J. clin. Path., 1975, **28**, 118-123

Pneumococcal antigen in lobar pneumonia

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SYNOPSIS This paper describes the value in diagnosis and the clinical implications of the detection of pneumococcal antigen in patients with lobar pneumonia. Ninety-eight patients with lobar pneumonia were investigated. Pneumococcal antigen was detected by counter-current immunoelectrophoresis in the sputum of 79% of patients with purulent sputum, in the serum of 29% of the patients, and in the urine of 54% of the patients. The diagnostic value of counter-current immunoelectrophoresis was not affected by prior antibiotic therapy. Patients with antigenaemia had a higher incidence of complications than those without as shown by an association between antigenaemia and jaundice, diarrhoea, and persistent pyrexia. Antigen persisted in the circulation for at least seven days in half the patients studied, possibly indicating the development of immunological tolerance to the polysaccharide antigen.

Lobar pneumonia continues to be a major medical problem throughout the world, despite the introduction of antibiotics. In many parts of the tropics pneumonia is the commonest cause of admission to hospital adult medical wards (Shaper and Shaper, 1958; Young, 1959; Gove, 1967; Riley, 1973). *Diplococcus pneumoniae* is the commonest causative organism in the tropics but in temperate countries infection with other organisms, which may be penicillin resistant, accounts for an increasing number of cases of lobar pneumonia (Barrett-Connor, 1971). Identification of the causative organism of lobar pneumonia is important in ensuring that an effective antibiotic is given. A certain diagnosis of pneumococcal pneumonia can at present only be made if the organism is cultured from the blood. The presence of numerous Gram-positive diplococci in the sputum is strongly suggestive of pneumococcal infection but is not often found; more usually a mixture of Gram-positive diplococci and other organisms is seen. Culture of sputum in lobar pneumonia is of little value because of contaminating pharyngeal organisms (Laurenzi, Potter, and Kass, 1961). Even when numerous Gram-positive diplococci are seen in the sputum the pneumococcus is not often grown (Lepow, Balassanian, Emmerich, Roberts, Rosenthal, and Wolinsky, 1968) and a sputum isolation rate of only 45% was obtained in a series of patients with proven bacteraemic pneumococcal pneumonia (Fiala, 1969). Transtracheal aspiration offers a useful means of differentiating between upper and lower respiratory tract organisms but is not completely without risk (Ries, Levison, and Kaye, 1974). Prior antibiotic treatment is a common cause of failure to make a definitive bacteriological diagnosis in patients with pneumonia (Spencer and Philp, 1973).

In 1917 Dochez and Avery demonstrated the presence of type-specific polysaccharide antigen in the serum of patients with pneumococcal pneumonia using precipitin tubes. Interest in this finding has been reawakened by the discovery that small amounts of polysaccharide bacterial antigens can readily be detected in biological fluids by counter-current immunoelectrophoresis (CIE). Pneumococcal antigen can be detected by counter-current immunoelectrophoresis in the cerebrospinal fluid of patients with pneumococcal meningitis (Coonrod and Rytel, 1972; Whittle, Tugwell, Egler, and Greenwood, 1974) and in the serum and urine of patients with pneumococcal lobar pneumonia (Dorff, Coonrod, and Rytel, 1971; Coonrod, and Rytel, 1973). In this study we have compared counter-current immunoelectrophoresis of sputum, blood, and urine with routine tests in the bacteriological diagnosis of lobar pneumonia. We have also studied the clinical implications of pneumococcal antigenaemia in patients with this disease.

Patients and Methods

PATIENTS

All patients with clinical lobar pneumonia confirmed

Received for publication 18 November 1974.

by radiography admitted to Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, during a four-month period were studied. Ninety-eight patients, who were all Nigerians, were investigated. Seventy patients were male and 28 female. Their mean age was 31·7 years with an age range of 10 to 74 years. Six patients had received penicillin injections before admission to hospital. Four patients died.

Forty patients with proven pulmonary tuberculosis and 30 healthy adult Nigerians attending hospital for routine medical examinations acted as controls.

SAMPLES

Sputum, blood, and urine samples were collected from the patients with lobar pneumonia on admission and from the patients with pulmonary tuberculosis shortly after admission. Blood, urine, and nasopharyngeal swabs were collected from the healthy controls on presentation for routine medical examination. Samples for counter-current immunoelectrophoresis were stored at −20°C until tested.

