Community-acquired pneumonia: a clinical approach to hospital admission, diagnosis and treatment
van der Eerden, M.M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 5

The value of intensive diagnostic microbiological investigation in low and high risk patients with community-acquired pneumonia

Eur J Clin Microbiol Infect Dis; accepted for publication


1) Department of Pulmonary Diseases and 2) Laboratory for Medical Microbiology, Medical Centre Alkmaar, The Netherlands. 3) Department of Pulmonary Diseases, Academic Medical Centre, the Netherlands
Abstract

In a prospective study, material for microbiological investigation was obtained from 262 patients with community-acquired pneumonia. These consisted of sputum samples for Gram’s staining, culture and pneumococcal antigen detection; blood cultures; urine for *Legionella pneumophila* serogroup 1 antigen test and pneumococcal antigen test; specimens from fiberoptic bronchoscopy and blood for serological determination. In 158 patients (60%) a pathogen was identified, with *Streptococcus pneumoniae* (n=97) as the most common causative agent of community-acquired pneumonia. In 82% of the patients a positive Gram’s stain on an adequate sputum specimen (n=44) was confirmed by positive sputum culture. *Streptococcus pneumoniae* infections were detected principally when sputum examination was performed on adequate specimens by Gram’s stain and culture and on adequate and non-adequate sputum specimens by pneumococcal antigen determination (n=58; 60%). The urinary pneumococcal antigen test was the most valuable single test for detection of *Streptococcus pneumoniae* infections (n=52; 54%), when sputum pneumococcal antigen determination was not performed. Fiberoptic bronchoscopy was of additive diagnostic value in 49% of the patients who did not expectorate sputum and in 52% of those who suffered treatment failure. Sputum investigation by Gram’s stain, culture, and pneumococcal antigen detection was the most useful method for establishing an aetiological diagnosis of community-acquired pneumonia, followed by urinary pneumococcal antigen test. Fiberoptic bronchoscopy may be of additional value when treatment failure occurs.
Introduction

The value of performing microbiological investigation in hospitalised patients with non-severe community-acquired pneumonia (CAP) is discussed in different guidelines. The main argument against the performance of microbiological research is the lack of sensitivity and specificity of the routine diagnostic methods currently employed. Furthermore, it is known that in 25-50 percent of patients with CAP the aetiologic agent cannot be identified. In an era in which the antibiotic resistance of certain pathogens is an increasing problem, careful use of antibiotics is recommended. In our opinion microbiological investigations can be used as an aid in choosing optimal therapy. Antibiotic therapy could be adapted and narrowed according to microbiological culture results.

Another advantage of performing microbiological investigation is that resistant strains of epidemiologically important organisms (e.g., *Legionella pneumophila*, drug-resistant *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus*) may be detected. Moreover, the identification of the causative agent may be important when failure on antibiotic treatment occurs.

Next to conventional methods such as sputum and blood cultures for identification of the causative agent, microbial yield could be increased by using the rapid immunochromatographic urinary pneumococcal antigen (PCA) test. The urinary PCA test yielded a sensitivity of 77-92% and a specificity of 97-100% in patients with bacteremic pneumococcal pneumonia. These results are encouragement towards application of the test in clinical practice for improved identification of undetected *S. pneumoniae* infections.

Fiberoptic bronchoscopy (FOB) is another diagnostic method, which could be used to improve microbiological yield, especially in patients who do not expectorate sputum. Two studies have demonstrated that FOB can be valuable in the case of non-resolving pneumonia or when treatment failure occurs.

In this study, we prospectively evaluated the diagnostic yield of different microbiological tests in hospitalised patients with CAP. Especially, we assessed whether the implementation of the urinary PCA test would improve the number of pneumococcal pneumonia. The second objective was to assess whether FOB could improve the diagnostic yield in patients who
did not expectorate sputum within 24 hours after admission, or in those in whom treatment had failed. In addition, we investigated the influence of the severity of CAP and that of outpatient antibiotic therapy on the diagnostic outcome of the various microbiological tests.

