A study of the agglutinin response in 40 cases of bacterial pneumonia

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SYNOPSIS Comparison of the results of cultures from blood and sputum with those of agglutinin response in 40 cases of bacterial pneumonia and 36 controls suggests that a provisional identification of causal bacteria, for purposes of antibiotic therapy, may be made by the serological tests with much greater rapidity and comparable accuracy. A combination of these methods is recommended.

The identification of the causal agent in pneumonia is important in the choice of antibiotic therapy. The present methods of identification, by sputum and blood culture, are often unsatisfactory, since in a significant percentage of patients the organism actually responsible for the pneumonia is not detected (Fraser and Pare, 1970).

Pre-admission antibiotic therapy can affect the sputum flora (Spencer and Philp, 1973), making it difficult to judge the significance of a particular organism in the sputum, even when it is present in large numbers; this is especially true of Gram-negative bacteria. Failure to isolate a specific bacterial pathogen from sputum culture usually leads to the supposition that the infective agent is a virus (Fraser and Pare, 1970).

The isolation of bacteria from the blood of pneumonia patients is usually considered to be sufficient evidence for the identification of the causative organism (Fraser and Pare, 1970; Reimann, 1971) but this technique is not always satisfactory, since less than half of all bacterial pneumonias are accompanied by bacteraemia (Holmes, 1956; Fisher *et al*, 1958; Morse *et al*, 1967; Reimann, 1971), and the result may take several days to obtain.

A rising antibody titre is indicative of active infection, and is a major diagnostic criterion in many diseases, but has not been extensively applied to the diagnosis of respiratory infections.

Reimann (1971), however, states that *Haemophilus influenzae* pneumonia can be reliably diagnosed only by the detection of agglutinins in the serum of convalescent patients. Doggett *et al* (1965) found

that agglutinin titres in excess of 1/32 to *Pseudomonas aeruginosa* were uncommon in controls although titres in excess of 1/1000 could be observed in the sera of patients with systemic infections, although Gaines and Landy (1955) reported that the failure to detect agglutinins to *Ps. aeruginosa* in normal human sera was due to unsuitable technique. Lund (1960), by contrast, considered that tests for precipitins against specific polysaccharides of *Streptococcus pneumoniae* were not useful in diagnosis.

The purpose of this study was to compare the results of sputum and blood culture with the presence of specific agglutinins to various bacteria in the sera of pneumonia patients, and thus to examine the value of the agglutination test as an aid to the diagnosis of bacterial pneumonia.

Methods

SUBJECTS

Forty patients were examined; these had been admitted to Selly Oak Hospital, Birmingham between January 1972 and March 1973 and were tested by us after pneumonia had been diagnosed from clinical and/or radiological evidence.

The control group consisted of 36 volunteer subjects (27 male, 9 female) between the ages of 21 and 75 years, with an average age of 48 years. None of the control subjects had suffered clinically apparent infection of any kind for a period of six months previously.

CULTURAL METHODS

Sputum digested with pancreatin buffer by the method of Rawlins (1953), prior to plating, was cultured on nutrient agar (Oxoid), horse-blood agar, and Fildes agar, prepared according to Cruickshank

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(1968) but with horse-blood in place of sheep blood.

Cultures were incubated at 37°C, both in normal atmosphere and with 10% added carbon dioxide.

Blood was cultured in triplicate bottles in two media: 0.1% glucose broth, and cooked meat broth, both with 0.05% Liquoid and penicillinase added, prepared according to Cruickshank (1968).

ANTIBIOTIC SENSITIVITIES

Antibiotic sensitivities were assessed by impregnated discs (Mast Laboratories) and controlled with organisms of known sensitivities (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418, and *Ps. aeruginosa* NCTC 10662) according to the recommendations of Stokes (1968) and Garrod and Waterworth (1969). All tests were carried out on diagnostic sensitivity test agar (Oxoid).