SPUTUM MORPHOLOGY

Sputum from 72 patients was examined immediately after collection by Gram stain. Fifty-three samples were considered purulent as they contained more than 4 white cells per high-power field; the remaining 19 specimens were considered unsatisfactory as they were unlikely to be representative of lower respiratory tract secretions. Purulent samples (by the above criterion) were obtained from all the patients with tuberculosis.

SPUTUM CULTURE

Sputum was cultured on blood agar in 5% carbon dioxide. Blood and pleural fluid samples were cultured on sheep blood agar and inoculated into Todd-Hewitt broth. *D. pneumoniae* was identified by colony morphology, Gram stain, and optochin disc inhibition (inhibition zone greater than 15 mm).

COUNTER-CURRENT IMMUNOELECTROPHORESIS

Counter-current immunoelectrophoresis was carried out in 0·75% agarose using a discontinuous tris-barbital buffer system (Greenwood and Whittle, 1974). Plates were read after electrophoresis for one hour. Serum samples were tested neat and, if positive, at dilutions of 1:10, 1:20, 1:40, 1:80, and 1:160. Sputa and purulent pleural fluids were homogenized with an equal volume of phosphate-buffered saline at pH 7·2 using a Whirlimixer (Fisons); the mixture was centrifuged and the supernatant tested. Urine was initially tested directly. Negative samples were concentrated 5-10 fold with Lyphogel (Gelman-Hawksley) and retested; 10 samples which were still negative were concentrated approximately 50-fold by negative pressure dialysis (UF/US microconcentrator, Biomed Instruments Inc) and then retested.

All samples were tested against Omniserum and type 3 pneumococcal antiserum (Statens Serum Institut, Copenhagen). Omniserum contains antibody activity against 82 pneumococcal capsular serotypes but has only weak activity against type 3. Antigen typing was carried out on positive specimens using group-specific and monospecific pneumococcal antisera (Statens Serum Institut, Copenhagen). The accuracy of typing by this system was confirmed by carrying out parallel tests with counter-current immunoelectrophoresis and the Neufeld-Quellung capsular reaction.

The specificity of counter-current immunoelectrophoresis for the detection of pneumococcal antigen in biological fluids was investigated by testing broth cultures of *Neisseria meningitidis*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli*, *Streptococcus viridans*, and diphtheroids. Precipitin reactions were obtained with two of three broth cultures of *S. viridans* but with none of the others. Reaction with *S. viridans* was abolished by absorbing Omniserum with a concentrated broth solution prepared from several cultures of this organism. Absorbed Omniserum was used for all the investigations described below apart from typing experiments in which non-absorbed monospecific antisera were used. Type 7 and type 14 organisms were satisfactorily detected by counter-current immunoelectrophoresis.

STATISTICS

Comparisons between patient groups have been considered to be statistically significant when these have reached the 5% level.

Results

COUNTER-CURRENT IMMUNOELECTROPHORESIS IN THE DIAGNOSIS OF PNEUMOCOCCAL LOBAR PNEUMONIA

Sputum

Sputa from patients with lobar pneumonia and tuberculosis and nasopharyngeal swabs from healthy controls were examined by routine bacteriological methods and by counter-current immunoelectrophoresis (table I).

A predominance of Gram-positive diplococci was seen in only nine direct smears of purulent sputum from patients with lobar pneumonia although Gram-

	Lobar Pneumonia		Pulmonary Tuberculosis		Controls	
	Number Tested	Percentage Positive	Number Tested	Percentage Positive	Number Tested	Percentage Positive
Gram stain Predominant diplococci	53	17	40	0	—	—
Gram stain Mixed flora including diplococci	53	77	40	43	—	—
Antigen in purulent sputum	53	79	40	8	—	—
Antigen in broth culture of sputum or nasopharyngeal swab	53	66	40	60	30	53

Table I *Counter-current immunoelectrophoresis and bacteriological examination of the sputum of patients with lobar pneumonia and tuberculosis and of nasopharyngeal swabs from controls*

positive diplococci mixed with other organisms were present in 42 of 53 purulent specimens. *D. pneumoniae* was cultured from 25 of 72 sputum specimens from patients with lobar pneumonia.