**Material and Methods**

**Patients**
A prospective randomised study was performed between December 1998 and November 2000 in the Departments of Pulmonary Diseases and Internal Medicine of the Medical Centre Alkmaar, which is a teaching hospital with 900 beds. The local medical ethics committee approved the study. Patients who fulfilled the following criteria were enrolled in the study after giving written informed consent: 1) aged 18 years or older 2) clinical presentation of an acute illness with one or more of the following symptoms suggesting CAP: presence of fever ($\geq 38.0^\circ\text{C}$), dyspnoea, coughing (with or without expectoration of sputum), chest pain; 3) presence of new consolidation(s) on the chest radiograph. Patients were excluded from the study if one of the following criteria applied: presence of severe immunosuppression (HIV infection, prednisolone $> 35$ mg/day or other immunosuppressive agents); presence of malignancy; pregnancy or lactation; documented severe allergy for antibiotics; presence of obstruction pneumonia; pneumonia developed within 8 days after hospital discharge.

The assessment of the severity of CAP was performed using the Pneumonia Severity Index (PSI)$^{23}$ and the CURB-65 severity score.$^{24}$ Patients stratified in PSI classes I and II or patients with a CURB-65 score 0 or 1 have a low risk of mortality. An intermediate risk of mortality can be considered for patients stratified in risk class III or patients with a CURB-65 score 2. Patients from risk classes IV and V or with a CURB-65 score 3-5 have a higher risk of mortality.$^{25}$

Antibiotic treatment ranged from a pathogen-specific therapy to broad-spectrum empirical treatment. The antibiotics most frequently used were: the combination of erythromycin and amoxicillin-clavulanate (43%); penicillin (21%); amoxicillin-clavulanate (18%); erythromycin (6%), amoxicillin (3%) and other (9%).
The value of intensive diagnostic microbiological investigation

**Microbiological investigations**

Sputum specimens were obtained at admission for Gram's stain (presence of > 25 polymorphonuclear leucocytes and < 10 squamous cells at 100x magnification), semi-quantitative culture and *S. pneumoniae* capsular antigen detection (latex agglutination test, Murex Diagnostics, Dartford, United Kingdom). Sputum PCA detection was performed on representative and non-representative sputum samples. Three sets of blood cultures were obtained within one hour after admission and a urine sample for *Legionella pneumophila* serogroup 1 antigen detection (Legionella Urinary Antigen EIA, Binax, Portland, Maine, USA) was obtained. Bronchoalveolar lavage (BAL) and protected specimen brush (PSB) were performed, after informed consent, if patients did not expectorate sputum within 24 hours after admission or in case of treatment failure. Treatment failure was present when signs and symptoms of pneumonia did not improve on antibiotic treatment within 72 hours of therapy and persisted or progressed thereafter. BAL fluids (BALF) and PSB specimens were processed for Gram's stain, semiquantitative culture and PCA detection (latex agglutination test). Thoracentesis was performed if pleural fluid was present; this material was investigated by Gram's stain, PCA detection (latex agglutination test) and culture for aerobic and anaerobic bacteria. If pleural fluid was detected at the emergency room, it was tapped for microbial investigation before the administration of antibiotic therapy. Urine samples obtained on all patients at admission were analysed for the presence of *S. pneumoniae* cell-wall antigen (NOW ICT *Streptococcus pneumoniae*, Binax, Portland, Maine, USA), and were further tested after a fifty times concentration. Serology was performed using enzyme linked immuno-assay (Serion ELISA classic, Virion GmbH, Würzburg, Germany). Blood samples were drawn on days 1 and 14 of treatment, for the detection of antibodies to *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *L. pneumophila* serogroup 1-7, influenza A and B virus, parainfluenza virus 1-3, respiratory syncytial virus and adenovirus. On day 28 a third blood sample for serology determination was taken when a not conclusive increase in antibody level was observed between the first two samples.

Definitive aetiology was defined as: a) identification of an aetiologic agent from blood and/or pleural fluid, b) the detection of *Legionella* spp. antigen in urine, c) the presence of *S. pneumoniae* antigen in pleural fluid,
d) a threefold increase in antibody level of *L. pneumophila* serogroup 1-7, *M. pneumoniae, C. pneumoniae*, influenza A and B, parainfluenza virus 1-3, respiratory syncytial virus or adenovirus.

