Diagnosis of Pneumococcal Pneumonia by Antigen Detection in Sputum

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Received for publication 4 January 1978

Pneumococcal polysaccharide was detected by counterimmunoelectrophoresis in the sputum of 20 of 26 (77%) adults with community-acquired pneumonia and a positive sputum culture for *Streptococcus pneumoniae*. The test was negative in 29 pneumonia patients with negative sputum culture for *S. pneumoniae*. Pneumococcal antigen was also detected in the sputum of six of nine adults with chronic bronchitis and a positive sputum culture, but was not detected in expectorated respiratory secretions of 22 pneumococcal carriers with colds. Pneumococcal antigen could also be detected in sputum by immunodiffusion; antigen titers varied from 1:2 to 1:256. These results strongly suggest that the detection of pneumococcal antigen in respiratory tract secretions indicates infection caused by *S. pneumoniae*.

The diagnosis of pneumococcal infection in patients with pneumonia is presumptive when it is based on the presence of gram-positive diplococci on smear and/or isolation of pneumococci on culture of expectorated sputum. Sputum culture may be contaminated by pneumococci that are found in the upper respiratory tract under normal conditions and thus give false positive results (5). A definitive diagnosis of pneumococcal pneumonia requires isolation of *Streptococcus pneumoniae* from pleural fluid, blood, lung, or transtracheal aspirates. Cultures of blood and pleural fluid are relatively insensitive, however, and routine use of lung or transtracheal puncture is impractical.

Counterimmunoelectrophoresis (CIE) has been used to detect pneumococcal polysaccharide in serum and urine in patients with pneumococcal disease (1, 5, 8). Whereas CIE of serum and urine has proven to be highly specific for the detection of pneumococcal polysaccharide, it is not sensitive enough to be useful routinely in the diagnosis of pneumococcal pneumonia. Recently, several studies have demonstrated that pneumococcal antigen could be detected with CIE in the expectorated sputum of patients with pneumonia (6, 9, 11, 13). The specificity of this test for pneumococcal pneumonia has not been conclusively established. In previous reports, control respiratory-tract secretions were collected from noninfected normals or patients with either tuberculosis or bronchitis. Whether secretions from patients with upper-respiratory-tract infections who carry pneumococci in the nasopharynx contain sufficient polysaccharide

to be detected by CIE has not been established. The current study was designed to determine the validity of this test in the diagnosis of pneumococcal pneumonia by examining respiratory-tract secretions from patients with pneumonia and from pneumococcal carriers with upper-respiratory-tract infections without pneumonia.

MATERIALS AND METHODS

Specimen collection. (i) Pneumonia patients. Adults admitted to the University of Virginia Hospital with acute community-acquired pneumonia were studied prospectively from January through September, 1975. Criteria for the diagnosis of pneumonia were an acute illness with fever, productive cough, an infiltrate on chest roentgenogram, and/or evidence of pulmonary consolidation on physical examination. Expectorated sputum, urine, and serum specimens were collected from each patient prior to initiation of therapy. The sputum was cultured for bacteria; sputum, urine, and serum were immediately refrigerated until tested with CIE.

(ii) Pneumococcal carriers with colds. Two hundred adult insurance company workers with acute coryzal infections were studied in an outpatient facility during the same time period. Pharyngeal cultures were obtained early in the cold and examined for the presence of pneumococci. Twenty-two patients (11%) were found to be pharyngeal carriers of pneumococci. A sample of respiratory tract secretions was then obtained from these 22 carriers, none of whom had pneumonia or chronic bronchitis, for testing with CIE.

(iii) Patients with chronic bronchitis. Sputum containing pneumococci was obtained from nine patients admitted to the hospital with chronic bronchitis. Diagnosis in these patients was chronic obstructive pulmonary disease with bronchitis in eight, and bronchiectasis in one. At the time the specimen was ob-

tained, all nine had chest roentgenograms which showed no change from previous examinations; specifically, an infiltrate suggestive of an acute pneumonia was not noted.

Cultures. Sputum specimens from pneumonia patients were inoculated immediately onto sheep blood agar and sheep blood agar containing 5 μ g of gentamicin per ml (3). Pharyngeal swabs from outpatients with colds and from patients with chronic bronchitis were inoculated onto the gentamicin agar alone. Isolates of *S. pneumoniae* were identified with the use of the Quellung reaction with Omniserum (Staten Serum Institute, Copenhagen, Denmark). Pneumococci were typed as previously described by a combinatorial pool method, using nine grouped antisera with confirmation with monospecific antiserum (5).

