likely to result in death, intensive care admission, and prolonged hospital length of stay. The latter appear less represented when cases of hospitalized CAP are selected through ICD-9-CM coded hospital discharge records.

Advantages of our study are the possibility to include pneumonia cases from seven different sites decreasing the chances of coding bias, the availability of microbiological data, and the ability to study differences in sensitivity between

primary and secondary discharge codes. Because CAP occurs frequently in combination with other diseases such as congestive heart failure, chronic bronchitis, or exacerbation of asthma, the pneumonia code may appear in a secondary position. In our study, extending the assessment from the first to secondary positions resulted in only 21 (7.1%) additional patients with a pneumonia-related code in a secondary position instead of the primary position. This important finding indicates that case selection based on principal diagnosis codes alone does not exclude large numbers of patients with CAP. Principal diagnosis codes may also have greater positive predictive value (PPV) for identifying pneumonia, although we were unable estimate the PPV in the present study because the population was limited to confirmed cases of pneumonia.

Our study also has some limitations, which need to be discussed. First, we used patients from a prospective clinical trial in which patients with pneumonia were included using a strict protocol. However, because the data collected in the CAP study were not available to medical records staff, we do not believe that the study had any effect on the coding practices. If the study had any impact on accuracy of coding practices, this was likely to lead to an underestimation of the real problem of inaccurate code assignment. Secondly, this study was conducted in seven hospitals in the Netherlands, which could prevent extrapolation of the findings to other countries due to potential differences in coding of hospital discharge records. In addition, our study provides only information on the sensitivity of ICD-9-CM code assignment for hospitalized CAP and not on pneumonia treated in primary care.

The findings from this study could have impact on the validity of studies using coded hospital discharge records for case selection. For example, when studying the effects of pneumococcal vaccination on pneumococcal pneumonia incidence, a considerable underestimated incidence has to be faced. Or, in case-control studies, as a result of disease misclassification, selection bias could occur when not all observed cases (or controls) are true cases (or controls). Especially in hospital-based case-control studies, this can cause dilution of any association under study.

In conclusion, ICD-9-CM codes showed modest sensitivity for detecting CAP in hospital administrative databases, leaving at least one quarter of pneumonia cases undetected. Sensitivity decreased with longer duration of hospital stay.

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# 5.2 | Validation of community-acquired pneumonia: high positive predictive value for ICD-9 codes

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Submitted for publication

## Abstract

Many epidemiological studies on community-acquired pneumonia select cases using the International Classification of Diseases (ICD-9-CM). Aim of this study was to assess the positive predictive value (PPV) of ICD-9-CM codes for pneumonia.

In a Dutch 600-bed teaching hospital, all hospital discharge records for pneumonia (ICD-9-CM 481-486) in 2003 were identified. Subsequently, one third of the records was randomly selected and reviewed by an independent physician, blinded for the coded discharge diagnosis, in order to confirm or reject the correct diagnosing of community-acquired pneumonia. Patients were categorized as having pneumococcal pneumonia, other bacterial pneumonia with pathogen specified, pneumonia with unspecified pathogen, hospital-acquired pneumonia or other diagnosis. For each code the PPV was calculated. Furthermore, pneumonia-related patient characteristics were compared between true and false positives.

Medical charts of 73 patients were reviewed. The overall PPV was 88% (95% CI 80-95). For pneumococcal pneumonia (ICD code 481) and pneumonia with specified organism (ICD code 482-483) the PPVs were 50% and 69% respectively. There were no significant differences in characteristics between true and false positives.

The positive predictive value of ICD-9-CM coded hospital discharge diagnoses of community-acquired pneumonia is sufficiently high to warrant its use in epidemiological database studies.

## Introduction

Community-acquired pneumonia (CAP) is a common and potentially fatal infection of lung tissue and is associated with high healthcare costs [1]. Therefore, the burden of CAP is subject to many epidemiological and economical studies [2, 3]. Despite substantial progress in standards of care and the availability of antibiotics the incidence of CAP is rising nowadays [3]. An explanation for this could be the disproportionate growth of the elderly population that is most vulnerable to this deadly disease. In the elderly, CAP is often a terminal event superimposed on their underlying chronic debilitating illnesses. This makes incidence of CAP an interesting marker for health status in general. Because admitted community-acquired pneumonia is most likely to result in complications and resource use, most studies on CAP use cases of hospitalised pneumonia. A common way to identify cases of community-acquired pneumonia in hospital administrative databases is on the basis of relevant ICD-9-CM codes [4]. For some studies, medical chart review provides an option to confirm or reject cases identified through ICD-9-CM codes. However, in studies where data are sampled from large numbers of patients, anonymous data are provided, or many medical centres collaborate such a strategy is time consuming, costly and or not feasible. Therefore, information on the validity of disease identification based on ICD-9-CM codes is essential to evaluate possible bias through misclassification. A previous validation study from the Netherlands showed that around 72% of patients with confirmed pneumonia are assigned an ICD-9-CM code for pneumonia [5]. This, however, provides no information that cases identified through ICD-9-CM codes are all cases of community-acquired pneumonia. The aim of this study was to estimate the positive predictive value of hospital discharge diagnoses of pneumonia, classified according to ICD-9-CM codes, as a marker for community-acquired pneumonia. Furthermore, we sought to identify determinants for possible misclassification.

## Patients and Methods

*Study subjects*

Data for this study were obtained from the St. Antonius Hospital, a 600-bed teaching hospital located in the centre of The Netherlands. All patients with a principal hospital discharge diagnosis of pneumonia (ICD-9-CM codes 481-486) between January 1st and December 31st 2003 were identified. One patient could represent more than one hospital discharge diagnosis of pneumonia. Subsequently, one third of these cases were randomly selected and divided into three

categories: pneumococcal pneumonia (481), pneumonia with other organism (482, 483), and pneumonia with no organism specified (485, 486). The study was approved by the Ethics Committee of the St. Antonius Hospital.

*Validation*

Medical records of the patients were retrieved and reviewed by an independent pulmonary physician, blinded for the coded discharge diagnosis. Hospital discharge letters, X-thorax, data on blood samples, as well as sputum cultures were evaluated. On the basis of the available data, the physician was asked to make a diagnosis and classify this diagnosis according to the ICD-9-CM. Possible outcomes were: (1) no diagnosis of pneumonia, (2) community-acquired pneumococcal pneumonia (481), (3) community-acquired pneumonia with other organism specified (482-483), (4) community-acquired pneumonia, no organism specified (485-486), or (5) hospital-acquired pneumonia (first positive X-thorax >2 days after hospitalisation). Criteria for pneumonia were a new or progressive infiltrate on a chest X-ray plus at least two of the following criteria: cough, sputum production, temperature >38°C or <35°C, ausculatory findings consistent with pneumonia, leucocytosis or -penia (>10 G/L, <4 G/L, or >10% rods in leucocyte differentiation), C-reactive protein >3 times the upper limit of normal.

