

Pneumococcal antigen detection in bronchoalveolar lavage fluid from patients with pneumonia

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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 fiberoptic 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.¹⁻⁴ 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^{3,5} 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^{6,7} and latex agglutination⁸ 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.^{5,7-10} 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 fiberoptic 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.¹¹ We therefore investigated the detection of *Str 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 fiberoptic bronchoscopy with BAL before starting antibiotics to ascertain the aetiology of pneumonia.¹¹ 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 fiberoptic 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.¹²

Patients were subsequently reclassified into three groups. (1) Pneumococcal pneumonia (n = 24) was diagnosed if blood cultures yielded *Str pneumoniae* or cultures of BAL fluid yielded $\geq 10^3$ cfu/ml *Str pneumoniae*. This group included 18 men and six women of mean age 40

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(range 18–88) years. Of these patients 10 were alcoholics, eight were smokers, three had chronic obstructive pulmonary disease (COPD), and one each had diabetes, asthma, gallstones, chronic renal failure, and long term use of corticosteroids. Two patients had had influenza in the previous two weeks and seven patients had no associated illness. Blood cultures were set up on admission and, when possible, a sputum sample was obtained for Gram stain and culture. All underwent fiberoptic bronchoscopy and BAL to obtain samples for quantitative culture.¹¹ (2) Pneumonia of other known aetiology occurred in 18 patients with isolation of a non-pneumococcal pathogen at counts $\geq 10^3$ cfu/ml from BAL fluid. Of these patients four had *Haemophilus influenzae* pneumonia (three had COPD) and one had *Haemophilus parainfluenzae* pneumonia. The remaining patients had non-resolving pneumonia and the organisms recovered were *Staphylococcus aureus* in four, Gram negative bacilli in six (one each of *H influenzae*, *Hafnia alvei*, *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus*, *Enterobacter* sp, and *K pneumoniae* plus *A calcoaceticus*), *Candida* sp in two, and *Staph epidermidis* in one. All were initially undiagnosed and were receiving antibiotic treatment when fiberoptic bronchoscopy and BAL was carried out. (3) Pneumonia of unknown aetiology occurred in 17 patients in whom no positive microbiological results were obtained including blood culture. Three of these patients did not expectorate, in five patients sputum culture yielded normal flora, and the remaining nine had non-resolving pneumonia with negative diagnostic tests on admission.

A control group of 13 patients with interstitial lung disease underwent fiberoptic bronchoscopy as part of their routine investigations. These patients had neither clinical nor radiographic signs of pneumonia, and BAL fluid cultures were negative or yielded normal flora with low colony counts.

All patients gave informed consent.

BRONCHOSCOPY

The bronchoscope was wedged in the most affected site according to the chest radiograph and 180 ml of sterile normal saline was instilled and gently aspirated.¹¹ The recovered fluid was pooled and quantitative cultures were performed under aerobic and anaerobic conditions.^{13,14} In addition an aliquot of BAL fluid was stored at -20°C until assayed for pneumococcal antigen.

ANTIGEN DETECTION

Countercurrent immunoelectrophoresis

Glass slides (6.9×6.9 cm) were coated with 7 ml of 0.75% agarose (Merck) in barbitone buffer 0.05 mmol/l (pH 8.6).¹⁵ Two rows of paired wells, each 4 mm in diameter, were punched immediately before use. Ten μl anti-serum (Omniserum, Statens Seruminstitut, Copenhagen, Denmark) was placed on the anode side and 10 μl BAL fluid was placed in a paired well. Slides were arranged in an electrophoretic chamber containing 500 ml

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 *Str 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 *Str 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 known aetiology.¹⁶

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 *Str 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*

Diagnosis	No. of positive cases/no. tested (%)					
	Sputum Gram stain	Sputum culture	Blood culture	Serum CIE	BAL antigen	
					CIE	LA
Pneumococcal pneumonia (n = 24)	5/18 (28)	8/18 (44)	6/24 (25)	1/24	12/24 (50)	13/24 (54)
Pneumonia of other known aetiology (n = 18)			0/18	0/5	0/5	0/18
Pneumonia of unknown aetiology (n = 17)	0/14	0/14	0/17	0/5	4/8 (50)	9/17 (53)

CIE = counter-current 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 counter-current 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 counter-current immunoelectrophoresis.

We have previously established the reliability of quantitative cultures of BAL fluid for the aetiological diagnosis of pneumonia in patients with moderately severe infection,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.^{8,17} 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 this 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 sputum⁸ 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.¹⁸ An alternative suggestion is that most patients with pneumonia of unknown aetiology have pneumococcal pneumonia.^{5,19} 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 aetiological 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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