Comparison of Sputum Counterimmunoelectrophoresis and Culture in Diagnosis of Pneumococcal Pneumonia

BARBARA A. DOWNES† AND PAUL D. ELLNER*
Department of Microbiology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Received for publication 3 August 1979

The diagnostic value of counterimmunoelectrophoresis performed on sputum was compared with that of sputum culture. The detection of pneumococcal polysaccharide in sputum showed a better correlation with the presence of pneumococcal pneumonia than the recovery of pneumococci by culture. The authors conclude that sputum counterimmunoelectrophoresis can provide diagnostic guidance to physicians awaiting the results of sputum culture and aid in the interpretation of cultural findings.

It is sometimes difficult to definitely assign pneumococcal etiology to clinical pneumonia. The presence of pneumococci in a sputum culture may reflect infection or colonization of the upper respiratory tract. Conversely, a sputum culture that is negative for pneumococci does not rule out pneumococcal pneumonia.

By counterimmunoelectrophoresis (CIE), Perlino and Shulman were able to detect pneumococcal polysaccharide in the sputa of patients with pneumococcal pneumonia (16). Their study indicated that polysaccharide detection was more rapid, more sensitive, and more specific than sputum culture in diagnosing pneumococcal pneumonia. The present study compares two techniques of CIE with sputum culture results and correlates findings with the clinical diagnosis.

MATERIALS AND METHODS

Sputum specimens from inpatients or patients presenting at the Outpatient Clinic of Presbyterian Hospital during February, March, and April were submitted to the Bacteriology Laboratory of the Clinical Microbiology Service.

All specimens were expectorated sputa and were received in Sputum Collection Systems (BBL Microbiology Systems). Upon arrival in the laboratory, sputa were liquefied by the addition of an equal volume of 10% dithiothreitol solution (Sputolysin, Calbiochem). Specimens were mixed in a Vortex mixer and permitted to stand for 15 min at room temperature. This homogenization procedure has been used for many years and has been shown not to interfere with the recovery of pneumococci. Immediately after mixing, a loopful was removed and used to prepare a smear for Gram stain. Specimens that did not contain any polymorphonuclear leukocytes were rejected, and a repeat specimen was requested.

† Present address: Roche Diagnostics, Nutley, NJ 07110.

Sputum culture. The homogenized sputum was streaked to the surface of colistin-nalidixic acid agar with 5% sheep blood, chocolate agar, and eosin-methylene blue agar (Scott or BBL). Cultures were incubated in 5% CO2 at 35°C for at least 18 h. Catalase-negative colonies of gram-positive cocci resembling Streptococcus pneumoniae were identified by the bile solubility test. Growth was categorized as few (less than 10 colonies), moderate (10 to 100 colonies), many (more than 100 colonies), or heavy (confluent growth). Specimens from which pneumococci were recovered were frozen and stored at -20°C.

Sputum CIE. CIE was performed by using a Hyland Electrophoresis Unit consisting of disposable base units, electrodes, and wicks. Barbitral buffer, pH 8.6, was used to prepare the agar and perform the procedure. The homogenized sputum was placed in the well nearest the cathode, and the anodic well was filled with polyvalent pneumococcal antiserum (Omniserauming, Statens serum Institut, Copenhagen). The system was run at room temperature for 1 h at 30 mA. Slides were examined immediately for the presence of precipitin lines.

Specimens negative by the above procedure were retested by using type 3 pneumococcal antiserum (Statens Serum Institut). These specimens were also run against the polyvalent antiserum, using agar gel prepared with pH 6.6 barbitral buffer, as described by El-Refaie and Dulake (8).

Protocol. During the first phase of the study, CIE was performed on sputum specimens from which S. pneumoniae had been cultured. Sputa giving negative results with pH 6.6 buffer and polyvalent antiserum were retested against type 3 antiserum and at pH 6.6. Charts were reviewed on patients whose sputa revealed S. pneumoniae by culture. The clinical diagnosis was supported by X-ray findings and Gram stain of the sputa and was the criterion used to assign patients to the diagnostic groups.

During the latter phase of the investigation, CIE at pH 8.6 against polyvalent antiserum was performed on random sputum specimens before the cultural re-
sults were known. The results from these random specimens were used to calculate the sensitivity (percent positive test results in the presence of disease), specificity (percent negative test results in the absence of disease), efficiency (percent of results that are true), and the predictive values (percent of positive test results that are diagnostic) for sputum culture and CIE as described by Galen and Gambino (9), using an estimated prevalence for pneumococcal pneumonia of 87 per 100,000.