Pneumococcal antigen was detected by counter-current immunoelectrophoresis in 42 of 53 purulent sputum specimens from patients with lobar pneumonia but in only three of 40 purulent specimens from patients with tuberculosis ($P = 0{\cdot}001$). Specimens from five patients who had received prior antibiotics were all positive. Ten negative sputa were sonicated, frozen and thawed six times and retested; none were positive. Antigen was detected in 35 of 53 Todd-Hewitt broth cultures inoculated with sputum from patients with lobar pneumonia but also in a high proportion of broth cultures from tuberculous sputa and in a high proportion of broth cultures from normal nasopharyngeal swabs (table I).

Blood

Pneumococci were isolated from blood cultures from 20 of 98 patients with lobar pneumonia. Pneumococcal antigen was detected by counter-current immunoelectrophoresis in 27 of 98 initial blood samples from patients with lobar pneumonia (table II) and in a second sample taken from a further patient whose first serum was negative. Antigen was present in the blood of three of six patients who has received antibiotics. Antigen was not detected in any of the sera of 30 healthy controls or of 40 patients with tuberculosis.

Serum samples from 23 patients with initial antigenaemia were retested one week after the start of antibiotic therapy; 12 were still positive. Serum was obtained from five of these 12 patients a week later; all were still positive. Three of these five patients were seen again three weeks after the start of treatment and were found to be still antigen positive.

Urine

Antigen was detected in the urine of 42 patients with lobar pneumonia (table II). Antigen was detected in 30 routine samples, in 11 more samples after concentration with Lyphogel, and in one of 10 further negative samples after negative pressure dialysis. Antigen was found in the urine of five of six patients who had received antibiotics. Antigen was not detected in the concentrated urine of patients with tuberculosis or in the urine of the normal controls.

Pleural fluid

Twelve patients with lobar pneumonia had a pleural effusion; pus was aspirated in eight and straw-

	Lobar Pneumonia		Pulmonary Tuberculosis		Controls	
	Number Tested	Percentage Positive	Number Tested	Percentage Positive	Number Tested	Percentage Positive
Antigen in purulent sputum	53	79	40	8	—	—
Antigen in blood	98	29	40	0	30	0
Antigen in concentrated urine	78	54	40	0	30	0
Antigen in pleural fluid	12	83	—	—	—	—
Antigen in any specimen	98	64	40	8	30	0

Table II *Prevalence of pneumococcal antigen in purulent sputum, blood, urine, and pleural fluid of patients with lobar pneumonia and pulmonary tuberculosis and in controls*

coloured fluid in four. Pneumococcal antigen was detected in all eight purulent specimens and in two of the four straw-coloured samples. All four of the patients with straw-coloured effusions were receiving antibiotics. Pneumococci were detected by routine bacteriological methods in only five of the 12 samples.

SEROTYPING BY COUNTER-CURRENT IMMUNOELECTROPHORESIS

Serotyping of sputum found to contain pneumococcal antigen was carried out by direct counter-current immunoelectrophoresis of homogenized sputum against type-specific antisera. A single precipitin line was obtained with 30 samples and one precipitin line predominated in seven of the remainder. Two antigenic types were detected in five samples. Antigen was detected in sputum and either blood or urine in 29 patients; in 28 the type of the antigen detected in the sputum was the same as that found in the blood or urine. The pneumococcal types found in the sputum of patients with lobar pneumonia are shown in table III. Positive blood, urine, and pleural fluid samples were also typed directly by counter-current immunoelectrophoresis. The antigenic types detected are shown in table III.

Pneumococcal Type	*Sputum*	*Blood*	*Urine*	*Pleural Fluid*	*Total*
1	15	6	15	2	38
2	3		1		4
3	6	8	9	4	27
4	4	1	1		6
5	4	5	4	2	15
6	1			1	2
9	2	2	1		5
10	1	1		1	3
12	2		1		3
19	5	3	4		12
20	2				2
37			1		1
45	1	1	1		3
46	1	1	1		3

Table III *Pneumococcal capsular serotypes detected in the sputum, blood, urine, and pleural fluid of Nigerian patients with lobar pneumonia by counter-current immunoelectrophoresis*

THE PROGNOSTIC SIGNIFICANCE OF PNEUMOCOCCAL ANTIGENAEMIA

Antigenaemia and complications

The presence or absence of pneumococcal antigen in blood or urine at the time of presentation has been correlated with the following clinical features of patients with lobar pneumonia: age, duration of symptoms, number of lobes affected, diarrhoea, pleural effusion, jaundice, persistent pyrexia, leucocytosis, and azotaemia. A significant correlation was found between the occurrence of antigenaemia and jaundice, diarrhoea, and persistent pyrexia.

