The value of signs and symptoms in differentiating between bacterial, viral and mixed aetiology in patients with community-acquired pneumonia

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Current diagnostics for community-acquired pneumonia (CAP) include testing for a wide range of pathogens, which is costly and not always informative. We compared clinical and laboratory parameters of patients with CAP caused by different groups of pathogens to evaluate the potential for targeted diagnostics and directed treatment. In a prospective study, conducted between April 2008 and April 2009, adult patients with CAP were tested for the presence of a broad range of possible respiratory pathogens using bacterial cultures, PCR, urinary antigen testing and serology. Of 408 patients with CAP, pathogens were detected in 263 patients (64.5%). Streptococcus pneumoniae and influenza A virus were the most frequently identified bacterial and viral pathogens, respectively. Age had a significant effect on the prediction of aetiology (P = 0.054), with an increase in the relative contribution of viruses with advancing age. Multivariate analyses further showed that the presence of cough increased the likelihood of detecting a viral pathogen [odds ratio (OR) 5.536, 95% confidence interval (CI) 2.130–14.390], the presence of immunodeficiency decreased the likelihood of detecting a bacterial pathogen (OR 0.595, 95% CI 0.246–1.437) and an increase in pneumonia severity index score increased the likelihood of detecting a pathogen in general. Although several variables were independently associated with the detection of a pathogen group, substantial overlap meant there were no reliable clinical predictors to distinguish aetiologies. Therefore, testing for common respiratory pathogens is still necessary to optimize treatment.

INTRODUCTION

Community-acquired pneumonia (CAP) is defined as pneumonia that is not acquired in a hospital or long-term care facility (Mandell et al., 2007; Wiersinga et al., 2012). Although the majority of patients with CAP have self-limiting disease and are treated as outpatients, CAP is still the most important cause of death from infectious causes in high-income countries (Mandell et al., 2007). Recent studies have demonstrated an increasing trend towards hospitalization, especially in the elderly population (Fry et al., 2005; van Gageldonk-Lafeber et al., 2009; Woodhead et al., 2011). In Europe, the cost of caring for patients with pneumonia is estimated to be around €10.1 billion annually (Welte et al., 2012). Streptococcus pneumoniae is the most common bacterial pathogen and influenza the leading viral pathogen, but up to 35% of patients with CAP have a polymicrobial aetiology (Johansson et al., 2010). In the past two decades, modern molecular biological techniques have been developed and more pathogens associated with CAP have been discovered, especially viruses (Ruuskanen et al., 2011). As a consequence, the diagnostic work-up has been expanded to include multiple pathogens.

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; hPIV, human parainfluenza virus; ICU, intensive care unit; InfA, influenza A virus; OR, odds ratio; PSI, pneumonia severity index; RT-qPCR, reverse transcriptase quantitative PCR.
Awareness of the likely causative pathogen is important to allow commencement of appropriate antimicrobial treatment. Use of clinical symptoms and signs to differentiate between bacterial and viral CAP could aid in choosing a targeted antimicrobial treatment and, therefore, decrease antibiotic consumption and reduce antimicrobial resistance (Albrich et al., 2004; Avdic et al., 2012). Choosing the right initial therapy is particularly important for patients with a mixed aetiology, who often develop severe CAP and have longer hospitalization and poorer outcomes (Jennings et al., 2008; Johansson et al., 2011; Woodhead et al., 2011). Moreover, by performing diagnostics, epidemiologically important organisms such as *Legionella pneumophila*, or an increased incidence of influenza A virus (InfA) or *Coxiella burnetii*, may be detected.

Most studies that have focused on the clinical characteristics of CAP in adults have not used extensive molecular diagnostics but a relatively limited range of viral and bacterial tools such as bacterial and viral culture or antigen assays, thereby compromising the ability to detect certain viruses and bacteria (Jokinen et al., 2001). We therefore performed a prospective study of CAP patients using extensive diagnostic microbiological methods and detailed clinical data collection. The purpose of this study was to try to differentiate pure bacterial, pure viral and mixed viral and bacterial aetiologies based on clinical signs and symptoms at admission and clinical severity score in patients with CAP.

