Counterimmunoelectrophoresis of Urine for Diagnosis of Bacterial Pneumonia in Pediatric Outpatients

Ronald B. Turner, MD, Frederick G. Hayden, MD, and J. Owen Hendley, MD

From the Departments of Pediatrics and Internal Medicine, University of Virginia School of Medicine, Charlottesville

ABSTRACT. Thirty-eight pediatric outpatients with pneumonia were studied by counterimmunoelectrophoresis for the presence of Haemophilus influenzae type b or pneumococcal antigenuria. Of the 38 patients eight (21%) had H influenzae type b antigenuria and two (5%) had pneumococcal antigenuria. H influenzae type b antigenuria was detected more frequently in patients <2 years of age than in older children. Urine counterimmunoelectrophoresis appears to be a useful tool for the etiologic diagnosis of bacterial pneumonia and should facilitate further studies of the epidemiology, pathogenesis, and clinical spectrum of this disease. Pediatrics 1983;71:780-783; counterimmunoelectrophoresis, bacterial pneumonia.

The etiology of pneumonia in children is not commonly determined. Blood culture is a specific but insensitive method for establishing an etiologic diagnosis in pneumonia. Lung puncture is a reliable means of making an etiologic diagnosis, but it is too invasive for routine use. Sputum for Gram stain and culture, commonly utilized for diagnosis in adults, is usually not available for examination in children <5 years of age. The detection of pneumococcal capsular polysaccharide in urine of adults with pneumococcal pneumonia has been found to be specific and more sensitive than blood culture for making an etiologic diagnosis.

In this study counterimmunoelectrophoresis (CIE) was performed to detect the capsular antigens of Streptococcus pneumoniae and Haemophilus influenzae type b in the urine of pediatric outpatients with pneumonia in order to determine the usefulness of this technique in uncomplicated pneumonias in children.

MATERIALS AND METHODS

Patients

Patients with pneumonia who were seen in the pediatric clinics of the University of Virginia Hospital and who were at least 2 months old were eligible for the study. Criteria for the diagnosis of pneumonia included (1) the presence of an infiltrate on chest roentgenogram or rales on physical examination and (2) a temperature ≥38°C or a history of fever. Patients with known chronic lung disease or immunodeficiency were excluded. Patients meeting the criteria for the study were identified by housestaff or attending physicians who either collected the appropriate specimens or notified one of the investigators. Specimens were solicited in October through March 1978/1979, 1979/1980, and 1980/1981. Of the 38 patients studied 26 were enrolled during the winter of 1979/1980. These 26 patients represented 20% of the 128 patients with pneumonia seen in the clinic during this time period.

Collection of Specimens

A throat swab, nasal wash, and urine were collected from each patient after informed consent was obtained. Pharyngeal secretions on the throat swab were inoculated onto sheep blood agar containing gentamicin, 5 μg/mL, for isolation of S pneumoniae.
After 24 hours of incubation at 37°C in a candle extinction jar, colonies tentatively identified by morphology as pneumococci were subcultured onto sheep blood agar for determination of ethylhydrocupreine (Optochin) sensitivity. Isolates were confirmed as pneumococci by quellung reaction with pneumococcal omniserum (Staten Seruminstitut, Copenhagen). Pneumococci were typed by a combinatorial pool method using nine antiserum pools and monospecific antisera.4,5

Nasal secretions from each patient were obtained by a nasal wash with 5 mL of phosphate-buffered saline. The nasal wash and urine specimens were concentrated 50- to 100-fold by ethanol precipitation6 before testing for bacterial antigen with CIE.

CIE

CIE was performed using 3-in x 1-in glass slides coated with 3 mL of 1% agarose (electrophoresis grade, Gibco, Grand Island, NY) in barbital buffer (pH 8.2, ionic strength 0.05). Wells, 3 mm in diameter and 4 mm apart (edge to edge), were filled with 5 μL of the appropriate sample and subjected to electrophoresis with the antigen at the cathodic end. The antisera used were pneumococcal omniserum and burro antiserum against H influenzae type b capsular polysaccharide (provided by Dr John Robbins, Bethesda, Md). CIE is more sensitive when performed with monospecific pneumococcal antiserum than with omniserum7; therefore, specimens that did not have a precipitin line with omniserum or H influenzae type b antiserum were tested with the monospecific pneumococcal antiserum corresponding to the pneumococcal type isolated from the throat. CIE was performed with a constant current of 5 mA per slide for one hour at room temperature. Slides were read for precipitin lines immediately and after at least one hour at 4°C in a moist environment. A few specimens produced diffuse lines that were not characteristic of antigen-antibody precipitin lines and which disappeared when the slides were soaked in saline overnight at 4°C. These specimens were interpreted as negative for bacterial antigen. Approximately 10 ng/mL of H influenzae type b antigen and 250 ng/mL of pneumococcal antigen could be detected by this method.