*Analysis*

Taking the independent physician's diagnosis as the 'gold standard', the positive predictive value was defined as the ratio of confirmed pneumonia cases and the total number of patients identified by an ICD-9-CM code. Analyses were performed at two levels: first, for all codes grouped together and, secondly, for all individual ICD-9-CM codes. Corresponding 95% confidence intervals (CI) were calculated according to Altman [6]. In addition, the following patient characteristics were evaluated for all subjects: gender, age, duration of hospital stay, and date of hospitalisation. Differences between characteristics of true and false positives were evaluated using the Students' T-test for continuous variables and Chi-2 test for proportions. Fisher's Exact test was used if not all expected numbers in a two-by-two table were 5 or higher.

## Results

In 2003, a total of 254 principal discharge diagnoses for pneumonia representing 229 unique patients were identified from the medical registration department. We randomly selected one third of these cases (n=84) of whom 73 (88%) medical records were retrieved and validated. Of these cases, 26 (35.6%) had an ICD-9-CM code for pneumococcal pneumonia, 13 (17.8%) had an ICD-9-CM code for pneumonia with other organism, and 34 (46.6%) an ICD-9-CM code for pneumonia with no organism specified.

**Table 1** *Validated diagnoses of community-acquired pneumonia*

| Observed ICD-9-CM code | n | Correct diagnosis | | | | PPV (95% CI) | |
|---|---|---|---|---|---|---|---|
| | | 481 | 482 | 485-486 | Other | A | B |
| 481 | 26 | 13 | 1 | 8 | 4 | 50 (31-69) | 85 (71-99) |
| 482 | 13 | 2 | 9 | 0 | 2 | 69 (44-94) | 85 (66-100) |
| 485-486 | 34 | 2 | 4 | 25 | 3 | 74 (59-89) | 91 (81-100) |
| Total | 73 | 17 | 14 | 33 | 9 | 88 (80-95) | 88 (80-95) |

*A=PPV for individual ICD code; B=PPV when any pneumonia diagnosis is considered valid*

Overall, 64 of these 73 selected discharge diagnoses were validated as being either pneumococcal pneumonia, pneumonia with other pathogen or unspecified pathogen, yielding an overall PPV of 88% (95% CI 80-95). For the individual ICD-9-CM codes the PPVs were 50% (95% CI 31-69) for 481, 69% (95% CI 44-94) for 482-483 and 73% (95% CI 58-88) for 485-486 (Table 1). When any diagnosis of pneumonia (categories 2 to 4) was considered valid, the PPVs for the individual ICD-9-CM codes were 85%, 85%, and 91%, respectively.

Nine patients with an ICD-9-CM code for pneumonia (12%) were categorised as having no pneumonia after chart review. Most of these patients experienced pulmonary complications, but did not have evident X-thorax abnormalities. None of the patients could be classified with a diagnosis of hospital-acquired pneumonia. Subsequently, we assessed whether patient characteristics or seasonality were associated with a higher or lower probability of correct coding. The mean age of the cases was 66 years for true positives and 69 years for false positives. Duration of hospital stay and seasonality were not statistically different between both groups with 12 and 14 days, and 78% and 56% winter season (November-March) respectively.

## Discussion

Our study showed an overall positive predictive value of 88% (95% CI 80-95) for ICD-9-CM coded hospitalisations for community-acquired pneumonia. The PPVs for the different ICD-9-CM codes individually were somewhat lower and should therefore be used with precaution when studying differences between different types of pneumonia. The PPV did not depend on age, gender, nor duration of hospital stay.

Assignment of the correct primary ICD-9-CM code to a hospital discharge record is the result from a long chain starting with hospitalisation, followed by diagnosing, medical record keeping, filling the discharge form by the treating physician and interpretation by the coding clerk. Our study showed that almost 88% of all identified cases were true cases of community-acquired pneumonia, leaving only 12% of cases misclassified. Most of these patients, however, had pulmonary symptoms. The most probable causes of disease misclassification are the complexity of setting a diagnosis of pneumonia and the risk of misinterpretation of other diseases with similar symptoms (such as bronchitis) as being pneumonia.

A previous study from the Netherlands showed that the sensitivity for identifying pneumonia on the basis of ICD-9-CM codes is 72% [5]. Combined with the findings from the present study, this means that when selecting cases of pneumonia based on ICD-9-CM codes an underestimated number is to be expected but with a high validity. Such a combination of modest sensitivity and high PPV has also been observed for other common hospital discharge diagnoses [7]. Based on our findings it is recommended to identify cases of pneumonia using combined ICD-9-CM codes 481-486, as the PPV is the highest when combined.

Our study has some limitations. First, data were obtained from one hospital only, what poses questions about the extrapolation to other hospitals. However, the fact that our results comply with previous validation studies on this subject [8, 9] supports the fact that ICD codes are appropriate to detect patients with community-acquired pneumonia in hospital administrative databases in general. Second, a factor which may have influenced our results is the fact that we were not able to use a more comprehensive medical history for diagnosis by the independent pulmonary physician. Although we believe that we have used the most relevant information needed to make the diagnosis, more detailed information

could have improved the accuracy of the "gold standard" diagnosis. It is therefore possible that some residual misclassification is still present in our validated outcomes. However, this will most likely have resulted in an underestimation of the true PPVs. Another limitation is that we only evaluated principal discharge diagnoses what leaves cases of pneumonia coded as secondary diagnosis untouched. Because community-acquired pneumonia occurs frequently in combination with other diseases such as congestive heart failure, chronic bronchitis, or exacerbation of asthma, it is not inconceivable that a pneumonia ICD-9-CM code might have appeared in as secondary diagnosis. The previously mentioned study, however, showed that in patients with confirmed community-acquired pneumonia only very small numbers of patients had a pneumonia-related ICD-9-CM code as secondary diagnosis instead of the principal diagnosis [5]. For this reason we evaluated principal discharge diagnoses only.

In conclusion, the positive predictive value of ICD-9-CM coded hospital discharge diagnoses of community-acquired pneumonia is sufficiently high to warrant its use in epidemiological database studies.

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# 6 | Summary and general discussion

## Introduction

Besides the intended therapeutic effects of drugs, medications also have different additional effects. Some of these additional effects are already known at the time of market approval and some are not. Rare or unexpected effects, not related to the considered mechanism of action, are often discovered after market approval. This thesis presents a series of studies exploring the hypothesis that both ACE-inhibitors and statins, besides their intended effects, reduce the risk of acquiring pneumonia. To begin, as the burden of pneumonia is not only determined by its incidence but also by a high mortality, another widely prescribed class of drugs, antibiotics, is evaluated in relation to the prognosis of pneumonia.

## Challenges in the treatment of pneumonia

The decision to treat a patient with suspected community-acquired pneumonia either in the community or to refer them to a hospital is complex and dependent on both clinical judgement and patients' social circumstances and wishes. Nowadays, many guidelines provide tools for identifying patients who are at low risk of death and who can usually be safely treated at home [1-4]. The two studies presented in this thesis, however, showed that in daily medical practice many patients still do not sufficiently respond to outpatient treatment and get hospitalised subsequently. In both studies almost twenty-five percent of the patients admitted to hospital for community-acquired pneumonia were treated at home initially.