Presumptive aetiology was defined as: a) a positive sputum culture, BALF or PSB culture by semi-quantitative methods (minimal presence of less-moderate number of bacteria: > $10^4$/ml-$10^5$/ml bacteria at 100x magnification), compatible with the organism(s) seen on a good Gram's stain specimen, b) detection of *S. pneumoniae* antigen in urine or sputum, BAL or PSB specimen, c) a single elevated IgM level of > 17 U/ml for *M. pneumoniae*.

*S. pneumoniae* resistance to penicillin was determined by an minimal inhibitory concentration (MIC) of > / = 2 µg/ml; and to macrolide antibiotics by an MIC of > / = 2 µg/ml.

**Statistical analysis**
The Chi-square test was used to compare categorical data. Data were analysed using SPSS Version 11.5 for Windows (Chicago, USA). All percentages were rounded. A result of $p < 0.05$ was considered to be significant.

### Results

**Patient characteristics.**
Two-hundred and sixty-two patients with CAP were included with a mean age of 64 years. Gender was almost equally distributed (54% men versus 46% women). Only 6 patients (2%) were admitted from nursing homes. The most common co-morbidity was chronic obstructive pulmonary disease, present in 96 patients (37%), followed by diabetes mellitus (10%), asthma (9%), and congestive heart failure (8%).

**Aetiology**
Overall, pathogens were identified in specimens from 158 patients (60%). The most common pathogen was *S. pneumoniae*, present in 97 patients (37%), followed by *M. pneumoniae* in 23 patients (9%) and *Haemophilus influenzae* identified in 19 patients (7%) (table 1). The prevalence of the three most common pathogens was not significantly higher in a high risk
The value of intensive diagnostic microbiological investigation

class (PSI class 4 or 5, or CURB-65 score 3-5) compared to a lower risk class (data not shown). No penicillin- or macrolide-resistant *S.pneumoniae* strains were identified. Mixed infection, consisting of the combination of a bacterial pathogen plus an atypical bacterial pathogen (*M. pneumoniae*, *L. pneumophila*, *C. pneumoniae*) or respiratory virus was present in 17 patients (6%).

**Table 1:** Results of microbiology research from 262 patients

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Definitive diagnosis, n</th>
<th>Presumptive diagnosis, n</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>31</td>
<td>66</td>
<td>97 (37)</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>23</td>
<td></td>
<td>23 (9)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>3</td>
<td>16</td>
<td>19 (7)</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>14</td>
<td></td>
<td>14 (5)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6</td>
<td>4</td>
<td>10 (4)</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td></td>
<td>7</td>
<td>7 (3)</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>1</td>
<td></td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Gram negative enterobacteriaceae</td>
<td>2</td>
<td>11</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Viral pathogens*</td>
<td>10</td>
<td></td>
<td>10 (4)</td>
</tr>
<tr>
<td>Other*</td>
<td>2</td>
<td>3</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Mixed infection*</td>
<td>9</td>
<td>27</td>
<td>36 (14)</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td>104 (40)</td>
</tr>
</tbody>
</table>

*In some patients more than one pathogen was present

*Definitive diagnoses: E-coli (1x); Enterobacter cloacae (1x). Presumptive diagnoses: E-coli (3x); Enterobacter aeruginosa (1x); Klebsiella pneumoniae (1x); Citrobacter freundii (1x), Klebsiella oxytoca (1x), Morganella morganii (1x); Pseudomonas aeruginosa (2x), E. cloacae (1x). *Adenovirus (n=7), Parainfluenza 1-3 virus (n=2), Respiratory syncytial virus (n=1).

*Definitive diagnoses: Arcanobacterium haemolyticum (1x) and Streptococcus group C (1x). Presumptive diagnoses: Streptococcus group B (1x), Haemophilus parainfluenzae (2x).

