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Prevalance of antibodies to 15 antigens of Legionellacae in patients with community-acquired pneumonia

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SUMMARY

Sera from 252 patients with community-acquired pneumonia were examined for the presence of antibodies to 15 antigens of 7 *Legionella* spp. by indirect immunofluorescent antibody testing. The sera had been collected as part of the British Thoracic Society/Public Health Laboratory Service study of community-acquired pneumonia in adults. We also examined sera from 20 patients with gram-negative sepsis.

Using a limited range of antigens of *L. pneumophila*, nine cases of legionellosis were diagnosed in the original study. However, using antigens to other *Legionella* spp., we identified two further cases, caused by *L. micdadei* and *L. gormanii* respectively. Twenty-six other patients had titres of 16 or 32 to one or more antigens, most commonly *L. bozemanii* serogroup 1, *L. micdadei* and *L. dumoffi*. None of the patients with non-legionella pneumonia, however, had significant changes in legionella antibody titres. All of the patients with Gram-negative sepsis had titres of < 16.

INTRODUCTION

The indirect immunofluorescent assay (IFA) has been the reference serological test for diagnosing legionellosis since it was first used in the investigation of the 1976 outbreak of Legionnaire's disease (1). During the following years, a large number of species and serogroups have been described (2) and consequently the number of antigens and antisera necessary for a comprehensive diagnosis of legionellosis had increased. A previous study (3) has shown that over half of the cases of legionellosis may be missed using *Legionella pneumophila* antigens alone for serodiagnosis.

Another problem in early reports was of parallel rises in titre to *L. pneumophila* and *Mycoplasma pneumoniae* (4, 5). This lead to concern about cross reactions between *Legionella* sp. and other microbial antigens. Analysis of *L. pneumophila* antigens by crossed immunoelectrophoresis has demonstrated a number of antigens that cross-react with a wide variety of Gram-negative organisms (6) and indeed false positive reactions appears to be a common problem in patients with cystic fibrosis (7).

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In order to determine the extent of legionellosis caused by non-pneumophila species and to evaluate the degree of serological cross-reactivity with other agents causing pneumonia, we examined sera from patients with community acquired pneumonia for antibodies to 15 legionella antigens.

MATERIALS AND METHODS

Sera from 252 patients with community-acquired pneumonia were examined for the presence of antibodies to *L. pneumophila* serogroups 1–8, *L. micdadei*, *L. dumoffii*, *L. bozemanii* serogroup 1, *L. gormanii*, *L. jordanis* and *L. longbeachae* serogroups 1 and 2 by indirect immunofluorescence using methods previously described (8). The formolized egg-yolk sac antigens were kindly supplied by Professor Fleurette and Doctor Bornstein (Lyons, France). The sera had been collected as part of the joint British Thoracic Society and Public Health Laboratory service (BTS/PHLS) study of community-acquired pneumonia in adults from November 1982 to December 1983, and stored at −20 °C. In the original study, the sera were tested for antibodies to influenza A and B, adenovirus, respiratory syncitial virus, *Chlamydia psittaci* and *Coxiella burnettii* by complement fixation test (CFT), *Legionella pneumophila* serogroups 1–6 by IFA, CFT for *Mycoplasma pneumoniae* and *Mycoplasma* IgM and IgG. Sputum, blood and urine specimens from these patients were also examined. The microbiological methods for diagnosis and the results of the study have been fully reported elsewhere (9). The standard criteria recommended by the Communicable Disease Surveillance Centre for the serological diagnosis of Legionnaires' disease by IFA using the formolized egg yolk sac antigen were used, these are a fourfold rise in titre in paired sera to at least 64 or a single titre of 128 with a relevant clinical history (10).

Paired sera from 20 patients with Gram-negative septicaemia or pneumonia were also examined, as detailed in Table 3.

RESULTS

Two hundred and twenty-eight paired sets of serum and 24 single convalescent samples of sera from the BTS/PHLS study were available for examination. Using the more limited number of antigens, nine patients were diagnosed in the original study as having definite or probable evidence of infection with *L. pneumophila*. The results of testing these patients serum with the wider range of legionella antigens are detailed in Table 1, only titres of $\geqslant 16$ are shown.