**METHODS**

During April 2008 through April 2009, all patients aged 18 years and older attending the emergency wards of two teaching hospitals in Tilburg, the Netherlands, with a suspected diagnosis of CAP were analysed. CAP was defined as the presence of a new or progressive infiltrate on a chest radiograph with clinical symptoms suggestive of a lower respiratory tract infection [i.e. fever (temperature $\geq 38.0$ °C) or hypothermia (temperature $<35$ °C), new cough with or without sputum production, abnormal percussion and altered breath sounds on auscultation, dyspnoea, tachypnoea or hypoxia, and leukocytosis or leukopenia]. Exclusion criteria included: (1) recent hospitalization (<2 weeks) or residence in a long-term care facility; (2) known bronchial obstruction or a history of post-obstructive pneumonia [with the exception of chronic obstructive pulmonary disease (COPD)]; (3) primary lung cancer or another malignancy metastatic to the lungs; (4) AIDS, or known or suspected *Pneumocystis jirovecii* pneumonia; and (5) known or suspected active tuberculosis.

**Case report.** A case report form was obtained from all patients, containing information on age, sex, current smoking, comorbidities, clinical symptoms, antimicrobial treatment prior to and at admission and blood analysis. Symptoms were categorized as fever, altered mental status, cough, sputum production, headache, chest pain, dyspnoea, gastrointestinal symptoms, chills and general discomfort. Recorded comorbidities were COPD, cardiac failure, cerebrovascular disease, diabetes mellitus, renal insufficiency or non-respiratory malignancy. Immunodeficiency was defined as clinically suspected or proven immunodeficiency, the use of immunosuppressive therapy or immunomodulating medication in the past 3 months, or the use of more than 10 mg prednisone or equivalent each day for the past 3 months.

**Ethics.** The study was approved by the local medical ethics committee. Written informed consent was obtained from all participants.

**Laboratory diagnostics.** A throat swab, sputum, urine and serum samples and two sets of blood samples were obtained in the emergency ward. The blood and sputum samples were cultured according to standard microbiological procedures. Urine samples were used to detect *S. pneumoniae* and *L. pneumophila* antigens with the BinaxNOW pneumococcal urinary antigen test and the BinaxNOW Legionella urinary antigen test (both from Binax).

All respiratory samples (i.e. throat swabs and sputum samples) were tested using real-time reverse transcriptase quantitative PCR (RT-qPCR) for the presence of respiratory viruses and bacterial pathogens, including human adenovirus, human parainfluenza virus (hPIV1, hPIV2, hPIV3, hPIV4), influenza A virus (van de Pol et al., 2007), human bocavirus (Allander et al., 2007), KI and WU polyomaviruses (Bialasiewicz et al., 2007), human metapneumovirus (van den Hoogen et al., 2001), human coronavirus (OC43, NL63, HKU1 and 229E) (van Elden et al., 2004), human rhinovirus (Andeweg et al., 1999), Chlamydomphila pneumonae (Maraha et al., 2001), *L. pneumophila* (Diederken et al., 2008), *Mycoplasma pneumonae* (Dorigo-Zetsma et al., 1999), *Chlamydomphila psittacci* (Heddem et al., 2006) and *Coxiella burnetii* (Tilburg et al., 2010). Sputum samples were also tested for the presence of *S. pneumoniae* (Greiner et al., 2001). Pair ed serum samples during the acute and convalescent phases of infection (separated by at least 2 weeks) were obtained from some patients for serological studies. The serum samples were analysed in parallel. In-house complement fixation tests were performed to detect antibodies to InfA, influenza B virus, respiratory syncytial virus, hPIV 1–4, human adenovirus, *M. pneumonae*, *L. pneumophila*, *Chlamydomphila psittacci* and *Coxiella burnetii* was detected.