*Mixed infection consisted of a typical bacterial pathogen and a definitive atypical bacterial or viral pathogen in 17 patients (7 definite and 10 presumptive); in 19 patients mixed infections consisted of double infections with typical bacterial pathogens.
**Diagnostic yield of microbiological investigations**

Sputum examination of representative samples (n=44) had the highest diagnostic yield for detecting the causative pathogen (table 2); cultures were positive in 36 cases (82%). In 52 of the 97 pneumococcal infections (54%) the diagnosis was established by positive urinary PCA test. In 6 of the 52 patients (12%) the test was positive only after the urine was concentrated 50 times. Blood cultures were positive in 40 patients (16%). Patients who were stratified in PSI class IV or V showed a higher diagnostic yield from sputum examination, consisting of Gram’s stain investigation, culture and PCA detection (p=0.03) than patients stratified in PSI classes I to III. A CURB-65 score of 3-5 did not show this significant difference compared to a CURB-65 score of 0-2. A urinary Legionella antigen test was positive in a statistically higher number of patients with a CURB-65 score of 3-5 compared to a score of 2 or lower (10% [9/94] versus 2% [3/168], respectively; p=0.004). Although the diagnostic yield of the other microbial investigations was not significantly different between the PSI risk classes or CURB-65 scores, more patients with an aetiological diagnosis were identified in the higher severity scores.

Pretreatment with antibiotics resulted in a significant lower detection of pathogens in PCA in sputum (p=0.01), urinary PCA test (p=0.05) and blood culture (p=0.03). In the other microbiological investigations the identification of microbial pathogens was not influenced by outpatient antibiotic therapy (table 3).

**Identification of Streptococcus pneumoniae infections**

The 97 pneumococcal infections were mainly identified by urinary PCA test (n=52; 54%), by sputum PCA test (n=50; 52%) or by blood culture (n=30; 33%) (Fig. 1). Combining sputum culture with sputum PCA detection and positive Gram’s stain determination increased identification of *S. pneumoniae* infection to 58 patients (60%). When the urinary PCA test was compared with blood culture and sputum examination (Gram’s stain, culture and PCA detection), urinary PCA test provided a sole diagnosis of *S. pneumoniae* infection in 31% of the positive urinary tests (16/52). The urinary PCA test was positive in 22 of the 30 patients (73%) with a positive blood culture of *S. pneumoniae* infection.
Table 2: Diagnostic yield of microbiological investigations according to pneumonia severity index and CURB-65 score

<table>
<thead>
<tr>
<th>Microbiological investigation, positive / total no. tests (%)</th>
<th>PSI I-III, n=146 (56%)</th>
<th>PSI IV-V, n=116 (44%)</th>
<th>p-value</th>
<th>CURB-65* (0-2 points), n=168 (64%)</th>
<th>CURB-65 (3-5 points), n=94 (36%)</th>
<th>p-value</th>
<th>Total (262)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sputum specimen / total no. of sputum samples</td>
<td>21/85 (25)</td>
<td>23/72 (32)</td>
<td>0.31</td>
<td>31/101 (31)</td>
<td>13/56 (23)</td>
<td>0.32</td>
<td>44/157 (28)</td>
</tr>
<tr>
<td>Positive Gram's stain / adequate sputum specimen</td>
<td>19/21 (90)</td>
<td>21/23 (91)</td>
<td>0.66</td>
<td>28/31 (90)</td>
<td>12/13 (92)</td>
<td>0.66</td>
<td>40/44 (91)</td>
</tr>
<tr>
<td>Positive sputum culture/ adequate sputum specimen</td>
<td>16/21 (76)</td>
<td>20/23 (87)</td>
<td>0.36</td>
<td>24/31 (77)</td>
<td>12/13 (92)</td>
<td>0.24</td>
<td>36/44 (82)</td>
</tr>
<tr>
<td>Sputum pneumococcal antigen/ total no. of sputum samples</td>
<td>22/85 (26)</td>
<td>28/72 (39)</td>
<td>0.08</td>
<td>31/101 (31)</td>
<td>19/56 (34)</td>
<td>0.68</td>
<td>50/157 (32)</td>
</tr>
<tr>
<td>Sputum*/ total no. of sputum samples</td>
<td>35/85 (41)</td>
<td>42/72 (58)</td>
<td>0.03</td>
<td>50/101 (50)</td>
<td>27/56 (48)</td>
<td>0.88</td>
<td>77/157 (49)</td>
</tr>
<tr>
<td>Blood culture</td>
<td>18/142 (13)</td>
<td>22/112 (20)</td>
<td>0.13</td>
<td>23/163 (14)</td>
<td>17/91 (19)</td>
<td>0.34</td>
<td>40/254 (16)</td>
</tr>
<tr>
<td>Urine pneumococcal antigen</td>
<td>30/146 (21)</td>
<td>22/116 (19)</td>
<td>0.75</td>
<td>36/168 (21)</td>
<td>16/94 (17)</td>
<td>0.39</td>
<td>52/262 (20)</td>
</tr>
<tr>
<td>Urine Legionella antigen test</td>
<td>5/146 (3)</td>
<td>7/116 (6)</td>
<td>0.32</td>
<td>3/168 (2)</td>
<td>9/94 (10)</td>
<td>0.004</td>
<td>12/262 (5)</td>
</tr>
<tr>
<td>Bronchoscopy*</td>
<td>17/38 (45)</td>
<td>15/26 (58)</td>
<td>0.31</td>
<td>17/37 (46)</td>
<td>15/27 (56)</td>
<td>0.45</td>
<td>32/64 (50)</td>
</tr>
<tr>
<td>Serology</td>
<td>21/146 (14)</td>
<td>13/116 (11)</td>
<td>0.45</td>
<td>25/168 (15)</td>
<td>9/94 (10)</td>
<td>0.22</td>
<td>34/262 (13)</td>
</tr>
<tr>
<td>No. of patients with aetiological diagnosis</td>
<td>81/146 (55)</td>
<td>77/116 (66)</td>
<td>0.07</td>
<td>96/168 (57)</td>
<td>62/94 (66)</td>
<td>0.16</td>
<td>158/262 (60)</td>
</tr>
</tbody>
</table>