The study in **chapter 2.1** aimed to assess whether non-responsiveness to initial outpatient antibacterial treatment is predictive for microbial aetiology of pneumonia. This was a hospital-based prospective observational study including all patients admitted with community-acquired pneumonia in the St. Antonius Hospital (Nieuwegein, The Netherlands) between October 2004 and August 2006. Microbial investigation included sputum, blood culture, sputum PCR, antigen testing, and serology. Multivariate logistic regression analysis was applied to assess whether prior outpatient antimicrobial treatment is predictive for microbial aetiology. Patient demographics, co-morbidities and pneumonia severity were considered as other potential predictors. The microbial aetiology was determined in 64% of the patients. The five most prevalent pathogens were *Streptococcus pneumoniae*, *Heamophilus influenzae*, *Legionella spp.*, *Mycoplasma pneumoniae*, and Influenza virus A+B. Forty-seven patients (23%) had received initial outpatient antimicrobial treatment. In multivariate analyses, initial outpatient

treatment was associated with a three-fold increased chance of finding atypical pathogens (OR 3.11, 95% CI 1.16-8.33) and a three-fold decreased probability of pneumococcal infection (OR 0.39, 95% CI 0.17-0.91). In conclusion, this study showed that in patients admitted to hospital for community-acquired pneumonia, whether the patient received initial outpatient treatment or not is very relevant information in the diagnostic work-up aiming to identify the causative pathogen and planning corresponding treatment.

There can be several explanations for the findings from the study described above. Only a small range of pathogens causes pneumonia, with *S. pneumoniae* being the most common. Based on this knowledge amoxicillin is the preferred empirical antibiotic choice in the Netherlands. Amoxicillin, however, does not cover the less frequent atypical pathogens (*L. pneumophilia, M. pneumoniae, C. psittaci*). Therefore, patients with pneumonia caused by these atypical pathogens will probably not respond the amoxicillin treatment which could cause deterioration of the clinical situation making hospital admission necessary. This provides one explanation for the higher prevalence of atypical pathogens in patients with prior outpatient antibacterial treatment. Another explanation could lie in the interference of the antimicrobial drugs with the microbial investigations. Presence of amoxicillin, for example, could have prevented growth of *S.pneumoniae* in blood or sputum culture. Such an effect might explain the reduced probability of pneumococcal infection, but not the increased likelihood of atypical pathogens.

Whether or not failure of initial outpatient treatment in turn compromises outcome was studied in **chapter 2.2**. Data for this study were obtained from the Dutch PHARMO Record Linkage System (RLS). Duration of hospital stay and in-hospital mortality were compared between patients admitted to hospital for pneumonia after outpatient antibacterial treatment (n=296) and patients admitted directly (n=794). The median duration of hospital stay was 10 days and was similar for both groups. In patients with a prior history of respiratory diseases or heart failure the median duration of hospital stay was 12 and 14 days, respectively. The overall in-hospital mortality was 7.2% and was not different between both groups. In patients with congestive heart failure the mortality was 9.8% for patients admitted directly and 23.3% for patients hospitalized after initial outpatient treatment (adjusted OR 2.78, 95% CI 1.01-7.81). In conclusion, failure of initial outpatient treatment does not compromise the outcome of pneumonia except for patients with heart failure. Patients with a combination of pneumonia

and heart failure are probably more susceptible for deterioration of the clinical situation. Non-responsiveness to initial outpatient treatment could delay the hospital treatment, which is essential to rescue this type of severe patients.

Altogether, both studies in chapter 2 showed that despite the availability of different prediction models to identify patients who can be safely treated at home many patients are still hospitalised after initial outpatient treatment. This finding illustrates the complexity of the treatment of pneumonia but also the need for further improvement of these prediction models. A focus for improvement can be better identification of patients with pneumonia of atypical origin. These patients could then be effectively treated in primary care (e.g. with macrolides) possibly preventing clinical deterioration requiring hospital admission. Furthermore, in-hospital treatment of pneumonia should not ignore the possibility of atypical pathogens as causative agents in patients treated at home initially.

### ACE-inhibitors affecting the risk of pneumonia

In this thesis, two different studies were presented on the possible association between ACE-inhibitor use and the risk of acquiring pneumonia. Both studies had a case-control design and were conducted with data originating from large and valid administrative databases. The studies, however, resulted in different findings.

The first study (**chapter 3.1**) was conducted with data extracted from the Dutch PHARMO RLS. Cases were defined as patients with a first hospital admission for CAP between 1995 and 2000. For each case, up to four population controls were matched by age and sex. In total, the study population comprised 1,108 patients with a first hospital admission for CAP and 3,817 matched controls. After adjusting for confounding, ACE-inhibitor use was not associated with the risk of pneumonia (adjusted OR 1.12, 95% CI 0.88-1.43). Additionally, no significant association was observed in patients with diabetes, respiratory diseases, heart failure, or patients with both of the last two conditions. Furthermore, adjustment of treatment effects on pneumonia risk using stratification on balancing score also showed no significant association between ACE-inhibitor use and pneumonia risk within the different strata (overall adjusted OR 1.09, 95% CI 0.87-1.36). Based on these findings, the conclusion was that the beneficial effect of ACE-inhibitors on pneumonia risk as observed in Asian populations could not be confirmed in a general, essentially white population.

The second study (**chapter 3.2**) was conducted in a population of patients from the United Kingdom General Practice Research Database (GPRD) who had a diagnosis of diabetes (both type 1 and type 2) between 1987 and 2001. Cases were defined as patients with a first diagnosis of pneumonia (n=4,719). For each case, up to four controls were matched by age, gender, practice, and index date (n=15,322). After adjusting for confounding, ACE-inhibitor therapy was associated with a significant reduction in pneumonia risk (adjusted OR: 0.72, 95% CI 0.64-0.80). The protective association was consistent across different relevant subgroups with the strongest association in patients with a history of stroke. There was a significant dose-effect relationship (p for trend <0.001). The conclusion from this study was that the use of ACE-inhibitors is associated with a significant reduction in pneumonia risk.

What can be learned from the different findings from the two studies described above? Contrasting findings often provide a unique opportunity to assess the adequacy of study designs [5]. Table 1 gives an overview of all currently available studies on the association between ACE-inhibitor use and the risk of pneumonia. When comparing the studies there are some items that could explain why some studies find an association between ACE-inhibitor use and risk of pneumonia and some do not.