Two of the remaining 243 patients had fourfold rises in titre to 256 to *L. micdadei* and 128 to *L. gormanii* respectively, suggestive of legionellosis. In the former case there was also a rise in titre to 16 to *L. bozemanii* serogroup 1, but no rise in titre to any *L. pneumophila* antigens were found. A pathogen had not been identified in either patient in the BTS/PHLS study.

Another 26 patients had titres of 16 or 32 to one or more antigens. Nine of these patients had titres of $\geqslant 16$ to multiple antigens (one patient had titres of 16 to five antigens *L. pneumophila* serogroup 1, 3 and 8, *L. dumoffii* and *L. bozemanii* serogroup 1).

Table 1. *Antibody titres in patients with known Legionnaires' disease*

	Case	Antibody titre			
		L. pn SG1			
1	A	< 16			
	C	256			
		L. pn SG1			
2	A	< 16			
	C	128			
		L. pn SG1			
3	A	< 16			
	C	128			
		L. pn SG1			
4	C	256			
		L. pn SG1			
5	A	< 16			
	C	128			
		L. pn SG1	*L. pn* SG3	*L. pn* SG6	
6	A	< 16	< 16	< 16	
	C	< 16	512	16	
		L. pn SG1	*L. pn* SG6	*L. dum*	
7	A	< 16	< 16	< 16	
	C	512	512	16	
		L. pn SG1	*L. pn* SG6	*L. boz* SG1	
8	A	< 16	< 16	< 16	
	C	128	64	16	
		L. pn SG1	*L. pn* SG6	*L. pn* SG7	*L. boz* SG1
9	A	128	32	32	64
	C	512	32	64	64

A, Acute sera; C, convalescent; *L. pn*, *Legionella pneumophila*; *L. dum*, *Legionella dumoffi*; *L. boz*, *Legionella bozemanii*; SG, serogroup.

Table 2 shows the distribution of convalescent antibody titres in the 241 patients with pneumonias other than those caused by *Legionella* spp. In 21 patients more than one pathogen was identified.

Antibody titres to all 15 legionella antigens were < 16 in the 13 patients with septicaemia and 7 patients with pneumonia caused by Gram-negative organisms (Table 3).

DISCUSSION

Wilkinson and colleagues (3) studied 444 patients with suspected legionellosis and found 14·9% patients with positive IFA titres against one or more of the 29 heat-killed legionella antigens tested. This proportion was more than twice the 6% detected with *L. pneumophila* antigens alone. In the present study one would therefore have expected to find a substantial number of new cases of legionellosis. We identified only two new cases which had not been diagnosed using *L. pneumophila* antigens alone. These findings could be explained by the larger number of antigens used by Wilkinson and colleagues, the different methods of antigen preparation or differences in the population groups examined. They studied a more highly selected group of patients with suspected legionellosis.

A number of workers have reported serological overlap between *L. pneumophila*

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Table 2. *Convalescent antibody titres in non-legionella pneumonias*

Microbial diagnosis (no.)*	Titre	*L. pneumophila* serogroup 1	2	3	4	5	6	7	8	*L. dumoffii*	*L. micdadei*	*L. bozemannii* SG1	*L. gormanii*	*L. jordanis*	*L. longbeachae* SG1	SG2
None (69)	< 16	68	69	68	69	69	69	69	68	67	63	63	69	68	69	67
	16	1	—	1	—	—	—	—	1	2	5	5	—	1	—	2
	32	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
Streptococcus pneumoniae (87)	< 16	87	87	87	87	86	87	87	87	85	86	84	87	87	87	87
	16	—	—	—	—	1	—	—	—	2	—	2	—	—	—	—
	32	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
Mycoplasma pneumoniae (45)	< 16	45	45	45	45	45	45	45	44	45	45	43	45	45	45	44
	16	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
	32	—	—	—	—	—	—	—	1	—	—	1	—	—	—	1
Influenza A *virus* (22)	< 16	22	22	22	22	22	22	22	22	22	22	22	22	21	22	22
	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	32	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Haemophilus influenzae (20)	< 16	20	20	20	20	20	20	20	20	18	20	19	20	19	20	20
	16	—	—	—	—	—	—	—	—	2	—	1	—	1	—	—
Chlamydia psittaci (9)	< 16	9	9	9	9	9	9	9	9	9	8	9	9	8	8	8
	16	—	—	—	—	—	—	—	—	—	1	—	—	1	1	1
Streptococcus spp. (other) (4)	< 16	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Gram-negative (3)	< 16	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Staphylococcus aureus (2)	< 16	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
	16	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Q fever (1)	< 16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total (262)	⩾ 16	1	—	1	—	1	—	—	2	6	9	12	—	4	1	4