*Pneumonia severity index: low risk patients are stratified in class I-III; high risk patients in class IV and V  
*CURB-65: low risk patients are stratified as 0-2 points, high risk patients as 3-5 points  
*Gram's stain + culture + pneumococcal antigen detection  
*Bronchoalveolar lavage and protected specimen brush culture + pneumococcal antigen detection
Table 3: Influence of outpatient antibiotic therapy on diagnostic outcome

<table>
<thead>
<tr>
<th>Diagnostic outcome</th>
<th>Outpatient antibiotic therapy (n=68)</th>
<th>No outpatient antibiotic therapy (n=194)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sputum specimen / total no. of sputum samples</td>
<td>8/39 (21)</td>
<td>36/118 (31)</td>
</tr>
<tr>
<td>Positive sputum Gram's stain specimen / adequate sputum specimen</td>
<td>7/8 (88)</td>
<td>33/36 (92)</td>
</tr>
<tr>
<td>Sputum culture / adequate sputum specimen</td>
<td>7/8 (88)</td>
<td>29/36 (81)</td>
</tr>
<tr>
<td>Sputum pneumococcal antigen / total no. of sputum samples</td>
<td>6/39 (15)</td>
<td>44/118 (37)*</td>
</tr>
<tr>
<td>Urinary pneumococcal antigen</td>
<td>8/68 (12)</td>
<td>44/194 (23)*</td>
</tr>
<tr>
<td>Blood culture</td>
<td>5/66 (8)</td>
<td>35/188 (19)*</td>
</tr>
<tr>
<td>Urinary Legionella antigen test</td>
<td>5/68 (7)</td>
<td>7/194 (4)</td>
</tr>
<tr>
<td>Serology</td>
<td>10/68 (15)</td>
<td>24/194 (12)</td>
</tr>
<tr>
<td>Bronchoscopy</td>
<td>10/21 (48)</td>
<td>22/43 (51)</td>
</tr>
</tbody>
</table>

*p=0.01; †p=0.05; ‡p=0.03

Bronchoalveolar lavage fluid and protected specimen brush culture + pneumococcal antigen detection

Fiberoptic bronchoscopic investigation

A FOB was performed, after consent, in 64 patients (24%), of whom 37 (58%) did not expectorate sputum within 24 hours after admission and 27 (42%) suffered treatment failure within 72 hours after admission (Fig. 2). FOB provided a microbial diagnosis in 18 patients (49%) who did not expectorate sputum and in 14 patients (52%) who presented with treatment failure. No important complications, such as worsening of respiratory failure and admission to intensive care unit, were observed during and after performing FOB.

