Evaluation of the Polymerase Chain Reaction Method for Detection of Streptococcus pneumoniae DNA in Pleural Fluid Samples*

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Study objective: Streptococcus pneumoniae is the most frequent causative agent of community-acquired pneumonia (CAP); however, an etiologic diagnosis by traditional techniques can be accomplished in only a small percentage of patients with CAP. Pleural fluid is present in approximately 40% of patients with CAP; therefore, we hypothesized that detection of S pneumoniae DNA in pleural fluid by polymerase chain reaction (PCR) may help to increase the rate of diagnosis of pneumococcal pneumonia.

Design: A prospective study of cases.

Setting: A university hospital in Lleida, Spain.

Patients and methods: One hundred two samples of pleural fluid (51 samples from consecutive adult patients with pneumonia and 51 samples from unselected control subjects) were tested by the nested-PCR method to detect selected pneumolysin gene of S pneumoniae, and the results were compared with those provided by alternative diagnostic methods.

Results: PCR in pleural fluid had a diagnostic sensitivity of 78% in patients with pneumococcal pneumonia, with positive results in 2 of 2 patients (100%) and 5 of 7 patients (71%) who had positive or negative pleural fluid culture findings, respectively. PCR results were also positive in 3 of 24 patients (12%) with pneumonia of unknown etiology and negative in all patients with pneumonia due to microorganisms other than S pneumoniae. Thus, the calculated specificity was 93%. Among control subjects, PCR gave positive results in two cases (4%).

Conclusion: The nested-PCR test, applied to pleural fluid samples from patients with CAP, showed a sensitivity of 78% and a specificity of 93% in the diagnosis of pneumococcal pneumonia.

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Key words: community-acquired pneumonia; pleural fluid; polymerase chain reaction; Streptococcus pneumoniae

Abbreviations: CAP = community-acquired pneumonia; PCR = polymerase chain reaction

Parapneumonic effusion is present in approximately 40% of patients with community-acquired pneumonia (CAP).1 In addition to microbiological tests, the biochemical examination of pleural fluid is essential to guide management of patients. In fact, it has been suggested that pleural fluid should be obtained from any patients with CAP in whom a sufficient amount of pleural effusion is detected.2 Certainly, thoracentesis constitutes an unquestionable recommendation, included in all recently published guidelines.3–5 However, pleural space has a close relation to lung parenchyma; consequently, pleural fluid appears to be a very attractive target for etiologic studies of pneumonia. Unfortunately, Gram stain and culture of pleural fluid are relatively insensitive; and antigen detection methods, which showed initial promising results, have not been subsequently validated.6

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Polymerase chain reaction (PCR) is a new diagnostic method that offers many potential advantages: results are positive early in the course of infection, it is unaffected by the prior administration of antibiotics, and it is not dependent on a host response. In addition, the sample can be stored and tested or retested months or years later.7 This test has been efficaciously employed in experimental or clinical studies to detect Streptococcus pneumoniae DNA in a variety of body fluids.8–11 We demonstrated that this test, applied to blood samples, is useful to diagnose bacteremic and nonbacteremic patients with pneumococcal pneumonia.12 However, the usefulness of PCR in pleural fluid has not been extensively evaluated. The purpose of the present study was to analyze, in a group of unselected control subjects and patients with CAP and pleural effusion, the utility of PCR in pleural fluid to diagnose pneumococcal pneumonia.

Materials and Methods

Study Subjects

Over a 3-year period (from January 1998 until the end of December 2000), 261 adult patients with a clinical and radiologic picture suggestive of CAP were admitted at our institution; a sufficient amount of pleural fluid to be aspirated was radiologically identified in 51 patients (19%), and they were enrolled into the present study. In addition, 51 unselected patients with pleural effusion due to other conditions were also included and used as control subjects. Informed consent was obtained from patients, and the study was approved by the ethical and the scientific committees of our institution.

Study Design

Collection and Study of Samples: For all patients, a sample of pleural fluid, obtained by thoracentesis, was bacteriologically identified in 51 patients (19%), and they were enrolled into the present study. In addition, 51 unselected patients with pleural effusion due to other conditions were also included and used as control subjects. Informed consent was obtained from patients, and the study was approved by the ethical and the scientific committees of our institution.

