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Pneumocystis carinii antibody testing

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SUMMARY Sera from blood donors and patients from all over Scotland were examined by indirect immunofluorescence using *Pneumocystis carinii* antigen from infected rat lung. Antibody was found in 76 of 488 (15·6%) of patients tested on clinical grounds but in only 13 of 148 (8·8%) blood donors. The antibody rates were higher in disease groups likely to have or develop *P carinii* pneumonia: in those with histologically confirmed or strongly suspected *P carinii* pneumonia the rate was 14 of 24 (58·3%); in those who had undergone transplantation eight of 24 (33·3%); in those who were immunosuppressed five of 16 (31·2%); in those who were human immunodeficiency virus antibody (HIV) positive 11 of 43 (25·6%); in those with malignancy 34 of 233 (14·6%); and in those with chest infection 10 of 85 (11·7%). *P carinii* pneumonia was confirmed or likely in four of 45 (8·8%) patients with titres of 1/8–1/16 and in three of seven (42·8%) in those with titres of $\geqslant$1/128. Seroconversion or rising titre was detected in seven of 13 (53·8%) cases of confirmed or likely *P carinii* pneumonia compared with 10 of 93 (10·7%) in other patients.

Diagnosis of *P carinii* infection can therefore be assisted by positive immunofluorescence results, but negative serology does not exclude infection.

Pneumocystis carinii is an important opportunist pathogen. Life threatening pneumonia can occur in patients who are immunocompromised, especially those with cancer or who have undergone transplantation[1] and those with acquired immune deficiency syndrome (AIDS).[2] Conventionally, diagnosis of *P carinii* pneumonia relies on direct demonstration of cysts in bronchoalveolar lavage fluid or biopsy tissue.[3] Better sampling and staining techniques and the development of monoclonal antibodies[4] have improved recognition of the parasite, but it can still be difficult to reach a definitive diagnosis.

Antibody testing provides a simpler method of investigating patients with suspected *P carinii* pneumonia, but reports on its usefulness for diagnosis have varied.[5] In vitro culture of *P carinii* is not routinely available and serology relies on antigens prepared from infected lung, either human lung obtained at necropsy or rat lung from immunosuppressed animals.[6] Although much of the published evidence has not favoured antibody detection as a worthwhile diagnostic procedure, our preliminary results[7] encouraged us to persist. This report records four years' experience of using rat antigen with immunofluorescence to examine sera from patients referred on clinical grounds from hospitals throughout Scotland.

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Material and methods

Specimens were submitted for investigation of *P carinii* from hospitals in Scotland and included HIV positive patients and those with malignancies and transplants. Over four years (1984–1987), 680 sera from 488 symptomatic patients (usually with chest infection or fever) were examined, with serial specimens from 106 patients. This included five patients who had histologically confirmed *P carinii* pneumonia but fell outside the study period. Bronchial lavage fluid, biopsy, or sputum specimens were submitted from only 42 of these patients and were examined by cresyl echt violet[8] (Raymond A Lamb, London) or toluidine blue O[9] (Sigma Chemicals, London). Touch imprints of rat lung infected with *P carinii* were used as controls. *P carinii* pneumonia was histologically confirmed in 15 patients and regarded as highly probable in nine others from whom pathology was unavailable. Serum was also examined from 148 blood donors.

SEROLOGY

Six week old Hooded Lister rats (Harlan Olac Ltd, Bicester, England) weighing 100–150 g were injected subcutaneously twice weekly with 1 ml cortisone acetate (25 mg/ml) (The Boots Company, Nottingham, England) to induce *P carinii* pneumonia. Tetracycline was added to the water supply (500 mg/

Table 1 *Prevalence of Pneumocystis carinii antibody in 488 symptomatic patients and 148 blood donors*

Clinical state	*Total*	*No (%) with immunofluorescence IgG of ⩾ 1/8*
Transplant	24	8 (33·3)
*Immunosuppressed	16	5 (31·2)
HIV positive	43	11 (25·6)
Malignancy	233	34 (14·6)
Chest infection	85	10 (11·7)
No information	87	8 (9·2)
Confirmed or likely *P carinii* pneumonia	24	14 (58·3)
Blood donors	148	13 (8·8)

*Patients receiving steroid or cytotoxic treatment, or immunosuppressed for unspecified reasons

l) to protect against bacterial infection. Animals were killed after six to eight weeks by intraperitoneal injection of pentabarbitone sodium (J M Laveridge, Southampton, England). Impression smears from cut surfaces of each lung were stained with cresyl echt violet[8] or toluidine blue O[9] to assess infection. Tissue was used immediately if possible or stored at −70°C.