**Classification of aetiology.** Cases were considered to be caused by a specific pathogen if one or more of the following criteria were met: (1) a micro-organism was cultured from blood samples and/or the urinary antigen test was positive for *S. pneumoniae* or *L. pneumophila*; (2) PCR of the throat swab or sputum sample yielded a positive result; (3) bacterial culture was obtained from sputum samples (presence of >25 polymorph nuclear leukocytes and <10 squamous cells per field) with a predominant organism and compatible results from Gram stain; (4) IgM antibodies for *M. pneumonae* (Serodia-Mycocard-Myco II kit; Fujirebio) were detected; and (5) a fourfold increase in IgG antibody titres for *M. pneumonae*, *L. pneumophila*, *Chlamydomphila psittacci* and *Coxiella burnetii* was detected.

**Assessment of illness severity.** The pneumonia severity index (PSI) (Fine et al., 1997) is a continuous score based on 20 established predictors that were prospectively registered for all patients. Missing data were recorded as normal findings, in accordance with the PSI protocol. PSI is often categorized into five classes (normal, $<70$; moderate, 71–90; severe, 91–130; and critical, $>130$ points). The mortality rate is expected to be less than 1% in classes I–III ($\leq 90$ points), almost 10% in class IV (91–130 points) and almost 30% in class V ($>130$ points). In the statistical analysis dealt with here, we used the continuous PSI score in order to take account of all information contained in this score.

**Statistical analysis.** Categorical nominal variables were compared among aetiology groups using a $\chi^2$ test or Fisher’s exact test. Categorical ordinal variables or numerical variables with a skewed distribution were compared among groups using a Mann–Whitney U test (two groups) or a Kruskal–Wallis test (more than two groups). Numerical variables with a Gaussian-shaped distribution were compared among groups using a t-test (two groups) or F-test (ANOVA for more than two groups).

Multinominal logistic regression analysis was used to examine whether certain characteristics on admission were simultaneously associated
with a pure bacterial, pure viral or combined viral and bacterial aetiology. We were particularly interested in understanding whether any characteristics could be used as clinical markers to distinguish the four aetiology groups, including the group with no viral or bacterial aetiology. Given a number of explanatory variables, the model can predict the probabilities of a subject’s membership of each of the four aetiology groups under the constraint that these four probabilities add up to unity as they should. The following explanatory variables, based on previous research, were initially and simultaneously included in a full model (File, 2003; Gutierrez et al., 2006; Wiersinga et al., 2012): age (in years), sex (male/female), frailty score, number of previous hospitalizations, COPD (y/n), heart failure (y/n), cerebrovascular disease (y/n), diabetes mellitus (y/n), renal failure (y/n), malignancies (y/n), immunodeficiency (y/n), recurrent pneumonia (y/n), an influenza-like illness in the 2 weeks before admission (y/n), prior use of antibiotics (y/n), duration of symptoms, general discomfort (y/n), altered mental status (y/n), cough (y/n), fever (y/n), dyspnoea (y/n), sputum production (y/n), gastrointestinal symptoms (y/n), headache (y/n), chest pain (y/n) and chills (y/n). Next, a backwards stepwise procedure was used to obtain a more parsimonious model that fitted almost equally well to the data as the initial full model. Deleting and re-entering variables from and into the model was based on a threshold of 0.30 for the P value.

As the definition of PSI is partly based on the above-mentioned explanatory variables, the relation between PSI and group membership was separately analysed in a univariable multinomial logistic regression model. Effects are presented as odds ratios (OR) with 95 % confidence intervals (CIs), as well as graphically by means of staggered lines of the four predicted group probabilities related to the covariables. The odds are defined as the probability that a patient belongs to a specific aetiological category (B for purely bacterial, V for purely viral and B + V for mixed), divided by the probability of membership to the no-aetiology group. Independence between the presence of bacteria and viruses is defined as the probability of the combination B + V being equal to the product of the marginal bacterial (B plus B + V) and viral (V plus B + V) probabilities. Independence without adjustment for covariables was simply tested using the \( \chi^2 \) test in a 2 × 2 table of both of these marginal outcomes. Using the predicted group membership probabilities from the multinomial logistic regression model, independence is defined as the ORs for the combination B + V being equal to the product of the separate ORs for B and V, which was tested by specifying the relevant linear restrictions on the model coefficients and using a Wald \( \chi^2 \) test. P values below 0.05 were considered to denote statistical significance. Analyses were conducted using PASW Statistics 18 (IBM).