Statistical Analysis

The final diagnosis of cases, according to criteria previously defined, was used as the standard for determining the diagnostic usefulness of the PCR method in terms of sensitivity, specificity, and predictive values. In consequence, patients with pneumonia of unknown etiology and patients with pneumonia caused by microorganisms other than S pneumoniae were considered as cases of nonpneumococcal pneumonia. Control subjects were not included in the statistical analysis.

Results

Patient Characteristics

One hundred two samples of pleural fluid (51 samples from patients with CAP and 51 samples from control subjects) were included in the study. For patients with CAP, mean age was 53 years (range, 20 to 87 years); 33 patients (65%) were men, and 35 patients (69%) had coexisting diseases. Before hospital admission, six patients had received antimicrobial therapy. The amount of pleural effusion, measured as the area of the hemithorax occupied by the collection in basis to the chest radiograph, could be considered as mild (<33%) in 25 patients, moderate (between 33% and 66%) in 22 patients, and massive (>66%) in 4 patients. During follow-up, two patients (4%) required admission to the ICU and five patients (10%) died.

For control subjects, mean age was 60 years (range, 18 to 86 years) and 35 of them (69%) were men. Predominant underlying diseases were neoplasm (15 patients), congestive heart failure (13 patients), and tuberculosis (9 patients). For both groups, relevant adverse events attributable to thoracentesis were not observed.

Etiologic Studies

Pleural fluid culture results were positive in 11 patients (22%) with CAP and pleural effusion, and negative in all control subjects, yielding S pneumoniae in 2 patients, Staphylococcus aureus in 2

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patients, and Streptococcus viridans in 2 patients, as the most frequent pathogens. The study of the 51 patients with CAP and pleural effusion included the following tests: (1) blood cultures were obtained from 49 patients, and results were positive in 11 patients (22%), with isolation of S pneumoniae in 4 patients; (2) a sputum sample for Gram staining and culture was available in 28 patients, results were positive in 5 patients (18%), and S pneumoniae was cultured in one case; (3) PCR on whole blood to detect S pneumoniae DNA was performed in 46 patients, with positive results in 5 patients (11%); (4) antigen detection test in urine for S pneumoniae was done in 12 patients, and results were positive in 1 patient; (5) antigen detection test in urine for L pneumophila was made in 20 patients, and results were negative in all cases; and (6) both serologic samples were obtained in 27 patients, demonstrating one case of Mycoplasma pneumoniae infection.

A diagnosis of pneumococcal pneumonia was made in nine patients, of whom two patients had positive pleural fluid culture result. (A detailed description of microbiological results from these patients with pneumococcal pneumonia is shown in Table 1.) A diagnosis of nonpneumococcal pneumonia was established in 18 cases, with the following etiologies: anaerobic and microaerophilic streptococci (n = 12); S aureus (n = 3); S viridans (n = 3); Streptococcus milleri (n = 1); Enterobacter aerogenes (n = 1); Pseudomonas aeruginosa (n = 1); Escherichia coli (n = 1); Enterococcus faecalis (n = 1); Staphylococcus epidermidis (n = 1); and M pneumoniae (n = 1). For the remaining 24 patients, the etiology was not found.

Results of PCR in Pleural Fluid Specimens for S pneumoniae DNA Detection

PCR findings in pleural fluid were positive in seven of nine patients with pneumococcal pneumonia; findings were positive in both patients with positive pleural fluid culture findings for S pneumoniae, and in five of seven patients with negative culture findings (Table 1). In consequence, the sensitivity was 78%.

PCR findings in pleural fluid were also positive in 3 of 24 patients (12%) with pneumonia of unknown etiology, and negative in all patients with pneumonia caused by nonpneumococcal species. Then, we can calculate a specificity of 93%, a positive predictive value of 70%, and a negative predictive value of 95%. Finally, test results were positive in 2 of 51 patients (4%) with pleural effusion related to other diseases; one patient had a pleural effusion secondary to thoracic traumatism, the other patient had Dressler syndrome, and both patients had no clinical manifestations of active infection.

**Discussion**

The results of the present study showed that PCR on pleural fluid samples can be a valuable test to diagnose pneumococcal pneumonia. The sensitivity and the specificity of the method were 78% and 93%, respectively, in patients with pneumonia, attending to results provided by alternative techniques. In comparison, the sensitivity of pleural fluid culture was only 22%. In addition, the PCR results were positive in 12% of patients with pneumonia of unknown etiology; although these cases were considered as false-positive results, they might certainly represent patients with pneumococcal pneumonia undetected by conventional methods. Unfortunately, a few unquestionably false-positive results were observed in the study of patients without pneumonia.