SEROLOGY

Sera were stored at -20°C without preservative. Antigens of *Staph. aureus* NCTC 6571, *Klebsiella pneumoniae* NCTC 9633, *Ps. aeruginosa* composite of Habs serotypes 3 and 6, *H. influenzae* composite of rough strains of Pittman capsules of types a-f, and *Str. pneumoniae* composite of types I, II, and III were prepared and titrated according to the method previously reported (Nicholls *et al.*, 1975).

Sputum and blood samples for culture were taken on admission before the start of hospital controlled antibiotic therapy; serum for agglutinin analysis was taken on days 1, 3, 7, 30, and 60.

Results

Nine patients, three female and six male, two of whom died as a result of the infection, had serological evidence of staphylococcal pneumonia; one was primary staphylococcal pneumonia, the others followed influenza, chronic bronchitis, and congestive cardiac failure. The average stay in hospital of the seven who recovered was ten days. Where possible, antibiotic therapy was given in accordance with the sensitivities of the causative organism; cloxacillin was employed in four cases, a combination of fusidic acid and lincomycin in three, gentamicin in one, and erythromycin in the ninth case. The highest agglutinin titres against *Staph. aureus* (1/1250) were recorded in the fatal cases. Rising titres were observed in five patients, and a maximum titre of 1/250 or more was recorded in all cases. *Staph. aureus* was isolated from the sputa of five patients and from the blood of four.

Str. pneumoniae was diagnosed as the cause of pneumonia in six male and two female patients, all of whom recovered with an average hospital stay of

eight days. Two of these were under 50 and there was no obvious predisposing condition. With one exception, the patients received antibiotic therapy with derivatives of the penicillin group, the eighth receiving tetracycline therapy. Four patients had rising titres to *Str. pneumoniae*; four patients, two of whom had rising titres, had positive blood cultures. Five had positive sputum cultures.

In 11 patients *K. pneumoniae* was diagnosed by serology as the cause of pneumonia, and of these one 62-year old man was admitted twice, the second pneumonia following two months after the first. The group consisted of four women, all over 60 years of age, and seven men aged between 42 and 68 years. All patients had a predisposing condition; in six cases chronic bronchitis was the underlying illness while influenza (two cases), bronchiectasis, and asthma were the predisposing conditions in four other cases. One 70-year-old woman suffered *K. pneumoniae* pneumonia as a sequel to abdominal surgery. The six patients who recovered had an average stay of 12 days in hospital; five infections proved fatal. The variation in the antibiotic sensitivity of *K. pneumoniae* is reflected in the diversity of the antibiotic therapy employed. Septrin was used alone in four cases and as a sequel to ampicillin in two cases; carbenicillin was employed in three cases and gentamicin in two cases, in one case replacing septrin. The agglutinin response to *K. pneumoniae* pneumonia was similar to *Staph. aureus* pneumonia in that the highest agglutinin titres were seen in fatal infections; three patients showed an agglutinin titre to *K. pneumoniae* of 1/1250 at death, while a fourth showed a restricted rise to a titre of 1/250, which over 30 days gradually fell to 1/50. This patient was hospitalized during the whole of this period and showed no clinical improvement at any point during those 30 days. One patient, who suffered two subsequent incidents of *K. pneumoniae* pneumonia, had a titre of 1/1250 in the second response, compared with 1/500 in the first. *K. pneumoniae* was isolated from six patients' sputa and from three patients' blood cultures, but only one yielded it from both.

Ps. aeruginosa was diagnosed as the cause of pneumonia in eight patients, six of whom were male, aged from 31 to 68, and there was only one case of primary pneumonia. Chronic bronchitis was regarded as the predisposing condition in four cases; viral or pneumococcal pneumonia and thoracic surgery preceded *Ps. aeruginosa* infection in the remaining four. Treatment was with carbenicillin or gentamicin although all but one patient had received previous therapy with penicillin, ampicillin or septrin. The average length of hospitalization of the five patients who recovered was 15 days; three died. The agglutinin response in fatal cases was different from

that seen in other fatal pneumonias in that there was a marked fall in titre immediately before death. *Ps. aeruginosa* was isolated from the sputa of three, one of whom, and two other patients, had positive blood cultures. Rising titres were observed in all eight.