RESULTS

A total of 408 patients were included. The median age of the patients was 68 years (range 20–94 years) and 250 patients (61.3 %) were male. CAP aetiology (as defined in Methods) was identified in 263 (64.5 %) of the 408 patients, with bacterial pathogens in 146 patients (36 %), respiratory viruses in 52 patients (13 %) and mixed bacterial and viral infections in 65 patients (16 %). For all patient groups combined, the most commonly identified pathogens were S. pneumoniae, Coxiella burnetii, human rhinovirus and InfA (Table 1).

Patients with a positive diagnostic test were less likely to have received prior antibiotic treatment (59/263 of patients [22.4 %]) than patients without a positive test result (50/145 of patients [34.5 %]; P = 0.010; \( \chi^2 \) test). The median length of hospital stay for patients with CAP was 10.9 days. The median length of hospital stay was 9.8 days for patients with no pathogen, 9.9 days for those with a viral pathogen, 11.5 days for those with a bacterial pathogen and 13 days for those with both viral and bacterial pathogens (P = 0.012; Kruskal–Wallis test).

Use of clinical signs and symptoms for triaging

We then grouped patients by aetiological diagnosis (bacterial, viral, mixed, or no pathogens detected) (Table 2) and performed multivariable analysis as described in Methods.

Most clinical signs and symptoms could not significantly discriminate patients with either a bacterial, viral, mixed, or no aetiology. Only age, cough and immunodeficiency were significantly associated with specific aetiologi cal categories (Table 3).

Coughing was significantly related to aetiology (P = 0.002), independently of the other signs and symptoms. If coughing was present on presentation, relatively more viral aetiologies were found (OR 5.536, 95 % CI 2.130–14.390).

With increasing age the probability of finding any pathogen became smaller, but relatively more viral aetiologies were found as the effect of age was less negative on viral aetiology (OR nearer to 1) than on the non-viral aetiologies.

Fig. 1 shows the effect of age ranging from 20 to 90 years, when setting the other explanatory variables at their means.

At presentation at the emergency room, median C-reactive protein (CRP) levels differed significantly among the aetiology groups (P < 0.0005; Table 4). In particular, patients with only a bacterial pathogen exhibited a higher median CRP than other patients.

PSI

On admission, 153 patients (37.5 %) were placed in the low-risk PSI group (I–III). However, the majority of the patients were placed in class IV (170 patients, 41.7 %) or class V (85 patients, 20.8 %). Patients without a pathogen had significantly lower mean PSI scores than patients with a pathogen (97 versus 105, P = 0.015 from t-test). Mortality rates were associated with higher PSI scores (130 versus 100 points; P < 0.0005 from t-test).

According to the univariable multinomial logistic regression model, PSI as continuous variable was significantly related to aetiology (P = 0.012) (Table 3). Each point increase in the continuous PSI score led to a higher likelihood of finding a viral (OR 1.011, 95 % CI 1.002–1.020) or mixed (OR 1.012, 95 % CI 1.004–1.021) aetiology. Fig. 2 shows how these multiplicative effects on the predicted aetiology probabilities worked out when letting PSI continuously increase from 0 to 250 points in the estimated univariable multinomial logistic regression model: with increasing PSI score, the purely viral and mixed aetiology components increased, while the other components decreased.
Impact of comorbidity

Immunodeficiency was significantly related to aetiology ($P=0.028$), independently of other signs and symptoms. Relatively more mixed aetiologies were found in patients with immunodeficiency (OR 2.323, 95% CI 1.006–5.363), while patients without immunodeficiency showed a larger bacterial aetiology component (Table 3).

Associations of various aetiologies with intensive care unit (ICU) admission and mortality

Of the 408 patients, 45 (11.0%) were treated in the ICU. An aetiological agent was found in 36 (80.0%) of these patients. Twenty-two patients had a bacterial pathogen, four patients had a viral pathogen and 10 patients had both viral and bacterial pathogens.

Twenty-six patients (6.4%) died during ICU admission. There was no mortality difference between patients with a bacterial and/or viral pathogen.