CIE and immunodiffusion. CIE of expectorated sputum for pneumococcal antigen was performed by a modification of the method of Perlino and Shulman (11). Sputum was mixed with an equal volume of 20% acetylcysteine (Mucomyst; Mead Johnson, Evansville, Ind.), shaken at 37°C for 30 min, and stored at 4°C until CIE was performed. Urine was concentrated 20-fold by ethanol extraction by the method of Coonrod and Rytel (3); serum was tested undiluted. Urine concentrates and serum were stored at -20°C until testing.

CIE of all samples was carried out in 1% agarose in Veronal buffer (pH 8.2, ionic strength 0.05, 25°C) (i) on 2-by-3-inch (ca. 51-by-76-mm) glass slides. Samples and pneumococcal antisera (Staten Serum Institute, Copenhagen, Denmark) in opposite wells, 3 mm in diameter, which were 2 mm apart (edge to edge), were subjected to electrophoresis in a chamber (Shandon Southern Instruments Ltd., Surrey, England) with the specimen at the cathode end. A constant voltage of 15 V was applied for 30 min. Slides were read for precipitin bands immediately and after refrigeration at 4°C for 30 min, 24 h, and 48 h. All samples were tested against Omniserum and against type-specific antisera directed against the serotype isolated from sputum or pharynx. Samples from one patient from whom a type 7 pneumococcus was isolated in sputum culture were tested by immunodiffusion, since type 7 polysaccharide does not move in an electrophoretic field (3).

Ten sputum samples from pneumonia patients that

were positive by CIE, and the nine samples from patients with chronic bronchitis, were also tested against Omniserum and type-specific antiserum, using serial twofold dilutions in immunodiffusion plates (Meloy, Springfield, Va.).

RESULTS

Fifty-four patients with pneumonia were studied, of whom 26 (48%) had positive sputum cultures for *S. pneumoniae* (Table 1). Pneumococcal polysaccharide was detected by Omniserum and/or type-specific sera in sputum from 20 (77%) of these 26 patients. One sputum sample was positive only with type-specific serum. All but one of the specimens that were positive with Omniserum were also positive with homotypic antiserum. Of the six patients with antigen-negative sputum samples, one had pneumococci cultured from a transtracheal aspirate and a positive serum CIE, and one had a positive urine CIE.

None of the patients in the pneumonia group with negative sputum cultures had a positive sputum, serum, or urine CIE. The etiology of the pneumonia in the 29 patients with negative pneumococcal cultures was varied. Ten patients were thought to have aspiration pneumonia on the basis of history; one patient had *Haemophilus influenzae* pneumonia (positive transtracheal aspirate); and in 17 a clear etiology could not be established.

Pneumococcal antigen was infrequently detected by CIE in concentrated urine or in serum from patients with pneumonia and sputum cultures positive for *S. pneumoniae*. Serum CIE was positive in only 1 of 26 (4%) specimens with Omniserum and in only 6 of 26 (23%) with homotypic antiserum. Urine was slightly more sensitive; 5 of 26 (19%) were positive with Omniserum and 7 of 26 (27%) were positive with homotypic antiserum. All positive serum and urine CIE samples were confirmed with type-specific

TABLE 1. Detection of pneumococcal polysaccharide in sputum, urine, and serum by CIE

No. and culture results of patients	No. (%) of patients positive for pneumococcal antigen by CIE								
	Sputum			Urine			Serum		
	Omniserum	Type-specific serum	Total	Omniserum	Type-specific serum	Total	Omniserum	Type-specific serum	Total
Pneumonia									
26 culture positive ^a	19 (73)	19 (73)	20 (77)	5 (19)	7 (27)	7 (27)	1 (4)	6 (23)	6 (23)
28 culture negative	0	0	0	0	0	0	0	0	0
Colds									
22 culture positive ^b	0	0	0	ND ^c	ND	ND	ND	ND	ND

^a *S. pneumoniae* isolated from sputum culture.

^b *S. pneumoniae* isolated from throat culture.

^c ND, Not done.

antibody for the type isolated in sputum culture.

All 10 sputum samples that were positive by CIE were also positive in the immunodiffusion plates to both Omniserum and homotypic antiserum. Titers of antigen in sputum were evenly distributed from 1:2 to 1:256. Each sample had identical titers to both antibody sources.