**Table 1** *The association between ACE-inhibitor use and the risk of pneumonia in different studies*

| | **Etminan [6]** | **Ohkubo [7]** | **Ohkubo [7]** | **Okaishi [8]** | **Sekizawa [9]** | **van de Garde [10]** | **van de Garde [11]** |
|---|---|---|---|---|---|---|---|
| Ethnicity | Caucasian | Asian | Caucasian | Asian | Asian | Caucasian | Caucasian |
| Mean age (yrs) | 72 | 64 | 64 | 81 | 76 | 67 | 73 |
| Subjects (n) | 34,981 | 2,352 | 3,753 | 275 | 440 | 4,925 | 20,041 |
| History of stroke | No | Yes | Yes | No | Yes | No | Yes / No |
| Study design | Case-control | Trial | Trial | Cohort | Cohort | Case-control | Case-control |
| Setting | Population | Population | Population | Hospital | Hospital | Population | Population |
| Outcome* | A | n.s. | n.s. | A | A | A | A + H |
| Prevention | No | Yes | No | Yes | Yes | No | Yes |

* *A=admitted to hospital; H=home treatment*

Firstly, the selection of the appropriate outcome measure can be of importance. In our PHARMO study, exposure to ACE-inhibitors was compared between patients admitted to hospital for pneumonia (diseased) and matched population controls (non-diseased). The GPRD study used a similar approach but used an expanded case definition that included patients treated in primary care as well. In the GPRD study, the association between ACE-inhibitor use and risk of pneumonia could only be established for pneumonia treated in primary care. An explanation for this finding can be that ACE-inhibitor treatment, through heart failure, indirectly predisposes for hospital admission [12]. In other words, the sample might have been biased toward overexposure among the cases admitted to hospital, thereby producing an overestimate of the relative risk or, in this case, the finding of a null-effect.

Secondly, there is the issue of the selection of the patients regarding underlying morbidity. In the GPRD study there was statistically significant modification of the effect through the presence or absence of a recent history of stroke. This finding supports the hypothesis that an enhanced cough reflex through ACE-inhibition is (in part) responsible for the protection against pneumonia (patients with a recent history of stroke are at increased risk for aspiration pneumonia due to dysphagia [13, 14]) but also provides an explanation why other studies could have missed the association between ACE-inhibitor use and pneumonia risk due to small numbers of patients with a history of stroke.

And finally, another issue regarding patient selection can be ethnicity of the study population. Except for the study presented in chapter 3.2, all other studies showing a protective association between ACE-inhibitor use and pneumonia risk were conducted in Asian populations. In addition, Ohkubo et al. showed that the protective effect of ACE-inhibitors on pneumonia risk was primarily present for Asian participants of the PROGRESS study and absent for non-Asian participants [7]. As the distribution of the ACE I/D polymorphism is known to differ between Asian and non-Asian populations [15] we studied whether the ACE I/D polymorphism is associated with the risk of acquiring pneumonia (**chapter 3.3**). The ACE I/D polymorphism distribution was compared between 200 patients admitted to hospital for pneumonia and 200 healthy control subjects. The distribution between both groups was almost identical (p=0.978) suggesting no association between the ACE I/D polymorphism and the risk of pneumonia. Furthermore,

there was no association between the ACE I/D polymorphism and the outcome of pneumonia in the 200 patients with pneumonia. This finding is interesting because Morimoto et al. previously showed that the DD genotype is associated with a two fold increased risk of pneumonia and a four fold increased risk of fatal pneumonia in Japanese [16]. Despite sufficient statistical power it was not possible to replicate these findings in an essentially white population. Possibly, the ACE I/D polymorphism is not a functional polymorphism [17] but rather a marker for a true functional polymorphism for which the linkage dysequilibrium with the true functional polymorphism is different between Asian and non-Asian populations. The latter could not be examined in the present study. Nevertheless, our finding reduced the possibility that the ACE I/D polymorphism could act as a confounder in the association between ACE-inhibitor use and pneumonia risk. The impact of the ACE I/D polymorphism on the effect size (i.e. interaction) remains to be elucidated in the white population.

Altogether, the findings from the two studies described in this thesis, in a predominantly white population, combined with earlier reports in Japanese stating that ACE-inhibitors protect against pneumonia, are in line with the proposed mechanism of action and suggest that differences in study population and outcome measurement rather than ethnicity can explain the finding of a null effect in previous studies with Caucasians. If indeed this conclusion is correct, here resides another among several reasons to favor the use of ACE-inhibitors in managing hypertension and preventing the complications of diabetes.

Because ACE-inhibitors have also been linked to the outcome of pneumonia, the role of serum ACE activity during an episode of pneumonia was studied in **chapter 3.4**. In a prospective hospital-based observational study, that included 134 patients with pneumonia, serum ACE activity was determined at admission, on days 2, 3, 5, and 10 of hospitalisation, and at recovery. We observed that serum ACE activity was significantly decreased during an episode of pneumonia and returned to normal range during recovery in all genotype groups (II, ID and DD). The decrease in ACE I/D corrected ACE activity did show a mild correlation with the severity of pneumonia quantified with the Acute Physiology Score. However, no significant association between the extent of decrease in serum ACE activity and clinical outcome could be observed. The pathophysiological mechanism behind the decreased ACE activity still is subject to speculation. A possible expla-

nation could be an increased demand for angiotensin II, leaving a depleted ACE pool. However, this could not be further examined in the present study. Furthermore, in the present study it was not possible to assess whether the decrease in serum ACE activity also represented a decrease in local ACE activity in the lungs. Nevertheless, the finding that serum ACE activity decreases during an episode of pneumonia is very interesting and suggests involvement of the renin-angiotensin system in the pathophysiology of pneumonia and that ACE-inhibitors may play a role [18]. In addition, the decrease in ACE I/D corrected serum ACE activity might also be of value in differentiating between pneumonia and nonpneumonic exacerbations of COPD, especially because serum ACE activity has shown to increase in patients with exacerbations of COPD [19]. The diagnostic applicability of serum ACE activity requires further study.

### Effects of statins on the risk of acquiring pneumonia

Another frequently prescribed class of drugs in relation to pneumonia risk was studied in **chapter 4.1** Nowadays, statins are not only considered the principal and most effective drugs in the management of coronary artery disease but also thought to provide positive pleiotropic effects. Statins appear to positively affect life-threatening infections associated with cytokine dysregulation, such as bacteraemia [20-22]. The study presented in this thesis aimed to assess whether this effect can be extended to pneumonia.

The study was conducted in the same population as described in chapter 3.2. A case-control study was performed with cases defined as patients with a first recorded diagnosis of pneumonia. For each case, up to four controls were matched by age, sex, practice, and index date. Patients were classified as current users when the index date was between the start and end date of statin treatment. Conditional logistic regression analysis was used to estimate the strength of the association between statin treatment and the occurrence of pneumonia. In total, statins were used in 1.1% of the cases and in 2.1% of the matched controls (crude odds ratio (OR) 0.51, 95% CI 0.37-0.68). After adjusting for potential confounders, treatment with statins was associated with a statistically significant reduction in the risk of pneumonia (adjusted OR 0.49, 95% CI 0.35-0.69). The effect was consistent among relevant subgroups (cardiovascular diseases, pulmonary diseases) and independent of the use of other prescription drugs. We concluded that the use of statins is associated with a considerable reduction in the risk of pneumonia in diabetic patients.