Overall, bacterial pathogens were identified by culture and PCA detection from BALF in 31 patients (48%). Culture of BALF resulted in a microbial diagnosis in 27 patients (42%). Pneumococci were identified by PCA in BALF in 8 patients (13%), confirmed by culture in 4 patients (6%). Protected specimen brush was performed in 25 patients (40%). In 7 of the 8 patients the same pathogen was identified by PSB as in BALF.
Figure 1: Comparison of different microbiological techniques for detection of Streptococcus pneumoniae infection

Sputum culture, $n=15$  
Sputum pneumococcal antigen test, $n=50$

![Figure 1: Comparison of different microbiological techniques for detection of Streptococcus pneumoniae infection](image)

Sputum positive Gram's stain, $n=19$

Blood culture, $n=30$

Sputum examination  
(positive Gram’s stain, culture and pneumococcal antigen), $n=58$

Urine pneumococcal antigen, $n=52$

Sputum culture, $n=15$  
Blood culture, $n=30$

Urine pneumococcal antigen test, $n=52$
**Figure 2:** Outcome of fiberoptic bronchoscopy as diagnostic intervention in patients with negative sputum culture or treatment failure

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Positive result</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOB* as diagnostic procedure</td>
<td>18 (49%)</td>
<td>n=37</td>
</tr>
<tr>
<td>BAL#, n=37</td>
<td>15 (41%)</td>
<td></td>
</tr>
<tr>
<td>PCA† positive:</td>
<td>5 (14%)</td>
<td></td>
</tr>
<tr>
<td>- 2 new established diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3 diagnoses similar to BAL culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOB in treatment failure, n=27</td>
<td>14 (52%)</td>
<td></td>
</tr>
<tr>
<td>BAL, n=27</td>
<td>12 (44%)</td>
<td></td>
</tr>
<tr>
<td>PCA positive:</td>
<td>3 (11%)</td>
<td></td>
</tr>
<tr>
<td>- 2 new established diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 diagnosis similar to BAL culture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fiberopti cc bronchoscopy; *Bronchoalveolar lavage; †Pneumococcal antigen; ‡Protected specimen brush. Characteristics for FOB as diagnostic procedure: no sputum production, n=23; inadequate sputum sample, n=13; adequate sputum specimen, n=1. Characteristics for FOB procedure in clinical failure: no sputum production, n=16; inadequate sputum sample, n=9; adequate sputum specimen, n=2

**Additional value of microbiological investigations**

In 14 patients (5%) the urinary PCA test provided an additional diagnosis compared to the other diagnostic tests. Sputum examination, combining the PCA detection in sputum with positive Gram's stain findings and culture, resulted in the highest yield as sole diagnostic test and provided a new diagnosis in 42 patients (27%). Fiberoptic bronchoscopy, which was performed in selected patients who did not expectorate sputum or who presented with treatment failure, provided an additional microbial diagnosis in 16 patients (25%) (table 4).
The value of intensive diagnostic microbiological investigation

Table 4: Additional value of microbiological investigations in patients with community-acquired pneumonia

<table>
<thead>
<tr>
<th>Microbiological Investigation</th>
<th>Sole positive diagnostic test/ no. of patients in which the test was performed (%)</th>
<th>Sole positive diagnostic test/ total no. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum Gram's stain*</td>
<td>17/44 (39)</td>
<td>17/262 (6)</td>
</tr>
<tr>
<td>Sputum culture*</td>
<td>15/44 (34)</td>
<td>15/262 (6)</td>
</tr>
<tr>
<td>Sputum PCA*</td>
<td>15/157 (10)</td>
<td>15/262 (6)</td>
</tr>
<tr>
<td>Total sputum†</td>
<td>42/157 (27)</td>
<td>42/262 (16)</td>
</tr>
<tr>
<td>Urinary PCA</td>
<td>14/262 (5)</td>
<td>14/262 (5)</td>
</tr>
<tr>
<td>Blood culture</td>
<td>7/254 (3)</td>
<td>7/262 (3)</td>
</tr>
<tr>
<td>Bronchoscopy†</td>
<td>16/64 (25)</td>
<td>16/262 (6)</td>
</tr>
</tbody>
</table>

* Adequate specimen.
† Pneumococcal antigen, determined in adequate and non-adequate sputum specimens.
‡ Sputum Gram's stain specimen + culture + pneumococcal antigen test.
§ Bronchoalveolar lavage and protected specimen brush culture + pneumococcal antigen detection.