RESULTS

*S. pneumoniae* was recovered from 218 (13%) of 1,680 sputum specimens. A total of 100 of these culture-positive specimens (46%) were also positive by CIE at pH 8.6 with polyvalent antiserum. Of the 118 specimens that gave negative CIE at pH 8.6, 9 gave positive CIE results when retested at pH 6.6. None of the 118 specimens gave positive results when tested against type 3 antiserum.

An additional 82 random sputum specimens were examined by CIE before the availability of the cultural results. Six of these specimens gave positive results by CIE; *S. pneumoniae* was subsequently cultured from three of them. Two of these three CIE and culture-positive specimens came from patients with pneumonia; the third patient had chronic obstructive pulmonary disease. The three CIE-positive, culture-negative specimens came from patients with diagnoses of pneumonia, viral pneumonia, and cholecystitis.

Pneumococci were recovered from 6 of the 76 CIE-negative specimens. None of these patients had pneumonia. Of the 70 CIE-negative, culture-negative specimens, 11 came from patients with diagnoses of pneumonia or questionable pneumonia, whereas the remainder had diagnoses unrelated to pneumonia.

Charts were examined for 188 of the 218 patients whose sputum cultures grew out *S. pneumoniae*. Table 1 shows the CIE results for the various clinical diagnoses associated with these patients. There is a high correlation between documented pneumococcal pneumonia and positive CIE results.

The correlation of the quantity of pneumococcal growth and CIE results is shown in Table 2. The probability of obtaining positive CIE results would appear to be related to the numbers of pneumococci present in the sputum specimen. However, as shown in Table 3, there does not appear to be any relationship between the quantities of pneumococcal growth on the primary plate and the clinical diagnosis.

The estimated relative values for sputum culture and CIE in the diagnosis of pneumococcal pneumonia are shown in Table 4.

### DISCUSSION

The direct Gram stain and culture of sputum have long been the mainstay of the laboratory diagnosis of pneumococcal pneumonia. A positive Gram-stained smear of sputum may strongly suggest the presence of pneumococci, but will miss 38% of the specimens containing *S. pneumoniae* (17). Direct Gram stains may be difficult to evaluate because of the presence of mouth organisms morphologically resembling pneumococci. The use of the direct quellung reaction of sputum specimens makes identifica-

### TABLE 1. CIE results and diagnosis on 188 culture-positive specimens

<table>
<thead>
<tr>
<th>Clinical diagnosis (no.)</th>
<th>CIE positive (%)</th>
<th>CIE negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable pneumococcal pneumonia (17)*</td>
<td>14 (82)</td>
<td>3</td>
</tr>
<tr>
<td>Non-pneumococcal pneumonia (48)*</td>
<td>23 (48)</td>
<td>25</td>
</tr>
<tr>
<td>Diagnoses other than above (123)</td>
<td>45 (37)</td>
<td>78</td>
</tr>
</tbody>
</table>

* Three of these were bacteremic.
* Aspiration pneumonia, necrotizing pneumonia, or viral pneumonia.

### TABLE 2. CIE results on 218 culture-positive sputa

<table>
<thead>
<tr>
<th>Pneumococcal growth on primary plate</th>
<th>Specimens</th>
<th>CIE positive (%)</th>
<th>CIE negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Few</td>
<td>3</td>
<td>1 (33)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>107</td>
<td>40 (37)</td>
<td>67</td>
</tr>
<tr>
<td>Many</td>
<td>82</td>
<td>42 (51)</td>
<td>40</td>
</tr>
<tr>
<td>Heavy</td>
<td>26</td>
<td>17 (65)</td>
<td>9</td>
</tr>
</tbody>
</table>

### TABLE 3. Amount of pneumococcal growth obtained on primary plates with CIE-positive sputa

<table>
<thead>
<tr>
<th>Clinical diagnosis (no.)</th>
<th>Growth on primary plate (% of specimens)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Few</td>
</tr>
<tr>
<td>Probable pneumococcal pneumonia (14)</td>
<td>0</td>
</tr>
<tr>
<td>Non-pneumococcal pneumonia (23)*</td>
<td>0</td>
</tr>
<tr>
<td>Diagnoses other than above (45)</td>
<td>2</td>
</tr>
</tbody>
</table>

* Aspiration pneumonia, necrotizing pneumonia, or viral pneumonia.