Interdependence of viral and bacteriological aetiology

There was no interdependence of viral and bacteriological aetiology, as the presence of a bacterial infection did not change with the absence or presence of a viral infection. This was tested as follows.
Table 2. Characteristics of patients with CAP

P values refer to the null hypothesis that the distribution of the variables does not differ among the four aetiology groups and is equal to that in all patients. For age, a Kruskal–Wallis test was used; for the other variables, a $\chi^2$ or Fisher’s exact test was used.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n=408)</th>
<th>No aetiology (n=145, 35%)</th>
<th>Bacterial aetiology (n=146, 36%)</th>
<th>Viral aetiology (n=52, 13%)</th>
<th>Bacterial and viral aetiology (n=65, 16%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>68</td>
<td>67</td>
<td>65</td>
<td>73</td>
<td>70</td>
<td>0.004</td>
</tr>
<tr>
<td>Range</td>
<td>20–94</td>
<td>20–94</td>
<td>20–92</td>
<td>21–91</td>
<td>24–89</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>250 (61.3)</td>
<td>88 (60.7)</td>
<td>93 (63.7)</td>
<td>34 (65.4)</td>
<td>35 (53.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>169 (42.4)</td>
<td>54 (38.8)</td>
<td>70 (48.3)</td>
<td>20 (38.5)</td>
<td>25 (39.7)</td>
<td>0.34</td>
</tr>
<tr>
<td>Comorbidity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>131 (32.1)</td>
<td>47 (32.4)</td>
<td>38 (26.0)</td>
<td>17 (32.7)</td>
<td>29 (44.6)</td>
<td>0.067</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>88 (21.6)</td>
<td>27 (18.6)</td>
<td>35 (24.0)</td>
<td>15 (28.8)</td>
<td>11 (16.9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>35 (8.6)</td>
<td>13 (9.0)</td>
<td>9 (6.2)</td>
<td>6 (11.5)</td>
<td>7 (10.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>78 (19.1)</td>
<td>27 (18.6)</td>
<td>21 (14.4)</td>
<td>16 (30.8)</td>
<td>14 (21.5)</td>
<td>0.074</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>12 (2.9)</td>
<td>3 (2.1)</td>
<td>5 (3.4)</td>
<td>2 (3.8)</td>
<td>2 (3.1)</td>
<td>0.92</td>
</tr>
<tr>
<td>Malignancy</td>
<td>46 (11.3)</td>
<td>13 (9.0)</td>
<td>20 (13.7)</td>
<td>5 (9.6)</td>
<td>8 (12.3)</td>
<td>0.61</td>
</tr>
<tr>
<td>Immunodeficiency</td>
<td>57 (14.0)</td>
<td>20 (13.8)</td>
<td>10 (6.8)</td>
<td>8 (15.4)</td>
<td>19 (29.2)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Antibiotics prior to hospital admission, n (%)</td>
<td>109 (26.8)</td>
<td>50 (34.7)</td>
<td>33 (22.6)</td>
<td>13 (25.5)</td>
<td>13 (20.0)</td>
<td>0.058</td>
</tr>
<tr>
<td>Admission to ICU, n (%)</td>
<td>45 (11.0)</td>
<td>9 (6.2)</td>
<td>22 (15.1)</td>
<td>4 (7.7)</td>
<td>10 (15.4)</td>
<td>0.052</td>
</tr>
<tr>
<td>Mortality rate, n (%)</td>
<td>39 (9.5)</td>
<td>14 (9.7)</td>
<td>11 (7.5)</td>
<td>4 (7.7)</td>
<td>10 (15.4)</td>
<td>0.32</td>
</tr>
</tbody>
</table>
The marginal probabilities were 51.7% \([146+65]/408\) for a bacterial aetiology and 28.7% \([52+65]/408\) for a viral aetiology. Under independence of presences of bacterial and viral infections, the expected probability for the combination by definition equals the product of these marginal probabilities: 14.8%. The observed percentage of a bacterial aetiology and 28.7% for a viral aetiology. Testing this null hypothesis in the multivariable model led to the same conclusion \(\chi^2=0.764\) with 2 degrees of freedom; \(P=0.68\).