Pneumonia caused by *H. influenzae* was diagnosed in four patients, aged from 41 to 71, two of whom were male. Two had histories of chronic bronchitis, one had pneumonia after influenza, and the fourth was a primary infection. Three patients received ampicillin, and the fourth, tetracycline. All patients recovered and were discharged after an average of 15 days. Three had rising agglutinin titres. *H. influenzae* was isolated from the sputa of three of the four and from one blood culture.

The agglutination titres observed in the 36 control subjects are recorded in table I. No patient had a

Causative Organism	No. of Cases	Organism Isolated from		Rising Titre	High Titre 1/250 or more
		Sputum	Blood		
<i>Staph. aureus</i>	9	5	4	5	9
<i>St. pneumoniae</i>	8	5	4	4	7
<i>K. pneumoniae</i>	11	6	3	6	11
<i>Ps. aeruginosa</i>	8	3	3	8	8
<i>H. influenzae</i>	4	3	1	3	4
Total	40	22	14	26	39

Sputum could not be obtained from a total of 11 patients

Table II Summary of culture results and agglutinin responses

(table I), when occasional subjects may have titres of 1/250 without apparent clinical infection. It seems likely that titres of 1/250 or above may be ascribed to a recent infection with that organism. The rate of fall of agglutinin titres in convalescence suggests that titres above 1/250 do not usually persist for longer than 30 days, nor titres above 1/500 for longer than three to seven days. Thus the presence of a raised agglutinin titre, together with clinical signs of infection, can provide information for the identification of an infecting organism from a single serological test. For example, one case of *Str. pneumoniae* pneumonia was diagnosed although *Klebsiella* was cultured from the sputum and the blood culture was negative. Treatment with penicillin was instigated and clinical improvement was noted in 48 hours. It is pertinent that the strain of *Klebsiella* isolated from the sputum was resistant to penicillin and could still be cultured after clinical improvement. Retrospective studies to discover a possible viral infection also proved negative.

High agglutinin levels were frequently found in fatal cases, indicating that the response was nevertheless insufficient to combat the infection. Two distinct types of response to fatal infection are suggested, the first showing rapid rise to a high titre which persisted at the time of death, while the second showed a rapid rise followed by a sharp decline before death. The former type of response is probably the consequence of the host failing to respond rapidly enough to infection while the second type may be associated with a paralysis of the immune system and a gradual removal of antibody by antigen excess (Nicholls, 1972). Antibiotic therapy did not control such infections, although the organisms isolated proved to be susceptible *in vitro* to the serum concentration of antibiotic achieved in the patient.

Titre	Antigen				
	<i>H. influenzae</i>	<i>Str. pneumoniae</i>	<i>Staph. aureus</i>	<i>K. pneumoniae</i>	<i>Ps. aeruginosa</i>
Rising	0	0	0	0	0
Below					
1/25	14 ¹	15	3	16	20
1/25	10	12	6	12	11
1/50	9	6	8	5	3
1/125	3	3	13	3	2
1/250	0	0	6	0	0
Above					
1/250	0	0	0	0	0

¹No. of Subjects

Table I Details of agglutinin response in 36 control patients

rising titre or a high static titre of more than 1/250 to any of the bacteria tested; 90% had titres of 1/50 or less to *H. influenzae*, *Str. pneumoniae*, *K. pneumoniae*, and *Ps. aeruginosa*; titres higher than 1/125 to these organisms were not found in any control; 84% had titres below 1/250 to *Staph. aureus*. The agglutinin titres recorded against *Staph. aureus* in controls were higher than those to any other antigen tested.