Our finding was replicated soon after by Schlienger et al. who reported an odds ratio of 0.47 (95% CI 0.25-0.88) for the association between statin use and risk of fatal pneumonia [23]. In addition to our study, they studied the effect in the general population instead of patients with diabetes and they sampled a more recent time frame (1995-2002). This suggests that the association is independent of trends in prescribing statins to patients. Also, Hak et al. showed an association between statin exposure and pneumonia risk in a recent time frame (1996-2004) [24]. More recently, Frost et al. examined the effects of statins on pneumonia related inpatient death in patients with COPD [25]. They found a dose-dependent reduced risk with an OR of 0.49 (95% CI 0.26-0.76) for an average statin dose of ≥4 mg per day. An advantage of their study was that they both applied a cohort design as well as a case-control design, reducing the possibility of artifacts of either study design or analysis.

Altogether, four different observational studies using different data sources all showed a strong association between statin exposure and pneumonia risk. The question remains, however, whether this protective effect is really caused by statins' intrinsic properties or by other factors related to statin usage. The Bradford-Hill criteria on causality indicate that it is unlikely that a strong association between exposure and outcome will be explained by other co-factors, because the confounding factor should in that case be associated with the outcome in the same order of strength to nullify the effect of the exposure factor [26]. On the other hand, the possibility of residual confounding cannot be excluded in all four observational studies addressed above.

### Methodological considerations

In observational studies there are endless ways a study can go awry. But the most difficult bias to detect and control for is confounding. Confounding is explained by the effect from one determinant being confused with another. In the case of preventive therapy, for example, the effect of treatment might be biased when drugs are more likely to be prescribed to relatively healthy or health seeking patients ("healthy users") and these patients are also more likely to see a doctor on a regular basis, eat a healthy diet, stop smoking, and adhere to treatments. Such selective prescribing might be applicable to statins. A survey among 35 general practitioners in the early 90s demonstrated that there is a moral dimension in the attitude towards the prescribing of lipid-lowering drugs [27]. A substantial number

of general practitioners were less likely to treat hyperlipidemia in obese patients and smokers. Another study from Glynn et al. suggested that asymptomatic conditions, including elevated cholesterol, are less likely to be treated vigorously in patients with the greatest risk of death [28]. The question resides what the impact of such selective prescribing can be on the findings from numerous observational studies suggesting that statins prevent cancer [29], reduce fractures related to osteoporosis [30], decrease venous thrombosis [31] and prevent cataract [32].
Recently, Majumdar et al. showed that health status indeed has impact on the association between statin use and the prognosis of pneumonia [33]. In their study, after adjustment for confounding by co-morbidity and health status, the odds ratios changed from 0.78 (adjusted for age and sex) to 1.10 (fully adjusted). What the contribution of such a healthy user effect is on our observed association between statin use and risk of pneumonia remains to be elucidated. The co-factors (age sex, smoking status, body mass index, pulmonary co-morbidity, heart failure, immunisation, number of GP visits/year) used for confounding adjustment in the study from Majumdar et al. were also included in both our study and the study from Schlienger et al., but did not cause a change in the point estimate. At this moment, given the magnitude of the observed potential benefit and the available literature, a randomised trial would probably be the only valid modality to finally resolve the ongoing debate about the pleiotropic effects of statins and the risk and outcome of infections, particularly given the dearth of preventive strategies for the increasing burden of pneumonia.

Besides confounding, another important cause of bias can be misclassification. Misclassification bias results from an incorrect determination of exposure or outcome and might be random (non-differential) or systematic (differential). Non-differential misclassification always results in a diminished degree of association between two variables, whereas the odds ratio, relative risk, or risk difference may be spuriously increased of decreased if classification errors depend on either case-control status or exposure (differential misclassification).

In this thesis, four case-control studies were presented where case selection was based on assigned ICD-9 codes for pneumonia. To study the possibility and magnitude of disease misclassification in these studies, two additional studies were conducted. **Chapter 5.1** reported on the sensitivity of ICD-9 code assignment in a population of patients with confirmed pneumonia. Based on microbiological

analysis data, 293 patients with a principal diagnosis of community-acquired pneumonia at seven hospitals in the Netherlands were assigned to three categories (pneumococcal pneumonia, pneumonia with another organism, or pneumonia with no organism specified). For these patients, the assigned principal and secondary ICD-9 codes in the hospital discharge record were retrieved and the corresponding sensitivity was calculated. The overall sensitivity was 72%. There were no significant differences between correctly and incorrectly coded subjects except for duration of hospital stay. This important finding implies that when cases of pneumonia are identified through ICD-9 codes an underestimation of patients with prolonged hospital stay is to be expected. This could in turn compromise the external validity of the findings due to a relative under representation of patients with prolonged and probably complicated hospital stay in the study population. Regarding our study on prior outpatient antibacterial therapy as prognostic factor for mortality in hospitalised pneumonia patients (chapter 2.2) this could have biased the findings towards the null when the number of patients with prior outpatient antibacterial treatment was underestimated due to prolonged duration of hospital stay. On the other hand, the proportion of patients hospitalised after initial outpatient antimicrobial treatment in that study was almost identical to that observed in the study on microbial aetiology (chapter 2.1) which makes such a selection bias less likely. In addition, the age distribution, co-morbidities and outpatient antibiotics utilisation profile were very similar, as were the median duration of hospital stay and in-hospital mortality.

The positive predictive value (PPV) of hospital discharge diagnosis of pneumonia was assessed in **chapter 5.2**. In case-control studies the PPV is the most important measure of overall validity as this indicates that cases are true cases. Data for this study originated from the St. Antonius Hospital, Nieuwegein, The Netherlands. The PPV of a discharge diagnosis of pneumonia was 88% (95% CI 80-95). There were no significant differences in age, gender, seasonality, and duration of hospital stay between correctly and incorrectly coded discharge diagnoses. Based on these findings it is concluded that the positive predictive value of coded hospital discharge records for pneumonia is sufficiently high enough to warrant its use in epidemiological research.

In summary, based on the two studies presented in chapter 5, it can be concluded that ICD coded hospital discharge diagnoses of pneumonia are useful for

identification of patients with pneumonia but that the impact of an underestimated number of patients with prolonged duration of hospital stay on the study findings should be addressed. Furthermore, the fact that the patient characteristics of both the PHARMO RLS cohort (chapters 2.2 and 3.1) as well as the St. Antonius cohort (chapters 2.1, 3.3 and 3.4) are very similar supports the idea that the cohort in the St. Antonius Hospital represents a reliable proxy for the Dutch population (present in PHARMO RLS) and that the PHARMO RLS is suitable to study community-acquired pneumonia as important outcome. More important, this finding can support the external validity of the findings from this thesis.

**Final considerations**

This thesis showed that the treatment of pneumonia is complex and surrounded by many uncertainties regarding microbial aetiology and appropriate site of care. Despite antimicrobial treatment and progression in supportive care pneumonia remains the seventh leading cause of death in the Netherlands [34]. The disproportionate growth of the elderly population, most vulnerable to this deadly disease, underlines the need for strategies to prevent pneumonia.

Currently, influenza and pneumococcal vaccination are two available modalities to prevent pneumonia. Influenza infection is often complicated by secondary bacterial pneumonia and *S.pneumoniae* is the most common aetiological cause of pneumonia. Influenza vaccination has proven to be an effective strategy to prevent pneumonia but the merits of pneumococcal vaccination still are subject to debate [35, 36]. The findings from this thesis provide two new possible strategies to prevent pneumonia.

