Pneumococcal antigen persistence in sputum from patients with community-acquired pneumonia

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The purpose of this study was to establish the diagnostic value of pneumococcal capsular antigen by comparing this with the results of Gram stain and culture in representative and nonrepresentative sputa during follow-up in patients with community-acquired pneumonia. Antigen was detected by a latex particle agglutination test. At the time of hospital admission, antigen was detected in 17 representative sputum specimens from 30 patients with pneumococcal pneumonia, which was comparable to the results of Gram stain and culture. In five additional patients, antigen was demonstrated in nonrepresentative specimens. During follow-up under antibiotic treatment, this number increased by six: three patients with representative and three patients with nonrepresentative sputum, respectively. Two of the 22 patients with pneumonia of other known cause had an antigen-positive sputum on admission and in another two patients, sputum antigen was detected during follow-up. Ten of 34 patients with pneumonia of unknown cause had detectable antigen in representative or nonrepresentative sputum on admission. During follow-up, antigen was detected in sputa of an additional seven patients. There was no difference in duration of antigen persistence between patients with pneumococcal pneumonia and pneumonia of unknown cause. It was observed that the first antigen-positive sputum specimen was always detected within the first five days of the hospital stay. We conclude that antigen detection in both representative and nonrepresentative sputum specimens at the time of hospital admission and during follow-up is of additional value for the diagnosis of pneumococcal pneumonia. It markedly increases the number of patients with pneumococcal pneumonia detected, who would otherwise be considered to have pneumonia of unknown cause. However, antigen-positive results should be interpreted carefully, especially in those pneumonia patients with chronic bronchitis, because detectable antigen may be caused by pneumococcal carriage of the lower respiratory tract.

Pneumococcal antigen detection has been established to be a reliable method for diagnosing pneumococcal pneumonia. The highest sensitivity for antigen detection was found in sputum specimens. Purulent specimens especially yielded the most positive results. The majority of investigators have studied antigen detection in sputum specimens obtained on hospital admission and compared these results with Gram stain or culture. Antigen detection has an additive role in diagnosing pneumococcal pneumonia, especially in those patients where sputum Gram stain and culture or blood culture are not conclusive. Patients pretreated with antibiotics before hospitalization or those who do not expectorate sputum at the time of hospital admission may be diagnosed by pneumococcal antigen detection in the later phase of the illness. Approximately 20 to 33 percent of the patients with a community-acquired pneumonia without microbial or serologic diagnosis have detectable pneumococcal antigen on admission, and can therefore be considered to have pneumococcal pneumonia.

Antigen persistence in sputum specimens during antibiotic treatment has not been studied extensively. Therefore, in the present study, detection of pneumococcal capsular antigen in sputum specimens from patients with community-acquired pneumonia was examined on admission and during follow-up under antibiotic therapy, and compared with the results of Gram stain and culture. Pneumococcal antigen persistence was also evaluated in relation to the quality of the sputum specimens.

Methods

Patients

Ninety consecutive patients were admitted to the University Hospital Groningen with a community-acquired pneumonia. All patients had an acute illness with fever and infiltrate(s) on the chest roentgenogram. Patients with a poststenotic infiltrate (eg, tumor) and those who were immunocompromised were excluded. All patients were investigated for the presence of causal agents, including Legionella species, Mycoplasma pneumoniae, Coxiella burnetii, Chlamydia species, and respiratory viruses.

In order to exclude potential cross-reactivity with nonpneumococcal microorganisms, patients with pneumonia caused by mixed
bacterial pathogens, which included *Streptococcus pneumoniae*, were excluded from analysis.

The included patients were subsequently classified into the following groups: (1) *Pneumococcal pneumonia* consisted of 30 cases (mean age, 60.9 years; range, 23 to 84 years). **Definite pneumococcal pneumonia**, in which blood culture yielded *S pneumoniae*, was diagnosed in 14 patients. **Probable pneumococcal pneumonia** (16 patients) was diagnosed if a Gram stain of washed representative sputum revealed only lancet-shaped Gram-positive encapsulated diplococci, confirmed by a culture that yielded a pure growth of *S pneumoniae*. The two groups were united because the number of patients as well as the number of specimens was too low for separate analysis. (2) **Pneumonia of other known cause** (22 patients with mean age 53.7 years; range, 27 to 89 years) was defined if a nonpneumococcal pathogen was predominant in Gram stain or culture of washed representative sputum, by a positive blood culture, or a fourfold or greater rise/fall in antibody titer of any of the serologic tests. (3) **Pneumonia of unknown cause** (34 patients) included patients (mean age, 59.2 years; range, 30 to 86 years) in whom no positive microbiologic or serologic results could be demonstrated.

Chronic bronchitis** was diagnosed in seven (23 percent) patients with pneumococcal pneumonia, four (18 percent) with pneumonia of other known cause, and seven (21 percent) patients with pneumonia of unknown cause.