**DISCUSSION**

In this prospective study, only the characteristics of age, coughing, PSI score and immunodeficiency could be used to predict a possible aetiology of CAP (bacterial, viral, mixed or no pathogen found). Furthermore CRP levels differed significantly in these patients, with higher CRP levels associated with the detection of a bacterial pathogen. Other studies have also shown that CRP and white blood cell counts are significantly raised in patients with bacterial pneumonia compared with those with viral pneumonia (Hohenthal et al., 2009; Jennings et al., 2008).

The ability to differentiate viral from bacterial pneumonia based on clinical signs and symptoms could have important implications for patient management. However, in this study and in others, substantial overlap and variability were seen in the clinical signs and symptoms of patients with viral and/or bacterial pneumonia (Angeles Marcos et al., 2006).
One of the most adopted hypotheses is that a viral infection is followed by secondary bacterial infection. Epidemiological and clinical studies, mostly otitis media studies, have shown that bacterial adherence and colonization of the nasopharynx is facilitated by viral infection through its effect on the respiratory epithelium; further invasion of the lung epithelium is also facilitated by prior viral infection (Peltola & McCullers, 2004). This has been best documented for InfA and S. pneumoniae (McCullers, 2006). However, another study has shown that the opposite is also possible. Ami et al. (2008) demonstrated that development of severe acute respiratory syndrome following infection with human coronavirus was facilitated by a low-virulence bacterial infection. In the current study, there appeared to be no significant evidence for interdependence between viral and bacterial infections. In our study, 16% of patients with CAP had a mixed infection, similar to in other studies (Diederen et al., 2009; Jennings et al., 2008). Some studies, including ours, have suggested that mixed infections can induce a more severe inflammatory and clinical disease (Cillóniz et al., 2012; Diederen et al., 2009; Jennings et al., 2008; Johansson et al., 2011; Templeton et al., 2005), but this has not been consistently observed (Angeles Marcos et al., 2006; Johnstone et al., 2008).

An interesting observation from this study is the impact of age on the various aetiologies, as the probability of finding a pathogen becomes smaller with advanced age, caused by a substantial decrease in the probability of finding a bacterial pathogen. In contrast to our findings, other studies have reported an increasing trend towards finding a pathogen in patients at older ages, with, similar to our study, viral infections occurring more frequently in older patients (Capelastegui et al., 2012; Jokinen et al., 2001).

Similar to our study, Ewig et al. (2002) reported that age was significantly associated with an undetermined aetiology. A hypothesis is that CAP in elderly patients may be caused by pathogens that are difficult to demonstrate, such as anaerobic pathogens because of silent aspiration, or because of the fact that elderly patients more often have incomplete sample collections (Ewig et al., 2002). Acute infections in the lower respiratory tract from residual DNA/RNA from a prior infection or asymptomatic carriage.
Table 4. Signs and symptoms of patients with CAP

P values refer to the null hypothesis that the percentage of symptoms or the distribution of clinical characteristics is the same in all four groups and identical to those in all patients. Symptoms were tested using a $\chi^2$ or Fisher’s exact test. Clinical characteristics were tested using a Kruskal–Wallis test (when median and range are presented) or F-test (when mean and SD are presented).

