Pneumococcal antigen detection in bronchoalveolar lavage fluid from patients with pneumonia

Patricio Jiménez, Mónica Meneses, Francisco Saldías, Maira Velásquez

Abstract

**Background** — Pneumococcal pneumonia can be diagnosed by the detection of capsular antigen in sputum, serum, pleural fluid, or urine using countercurrent immunoelectrophoresis and latex agglutination. In addition, quantitative cultures of bronchoalveolar lavage (BAL) fluid are also reliable for establishing the aetiology of pneumonia. This study investigated the value of rapid detection of pneumococcal antigen in BAL fluid from patients with pneumonia.

**Methods** — Pneumococcal antigen was detected by countercurrent immunoelectrophoresis and latex agglutination. Patients were grouped according to BAL quantitative culture results into pneumococcal pneumonia (n = 24), other known aetiology (n = 18), and unknown aetiology (n = 17). Thirteen patients with interstitial lung disease and without pneumonia served as a control group.

**Results** — In patients with pneumococcal pneumonia, antigen was detected by countercurrent immunoelectrophoresis in 50% and by latex agglutination in 54% of cases. In patients with pneumonia of unknown aetiology pneumococcal antigen was detected by latex agglutination in 53% of cases. Antigen was not detected in patients with pneumonia of other known aetiology or in control patients, yielding a specificity of 100%.

**Conclusions** — In patients with pneumococcal pneumonia requiring fibroptic bronchoscopy detection of pneumococcal antigen in BAL fluid may rapidly and accurately confirm the aetiology. Furthermore, in nearly half the cases of pneumonia of unknown aetiology antigen can be detected, suggesting that *Streptococcus pneumoniae* is a major causative agent in such patients.

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The establishment of an aetiological agent in community acquired pneumonia remains an unresolved problem. Despite extensive investigations the aetiology of pneumonia remains undetermined in up to 50% of patients. Diagnostic investigations are usually limited to examination of sputum by Gram stain and culture, and sometimes blood cultures. Unfortunately sputum is not available in one third of patients early in the illness and, when it is obtained, the results may not establish the cause. Furthermore, the use of antimicrobials before culture often precludes the growth of some organisms. Even when pathogens are isolated results may not be available for 48 hours or longer after hospital admission.

To overcome these deficiencies methods of detecting capsular antigens by using countercurrent immunoelectrophoresis and latex agglutination have been developed. These techniques are not affected by prior antibiotic treatment and provide rapid results. Sputum, serum, and urine have been studied with the aim of detecting pneumococcal antigens.

The results have been variable depending on the population studied and the kind of specimens used.

Bronchoalveolar lavage (BAL) has been used as an additional technique to fibreoptic bronchoscopy for investigating the aetiology of pneumonia. There is little information about the reliability of pneumococcal antigen detection in BAL fluid from patients with pneumonia. We have shown previously that *Streptococcus pneumoniae* was the main agent in our patients. We therefore investigated the detection of *S. pneumoniae* antigen in the BAL fluid from patients with pneumonia and compared the findings with conventional culture techniques.

**Methods**

**Patients**

Patients with community acquired pneumonia were selected for inclusion into two studies designed to investigate the value of BAL in the diagnosis of bacterial pneumonia, and the evaluation of non-resolving pneumonia. A group of patients with community acquired pneumonia underwent fibreoptic bronchoscopy with BAL before starting antibiotics to ascertain the aetiology of pneumonia. Community acquired pneumonia was defined as an acute febrile illness with transient shadows on the chest radiograph. A further group of patients with non-resolving pneumonia was also studied with fibreoptic bronchoscopy and BAL.

Non-resolving pneumonia was defined as the persistence of fever, clinical, and radiographic signs of pneumonia beyond five days of antibiotic treatment.

Patients were subsequently reclassified into three groups. (1) Pneumococcal pneumonia (n = 24) was diagnosed if blood cultures yielded *S. pneumoniae* or cultures of BAL fluid yielded ≥10⁵ cfu/ml *S. pneumoniae*. This group included 18 men and six women of mean age 40.
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Barbitone buffer (pH 8.6) and a constant current of 20 mA was applied for 45 minutes at room temperature. Slides were assessed immediately after electrophoresis for visible bands. A positive control was prepared by extracting a sonicated suspension of *Streptococcus pneumoniae* with ethanol and redissolving the precipitate in saline phosphate buffer.

Latex agglutination

The Wellcogen ZL22 kit (Murex Diagnostics, Dartford, UK) was used following the manufacturer's instructions. BAL fluid samples were thawed and immersed in boiling water for five minutes; 20 μl BAL fluid was mixed with reagents on an appropriate card. Agglutination was assessed with the naked eye three minutes after mixing.