### TABLE 4. Estimated comparative values for sputum culture and CIE in the diagnosis of pneumococcal pneumonia

<table>
<thead>
<tr>
<th>Determination</th>
<th>Sputum culture (%)</th>
<th>CIE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>50</td>
<td>82</td>
</tr>
<tr>
<td>Specificity</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>Efficiency</td>
<td>50</td>
<td>66</td>
</tr>
<tr>
<td>Predictive value</td>
<td>8.7</td>
<td>17.4</td>
</tr>
</tbody>
</table>
tion of the organism more definitive, but does not provide reliable information as to the presence of pneumococcal pneumonia.

Pneumococci are normal inhabitants of the upper respiratory tract and may be found in 5 to 60% of the population, depending upon the season (1). The recovery of pneumococci from 13% of the sputum specimens during the months in which our study was conducted is not unusual.

Attempts to distinguish pneumococcal pneumonia from simple colonization by quantitative sputum culture (10, 14) have not proven reliable. Our study has not demonstrated any correlation between the numbers of pneumococci present in the sputum and the presence of pneumonia. The careful selection of sputum specimens by eliminating those lacking polymorphonuclear leukocytes was shown to provide results closely resembling that of transtracheal aspirates (15). A similar system of specimen selection was used in our study. Transtracheal aspirates have not been shown to provide uncontaminated cultures or to increase the yield of pneumococci compared with that obtained by sputum culture (19, 20).

Negative sputum cultures certainly do not rule out pneumococcal pneumonia. Barrett-Connor (2) failed to recover S. pneumoniae from 50% of cases of bacteremic pneumococcal pneumonia. The negative sputum cultures in such cases may be the result of previous antimicrobial therapy (18), the die-off of pneumococci in sputum during transport due to autolysis, or the overgrowth of commensals (13).

The utility of CIE to detect pneumococcal polysaccharide antigen was demonstrated by Coonrod and Rytel (5), who detected the antigen in serum and urine in 20 to 30% of cases (6) and showed the relationship of antigenemia in bacteremic and severe, protracted pneumococcal pneumonia (4). In a similar manner, the detection of pneumococcal antigen in pleural fluid was found to be a useful diagnostic procedure in partially treated intrapleural empyema associated with negative cultures (7, 11). CIE has been performed on the nasopharyngeal secretions of children and is able to distinguish between pneumonia and the carrier state (3). Perlino and Shulman (16) found the detection of pneumococcal polysaccharide in sputum to provide more rapid, specific, and sensitive diagnostic information than sputum culture in pneumococcal infections of the lung. Leach and Coonrod (12) detected pneumococcal antigen in the sputum of 74% of their patients with evidence of pneumococcal pneumonia and found good correlation between the detection of antigen and the degree of certainty of the clinical diagnosis.

Our results indicate a relationship between the number of pneumococci in sputum and the probability of obtaining positive CIE results. A total of 50% of CIE-positive sputa yielded pneumococci; cultures were negative for pneumococci in 92% of CIE-negative specimens.

El-Refaie and Dulake (8) reported a 1.5- to 2-fold increase in CIE sensitivity by running at pH 6.6; we detected only 7.6% additional positives by that system. The system may fail to detect pneumococci of types 7 and 14 because these polysaccharides are neutral and migrate in the opposite direction due to endosmotic flow (3, 8). A total of 82% of culture-positive patients with positive CIE were found to have pneumococcal pneumonia, whereas only 18% of culture-positive patients with negative CIE had that disease. Our study suggests that the predictive value of CIE may be twice that of sputum culture.

The authors do not consider that CIE replaces the Gram stain or culture of sputum. However, the detection of pneumococcal polysaccharide in sputum by this rapid and relatively simple method can assist physicians in the evaluation of sputum culture results or provide them with diagnostic guidance while awaiting those results. The value of CIE in partially treated disease was exemplified by a case of lobar pneumonia in which polysaccharide was detected in three sputum specimens several days after the initiation of penicillin therapy, whereas sputum cultures remained negative.

LITERATURE CITED

SPUTUM COUNTERIMMUNOELECTROPHORESIS