<table>
<thead>
<tr>
<th>Symptoms, n (%)</th>
<th>All patients (n=408)</th>
<th>No aetiology (n=145)</th>
<th>Bacterial aetiology (n=146)</th>
<th>Viral aetiology (n=52)</th>
<th>Bacterial and viral aetiology (n=65)</th>
<th>$P$ value $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>286 (70.1)</td>
<td>96 (66.2)</td>
<td>107 (73.3)</td>
<td>40 (76.9)</td>
<td>43 (66.2)</td>
<td>0.33</td>
</tr>
<tr>
<td>Altered mental status</td>
<td>64 (16.0)</td>
<td>16 (11.2)</td>
<td>26 (18.2)</td>
<td>10 (20.0)</td>
<td>12 (18.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>Cough</td>
<td>259 (63.5)</td>
<td>83 (57.2)</td>
<td>93 (63.7)</td>
<td>42 (80.8)</td>
<td>41 (63.1)</td>
<td>0.027</td>
</tr>
<tr>
<td>Sputum</td>
<td>66 (16.2)</td>
<td>20 (13.8)</td>
<td>22 (15.1)</td>
<td>8 (15.4)</td>
<td>16 (24.6)</td>
<td>0.24</td>
</tr>
<tr>
<td>Headache</td>
<td>30 (7.4)</td>
<td>7 (4.8)</td>
<td>17 (11.6)</td>
<td>2 (3.8)</td>
<td>4 (6.2)</td>
<td>0.095</td>
</tr>
<tr>
<td>Chest pain</td>
<td>81 (19.9)</td>
<td>29 (20.0)</td>
<td>37 (25.3)</td>
<td>6 (11.5)</td>
<td>9 (13.8)</td>
<td>0.090</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>263 (64.5)</td>
<td>90 (62.1)</td>
<td>86 (58.9)</td>
<td>38 (73.1)</td>
<td>49 (75.4)</td>
<td>0.060</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>38 (9.3)</td>
<td>12 (8.3)</td>
<td>17 (11.6)</td>
<td>3 (5.8)</td>
<td>6 (9.2)</td>
<td>0.59</td>
</tr>
<tr>
<td>Chills</td>
<td>66 (16.2)</td>
<td>22 (15.2)</td>
<td>25 (17.1)</td>
<td>8 (15.4)</td>
<td>11 (6.9)</td>
<td>0.96</td>
</tr>
<tr>
<td>General discomfort</td>
<td>89 (21.8)</td>
<td>30 (20.7)</td>
<td>34 (23.3)</td>
<td>11 (21.2)</td>
<td>14 (21.5)</td>
<td>0.95</td>
</tr>
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**Clinical characteristics**

| Duration of symptoms (days), median (range) | 3.0 (0–60) | 4.0 (0–40) | 3.0 (0–60) | 3.5 (0–30) | 3.0 (0–60) | 0.81 |
| Temperature (°C), mean ± SD | 38.2 ± 1.19 | 38.1 ± 1.24 | 38.4 ± 1.19 | 38.1 ± 1.20 | 38.4 ± 1.05 | 0.085 |
| Respiratory rate (min$^{-1}$), median (range) | 23 (10–40) | 23 (10–40) | 23 (12–40) | 23 (14–40) | 23 (12–39) | 0.94 |
| Heart rate (min$^{-1}$), mean ± SD | 101 ± 21.0 | 99 ± 20.5 | 103 ± 21.1 | 97 ± 21.4 | 103 ± 21.4 | 0.21 |
| Sodium (mmol L$^{-1}$), mean ± SD | 138 ± 6.27 | 138 ± 6.95 | 137 ± 4.83 | 137 ± 5.83 | 139 ± 7.60 | 0.10 |
| CRP mg dl$^{-1}$, median (range) | 128 (0–690) | 116 (0–528) | 219 (0–658) | 77.5 (0–341) | 103 (12–690) | <0.0005 |
| White blood cell count ($\times 10^3$ ml$^{-1}$), median (range) | 13.2 (0.2–52.4) | 12.7 (4.1–31.1) | 13.9 (0.2–36.2) | 12.7 (2.4–23.2) | 14.3 (0.6–52.4) | 0.062 |
cough is frequently treated with antibiotics, which are often ineffective (Butler et al., 2010). In this study, the presence of coughing was associated with the detection of relatively more viral aetiologies. By taking this symptom into consideration, inappropriate antibiotic prescribing could be reduced.

Despite improved diagnostic methods, a causative agent remains undetected in a large proportion of patients with CAP. The use of antibiotics prior to admission is an important factor that decreases the diagnostic yield (Cillo´niz et al., 2012; Ewig et al., 2002; Mortensen et al., 2008; van de Garde et al., 2008). In our study, 109 (26.8%) patients with CAP received antibiotics prior to sampling and 45.8% of those remained without aetiology. Compared with other studies, fewer patients in the current study were categorized as PSI risk group I or II. This may be because CAP patients showing only mild symptoms in the Netherlands are often seen by their general practitioner. Whereas CAP affects about 5–10 persons per 1000 inhabitants, only 5–20% of these cases are admitted to hospitals (van Gageldonk-Lafeber et al., 2009; Wiersinga et al., 2012).