RESULTS

A total of 38 outpatients, including 19 infants aged 2 to 24 months and 19 children >2 years old, were studied; 13 of the patients, 11 infants and two children, were subsequently admitted to the hospital.

Of the 38 patients, ten (26%) had bacterial antigen detected in their concentrated urine: H influenzae type b antigenuria was detected in eight (21%) and pneumococcal antigenuria was detected in two (5%). One of the two patients with pneumococcal antigen in the urine had a positive CIE with pneumococcal omniserum. The antigenuria in the second patient was detected only when the CIE was performed with antiserum to type 1 pneumococcus, the serotype present in the patient’s pharyngeal secretions.

The type of antigenuria varied with the age of the patient (Figure). H influenzae type b antigenuria was detected in seven (37%) of 19 infants <2 years old but in only one (5%) of the 19 older children, a 6 year old (P = .02, Fisher exact test). The two children with pneumococcal antigenuria were 9 and 10 years old.

Nasal wash specimens from five of the patients with H influenzae type b antigen in the urine were tested for antigen by CIE. H influenzae type b antigen was detected in two of the five nasal washes. No antigen was detected in the nasal secretions from the one patient with pneumococcal antigenuria for whom a nasal wash specimen was available. Nasal wash specimens from 20 patients who did not have detectable antigenuria were tested; pneumococcal antigen was detected in one of the 20 and

![Figure](http://example.com/figure.png)

Figure. Antigenuria in 38 pediatric outpatients with pneumonia.

| Age of Patients | No Antigenuria Detected | Hib Antigenuria | Pneumococcal Antigenuria |
|-----------------|-------------------------|----------------|
| 2-12 mo.        | 15                      | 15             | 15                      |
| 1 yr            | 15                      | 15             | 15                      |
| 2 yr            | 15                      | 15             | 15                      |
| 3 yr            | 15                      | 15             | 15                      |
| 4 yr            | 15                      | 15             | 15                      |
| 5 yr            | 15                      | 15             | 15                      |
| 6 yr            | 15                      | 15             | 15                      |
| 7 yr            | 15                      | 15             | 15                      |
| 8 yr            | 15                      | 15             | 15                      |
| 9 yr            | 15                      | 15             | 15                      |
| 10 yr           | 15                      | 15             | 15                      |
| 11 yr           | 15                      | 15             | 15                      |
| 12 yr           | 15                      | 15             | 15                      |

TABLE. Clinical Features of Pneumonia in 19 Infants <2 Years Old With or Without Haemophilus influenzae Type b Antigenuria*

<table>
<thead>
<tr>
<th>Feature</th>
<th>Antigen Positive n = 7</th>
<th>Antigen Negative n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concurrent otitis media</td>
<td>2/6*</td>
<td>3/11</td>
</tr>
<tr>
<td>Temperature ≥39°C</td>
<td>4/6</td>
<td>6/10</td>
</tr>
<tr>
<td>Respiratory rate ≥40 breaths/min</td>
<td>3/5</td>
<td>7/9</td>
</tr>
<tr>
<td>WBC ≥15,000/μL</td>
<td>5/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>4/7</td>
<td>7/12</td>
</tr>
</tbody>
</table>

* Values shown are number of patients with feature/number with information available.
no H influenzae type b antigen was detected. Blood cultures were performed in ten (26%) of the 38 patients and were negative in nine, including two patients with bacterial antigenuria. One patient with H influenzae type b bacteremia had H influenzae type b antigen detected in the urine.

The infants <2 years old with pneumonia who had H influenzae type b antigenuria could not be distinguished clinically or by roentgenographic findings from those infants without detectable H influenzae type b antigenuria (Table). Total leukocyte counts and absolute band counts were significantly higher among antigen-positive children than among antigen-negative children (P = 0.05, Wilcoxon rank sum test). The mean leukocyte counts were 27,600/μL for antigen-positive and 14,600/μL for antigen-negative patients, and the mean absolute band counts were 4,900/μL and 1,300/μL in the two groups. However, the range of values for the two groups overlapped, limiting the clinical usefulness of both tests.