* Including multiple diagnosis.

Table 3. *Cases of Gram-negative septicaemia and pneumonia*

Patient number	Organism
Septicaemia	(13 episodes; 3 mixed)
1–3	*Pseudomonas aeruginosa*
4–5	*Escherichia coli*
6	*Proteus mirabilis*
7	*Cardiobacterium hominis*
8	*Klebsiella oxytoca*
9	*Pseudomonas* sp.
10	*Bacteroides fragilis*
11	*Escherichia coli* and *Enterbacter cloacae*
12	*Pseudomonas aeruginosa* and *Escherichia coli*
13	*Enterobacter cloacae* and *Proteus mirabilis*
Pneumonia	(7 episodes)
1–4	*Pseudomonas aeruginosa*
5	*Pseudomonas maltophilia*
6–7	*Klebsiella pneumonia*

and *M. pneumoniae* (4, 5). Wilkinson and colleagues (11) found 22% of sporadic cases of pneumonia with parallel rises in titre to *M. pneumoniae* and *L. pneumophila*. These findings they attributed to a variety of reasons including polyvalent activation of the immune system, an adjuvant effect by legionellae, or possibly dual infection.

In this study we found that only 3 (6·7%) of the 45 patients with mycoplasma pneumonia had developed antibodies to *Legionella* sp. and only with titres to 16 or 32.

Overall 26 patients (10·8%) with non-legionella pneumonias had titres of 16 or 32 to one or more antigens, most commonly *L. bozemanii* serogroup 1 (4·6%), *L. micdadei* (3·4%) and *L. dumoffii* (2·3%). Only three patients had titres $\geqslant 16$ to *L. pneumophila* antigens. A higher prevalence of antibodies with titres $\geqslant 16$ to non-pneumophila antigens has been observed previously in healthy blood donors (12). However, in the present study significant rises in titre were not seen in patients with pneumonias caused by other agents. These findings are in agreement with other workers using the formolized egg-yolk sac antigen (13). In addition, no rises in antibody titre to legionella antigens were seen in patients with Gram-negative sepsis despite the reported presence of cross-reacting antigens.

Although IFA for diagnosis of legionellosis caused by *L. pneumophila* serogroup 1 has been fully validated (14), problems remain with serodiagnosis of legionellosis caused by other species. It has been difficult to obtain sufficient numbers of matched sera from culture proven cases, or epidemiologically documented cases from non-pneumophila outbreaks of legionellosis, to establish the sensitivity and positive predictive value of these tests. More data therefore is needed to determine the best combination of antigens, and the most appropriate criteria for the diagnosis of non-pneumophila legionellosis in view of the higher prevalence of these antibodies in the healthy population.

In the original BTS/PHLS study, legionella infection was diagnosed in 2% of the 453 patients overall or 2·8% of those actually tested. Using the additional

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antigens, two further cases were identified. If the figures are corrected for availability of sera this would give an incidence of legionella infections of 2·6% overall or 3·6% of those actually tested. In this context a polyvalent antigen with additional *Legionella* spp. might prove useful as an extra screening test in suspected cases of legionellosis. However in a low prevalence disease where these tests have not yet been fully validated it is equally, if not more important to make greater efforts to isolate the organism. In these ways a sound basis for improving diagnosis of legionellosis caused by other species will be achieved.

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