**Sputum Collection and Examination**

In all patients, antigen detection, Gram stain, and culture were all performed simultaneously on the same sputum specimens at the time of hospital admission and on consecutive days during follow-up. The observation period was usually 14 days. From each patient, only one specimen a day was tested for the presence of pneumococcal antigen. All sputum samples were sequentially washed three times in Petri dishes containing physiologic saline solution if sufficient specimen was available. The resultant purulent fragments from each sputum specimen were used for Gram stain, culture, and antigen detection concurrently. **Representative sputum** originating from the lower respiratory tract was defined as that containing ≥50 leukocytes and ≤5 squamous epithelial cells, with the examination performed at ×100 magnification (×10 objective). Specimens deviating from this standard were considered to be **nonrepresentative**. Bacteria were examined by oil-immersion (×1,000 magnification) and their identification was based on morphology and standard cultural characteristics. Clusters of leukocytes and bacteria in the absence of squamous epithelial cells were considered a typical feature of infection. Cultures were performed semiquantitatively.

**Antigen Detection**

Pneumococcal capsular antigen detection in sputa was performed by latex particle agglutination (using the Wellcome Kit, Wellcome Diagnostics, Dartford, England) according to the manufacturer’s instructions.

The duration of antigen persistence was defined as the period between the first and last expectorated sputum specimen in which antigen could be detected during the observation period.

**Statistical Analysis**

Continuous variables with abnormal distribution were analyzed by the Mann-Whitney *U* test.

**RESULTS**

**Pneumococcal Pneumonia**

The relationship between Gram stain, culture, and pneumococcal antigen-positive representative and nonrepresentative sputum specimens is shown in Figure 1. Of the 18 patients who expectorated representative sputum on admission, 17 had detectable antigen in these specimens. During the course of the illness, antigen was detected in sputum specimens from three additional patients who subsequently expectorated representative sputum (Table 1). In most of the representative sputa, antigen was demonstrated for a longer period, sometimes for more than one week, while they were being treated with adequate antibiotic therapy (Fig 1). After two days of antibiotic treatment, almost no pneumococci were detected by Gram stain or culture. On admission, five of the remaining 12 patients expectorated nonrepresentative sputum, of which antigen could be detected in all five specimens (Table 1). In only one of these pneumococci could be demonstrated in Gram stain and culture. None of these five patients had been pretreated with

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**Figure 1.** The relationship between Gram stain, culture and antigen detection during follow-up in sputum specimens from 30 patients with pneumococcal pneumonia (only one specimen a day from each patient). **•** Gram stain (+); **••** Culture (+); **•••** Antigen (+); **••••** No. of specimens tested.
antibiotics. During follow-up, antigen was also demonstrated in representative specimens from three of these patients. Another three patients who did not expectorate sputum on admission expectorated nonrepresentative antigen-positive sputa during the following days. The distribution pattern of antigen-positive specimens during follow-up was comparable to that of representative specimens (Fig 1).

**Pneumonia of Other Known Etiology**

Sputum obtained at the time of hospital admission or during follow-up yielded pneumococcal antigen in four of 22 patients with nonpneumococcal pneumonia (Table 1): two had a chlamydial infection, and two others were infected with respiratory viruses only. During follow-up, five antigen-positive specimens were collected from the same patient, who was receiving antibiotics prior to hospitalization. On admission and during follow-up, antigen could be detected in only a very low number of representative sputum specimens, whereas nonrepresentative specimens were false positive relatively more frequently (Fig 2).

**Pneumonia of Unknown Etiology**

Twenty of 34 patients with pneumonia of unknown etiology expectorated sputum at the time of hospital admission. Antigen could be detected in ten of these specimens: in six representative and in four nonrepresentative sputa (Table 1). In another seven patients with representative or nonrepresentative spu, antigen was demonstrated only during follow-up. Although the number of antigen-positive specimens is smaller, antigen persistence in patients with pneumonia of unknown cause is comparable to those with pneumococcal pneumonia (Fig 3). Thirteen of the 34 patients of this group had already received antibiotic treatment before hospital admission. This number is higher than that of the patients with pneumococcal pneumonia. (four of 30).

The diagnostic value of antigen testing in sputa in pneumococcal pneumonia and pneumonia of unknown etiology is shown in Figure 4. In nearly all patients, the first sputum with demonstrable antigen was obtained within the first five days of hospital admission. In seven (44 percent) patients with pneumonia of unknown cause, antigen was detected during follow-up, while they were receiving antibiotic therapy.

**Duration of Antigen Persistence**

In the group with pneumococcal pneumonia, the median antigen persistence was 4.5 days (range, one...
to 33 days). The median duration in the antigen-positive group of unknown cause was 5 days (range, one to 22 days). The duration of antigen persistence was not significantly different (p = 0.96) between the two groups despite the difference in antibiotic pretreatment.