Discussion

Performing the described microbiological investigations in patients with CAP resulted in the detection of pathogens in 60% of the study population. This percentage was comparable to results reported by others, but considerably lower than in two other studies where extensive microbiological investigation was performed to identify microorganisms in CAP (71%-75%). A lower microbial yield in the present study may be explained, by not using microbial investigations as viral culture, polymerase chain reaction to viral pathogens and atypical bacterial pathogens, as Legionella, serological testing for pneumococcal infection and for Legionella species other than L. pneumophila serogroup 1-7. Some pathogens, as Legionella, which may develop rise of antibody level after many weeks of infection, might not be detected in the study.

No important differences concerning the frequency of detection of various causative agents were noted in these studies. In the present study S. pneumoniae was the most common pathogen of CAP, as has also been demonstrated by others. The other important causes of CAP in this
study were infections with *H. influenzae* and *M. pneumoniae*. Remarkable was the low percentage of *C. pneumoniae* found in our study (<1%) and in the study performed by Bohte *et al.* (3%).

Lim *et al.* detected this microorganism in 13% of a British study population [12]. The proportion of mixed infections consisting of “typical” and “atypical” infections also differed between our results (6%), those of Bohte *et al.* (10%) and the results reported by Lim *et al.* (27%).

This difference in prevalence could be explained by the use of elaborate serological investigation by the latter group.

In the present study we demonstrated that sputum investigation, which consisted of culture combined with Gram’s stain and PCA detection, showed the highest diagnostic yield (n=77; 49%); followed by urinary PCA test (n=52; 20%) and blood culture (n=40; 16%). The value of sputum investigation is generally underestimated, perhaps due to the fact that sputum specimen could be obtained in only 60% of the study population, a figure which is similar to other studies. The quality of the Gram’s stain sputum sample could assist in the later interpretation of sputum culture. In our study sputum samples suitable for an adequate Gram’s stain were followed by a positive sputum culture in 82% of the cases. This result is comparable to results described in the study by Rosón *et al.* (83%).

Gleckman *et al.* showed that sputum Gram’s stain predicted 40 of 47 (85%) positive blood cultures. These results suggest that a good Gram stain on an adequate sputum specimen can be used as a reliable indicator to guide initial antibiotic therapy.

To increase detection of *S. pneumoniae*, we investigated the value of sputum PCA test and urinary PCA test. Cross-reactions with oral streptococci may theoretically occur in sputum samples and this should be considered when interpreting sputum PCA test results. However, false positive PCA results in sputum due to cross-reacting oral flora is in any event unlikely, because the amount of antigen produced by these microorganisms is usually far too low.

The PCA test was performed in all 157 representative and non-representative sputum specimens. In 32% of these specimens the antigen test turned out to be positive. This percentage was also observed in two other studies, in which 36% and 37%, respectively, of all expectorated sputa by CAP patients yielded a positive PCA test. By performing PCA detection in sputum, in addition to culture, the diagnostic yield increased by 24%.
The urinary PCA test was the most valuable investigation in identifying *S. pneumoniae* infections compared to blood culture and sputum culture. The results of the urinary PCA test in patients with a definitive diagnosis of pneumococcal pneumonia showed a lower sensitivity (73%) than described in other studies (77-92%).\textsuperscript{16-20} This difference in sensitivity for patients with a definite diagnosis of pneumococcal pneumonia is unclear, considering also that severity of pneumonia had no influence on positive results for the urinary test. In a study performed by Gutiérrez et al.,\textsuperscript{19} no differences in the predictive value of the urinary PCA test according to the PSI classification were observed. However, Rosón et al.\textsuperscript{20} showed that the urinary PCA test was more sensitive for patients with high-risk pneumonia. Our study showed that outpatient antibiotic therapy resulted in a significant lower percentage of positive urinary PCA test, a result which was also observed by Gutiérrez et al.\textsuperscript{19} Prior antibiotic therapy also influenced the outcome of blood culture in our study. This could explain the influence of outpatient antibiotic therapy on the result of the urinary PCA test, considering that a urinary PCA test is more sensitive for bacteremic than for nonbacteremic patients.\textsuperscript{16,19,20} A possible limitation of the urinary test could be that cultures are needed in order to determine susceptibility and guide therapy in countries with a high percentage of drug-resistant *S. pneumoniae*.