Firstly, ACE-inhibitors were shown to prevent pneumonia in a dose-dependent manner most likely through enhancement of the cough reflex. Especially a risk reduction of almost 45% for dosages above 1.5 daily defined doses is very promising. At this moment, however, the blood pressure lowering effects of ACE-inhibitors prevent the use of ACE-inhibitors in patients without elevated blood pressure. A challenge would be to develop a new drug specifically for enhancement of the cough reflex in elderly persons.

Secondly, this thesis showed an association between statin use and reduced risk of pneumonia. The probable mechanism behind this protective effect is an at-

tenuation of the inflammatory response to pathogens in the lung. Although the finding from this thesis has been replicated in other recent observational studies, the possibility of confounding due to selective prescribing of statins to patients with longer life expectancy cannot be excluded. Nevertheless, given the magnitude of the estimated effect, a randomised trial is more than warranted to confirm these findings. In the meanwhile, this potential additional beneficial effect of statins suggests that statin treatment should not be withheld from any patient with an indication for statin therapy, not even the elderly and frail.

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# 7 | Summary in Dutch

## Inleiding

De buiten het ziekenhuis opgelopen pneumonie (ook wel community-acquired pneumonie genoemd) behoort wereldwijd tot de meest voorkomende infecties. Voorzichtige Europese schattingen noemen incidenties van tussen de 1 en 10 gevallen per 1.000 personen per jaar. Er wordt geschat dat in Nederland jaarlijks 30.000 in het ziekenhuis worden opgenomen vanwege een pneumonie. Dit betreft voornamelijk ouderen. Ouderen lopen het grootste risico op een pneumonie vanwege een verminderde werking van het immuunsysteem op oudere leeftijd en vaak de aanwezigheid van onderliggende ziekten zoals diabetes, hartfalen en COPD.

Sinds de toepassing van antibiotica is de sterfte aan luchtweginfecties afgenomen, maar desondanks blijft een pneumonie in Nederland nog altijd een belangrijke oorzaak van sterfte. Pneumonie bezet de zevende plaats op de ranglijst van meest voorkomende oorzaken van overlijden in ons land. Dit gegeven in combinatie met de verwachte vergrijzing onderschrijft een grote behoefte aan strategieën ter preventie van een pneumonie. In dit proefschrift zijn twee bestaande geneesmiddelen onderzocht voor wat betreft hun mogelijkheden het risico op een pneumonie te verlagen: angiotensine-converting enzyme (ACE) remmers en statines. ACE-remmers zijn onderzocht omdat bekend is dat deze geneesmiddelen de hoest -en slikreflex verbeteren en hierdoor aspiratie van keelflora naar de longen zouden kunnen voorkomen. Verschillende Aziatische studies hebben al een beschermend effect laten zien. Statines zijn onderzocht omdat recentelijk duidelijk is geworden dat statines naast cholesterolverlaging ook een positief effect hebben op sepsis en bacteriemie. Sepsis en bacteriemie zijn veelvoorkomende complicaties van een pneumonie. Omdat de prognose van een pneumonie in verregaande mate wordt bepaald door de gekozen antimicrobiële therapie, is hiernaast antibioticagebruik geëvalueerd in relatie tot het overlijden aan een pneumonie.

## Antimicrobiële therapie van een pneumonie

In **hoofdstuk 2.1** is onderzocht of het niet reageren op initiële antimicrobiele therapie in de eerste lijn voorspellend kan zijn voor de verwekker van de pneumonie. Hiertoe is bij alle patiënten opgenomen vanwege een pneumonie in het St. Antonius Ziekenhuis Nieuwegein tussen oktober 2004 en augustus 2006 vastgesteld of zij in de dagen voor ziekenhuisopname al dan niet een antibioticum hebben gebruikt. De verwekker is vastgesteld door middel van verschillende microbiologische technieken (bloed en sputum culturen, sputum

PCR, antigeen testen en serologie). Vervolgens is met behulp van multivariate logistische regressie onderzocht of antibiotica gebruik voor opname onafhankelijk voorspellend is voor de gevonden verwekker. Het blijkt dat patiënten die eerst thuis zijn behandeld een driemaal zo grote kans hebben op een atypische verwekker (OR 3.11, 95% CI 1.16-8.33) en een driemaal zo kleine kans hebben op een pneumococcenpneumonie (OR 0.39, 95% CI 0.17-0.91). Deze resultaten geven aan dat informatie over antibiotica gebruik voor opname zeer relevant is binnen de diagnostiek en het plannen van bijpassende therapie.

Of initiële thuisbehandeling ook iets zegt over het verdere beloop van een pneumonie is onderzocht in **hoofdstuk 2.2.** De gegevens voor deze studie zijn verkregen uit het PHARMO Record Linkage System (RLS). De duur van ziekenhuisopname en overlijden in het ziekenhuis is vergeleken tussen patiënten met (n=296) en zonder (n=794) antimicrobiële behandeling voor ziekenhuisopname. De mediane opnameduur was 10 dagen en vergelijkbaar voor de 2 groepen. Voor patiënten met een longaandoening of hartfalen was de mediane opnameduur respectievelijk 12 en 14 dagen. De ziekenhuissterfte was 7,2% en niet verschillend tussen de groepen. Binnen patiënten met hartfalen was de mortaliteit 9.8% voor patiënten direct opgenomen en 23.3% voor patiënten met behandeling thuis voorafgaand aan de ziekenhuisopname. Dit suggereert dat, behalve voor patiënten met hartfalen, antimicrobiële therapie voor ziekenhuisopname geen invloed heeft op ziekenhuissterfte aan een pneumonie. Patiënten met een combinatie van hartfalen en een pneumonie zijn mogelijk gevoeliger voor een snelle achteruitgang van de klinische situatie. Het niet reageren op thuisbehandeling zou in deze gevallen de ziekenhuisopname kunnen uitstellen die wel nodig is voor een goede prognose.
Samengevat laten beide studies in hoofdstuk 2 zien dat ondanks de beschikbaarheid van diverse richtlijnen voor het identificeren van patiënten die veilig thuis behandeld kunnen worden er nog steeds veel patiënten alsnog worden opgenomen in het ziekenhuis. Deze bevinding illustreert de complexiteit van de behandeling van een pneumonie maar ook een behoefte aan verdere verbetering van eerder genoemde richtlijnen. Een verbeterpunt kan zijn het beter opsporen van patiënten met een pneumonie veroorzaakt door een atypische verwekker. Deze patiënten zouden dan eerder effectief kunnen worden behandeld in de eerste lijn. Eerste lijn; iets dat een ziekenhuisopname mogelijk kan voorkomen. Hiernaast dient de aanwezigheid van een atypische verwekker niet te worden onderschat in patiënten opgenomen in het ziekenhuis na eerst thuis te zijn behandeld met antibiotica.

**ACE-remmers en het risico op een pneumonie**

In dit proefschrift zijn twee studies opgenomen naar de mogelijke relatie tussen ACE-remmer gebruik en het risico op een pneumonie. Beide studies zijn uitgevoerd in grote databases echter met verschillende uitkomsten.