Antigen titre and prognosis

Initial serum samples from 27 antigenaemic patients gave the following titres: neat—14, 1:10—5, 1:20—2, 1:40—2, 1:80—2, and 1:160—2. Both patients with an initial titre of 1:160 died. Another patient who died had a titre of 1:10; the remaining patient who died did not have detectable antigenaemia. A high antigen titre (1:10 or greater) was found significantly more frequently in jaundiced than in non-jaundiced patients but no correlation was found between initial antigen titre and any other clinical features.

Persistent antigenaemia and prognosis

The clinical features of patients with antigenaemia persisting for longer than a week have never been compared with those of patients with only transitory antigenaemia. Pyrexia persisting for more than three days and jaundice occurred significantly more frequently in patients with persistent antigenaemia than in patients without this feature. However all except one of the 12 patients with persistent antigenaemia made a satisfactory clinical recovery. The one exception was a patient with jaundice complicating right middle lobe pneumonia whose serum antigen disappeared on the sixth day after the start of treatment to reappear three days later. Return of antigenaemia was associated with a return of fever, deepening jaundice, and signs of hepatic encephalopathy. His condition gradually improved and eventually he made a complete recovery (see fig).

Discussion

Bacteriological diagnosis of pneumococcal pneumonia continues to present difficulties and, at present, can be made only by blood culture which is positive

in about one-third of cases. Using counter-current immunoelectrophoresis we have been able to establish a diagnosis of pneumococcal infection in 64% of a series of patients with lobar pneumonia. Counter-current immunoelectrophoresis is a simple technique giving a result within one hour of setting up the test. Counter-current immunoelectrophoresis of sputum proved to be the most valuable diagnostic technique, being positive in 79% of patients with a purulent sputum. Infection with another organism may have been present in the nine patients with purulent sputum in which pneumococcal antigen could not be detected, for, although *D. pneumoniae* is the commonest cause of lobar pneumonia in adult patients seen at Zaria, we have seen patients from this community with lobar pneumonia due to *S. aureus*, *Klebsiella* spp. and *H. influenzae*. Counter-current immunoelectrophoresis is more efficient than Gram stain or culture in distinguishing between upper and lower respiratory trace colonization by pneumococci, perhaps because antigen can only be detected in the sputum if heavy multiplication of pneumococci in the lung is occurring. However, pneumococcal antigen has recently been detected in the sputum of patients with chronic bronchitis (Verhoef and Jones, 1974). The discriminant value of counter-current immunoelectrophoresis is lost if the test is applied to broth cultures, presumably due to the high prevalence of small numbers of pneumococci in the upper respiratory tract of normal subjects.

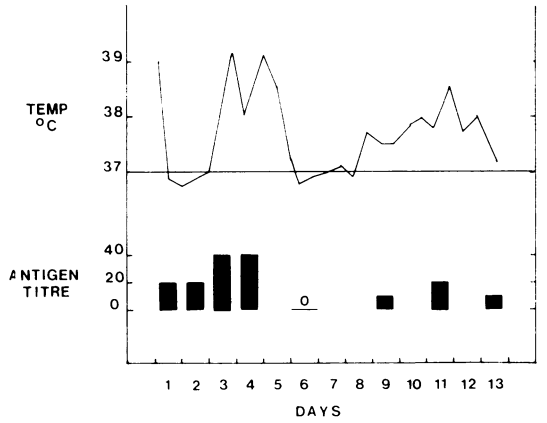

Fig *Antigen titre and pyrexia in a patient with lobar pneumonia*

Twenty-nine per cent of our patients with lobar pneumonia had pneumococcal antigen in the serum and 54% had antigen in the urine, similar figures to those obtained in a series of 30 patients studied in the United States (Coonrod and Rytel, 1973). Ten of our patients with antigenaemia had a negative blood culture (taken before the administration of antibiotics) in contrast to the findings in another study in which all antigenaemic patients had a positive blood culture (Kenny, Wentworth, Beasley, and Foy, 1972). Antigenaemia without a positive blood culture was, however, found by Coonrod and Rytel (1973). It seems likely that antigen may sometimes reach the circulation from bacteria rapidly multiplying within consolidated lung without there necessarily being division of bacteria within the circulation. Unlike conventional bacteriology the diagnostic efficiency of counter-current immunoelectrophoresis is not impaired by administration of antibiotics before presentation at hospital—a common occurrence in developed countries. Antigen was detected in purulent sputum from five of our six patients who had received penicillin before reaching hospital and was found in pleural effusions from patients developing this complication whilst receiving antibiotic therapy.