Two patients with H influenzae type b antigenuria were at opposite ends of the clinical spectrum of pneumonia. One was severely ill with a pleural effusion and a blood culture positive for H influenzae type b. This patient recovered after initial treatment with intravenous chloramphenicol. On the other end of the spectrum, a 7-month-old infant who had had tachypnea for two days was brought to the clinic because of a bloody nasal discharge. The patient was afebrile but had a respiratory rate of 80 breaths per minute with grunting. A chest roentgenogram revealed bilateral infiltrates; the WBC count was 15,700/μL with 16% band forms. This patient was observed without antibiotic therapy and had resolution of symptoms within 24 hours. The patient was discharged from the hospital without antibiotic therapy and recovered uneventfully. The remaining patients with H influenzae type b antigenuria were treated with ampicillin or erythromycin.

**DISCUSSION**

In this study, H influenzae type b or pneumococcal antigenuria was detected in 26% of outpatients with pneumonia. The critical issue, however, is whether bacterial antigenuria establishes that organism as the cause of the pneumonia. In adults, pneumococcal antigenuria has not been detected among: patients with pneumonia who had negative sputum cultures for S pneumoniae; patients with pneumonia known to be caused by other organisms; or patients without pneumonia. Comparable information about the specificity of H influenzae type b antigenuria in the diagnosis of H influenzae type b pneumonia is not available. However, in a recent study in which latex particle agglutination was used to detect H influenzae type b antigenuria, no antigen was detected in urine specimens from 55 adult control subjects or from 38/39 children with infections caused by other organisms. Other studies have also found that H influenzae type b antigenuria is generally absent in patients with pneumonia caused by other organisms, although antigenuria has been detected in two patients with no apparent H influenzae type b infection. On the basis of these studies showing high diagnostic specificity, pneumococcal or H influenzae type b antigenuria in a patient with pneumonia provides strong presumptive evidence of an etiologic association. The sensitivity of bacterial antigenuria in the diagnosis of bacterial pneumonia is not known, however, so a negative urine CIE does not rule out a bacterial etiology.

The identification of eight patients with H influenzae type b antigenuria and only two with pneumococcal antigenuria may be explained by two factors. (1) H influenzae type b antigen was detected at lower concentrations than pneumococcal antigen by the method used in this study, and (2) polysaccharide antigen from pneumococcal types 7 and 14 may not be detected by routine CIE. Although no conclusion can be drawn from this study about the relative frequencies of H influenzae type b and pneumococcal pneumonia in children, a surprisingly high proportion of patients <2 years of age in this study had H influenzae type b antigenuria. The frequency with which antigenuria was detected may be due in part to selection of the more seriously ill outpatients for inclusion in the study.

Detection of bacterial antigen in urine appears to be a useful tool for the etiologic diagnosis of bacterial pneumonia and should facilitate further studies of the epidemiology and pathogenesis of this disease. Furthermore, the clinical spectrum of H influenzae type b pneumonia appears to have been expanded by recognition of the disease in outpatients who were not severely ill as well as in patients with a clinical picture similar to that described in patients hospitalized with H influenzae pneumonia and bacteremia. The utility of this technique in a clinical setting is enhanced by the fact that an etiologic diagnosis may be provided within 24 hours using the method described.

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PERINATAL PATHOLOGIST AS SKEPTICAL INQUIRER

Protagonists in the life-and-death dramas of modern perinatal medicine do not take kindly to carping remarks from pessimists in the wings who are remote from the scenes of action. But, I will argue, such detached adversaries are indispensable. For it has been observed that the evils of controversy are temporary, while the benefits are permanent.

Semanticist Paul Lippert recently pointed out that in many societies there has persisted an irritating group of people who are in the habit of asking more questions than they answer. They are called by various names: artists, philosophers, troublemakers. In the Middle Ages, some of these irksome people caused quite a stir, but enough time has now passed so that we call them scientists. And we can see that the role of scientist is to doubt the certainty of unquestioned beliefs.

As modern medicine struggles to become scientific, the central role of doubt as a driving force has become better appreciated. Scientific medicine is not seen as having the property of closure or finality—a fixed body of undoubted knowledge and a limited set of unquestioned concepts. It is envisioned, rather, as an evolving, open-ended search. This field of human activity, then, is like all others that seek to make sense out of the natural world. The only way we have of detecting our blunders and of harnessing them for useful purposes is by critical scrutiny of knowledge claims. Modern epistemologists emphasize the importance of encouraging pesky doubters. Bronowski advised: “Ask an impertinent question! That is the essence of science.”

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