Prospective studies evaluating the etiology of CAP in adults have failed to identify the cause in 40 to 60% of patients. Blood cultures are very specific, but they have a low sensitivity, and the significance of Gram stain and culture of sputum is uncertain. In this context, pleural fluid, when present, can be

<table>
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<th>Patient No.</th>
<th>Blood Culture Findings</th>
<th>Pleural Fluid Culture Findings</th>
<th>Sputum Culture Findings</th>
<th>Antigen Detection in Urine</th>
<th>PCR in Blood</th>
<th>PCR in Pleural Fluid</th>
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*NA = not available.*
considered as an attractive target to perform etiologic studies for several reasons. First, a significant amount of parapneumonic effusion is detected by chest radiography in approximately 40% of patients with CAP.\textsuperscript{1} Second, in addition to the microbiology, biochemical examination is very useful for guiding therapeutic decisions; therefore, when a clinically important effusion is present, the pleural aspiration should be done routinely. And finally, the technique is not uncomfortable for patients, is associated with a low risk of complications, and always provides a valuable and specific sample, if we exclude a potential contamination by the habitual skin flora. However, the bacteriologic study of pleural fluid, based on Gram stain and cultures, is limited by the lack of sensitivity, particularly when criteria for empyema or complicated parapneumonic effusion are absent.\textsuperscript{14} Therefore, investigations with the use of new and more sensitive tests are needed.

To date, the value of these novel diagnostic tests on pleural fluid has not been sufficiently evaluated. The usefulness of pneumococcal antigen detection in pleural fluid, by latex particle agglutination, was studied by Boersma et al\textsuperscript{6} in 1993; they found, in comparison with culture, a higher sensitivity and a specificity of 92%. Unfortunately, further articles on this subject have not been published. Similarly, studies with PCR to detect \textit{S. pneumoniae} DNA have been assayed on a diversity of samples such as middle ear fluid,\textsuperscript{8} cerebrospinal fluid,\textsuperscript{9} and serum or blood.\textsuperscript{10} We also analyzed the value of the method in samples obtained by transthoracic needle aspiration\textsuperscript{15} and in whole-blood samples from bacteremic and nonbacteremic patients with pneumonia,\textsuperscript{12} with promising results. However, experiences with PCR on pleural fluid samples are very scarce, and are addressed to detect other pathogens such as \textit{M. pneumoniae} and particularly \textit{Mycoplasma tuberculosis}.\textsuperscript{16–18} In fact, studies of pneumococcal pneumonia have not been reported.

A positive Gram stain or pleural fluid culture finding suggests the presence of a complicated parapneumonic effusion; it rarely resolves with antibiotics alone and has a high likelihood of requiring pleural drainage.\textsuperscript{19} We could speculate about the real significance of a positive PCR test result without isolation of the microorganism. Certainly, the threshold number of pneumococci required for PCR to be detectable is considerably lower than culture. Studies have shown that approximately $10^8$ microorganisms per milliliter are required before a culture yields colonies on an agar plate\textsuperscript{20}; for nested PCR, the presence of only 10 cfu/mL can be detected.\textsuperscript{21} Thus, it is not surprising that patients with positive PCR findings in pleural fluid but negative culture results can be successfully managed with more conservative measures.

Although the specificity of the PCR in pleural fluid was high in patients with parapneumonic effusion, we found a 4% rate of positive results among control subjects, whose pleural effusions were caused by diseases other than CAP. The presence of some false-positive results has been previously reported by others, with a prevalence between 4% and 17%,\textsuperscript{9,21–22} as could be the case of our control patients with positive results. We also described, in evaluating whole-blood samples, a similar frequency of false-positive PCR results in control subjects.\textsuperscript{12} This fact, attributable to asymptomatic pneumococcal nasopharyngeal carriage of patients or to contamination of sample or reagents in the laboratory during the processing period, constitutes an important handicap of the test. However, inhibitors can be present in clinical specimens, particularly in blood-containing samples, leading to some surprising false-negative PCR results.\textsuperscript{12,23} Therefore, in the future, technical refinements need to be included in order to eliminate these false results.

**Conclusion**

Our study constitutes a preliminary report showing the potential use of this test in pleural fluid. The high sensitivity observed supports the evaluation of the method in a larger group of patients with pneumonia caused by \textit{S. pneumoniae}.

**REFERENCES**

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