In 16% of the study population a pathogen could be identified in blood culture, which is similar to results by others.\textsuperscript{10,32} Some authors have debated the value of blood cultures in CAP.\textsuperscript{32-34} The main argument for being more reserved in the taking of blood cultures consisted of the observation that, besides the low prevalence of positive cultures, positive cultures rarely lead to adaptation and narrowing of antibiotic therapy. It was suggested that blood cultures should only be obtained in selected patients with the greatest risk of mortality. To emphasise this assumption Waterer et al.\textsuperscript{32} showed that the yield of pathogens from blood cultures increased with PSI score. We did not observe a significant difference in yield from blood cultures between the low PSI risk classes I-III and the high risk classes IV-V, or in low and high severity assessment according to the CURB-65 score. For this reason we believe that blood cultures should be obtained at admission in all hospitalised patients.

Apart from epidemiological reasons, serological research has no important clinical function, because results cannot usually be obtained in
less than two weeks. The clinical significance of the lack of demonstrating atypical microorganisms, with the exception of *L. pneumophila*, has to be questioned. In our study mixed infections consisting of typical and atypical pathogens were observed in only 6% of the study population. However, this low number could partially be explained by the use of less elaborate serological investigation. Furthermore, from different studies it has been reported that inadequate treatment against atypical microorganisms did not result in a significantly higher mortality or clinical failure percentage.²⁷ ³⁵ ³⁶

In patients suffering treatment failure the use of FOB resulted in a microbial diagnosis in 14 patients (52%). This percentage is comparable to the result obtained by Örtqvist *et al.*,²² who reported a diagnosis by FOB in 54% of the patients with treatment failure. In patients who did not expectorate an adequate sputum specimen within 24 hours (n=37) FOB provided an additional diagnosis in 49% of the cases. BAL, in contrast to PSB, was responsible for the detection of bacterial pathogens in the majority of cases (97%). The results could have clinical implications for performing FOB, when treatment failure occurs and no pathogen has been identified from routine microbiological investigations. Moreover, the present results showed that prior antibiotic therapy did not have an influence on the outcome of FOB and that no important complications were observed.

Antibiotic therapy can be narrowed, and consequently antimicrobial resistance can remain limited, when a microbiological diagnosis is obtained. This should be an important goal in the ideal management of CAP. In this study no penicillin resistant pneumococcal strains were identified. In the Netherlands antibiotic usage is limited,³⁷ which is reflected by a low prevalence of penicillin resistant pneumococcal strains (1.5%) in 2002 (European Antimicrobial Resistance Surveillance System; www.earss.rivm.nl) in contrast to the United States of America [38]. Therefore, it is surprising that some authors observed that a specific microbiological diagnosis did not usually result in adaptation of the initial empirical antibiotic treatment.³³–³⁴

In this study we showed that several microbiological investigations provided additional useful information in patients with CAP. The results indicate that sputum investigation by Gram’s stain, culture, and PCA detection was the most useful test for the (rapid) diagnosis of CAP, followed by urinary PCA test and blood cultures. The urinary PCA test deserves
The value of intensive diagnostic microbiological investigation

implementation as routine diagnostic investigation in hospitalised patients with CAP. It may be useful in guiding pathogen-directed antimicrobial therapy besides conventional microbiological investigation, especially when sputum specimens are not available. Invasive procedures, such as FOB, can be of value in addition to blood cultures and sputum studies when treatment failure has occurred.

**Acknowledgments**
We would like to thank Mrs. Y. Holloway for her assistance in editing this manuscript. No potential conflicts of financial interest are present. The experiments comply with the current laws of the country in which they were performed.
Reference List


The value of intensive diagnostic microbiological investigation