The type of pneumococcus causing lobar pneumonia is of interest to the clinician because the course of the infection varies with different pneumococcal types (Austrian, 1968). Typing is also of value in epidemiological studies. Serotyping by counter-current immunoelectrophoresis offers a quick and cheap method of typing that does not require isolation of the causative organism. Direct typing of sputum was occasionally difficult because of the appearance of several precipitin lines but this was rarely a problem with serum or urine. In 28 of 29 patients identical serotypes were identified in sputum and in blood or urine suggesting that direct counter-current immunoelectrophoresis of sputum gives a true indication of the type of pneumococcus causing lobar pneumonia in a particular patient. Types 1, 3, and 5 were found most frequently in our patients.

The sensitivity and specificity of counter-current immunoelectrophoresis in the diagnosis of pneumococcal infections are dependent upon the qualities of the antisera used. At present Omniserum, prepared for use in the Neufeld-Quellung reaction, is the most satisfactory reagent available commercially but it does cross-react with some strains of *S. viridans* and should be absorbed with this organism before being used for the diagnosis of pneumococcal infections. The wide range of reactivity of Omniserum has, of necessity, led to some loss of potency and Kenny *et al* (1972) found that only five of 14 sera positive with monovalent pneumococcal antisera gave a reaction with Omniserum. Production of a multivalent antiserum with a high content of precipitating antibody specifically for use in counter-current immunoelectrophoresis might further increase the success rate of this test in the diagnosis of pneumococcal lobar pneumonia.

Previous studies (Dochez and Avery, 1917; Bukantz, DeGara, and Bullowa, 1942; Coonrod and Rytel, 1973) have suggested that the presence of large quantities of pneumococcal antigen in the blood or urine is a poor prognostic sign. Our findings are in agreement with these observations, for the two patients with the highest initial antigen titres died, and a positive correlation was found between antigenaemia and jaundice, diarrhoea, and prolonged pyrexia. Persistent antigenaemia was observed in some of our patients but not as frequently as in a group studied in the United States (Kenny *et al*, 1972). Persistent antigenaemia in pneumococcal infection contrasts with the situation observed in meningococcal infection in which we have never observed persistence of antigen for longer than a week after the onset of the illness (Whittle, Greenwood, Davidson, Tomkins, Tugwell, Warrell, Zalin, Bryceson, Parry, Brueton, Duggan, Oomen, and Rajkovic, 1974). It is possible that antigen is slowly released into the circulation over a period of weeks from sequestered sites in the lungs. However, these findings suggest that in man, as in experimental animals, pneumococcal polysaccharides can induce tolerance. It has been shown that injection of large doses of type 3 pneumococcal polysaccharide into mice leads to the persistence of antigen in the circulation and the production of almost complete immune paralysis (Howard, 1969). However it was not possible to detect tolerance to the antigen at a cellular level. It is possible that initially some free antibody is formed but that this is rapidly complexed with the persistent antigen. It would be interesting to know whether a similar form of tolerance occurs in man and we are currently investigating the antibody response of patients with and without persistent antigenaemia. It is of interest that the apparent development of tolerance to pneumococcal polysaccharide does not prevent a slow but satisfactory clinical recovery.

In spite of the frequent occurrence of antigenaemia in patients with pneumococcal pneumonia immune complex disease, with the possible exception of the nephrotic syndrome (Cameron, 1972), is not a feature of this infection. Pneumococcal disease thus contrasts sharply with meningococcal infection in which antigenaemia is less frequent but often associated with the development of allergic arthritis and vasculitis (Whittle, Abdullahi, Fakunle, Greenwood, Bryceson, Parry, and Turk, 1973) indicating important differences in the immune response of man to meningococcal and pneumococcal polysaccharides.

We wish to thank Mr Moses Damisah for his skilled technical assistance. This study was supported by a grant from the United Kingdom Medical Research Council.

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