Respiratory tract secretions from all 22 insurance workers with colds and positive cultures for *S. pneumoniae* were negative by CIE with both Omniserum and homotypic antiserum.

Pneumococcal antigen was detected in the sputum of six of nine patients with chronic bronchitis who had pneumococci in their sputum (Table 2). Although four of these patients had fever and six had increased sputum production, none had radiological evidence of acute pneumonia. Antigen titer was 1:8 in five of the CIE positive samples and 1:128 in one.

DISCUSSION

Several studies have demonstrated a correlation of pneumococcal polysaccharide antigen detected by CIE and *S. pneumoniae* isolated by culture in expectorated sputa of patients with acute pneumonia (6, 9, 11, 13). Recently Leach and Coonrod found antigen in sputum of 29 of 39 patients with evidence of pneumococcal pneumonia, and suggested that the test was more reliable than sputum culture in diagnosing this infection (9). Tugwell and Greenwood (13), who first suggested antigen detection in sputum as a potential diagnostic tool, controlled their observation with broth cultures of nasopharyngeal swabs from healthy volunteers and sputum from patients with tuberculosis. Antigen was detected in one-half of the broth cultures and in 8% of the sputum specimens. El-Rafaie and Dulake (6) found that 16 of 18 patients with lobar pneumonia had antigen detected by CIE, while only

3 had pneumococci isolated by culture. Many of these patients had received antibiotics prior to specimen collection. In addition, 38 of 100 unselected sputum samples submitted for routine AFB culture yielded a few colonies of pneumococci on culture. In only four was antigen detected; however, the clinical status of the donors was not described. Perlino and Shulman found antigen in 18 of 19 patients with "definite" or "probable" pneumococcal pneumonia (11). Antigen was not detected by CIE in saliva from 83 normals; however, only one specimen yielded *S. pneumoniae* on culture. In addition, 5 of 18 patients with clinical evidence of upper respiratory tract infection and pneumococci isolated by culture had antigen detected in respiratory secretions by CIE. This finding raises the possibility that the presence of the pneumococcal carrier state in patients with nonbacterial upper respiratory tract infections might result in antigen detection. Straker et al. (12) and Brimblecombe et al. (2) found that rates of pneumococcal carriage increase after the onset of coryzal illness, suggesting that the number of organisms and possibly the amount of polysaccharide might increase during colds. Our failure to detect pneumococcal antigen in respiratory secretions from 22 pneumococcal carriers with colds indicates that the antigen level remained below the threshold of detection by CIE. It can thus be concluded that detection of pneumococcal polysaccharide in respiratory secretions indicates pneumococcal infection of the respiratory tract. Although not as sensitive as sputum culture in detecting the presence of pneumococci, the apparent specificity of CIE should make it a valuable test for use in clinical diagnosis.

In the current study, pneumococcal antigen in sputum was also detected by immunodiffusion. The broad range in titers observed indicates that a spectrum of antigen concentrations from undetectable (culture positive but CIE negative) to strongly positive (titers to 256) occurs in patients with pneumococcal infections of the lower respiratory tract. We have found titers detected by immunodiffusion of up to 1:8 in pus aspirated directly from the sinus cavity of patients with acute pneumococcal maxillary sinusitis when bacterial counts in the sinuses ranged from 10^6 to 10^8 bacteria per ml of aspirate (personal observation). The concentration of the antigen may be influenced by the patient's state of hydration, the amount of saliva mixed with the sputum, and the bacterial counts in the respiratory tree. All of these factors undoubtedly help to explain why CIE fails to detect antigen in sputum from 5 to 25% of patients with pneumococcal pneumonia.

TABLE 2. *Pneumococcal antigen in sputum from patients with chronic bronchitis without pneumonia*

Patient	Clinical findings		Antigen in sputum		
	Fever ($\geq 38^\circ\text{C}$)	Increased sputum production	Pneumococcal type	CIE	Antigen titer (immunodiffusion)
G.B.	0	+	22	+	1:8
M.C.	0	+	10	+	1:8
G.H.	0	+	22	0	0
M.M.	0	0	23	+	1:8
J.P.	0	+	19	+	1:128
G.R.	+	0	Not typed	0"	0"
R.O.	+	+	23	+	1:8
M.O.	+	+	3	+	1:8
K.K.	+	0	8	0	0

" Only Omniserum was used.

ACKNOWLEDGMENT

We thank Sylvie Cousar for her expert technical assistance.

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