A limitation of our study is that a pathogen could not be found in all patients. Despite our best efforts, not all materials (blood cultures, sputum cultures and convalescent serum samples) were available for each patient, thereby potentially leading to an underestimation of the number of bacterial and/or viral pathogens that were present. However, extensive molecular diagnostic assays were used to increase the yield, in particular to detect viruses. All patients were categorized based on viral, bacterial, mixed or no aetiology. The heterogeneity of clinical signs and symptoms among patients with various bacterial and viral CAP pathogens may have restricted the ability to differentiate these predictors. However, the majority of patients with CAP in daily clinical practice undergo limited diagnostic tests to demonstrate an aetiological agent, other than urine antigen tests, blood cultures and, only if available, a bacterial sputum culture, with the main focus on bacterial pathogens. We demonstrated that despite an overlap in the clinical presentation of patients with CAP, other aetiologies (i.e. mixed) are of importance, as they can induce more severe clinical disease.

In this study, Chlamydophila psittaci was detected in seven patients by serology and qPCR on respiratory samples. Diagnosis of psittacosis is often based on clinical signs and serological tests. The main disadvantages of serology are cross-reactivity with other Chlamydophila species and the need for a convalescent serum pair. By using a qPCR method on all respiratory samples Chlamydophila psittaci can be detected more often, as reported by others (Branley et al., 2008; Stewardson & Grayson, 2010). In contrast, Chlamydophila pneumoniae was not found despite the use of qPCR, possibly because infection usually occurs in children and adults older than 70 years, and is generally
mild (Marrie et al., 2012). This low prevalence is supported by Templeton et al. (2005), who investigated the epidemiology of respiratory pathogens in acute respiratory infection in adults in the Netherlands. In addition, in contrast with the published literature, Coxiella burnetii was detected more often; this was due to a large Q fever outbreak in the Netherlands between 2007 and 2010 that affected more than 4000 people (Delsing et al., 2010). Q fever has generally been associated with transient outbreaks in animals and humans. Of those who become infected with Coxiella burnetii, an estimated 60% remain asymptomatic while the other 40% present with clinical symptoms ranging from a mild febrile illness to pneumonia or hepatitis (Porten et al., 2006).

Few M. pneumoniae infections were found in our study, even though M. pneumoniae is among the most common respiratory pathogens in the literature (Arnold et al., 2007; Mandell, 2004). This might be because M. pneumoniae infections occur in cyclic epidemics every 3–5 years, and are generally mild and more common in children. Many adult cases may be asymptomatic and not in need of medical attention (Waites & Talkington, 2004). hPIV1 was more often detected in this study than previously has been described in adults (Fry et al., 2006; Liu et al., 2013). This was the result of a seasonal peak in the Netherlands in 2009. Biennial autumn epidemics of hPIV1 have been reported in previous studies (Fry et al., 2006). hPIVs are common respiratory pathogens and can cause respiratory tract infections, including CAP (Liu et al., 2013).

Our findings indicate that about a quarter of adults with CAP have evidence of infection with respiratory viruses, and in half of these only a virus is detected as the possible causative agent. In the literature, the proportion of adult patients with a viral infection has been reported to be as high as 56%, depending on the method used (molecular diagnostics or viral culture) (Templeton et al., 2005). This stresses the importance of performing viral diagnostics, especially as we found that the detection of both viral and bacterial pathogens in patients with CAP was associated with increased disease severity. Furthermore, performing proper microbiological diagnostics may prevent the unnecessary use of antibiotics and thereby reduce adverse events in those receiving therapy and decrease selective antibiotic pressure on micro-organisms that might otherwise result in the development of antimicrobial resistance among respiratory pathogens.

In summary, this study could identify no reliable clinical predictors to guide microbiological evaluation, although several variables such as cough, age, PSI and CRP were associated with a bacterial and/or viral aetiology. Moreover, it was shown that mixed infections were associated with increased disease severity and a longer hospital stay. Our findings suggest that routine testing for common respiratory pathogens is warranted for all adults with CAP.

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