IMMUNOFLUORESCENCE

Only heavily infected tissue was processed. Lungs were finely minced, placed in a flask with 20 ml sterile phosphate buffered saline, pH 7·3, containing mixed antibiotics (penicillin, mycostatin, streptomycin 100 units/ml) (PBSM) and agitated for 30 minutes. The supernate was removed and the procedure repeated three to four times. Each extract was washed three times in PBSM, resuspended in 5 ml volumes, stained, and the cysts counted. Multispot slides (Henley, Middlesex, England) were prepared with 50 μl drops of the optimum dilution of rat antigen in PBSM (about 10^6 cysts/ml), air dried, and stored at −20°C. Control slides were prepared from similar extracts of normal rat lung. Sera were tested at dilutions from 1/8 to 1/256 by standard immunofluorescence using incubation periods of 40 minutes at 37°C, first with serum dilution, followed by fluorescein labelled anti-human IgG (Scottish Antibody Production Unit, Carluke, Scotland). A positive control serum with a recommended titre of 1/32–1/128 kindly provided by Dr A J Sulzer, Bureau of Laboratories, Centers for Disease Control, Atlanta, USA, gave a titre of 1/32. Antibody titres of ⩾1/8 were considered to be positive. Sera from patients with confirmed or strongly suspected *P carinii* pneumonia were also examined using fluorescein labelled anti-human IgM (Scottish Antibody Production Unit, Carluke, Scotland).

Table 3 *Correlation of P carinii pneumonia with immunofluorescence titre*

Immunofluorescence IgG	*No of patients*	*Examined for cysts**	*P carinii pneumonia positive† (%)*
Total	488	42	15
<8	412	31	7 (22·6)
8–16	45	5	3 (60)
32–64	24	4	3 (75)
⩾128	7	2	2 (100)
Rising titre/ seroconversion	17	5	5 (100)

*Sputum, lavage, biopsy, or necropsy tissue examined for *P carinii*.
†*P carinii* shown.

Results

P carinii IgG antibody was detected in 13 of 148 (8·8%) blood donors. Patients with clinical symptoms had a seropositive rate of 76 of 488 (15·6%). The antibody prevalence varied when patients were grouped according to clinical condition (table 1). Antibody titres were generally low; seroconversions and rising titres (17 of 106) were uncommon (table 2). Of the 15 cases with histologically confirmed *P carinii* pneumonia (10 with malignancies, three HIV positive, one with a transplant, one immunocompromised), eight (58·3%) were antibody positive. Serial specimens were available from nine of these 15 patients; four

Table 2 *Antibody titres in 488 patients examined for P carinii pneumonia*

	No	*Immunofluorescence IgG (highest titre for each patient)*						
		<8	*8*	*16*	*32*	*64*	*⩾128*	*Rising titre/ seroconversion*
*Examined for cysts**								
Positive	15	7	2	1	2	1	2	5
Negative	27	24	1	1	1	–	–	—
Total	42	31	3	2	3	1	2	5
Clinical evidence only								
Strong	9	3	0	1	1	3	1	2
Insufficient	437	378	14	25	10	6	4	10
Total	446	381	14	26	11	9	5	12

*Sputum, lavage, biopsy, or necropsy tissue examined for *P carinii*.

Table 4 *Published results of testing for Pneumocystis carinii antibodies by immunofluorescence*

Reference	*Antigen source*	*Preparation*	*Groups examined*	*Results and conclusions*
Walzer *et al*, 1974[1]	Human	Cyst suspension, gradient separation	167 healthy subjects, 45 with *P carinii* pneumonia	Titres ⩾1/8 in ≃ 40% *P carinii* pneumonia; false negative results; limited value
Kagan and Norman, 1976[11]	Rat or human	Cyst suspension, gradient separation as [1]	184 healthy subjects, 191 clinical *P carinii* pneumonia	Not sensitive, but titres ⩾1/16 specific; positive in 33% of cases; useful
Meuwissen *et al*, 1977[18]	Human	Cyst suspension pronase treated filtration	281 healthy children, 29 clinical *P carinii* pneumonia children	≃100% children titres ⩾1/40 results at 2 years old; false negative; changes in titre may be useful
Meyers *et al*, 1979[15]	Human	Cyst suspension, gradient separation as [1]	33 marrow transplant/*P carinii* pneumonia	Antibody present in 50% of all patients; changes in titre not helpful
Shepherd *et al*, 1979[12]	Human	Paraffin wax sections	91 healthy subjects, 23 immunosuppressed/*P carinii* pneumonia	Titres > 1/32 or decisive rise suggests *P carinii* pneumonia; confirms diagnosis
Tanabe *et al*, 1985[13]	Rat	Cyst suspension, collagenase treated filtration	100 healthy adults, 13 *P carinii* pneumonia/adults, 25 other pneumonia	94% healthy subjects < 1/20; 90% *P carinii* pneumonia patients > 1/40 but 84% other pulmonary infections ⩾1/40; serial monitoring useful
Elvin *et al*, 1988[4]	Human	Paraffin wax sections	18 HIV/*P carinii* pneumonia	27·7% seropositive ⩾1/20; no diagnostic value