De eerste studie (**hoofdstuk 3.1**) is een patiëntcontrole onderzoek uitgevoerd met data afkomstig uit het PHARMO RLS. Patiënten waren personen met een ziekenhuisopname vanwege een pneumonie tussen 1995 en 2000. Voor iedere patiënt zijn tot vier controle personen gematcht op basis van leeftijd en geslacht. In totaal bestond de studie populatie uit 1.108 patiënten en 3.817 controles. Na correctie voor confounding was er geen associatie tussen ACE-remmer gebruik en het risico op een pneumonie (OR 1.12, 95% CI 0.88-1.43). De associatie was ook niet aanwezig in subgroepen van mensen met diabetes, longziekten, hartfalen of met zowel hartfalen als longziekten. Dit resultaat suggereert dat er geen beschermende relatie is tussen ACE-remmer gebruik en het risico op een pneumonie.

De tweede studie (**hoofdstuk 3.2**) is een patiëntcontrole onderzoek uitgevoerd in een populatie diabeten afkomstig uit de Britse General Practice Research Database (GPRD). Patiënten waren gedefinieert als personen met een pneumonie diagnose (n=4.719). Per patiënt zijn tot vier controles gekoppeld op leeftijd, geslacht en index datum. Na correctie voor confounding was ACE-remmer gebruik significant geassocieerd met een verlaagd pneumonie risico (OR 0.72, 95% CI 0.64-0.80). De associatie was consistent aanwezig in verschillende subgroepen, maar het meest uitgesproken in patiënten welke recent een beroerte hebben doorgemaakt. Hiernaast was er een significante dosis-effect relatie (p voor trend<0.001). Deze resultaten suggereren dat ACE-remmer gebruik wel is geassocieerd met een verlaagd pneumonie risico.

Welke conclusies kunnen worden getrokken uit deze verschillende bevindingen? Wanneer we de twee studies hierboven vergelijken met andere studies naar het beschermende effect van ACE-remmers op een pneumonie dan vallen enkele verschillen tussen de studies op (zie ook Tabel 1 in de general discussion). Als eerste kan de keuze van de studie-uitkomst van belang zijn. In de PHARMO studie is het gebruik van ACE-remmers vergeleken tussen patiënten opgenomen in het ziekenhuis vanwege een pneumonie en populatie controles. De GPRD studie

had een gelijkende benadering, maar selecteerde ook patiënten behandeld in de eerste lijn vanwege een pneumonie. In de GPRD studie bleek de associatie uitsluitend aanwezig voor patiënten die thuis waren behandeld. Een mogelijke verklaring hiervoor kan zijn dat ACE-remmer gebruik indirect predisponeert voor ziekenhuisopname. ACE-remmers worden vaak voorgeschreven voor hartfalen en hartfalen is een reden voor ziekenhuisbehandeling. In andere woorden, er kan een overschatting van ACE-remmer gebruik zijn opgetreden in de groep opgenomen patiënten en hierdoor een overschatting van het relatieve risico of in dit geval de afwezigheid van een associatie. Als tweede kunnen ook onderliggende ziekten een rol spelen. In de GRPD studie vonden we dat de associatie voornamelijk aanwezig is in patiënten met een recent doorgemaakte beroerte. Dit is interessant, omdat dit het hypothetische werkingsmechanisme dat ACE-remmers aspiratie kunnen voorkomen ondersteunt. Patiënten met een beroerte hebben een verhoogd risico op een aspiratie pneumonie als gevolg van een verminderde slikreflex.

Een ander belangrijk punt kan zijn de etniciteit van de studiepopulatie. Behalve de studie in hoofdstuk 3.2 is de associatie tussen ACE-remmer gebruik en pneumonie risico voorheen uitsluitend gevonden in Aziatische studies. Bovendien hebben Ohkubo en collegae laten zien dat de associatie voornamelijk aanwezig was binnen de Aziatische deelnemers van de PROGRESS studie en afwezig binnen de niet-Aziatische deelnemers. Omdat bekend is dat de verdeling van het ACE insertie/deletie polymorfisme aanzienlijk verschilt tussen Aziatische en niet-Aziatische populaties zou dit mogelijk kunnen verklaren waarom de associatie verschillend is tussen populaties. In **hoofdstuk 3.3** is bestudeerd of het ACE I/D polymorfisme is geassocieerd met het risico op en het beloop van een pneumonie. Hiervoor is de genotypenverdeling vergeleken tussen 200 patiënten met een pneumonie en 200 gezonde controle personen. De verdeling bleek bijna identiek ($p=0.978$). Verder was er geen associatie tussen het genotype en het klinische beloop van de pneumonie. Dit is interessant, omdat in een Japanse studie wel een relatie tussen het ACE DD genotype en het overlijden aan een pneumonie is gevonden. Ondanks voldoende statistische power hebben we dit niet kunnen reproduceren in een blanke populatie. Mogelijk is het ACE I/D polymorfisme geen functioneel polymorfisme, maar een marker voor een wel functioneel polymorfisme waarvoor het linkage dysequilibrium verschilt tussen Aziatische en niet-Aziatische populaties.

Omdat ACE-remmers ook in verband zijn gebracht met het beloop van een pneumonie is in **hoofdstuk 3.4** gekeken naar serum ACE activiteit tijdens een pneumonie. In een prospectieve studie is van alle patiënten opgenomen vanwege een pneumonie in het St. Antonius Ziekenhuis (n=134) de serum ACE activiteit bepaald tijdens ziekenhuisopname en na ontslag. Het bleek dat de serum ACE activiteit significant lager is tijdens een pneumonie en weer normaliseert bij herstel. Het pathosfysiologische mechanisme achter deze daling in ACE activiteit is nog onduidelijk maar een mogelijke verklaring kan zijn een verhoogde vraag naar angiotensine II waardoor de hoeveelheid ACE afneemt. Dit kon echter niet onderzocht worden in deze studie. Desalniettemin suggereert de geobserveerde daling in ACE activiteit wel dat het renine-angiotensine systeem betrokken is bij de pathosfysiologie van een pneumonie en dat ACE-remmers hier mogelijk een effect op kunnen hebben.

### Effecten van statines op het pneumonie risico

Een andere klasse geneesmiddelen die onderzocht is in dit proefschrift zijn de statines. Tegenwoordig wordt steeds meer duidelijk dat statines naast hun cholesterolverlagende werking ook een effect hebben op het immuunsysteem. Het onderzoek in dit proefschrift (**hoofdstuk 4.1**) kijkt of statines het risico op een pneumonie kunnen verlagen.

De studie is uitgevoerd in dezelfde populatie als de studie in hoofdstuk 3.2. Een patiëntcontrole studie is uitgevoerd met 4.719 patiënten en 15.322 gekoppelde controles. In totaal werden statines gebruikt door 1.1% van de patiënten en 2.1 % van de controles (ruwe OR 0.51, 95% CI 0.37-0.68). Na correctie voor confounding bleef de associatie statistisch significant aanwezig (OR 0.49, 95% CI 0.35-0.69). Verder was de associatie consistent aanwezig in verschillende subgroepen en onafhankelijk van het gebruik van andere geneesmiddelen. De conclusie van deze studie is dat statine gebruik is geassocieerd met een aanzienlijke reductie in het risico op een pneumonie.