In all cases of pneumonia agglutinin titres had fallen to 1/250 or less by day 30. The results of the titrations of sera taken on day 60 were not significantly different from those in the control group, indicating that the agglutinin response subsided rapidly in the post-infection period.

Discussion

Whereas a rising antibody titre can provide evidence of active infection, the implication of a high static agglutinin titre is more difficult to assess. Titres above 1/125 are, in the authors' experience, rarely seen in control subjects except to *Staph. aureus*

A comparison of the serological and culture results (table II) also suggests that the former gives a more reliable identification of the pathogen. The advantages of the serological technique are that blood is often easier to obtain than sputum (sputum could not be obtained from 11 of the 40 patients in this investigation), and that the results can be obtained within two hours. The disadvantages are that only a general indication of antibiotic sensitivity can be obtained from serological results alone. Also, it may be necessary to test several antigens in order to detect the response. However, the technique could provide a rapid bacteriological diagnosis in a great majority of cases of bacterial pneumonias, with at least as great initial accuracy as cultural methods alone. A combination of both techniques would lead to the use of specific antibiotic therapy with less reliance on the use of broad spectrum antibiotics.

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References

- Cruickshank, R. (1968). *Medical Microbiology*, 11th edition, pp. 748-909. Livingstone, Edinburgh.
- Doggett, R. G., Harrison, G. M., Stillwell, R. N., and Wallis, E. S. (1965). Antibody response to *Pseudomonas aeruginosa* infections associated with cystic fibrosis of the pancreas (Abstr.). *South. med. J. (Bghm, Ala)*, **58**, 1595.
- Fisher, A. M., Trever, R. W., Curtin, J. A., Schultze, G., and Miller, D. F. (1958). Staphylococcal pneumonia: a review of 21 cases in adults. *New Engl. J. Med.*, **258**, 919-928.
- Fraser, R. G. and Pare, J. A. P. (1970). *Diagnosis of the Diseases of Chest*, Vol 1, pp. 589-611. Saunders, Philadelphia.
- Gaines, S. and Landy, M. (1955). Prevalence of antibody to *Pseudomonas* in normal human sera. *J. Bact.*, **69**, 628-633.
- Garrod, L. P. and Waterworth, P. M. (1969). Effect of medium composition on the apparent sensitivity of *Pseudomonas aeruginosa* to gentamicin. *J. clin. Path.*, **22**, 534-538.
- Holmes, R. B. (1956). Friedländer's pneumonia. *Amer. J. Roentgenol.*, **75**, 728-747.
- Lund, E. (1960). Laboratory diagnosis of *Pneumococcus* infections. *Bull. Wld Hlth Org.*, **23**, 5-13.
- Morse, L. J., Williams, H. L., Grenn, F. P., Jr., Eldridge, E. E., and Rotta, J. R. (1967). Septicemia due to *Klebsiella pneumoniae* originating from a hand-cream dispenser. *New Engl. J. Med.*, **277**, 472-473.
- Nicholls, A. C. (1972). Agglutinin response in ten cases of *Pseudomonas* pneumonia. (Abstr.) *J. gen. Microbiol.*, **73**, xiv.
- Nicholls, A. C., Pease, P. E., and Green, I. D. (1975). Agglutinin response to bacterial infection in acute exacerbations of chronic bronchitis. *J. clin. Path.*, **28**, 279-283.
- Rawlins, G. A. (1953). Liquefaction of sputum for bacteriological examination. *Lancet*, **2**, 538-539.
- Reimann, H. A. (1971). *The Pneumonias*. A. Hilger, London.
- Spencer, R. C. and Philp, J. R. (1973). Effect of previous antimicrobial therapy on bacteriological findings in patients with primary pneumonia. *Lancet*, **2**, 349-351.
- Stokes, E. J. (1968). *Clinical Bacteriology*, 3rd edition. E. Arnold, London.