seroconversions and one rising titre were identified. Antibody was detected in six of nine (66%) patients with good presumptive evidence of *P carinii* pneumonia (four with malignancies, one HIV positive, one with a transplant, and three with chest symptoms), five of five (100%) in those with clinical evidence supported by good response to anti-pneumocystis treatment; and one of four (25%) of those with supportive radiological results. Paired sera were tested in four of nine patients and two showed seroconversion. In patients examined for the presence of cysts the correlation with confirmed *P carinii* pneumonia improved at higher titres (table 3). *P carinii* IgM antibody was not detected in any of the sera from patients with confirmed or likely *P carinii* pneumonia.

Discussion

Pneumonia due to *P carinii* almost exclusively affects immunocompromised patients, and despite its increasing importance, diagnosis remains difficult. Cysts have been shown in sputum specimens[4] particularly in patients with AIDS, but biopsy or bronchoalveolar lavage is generally preferred for confirmation of the diagnosis. False negative results can occur due to sampling,[3] or staining failure, and the parasite may also be found in cases of pneumonia due to other causes.[10]

The value of serological testing is controversial but it should not be dismissed too readily. Patients who develop *P carinii* pneumonia as a result of immunosuppression may still make a serological response. Most of the patients with confirmed or likely *P carinii* pneumonia had some cause for immune deficiency (21 of 24) yet more than half had detectable antibody compared with less than 10% of healthy controls. When paired specimens were available, again more than half showed seroconversion or a rising titre. Other studies have also reported a higher incidence of antibody, seroconversion, and rising titres in patients with *P carinii* pneumonia.[11-13] The response may be limited, however, and titres low[12]; in two of our patients a titre of only 1/8 was achieved. We found, like others,[12 13] that testing for specific IgM was not helpful. While this suggests that disease results from reactivation rather than primary infection, immunological deficiencies may also make such patients poor IgM producers.

Most of the patients we studied were not investigated for the presence of cysts in respiratory secretions. In 437 such patients we found antibody in 59 (13·5%); 10 showed seroconversion or rising titre. In one case a rising titre from 1/8 to 1/256 was evident, but clinical evidence subsequently did not support a diagnosis of *P carinii* pneumonia; a presumptive serological diagnosis remained in the nine others. Positive titres or even seroconversions and rising titres have been reported in patients in whom pathological investigations proved negative,[14 15] and we found three seropositive results in 27 such patients. Demonstration of antibody may therefore reflect past infection not active disease, but we did find that higher antibody titres, seroconversions, and rising titres tended to correlate with confirmed and likely infection.

Although serological examination has been extensively used for the diagnosis of *P carinii* pneumonia (table 4), comparison of results is difficult. Patients have differed in age, clinical condition, and immunocompetence and sources and preparation of antigen have varied. The antigenic similarity of rat and human

cysts has been established,[16] but cyst and trophozoite antigens seem distinct.[17] This might explain the widely differing incidence of antibody reported in healthy populations; less than 10% in some studies[11 13] and more than 50% in others.[12 18] Such anomalies have hampered the evaluation of serological testing. An extensive review of serology in the diagnosis of *P carinii* pneumonia recorded conflicting conclusions on its efficacy.[5] A test such as the one described with apparently little background antibody is more likely to have diagnostic value. Three out of seven reported serological studies have similarly concluded that antibody testing could be useful[11-13] while two found high background levels limited test value[1 18] and two found that antibody testing was not helpful.[4 15]

Diagnosis of *P carinii* pneumonia requires further development. Although antibody tests are as yet far from satisfactory, they are relatively simple, and seroconversions and rising titres can provide a presumptive diagnosis. Thus we feel that serology does have a valuable role in investigating possible *P carinii* pneumonia in immunosuppressed patients.

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