Deze conclusie werd recentelijk ook getrokken in drie andere studies. Schlienger en collegae vonden een odds ratio van 0.47 (95% CI 0.25-0.88) en ook Hak en collegae vond een beschermende associatie tussen statines en optreden van een pneumonie. Nog recenter onderzochten Frost en collegae het effect van statines in een groep van patiënten met COPD. Zij vonden een dosisafhankelijk effect.

Samengevat zijn er nu vier studies die een sterke beschermende associatie laten zien tussen statine gebruik en het risico op een pneumonie. De Bradford-Hill criteria over causaliteit zeggen dat het onwaarschijnlijk is dat een sterke associatie wordt verklaard door onbekende co-factoren. Aan de andere kant kan de aanwezigheid van rest-confounding niet uitgesloten worden in geen van de bovengenoemde studies.

**Methodologische overwegingen**

In observationeel onderzoek zijn er tal van manieren waarop een studie de verkeerde kant op kan gaan. Het meest complex om te bannen is de mogelijke aanwezigheid van confounding. Confounding betekent verwarring van het effect van de ene determinant met de andere. In het geval van preventieve therapie kan dit optreden. In het bijzonder wanneer de behandeling wordt gegeven aan mensen die sowieso meer bezig zijn met een gezonde leefstijl en meer therapietrouw zijn. Zo'n effect kan ook van toepassing zijn op statine gebruik. Meerdere onderzoeken hebben laten zien dat statines minder worden voorgeschreven aan mensen met overgewicht en mensen die roken. Ook mensen met een korte levensverwachting krijgen minder vaak een statine voorgeschreven.

Majumdar en collegae hebben recent laten zien dat gezondheidstatus van de patiënten inderdaad invloed heeft op de associatie tussen statine gebruik en de prognose van een pneumonie. Wat de bijdrage van gezondheidsstatus op de associatie tussen statine gebruik en pneumonie risico blijft onduidelijk. De markers voor gezondheidstatus uit de studie van Majumdar waren ook meegenomen in onze studie en die van Schlienger en collegae, maar hadden geen invloed op de eindconclusie. Op dit moment is een gerandomiseerde studie waarschijnlijk de enige manier om vast te stellen wat de bijdrage van statines kan zijn bij het voorkomen van luchtweginfecties.

Een andere vorm van vertekening die kan ontstaan in observationeel onderzoek is misclassificatie van de uitkomst. Misclassificatie betekent dat personen onterecht worden bestempeld als patiënt of gezond. Omdat in dit proefschrift vier keer gebruik wordt gemaakt van ICD codes voor het identificeren van patiënten met een pneumonie is in hoofdstuk 5 onderzocht hoe betrouwbaar deze codes zijn. In **hoofdstuk 5.1** is de sensitiviteit vastgesteld van ICD code toewijzing in een groep patiënten van wie vastgesteld is dat deze een pneumonie hadden.

Het bleek dat 72% van de patiënten een juiste code toegewezen heeft gekregen. Er waren geen significante verschillen tussen patiënten met de juiste code en patiënten met de onjuiste code behalve voor wat betreft opnameduur. De sensitiviteit liet een negatieve correlatie met opnameduur zien. Dit betekent dat als patiënten worden geselecteerd met behulp van ICD codes er een ondervertegenwoordiging is van patiënten die lang in het ziekenhuis hebben gelegen. In **hoofdstuk 5.2** is de positief voorspellende waarde (PVW) van ICD codes voor een pneumonie onderzocht. In patiëntcontrole studies is de PVW een belangrijke maat omdat dit aangeeft dat de patiënten inderdaad de ziekte hadden. De gegevens voor dit onderzoek waren afkomstig uit het St. Antonius Ziekenhuis. De PVW van een ontslagdiagnose voor een pneumonie was 88% (95% CI 80-95). Er waren geen verschillen in patiëntenkarakteristieken tussen juist en onjuist gecodeerde ontslagdiagnoses.

Samengevat kan op basis van de studies in hoofdstuk 5 worden geconcludeerd dat ICD gecodeerde ziekenhuisdiagnoses bruikbaar zijn voor het identificeren van patiënten met een pneumonie maar dat de impact van een onderschat aantal "lange" liggers op de studie-uitkomst dient te worden bepaald.

### Tot besluit

Op basis van de bevindingen in dit proefschrift kan gesteld worden dat de behandeling van een pneumonie complex is en omgeven is met allerlei onderzekerheden rondom verwekker en keuze voor wel of geen ziekenhuisopname. Ondanks de beschikbaarheid van antibiotica blijft de pneumonie de zevende directe doodsoorzaak in Nederland. De aankomende vergrijzing onderstreept de behoefte aan mogelijkheden voor de preventie van een pneumonie.

Dit proefschrift suggereert twee nieuwe strategieën om het risico op een pneumonie te verlagen. Allereerst laat dit proefschrift een dosis-afhankelijke beschermende associatie zien tussen ACE-remmer gebruik en het risico op een pneumonie. Deze bevinding suggereert dat de ACE-remmer geïnduceerde hoest- en slikreflex bijdraagt aan een verlaging van het risico op een pneumonie. De bloeddrukverlagende eigenschappen van ACE-remmers staan op dit moment een uitgebreide toepassing van ACE-remmers echter in de weg. Het zou een uitdaging zijn om een geneesmiddel te ontwikkelen speciaal voor inductie van de hoestreflex.

Een andere mogelijkheid een pneumonie te voorkomen kan zijn door het gebruik van een statine. Statine gebruik blijkt geassocieerd met een 50% risico reductie. Het mogelijke werkingsmechanisme hierachter is immuunmodulatie door statines. Omdat het observationeel onderzoek betreft kan de mogelijkheid van selectief voorschrijven van statines aan relatief gezondere mensen niet worden uitgesloten. Een prospectief gerandomiseerd onderzoek is daarom waarschijnlijk de enige manier om deze bevinding te bevestigen. In de tussentijd zouden statines niet mogen worden onthouden aan iedere patiënt met een indicatie voor een statine, zelfs niet de meest oude en kwetsbare patiënten.

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# 10 | About the author

Ewoudt van de Garde was born on April 6, 1978 in Breda. He studied pharmacy at Utrecht University where he obtained his PharmD in 2002. During his study he did a research project at the department of Biotechnology Development and Production of Novartis Pharma AG, Basel, Switzerland (supervisors: Prof. dr. T. Arvinte and Prof. dr. D.J.A. Crommelin). In 2003 he started working at the department of Clinical Pharmacy of the St. Antonius Hospital Nieuwegein as a resident in hospital pharmacy.

In 2004 he started the research described in this thesis under supervision of Prof. dr. H.G.M. Leufkens and Prof. dr. J.M.M. van den Bosch. His PhD research was combined with training in hospital pharmacy (ZAPIKO) and a MSc in Epidemiology.

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