Statistical analysis

Sensitivity was defined as the number of positive countercurrent immunoelectrophoresis or latex agglutination tests divided by the number of tests in confirmed *Streptococcus pneumoniae* pneumonia. Specificity was defined as the number of negative countercurrent immunoelectrophoresis or latex agglutination tests divided by the number of tests in pneumonia of other aetiology.

Results

*Streptococcus pneumoniae* was detected in sputum by Gram staining and culture in five and eight cases respectively of the 18 patients with pneumococcal pneumonia. In the remaining six patients sputum could not be obtained. Blood cultures yielded *S. pneumoniae* in six of the 24 patients and serum countercurrent immunoelectrophoresis was positive in only one patient, whereas pneumococcal antigen was detected in BAL fluid in 12 patients by countercurrent immunoelectrophoresis and in 13 patients was detected by latex agglutination, yielding a sensitivity of 50% and 54% respectively (table).

In none of the patients with pneumonia caused by other known aetiological agents was pneumococcal antigen detected, yielding a specificity of 100% for pneumococcal infection. Furthermore, in all 13 patients with interstitial lung disease studied as controls negative results were obtained.

In patients with pneumonia of unknown aetiology pneumococcal antigen was detected in BAL fluid from four of eight cases by countercurrent electrophoresis and in nine of 17 cases by latex agglutination. All these patients had negative results by sputum Gram stain and culture, blood culture, and serum countercurrent electrophoresis.

Discussion

Various methods have been used to detect pneumococcal antigen in serum, sputum, urine, and pleural fluid from patients with pneumonia, though there are few data on the value of...
Results of antigen detection in BAL fluid and conventional cultures from patients with community acquired pneumonia*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of positive cases/no. tested (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sputum Gram stain</td>
</tr>
<tr>
<td>Pneumococcal pneumonia (n = 24)</td>
<td>5/18 (28)</td>
</tr>
<tr>
<td>Pneumonia of other known aetiology (n = 18)</td>
<td>0/18</td>
</tr>
<tr>
<td>Pneumonia of unknown aetiology (n = 17)</td>
<td>0/14</td>
</tr>
</tbody>
</table>

CIE = countercurrent immunoelectrophoresis; LA = latex agglutination.

* Patients classified according to BAL quantitative cultures.

detection in BAL fluid. Our results show that pneumococcal capsular antigen can be detected in BAL fluid from patients with pneumonia, and that latex agglutination and countercurrent immunoelectrophoresis have a comparable sensitivity. We suggest that latex agglutination is the technique of choice because it is faster to perform and requires less equipment than countercurrent immunoelectrophoresis.

We have previously established the reliability of quantitative cultures of BAL fluid for the aetiologic diagnosis of pneumonia in patients with moderately severe infection,\(^\text{11}\) although detection of pneumococcal antigen occurred in only 54% of patients with established pneumococcal infection, a sensitivity lower than in sputum or pleural fluid.\(^\text{12,13}\) We do not know the reasons for this discrepancy. There was no relation between the number of colony forming units and the results of latex agglutination. Performing antigen detection immediately after BAL and not in frozen samples as was the case in the study might improve antigen detection.

In our series pneumococcal antigen detection in BAL fluid was highly specific, with no detection of antigen in patients with pneumonia of other known aetiology, or in the control subjects with interstitial lung disease. The specificity obtained was better than that reported for sputum\(^\text{14}\) and may result from contamination of sputum with oropharyngeal secretions causing false positive results.

In up to 50% of patients with community acquired pneumonia the microbial aetiology is never determined despite extensive studies. It has been postulated that other agents causing pneumonia remain to be discovered.\(^\text{15}\) An alternative suggestion is that most patients with pneumonia of unknown aetiology have pneumococcal pneumonia.\(^\text{16,17}\) Our results are in accord with the latter explanation since 53% of our patients with pneumonia of unknown aetiology had pneumococcal antigen detected in BAL fluid – a similar proportion to that of patients with pneumococcal pneumonia.

The clinical implications of our results suggest that patients with pneumonia first undergo non-invasive investigations – for example, blood culture and sputum Gram staining and culture. If patients do not produce sputum, as happens in up to half the cases, the severity of illness needs to be determined. In mild cases an antibiotic for the pathogen considered the most likely aetiologic agent may be prescribed on an empirical basis. In more severe cases fiberoptic bronchoscopy with quantitative cultures of BAL fluid and antigen detection may be of value for establishing pneumococcal aetiology with accuracy. Antigen detection in BAL fluid may be of value, not only for the patients with pneumococcal pneumonia and positive cultures, but also for more than half the patients whose cultures will not be diagnostic.

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