Counterimmunoelectrophoresis is in the diagnosis of
Hemophilus influenzae Pleural Effusion

Douglas S. Holsclaw, Jr., M.D.,† and David A. Schaeffer, M.D.

A child with a sterile pleural effusion resulting from an infection with Hemophilus influenzae type b (Hi b) is described. The diagnosis was established by use of counterimmunoelectrophoresis (CIE). The alarming increase in incidence of pneumonia due to Hi b is noted, as is the large number of associated pleural effusions. CIE provides a rapid, reliable, and sensitive means by which to establish the exact etiology of such bacterial infections so that optimal antibiotic therapy can be started promptly.

Recent attention has been called to the increasing frequency in children of pneumonia caused by Hemophilus influenzae type b (Hi b). In 1978, Asmar et al1 noted that only 77 cases have been reported in the American literature since 1954, to which they added 43 cases diagnosed in the previous 43-month period. Others have reported an additional 112 cases in the past year.2-4 In Hawaii, over the past five years, Hi b has become the most common cause of bacterial pneumonia in children.5

Pleural effusion is found frequently in pneumonia caused by Hi b with reported incidences of 9, 31, 75 and 30 percent.1-4 However, routine bacterial cultures of pleural fluid obtained by thoracocentesis often fail to grow the organism. Lampe et al5 have shown that analysis of pleural fluid by counterimmunoelectrophoresis (CIE) can provide a presumptive etiologic diagnosis in more than half of samples with negative bacterial cultures. As the following case report illustrates, the use of CIE may increase the diagnostic yield of pleural fluid specimens by identifying the infecting organism.

CASE REPORT

A three-year-old boy was admitted to Hahnemann Hospital because of fever of two weeks’ duration and a left pleural effusion.

He had been well until two weeks before admission, when he fell and struck the right side of his face. The next day he had a fever and a swollen, ecchymotic right eye. The fever persisted, and examination 11 days before admission revealed exudative pharyngitis, which was treated with cephalixin. Throat culture was negative for β-hemolytic streptococci. The fever persisted (39.4° C), and the patient’s appetite and fluid intake decreased.

After two days of cephalixin therapy, he was admitted to another hospital because of pain in his left knee and inability to bear weight on his left leg. An x-ray film of the knee was normal, and a technetium bone scan revealed no evidence of osteomyelitis. Chest x-ray film showed left lower lobe pneumonia with a left pleural effusion. Thoracocentesis was attempted unsuccessfully. Cultures of blood, CSF, and urine were negative. Results of a PPD skin test, gastric aspirate for AFB, heterophile antibodies, and cold agglutinins were negative. His WBC count was 53,000/μm, with a left shift; ESR was 110 mm/hr. Antistreptolysin O titer was 240 Todd units (normal = less than 100). Antistreptococcal deoxyribonuclease B titer was 2750 units (normal = 0 to 60). Treatment included ampicillin, amoxicillin, and methicillin. Because the chest x-ray findings of pneumonia and pleural effusion persisted, on the 14th day of his illness the patient was transferred to Hahnemann Hospital.

Further history revealed that he had an eight-year-old sibling who had developed chickenpox two days before the onset of the patient’s present illness, and a ten-month-old sibling was currently hospitalized with pneumonia of unknown cause. The patient’s father had traveled in the.
caused childhood infections, including pneumonia, empyema, meningitis, epiglottitis, septic arthritis, facial cellulitis, osteomyelitis, and pericarditis. The reason for the increasing incidence of Hi b in childhood pneumonia is not clear. Some possibilities include the increased use of blood cultures in the diagnostic work-up of febrile children with pneumonitis, the relative lack of recognition of Hi b in the past due to masking by prior antibiotic treatment with ampicillin, and failure to utilize the fastidious culturing techniques required to detect this organism. In addition to these relative possibilities, we may be undergoing an absolute increase due to a change in the flora ecology of H influenzae.

Given the high incidence of pleural effusions complicating Hi b pneumonia, another reason for the past lack of appreciation of the frequency of this organism may, in part, be the reluctance of physicians to perform pleural taps in children. This is also true for lung punctures, which others have shown to have an excellent yield with infrequent complications.9-11

The principle underlying CIE is that precipitin lines form at points of antigen-antibody equilibrium under the proper conditions of buffer pH and diffusion media. When an electric field is applied, antigens become negatively charged and migrate toward the anode while the weakly negatively charged antibodies in association with the endosmotic flow of positive buffer ions migrate in the opposite, ie, counter, direction toward the cathode.7 CIE is especially helpful in the rapid presumptive diagnosis of bacterial infection, since the results can be obtained in approximately one hour, thus allowing effective and specific antibiotic treatment to be initiated quickly.12 CIE can provide a specific etiology in the face of a negative bacterial culture and Gram stain, and when prior antibiotic therapy may mask the underlying organism, as noted here. Our patient's condition was puzzling because he had serologic evidence of a recent streptococcal infection, joint swelling and pain suggesting possible rheumatic fever, recent facial trauma, a varicella prodrome, and exposure to a younger sibling with pneumonia and to a father who had traveled to tropical areas. The detection of the antigen to Hi b in the sterile pleural fluid enabled us to select the optimal antibiotic.

Although CIE has been the immunologic method most widely reported for the detection of bacterial antigens, another promising approach is afforded by the use of the latex particle agglutination (LPA) technique. Ward et al13 compared CIE with LPA in the rapid diagnosis of Hi b infections and demonstrated that LPA was quicker, cheaper, more sensi-

**Discussion**

Clinical applications of CIE have aided in the detection of antigens of encapsulated bacteria such as Streptococcus pneumoniae, H influenzae type b, Escherichia coli, and Pseudomonas sp in various body fluids.7 Specifically, CIE has been applied to the diagnosis of various H influenzae-
tive and specific, and technically easier than CIE. LPA does not require a specialized laboratory as does CIE, and a simplified yet effective LPA system may be set up for performance by house officers.

The need for a rapid specific etiologic diagnosis of Hi b pneumonia is underscored by the increasing existence of strains that are resistant to ampicillin. Until the causative organism is revealed to be sensitive to ampicillin, the initial treatment of a serious infection in which Hi b is suspected should also include chloramphenicol. Chloramphenicol is currently the drug of choice for infections caused by ampicillin-resistant isolates of Hi b. However, chloramphenicol-resistant isolates have been described, and failure of chloramphenicol treatment should alert the clinician to this possibility. Cefamandole may prove to be better and safer than chloramphenicol for treatment of ampicillin-resistant Hi b infections, excluding CNS infections. This drug may also prove useful in cases of Hi b that are resistant to both ampicillin and chloramphenicol.

In summary, with the rapid increase in cases of Hi b pneumonia, many of which have associated pleural effusions and the inadvisability of using ampicillin routinely when Hi b is suspected, the need for a quick, reliable, and sensitive test to provide an exact etiology is paramount. CIE would appear to fit this need, and we urge its more widespread use as part of the routine diagnostic tests ordered at the time of performing a pleural tap.

REFERENCES