Rapid detection of pneumococcal antigen in pleural fluid of patients with community acquired pneumonia

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Abstract

Background Detection of pneumococcal antigen may help to increase the rate of diagnosis of pneumococcal pneumonia. This study was designed to determine the value of rapid detection of pneumococcal antigen in pleural fluid from patients with community acquired pneumonia.

Methods Thoracentesis was performed in patients suspected of having empyema and in patients with pneumonia of unknown aetiology. Pneumococcal capsular antigen was detected by latex agglutination and this method was compared with Gram staining and culture, specimens of pleural fluid being examined in parallel by the three methods.

Results Pleural fluid was radiographically identified in 63 of 135 patients with community acquired pneumonia. In nine of 45 patients with pneumococcal pneumonia and pleural fluid pneumococci were identified by Gram stain in two and by culture in one specimen of pleural fluid, whereas antigen was detected in eight of these specimens. In 12 of 33 patients with pneumonia of other known aetiology only one pleural fluid specimen was antigen positive, providing a specificity of 92% for this test. Pleural fluid obtained from 12 of 58 patients with pneumonia of unknown aetiology yielded detectable antigen in seven cases.

Conclusions Detection of pneumococcal antigen by latex agglutination in pleural fluid may yield important and rapid information in patients with community acquired pneumonia.

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Detection of pneumococcal capsular antigen in sputum, serum, and urine has been shown to be of additive value for diagnosing pneumococcal pneumonia, especially in patients who have received antibiotic treatment before hospital admission, which often results in negative cultures.

Immunological techniques such as counterimmunoelectrophoresis, latex agglutination, and coagglutination detect the presence of pneumococcal antigen in sputum with a high sensitivity.^{1,2} The usefulness of sputum examination in pneumonia has been extensively discussed and criticised in the past, however. In contrast to sputum specimens,

pleural fluid can be obtained without contamination and thoracentesis is less uncomfortable for the patient than other invasive techniques, such as bronchoscopy or trans-thoracic needle aspiration.

In the present study pleural fluid was systematically investigated by a rapid method (latex agglutination) for the presence of pneumococcal capsular antigen in patients admitted to hospital with community acquired pneumonia.

Methods

One hundred and forty two patients admitted to hospital with community acquired pneumonia were enrolled into the study. Community acquired pneumonia was defined as an acute illness with fever and one or more infiltrates on the chest radiograph. Immuno-compromised patients and those with lung cancer or lung infarction were excluded. To exclude potential cross reactivity with non-pneumococcal microorganisms, we excluded from analysis seven patients with pneumonia caused by mixed bacterial pathogens that included *Streptococcus pneumoniae*.

Patients (n = 135) were subsequently classified into three groups. *Pneumococcal pneumonia* (n = 45) was diagnosed if blood culture yielded *S pneumoniae* or Gram staining of washed representative sputum (≥ 50 leucocytes and $\leq$ six squamous epithelial cells per low power field, $100\times$ magnification) showed pneumococci, confirmed by culture. The criterion for *pneumonia of other known aetiology* (n=32) was predominance of a non-pneumococcal pathogen in Gram stained or cultured washed representative sputum or a positive blood culture or a fourfold or greater rise or fall in antibody titre in any of the serological tests. The group with *pneumonia of unknown aetiology* (n=58) consisted of patients in whom no positive microbiological or serological results were obtained.

Pleural fluid was aspirated for diagnostic purposes only from patients suspected of having an empyema and from patients with pneumonia in whom no causative pathogen could be identified. Pleural effusion was identified radiographically if at least the costophrenic angle was blunted on the posteroanterior or lateral chest radiograph. Thoracentesis was performed with a 20 gauge needle attached to a 20 ml syringe with the patient sitting in the upright position.

Gram staining was performed routinely, and an acid fast smear only if clinically

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Results of diagnostic tests for pneumococci in pleural fluid from patients with community acquired pneumonia

Diagnosis (n)	Pleural fluid radiographically present (%)	No of positive cases/ No tested (%)		
		Pleural fluid		
		Gram stain	Culture	Antigen
Pneumococcal pneumonia (45)	22 (49)	2/ 9 (22)	1/ 9 (11)	8/ 9 (89)
Pneumonia of other known aetiology (32)*	16 (50)	0/12	0/12	1/12 (8)
Pneumonia of unknown aetiology (58)	25 (43)	0/12	0/12	7/12 (58)

**Chlamydia psittaci* (2 cases), *Haemophilus influenzae* (2), *Chlamydia psittaci* and *Mycobacterium tuberculosis* (1), *M. tuberculosis* (1), β haemolytic streptococcus group G (1), Gram negative rods—2 spp (1), *Escherichia coli* and *Streptococcus milleri* (1), *Bacteroides* spp and anaerobic Gram positive rods (1), *Fusobacterium* spp (1), mixed aerobic and anaerobic bacteria (1).

indicated. All specimens were cultured under aerobic and anaerobic conditions according to conventional methods. In cases of suspected tuberculosis Löwenstein-Jensen culture medium was inoculated.