Discussion

Pneumococcal antigen detection is an important diagnostic tool for the determination of pneumococcal infection. The sensitivity of the rapid antigen detection tests (latex agglutination and coagglutination), determined in sputum from pneumococcal pneumonia patients, varies from 63 to 94 percent.5,7,13,15,16 The specificity for detection of pneumococcal capsular antigen in sputa collected from patients with pneumonia caused by another known microorganism is highly acceptable (82 to 96 percent).5,7,13,16,17 Patients with community-acquired pneumonia are often treated with antibiotics by their general practitioner before being admitted to the hospital. Because S. pneumoniae is generally susceptible to penicillin, this pretreatment decreases the chance of sputum Gram stain and culture being positive in patients with pneumococcal pneumonia. Moreover, there is also a category of patients who do not expectorate any sputum initially due to pleuritic pain, bronchial obstruction or confusion, but do so in a later phase of the illness. This sputum can be used for antigen detection when Gram stain and culture results are (already) negative.

In an attempt to increase the diagnostic yield, this study was set up to investigate pneumococcal antigen persistence in representative and nonrepresentative sputa originating from patients with community-acquired pneumonia.

At the time of hospital admission, the number of antigen-positive representative sputum samples from pneumococcal pneumonia patients was comparable to the results of Gram stain and culture. After two days of antibiotic treatment, the conventional cultural techniques yielded negative results, whereas antigen still persisted in a significant percentage of patients. Five nonrepresentative sputum samples were antigen-positive on admission, whereas conventional microbiologic methods were positive in only one patient. Six additional patients were diagnosed during follow-up, when cultures were negative. The rate of antigen-positive results during follow-up in nonrepresentative specimens was comparable to that of representative specimens.

It was observed that after a few days, representative sputum was replaced by nonrepresentative sputum in approximately half of the patients. This is due to the gradually decreasing number of leukocytes in sputum specimens after initiation of antibiotic therapy. These
data confirm the results of earlier studies that sputa were not suitable for conventional microbiologic examination can additionally be used for antigen detection.

Antigen persistence in sputum from patients with pneumococcal pneumonia has been demonstrated in two other reports, but no reference was made to the quality of the sputa. The same trend of antigen persistence was found in the present study. It was also shown that pneumococcal antigen is found in a lower frequency in antibiotic pretreatment specimens, confirming the decline of antigen during therapy.

Antigen was detected in only a small number of sputum specimens of patients with pneumonia of other known cause. During follow-up, the high specificity was also confirmed. Nonrepresentative specimens were relatively more frequently antigen positive. Five of the 11 nonrepresentative specimens with detectable antigen were obtained from one patient with paramyxovirus 3 infection. Concomitant pneumococcal infection could not be excluded, because this patient had received antibiotics before hospital admission.

The value of antigen detection during follow-up was especially demonstrated in the group pneumonia of unknown cause. In 17 (50 percent) of these patients, antigen was detected in one or more of their sputum specimens (representative and nonrepresentative). This percentage is higher than that noted in other studies (8 to 23 percent), and is probably due to consistent sputum collection during follow-up. The same trend of antigen persistence was seen as in pneumococcal pneumonia, although the number of positive specimens was lower on the first day. No obvious difference in antigen persistence was seen between representative and nonrepresentative specimens. The duration of antigen persistence was also comparable to that of pneumococcal pneumonia. Considering the high specificity of the test and the observation that pneumococcal carriage in the oropharynx and nasopharynx seldom leads to a positive antigen test, detectable antigen in sputa of patients with a pneumonia of unknown cause can be considered indicative for pneumococcal pneumonia (W. G. Boersma, et al. Unpublished results). Some restrictions should be made for those patients with chronic bronchitis. We realize, as also emphasized in other reports, that colonization of the lower respiratory tract with S pneumoniae in patients with chronic bronchitis may be a disturbing factor for the interpretation of the usefulness of antigen detection in pneumonia. In the present study, approximately 20 percent of all patients with pneumonia had chronic bronchitis with an equal distribution among the three groups. In these patients, positive antigen results may be misleading and should be interpreted carefully.

For diagnostic purposes, we examined the point at which antigen was detected for the first time in sputum. With one exception, all patients with pneumococcal or unknown pneumonia had detectable antigen in sputum specimens during the first five days of their hospital stay.

We conclude that pneumococcal capsular antigen detection in sputum specimens during follow-up is of additional diagnostic value in patients with community-acquired pneumonia, especially in patients with negative Gram stain and culture. Apart from the fact that it yields rapid diagnostic information, it increases the number of patients diagnosed as having pneumococcal pneumonia by detecting antigen in the group otherwise classified as unknown cause. In patients with chronic bronchitis, detectable antigen may be misleading, because the lower respiratory tract may be colonized by pneumococci, and the results should be interpreted carefully. Although nonrepresentative sputum specimens are unreliable for bacteriologic examination, antigen detection in these specimens is of additional diagnostic value. To increase the yield of positive pneumococcal pneumonia antigen detections, sputum specimens should be examined for at least five consecutive days.

References