All pleural fluid specimens for antigen detection were handled as described previously.³ Pneumococcal capsular antigen detection was performed by latex particle agglutination, the Wellcogen Kit (Wellcome Diagnostics, Dartford, England) being used according to the manufacturer's instructions.

Results

Pleural fluid was radiographically visible in 63 (47%) of the 135 patients admitted with community acquired pneumonia and was obtained in 33 cases.

Samples of pleural fluid were collected from nine of the 45 patients with pneumococcal pneumonia, 12 of the 32 patients with pneumonia of other known aetiology, and 12 of the 58 patients with pneumonia of unknown aetiology.

S pneumoniae was detected in pleural fluid by Gram staining or culture in only two of the nine patients with pneumococcal pneumonia we studied, whereas pneumococcal capsular antigen was detected by latex agglutination in eight of these patients, yielding a sensitivity of 89% (table).

Detection of pneumococcal antigen in pleural fluid from patients with pneumonia caused by other known aetiological agents was highly specific (92%) for pneumococcal disease, only one of the 12 patients being antigen positive. This patient, with a chlamydial infection, had been treated with antibiotics before admission, suggesting that a mixed infection including *S pneumoniae* could not be excluded.

In patients who were classified as having pneumonia of unknown aetiology antigen was detected in seven of 12 pleural fluid samples, all of which were negative according to Gram staining and culture.

Only one patient developed a pneumothorax as a complication of the thoracentesis, and a chest tube was required. No other complications were noted.

Discussion

Pleural fluid is detected by chest radiography

in 16–57% of patients with community acquired pneumonia.^{4–7} Although radiographically present in 63 (47%) of the patients in this study, pleural fluid was examined in only 33.

Bacterial pneumonia has a higher incidence of parapneumonic effusion than "atypical" or viral pneumonia.^{5,8} Parapneumonic effusion develops in about half of all patients with pneumococcal pneumonia, the highest rate being seen in patients with bacteraemia.^{5–7}

Although more cases of bacterial pneumonia are caused by *S pneumoniae* than by any other bacterium, pneumococcal empyema is rare.^{6,7} In the study by Bartlett and associates⁹ pneumococci were identified by culture in only five of 83 pleural fluid specimens from patients with empyema, whereas in the present study pneumococci could be identified in two of nine specimens.

Once patients have received antibiotics the clinical manifestations and the bacteriology of the pleural fluid may be altered.¹⁰ In pneumococcal pneumonia antibiotic treatment has an enormous impact on the results of Gram staining and culture of clinical specimens, such as sputum and pleural fluid.^{11–15} Soluble bacterial antigens may persist in clinical specimens, including pleural fluid, even when bacteria can no longer be found. Previous studies have investigated pleural fluid for pneumococcal antigen by counterimmunoelectrophoresis.^{14,16} There is no published assessment of the value of more rapid and simpler methods, such as latex agglutination and coagglutination, for detecting capsular antigen.

In our study detection of pneumococcal antigen in pleural fluid was far more sensitive (89%) than Gram staining and culture. The same sensitivity of latex agglutination has been reported in sputum specimens.^{3,17,18} Tugwell *et al*¹⁶ reported that antigen could be detected by counterimmunoelectrophoresis in pleural fluid in 10 of 12 adults with lobar pneumonia, whereas routine bacteriological methods identified pneumococci in only five of these specimens. In a study among children counterimmunoelectrophoresis detected pneumococcal antigen in all 19 pleural fluid specimens in which *S pneumoniae* was identified.¹⁴ No false positive results were reported in children with non-pneumococcal pneumonia. In culture negative pleural fluid pneumo-

coccal antigen was detected in 15 of the 45 cases.

Antigen detection in pleural fluid may provide a definite aetiological diagnosis, as shown in seven of the 12 patients with pneumonia of "unknown" aetiology in the present study.

We observed that despite adequate antibiotic treatment antigen sometimes persisted in pleural fluid for at least one week, while Gram staining and culture gave negative results. This is in accordance with the findings of others.¹⁴

Our results suggest that antigen detection in pleural fluid in patients with community acquired pneumonia is a reliable method for establishing that *S pneumoniae* is definitely the cause. In contrast to pneumococci in sputum specimens, detectable antigen in pleural fluid is highly specific for the presence of pneumococcal pneumonia. The results of latex agglutination are available within one hour and antibiotic treatment can be directed at the pneumococcus.

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