Diagnostic efficacy of a DNA probe in pneumonia caused by *Legionella* species

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Summary. A commercial DNA probe kit (Gen-probe) for the detection of rRNA from legionellae was evaluated for its accuracy in diagnosing Legionnaires' disease in 167 patients with pneumonia. The test was performed on freshly obtained clinical respiratory tract samples. Cultures and direct immunofluorescence antibody (DFA) staining of the samples and serological tests were performed simultaneously for all patients. The probe assay result was positive in six patients; five of them had other laboratory evidence of disease (positive cultures or positive serological results or both). Depending on the diagnostic criteria, the probe test had a sensitivity of 31–67%, a specificity of 99% and positive predictive values of 67–83%. The diagnostic performance of the DNA probe assay in this study was superior to that of the DFA test. The results indicate that the examination of respiratory tract secretions by the Gen-probe kit is a suitable screening test for the diagnosis of Legionnaires' disease.

Introduction

The clinical and radiological presentation of legionella pneumonia is non-specific and specialised laboratory tests are necessary to establish the diagnosis. The definitive and most reliable method for diagnosis is isolation of the organism from respiratory secretions. The sensitivity of a positive culture has been reported from various laboratories as 50–80% and the specificity as 100%.¹ Two or 3 days are required usually for cultures to grow and most are positive within 5 days. Therefore, a more rapid diagnostic method has been sought. In July 1986, a DNA probe (Gen-probe, Inc., San Diego, CA, USA) for the detection of legionellae in clinical specimens became commercially available. Pre-release testing of the probe for confirmation of *Legionella* spp. in pure cultures revealed a specificity of 100%.^{2,3} A retrospective clinical study of frozen respiratory tract secretions showed the legionella probe kit to have a sensitivity of 75% and a specificity of 100%.⁴ However, experience with the test is still limited to a few clinical studies. Doebbeling *et al.*⁵ compared the probe assay with culture and the direct fluorescent antibody (DFA) test on all respiratory specimens submitted for legionella testing over an 18-month period; clinical features of the patients enrolled in this study were not

reported. In another similar study,⁶ the review of patient records included only those who had positive DNA probe tests. Both studies showed good overall performance of the DNA probe assay and concluded that the test is a satisfactory replacement for the DFA test for the laboratory diagnosis of legionella infections. In the present study, we evaluated the diagnostic efficacy of the DNA probe assay in freshly obtained respiratory secretions of patients with pneumonia.

Material and methods

Patients and investigations

Patients hospitalised at Harper Hospital, an 800 bed tertiary care hospital in Detroit, MI, who were suspected of having pneumonia on clinical grounds, were evaluated prospectively for the diagnosis of legionella pneumonia. Most of the patients were seen by members of the Infectious Disease Service and the charts and chest X-rays of all patients were reviewed by one of the authors. Pneumonia was defined as the presence of temperature > 38.5°C, compatible clinical signs and definite radiographic evidence of new pulmonary infiltrates. A laboratory diagnosis of legionella pneumonia was sought prospectively by examination of all fresh sputum samples by culture, direct immunofluorescence antibody (DFA) staining and the DNA probe test. Sputum samples were obtained by spontaneous expectoration or by deep suction through an endotracheal tube in mechanically ventilated patients.

Received 14 Jan. 1992; revised version accepted 12 Aug. 1992.

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Table I. Diagnostic criteria for the diagnosis of legionella pneumonia

Criteria	Legionella pneumonia, positive results	Control group, negative results
A	Culture or serology or both	Culture and serology
B	Culture	Culture and serology
C	Culture	Culture
D	Serology	Culture and Serology

Culture for Legionella spp.

Specimens were cultured on buffered charcoal-yeast extract selective agar containing α -ketoglutarate (BCYE- α) and supplemented with polymyxin B, anisomycin and vancomycin. Specimens were decontaminated by the acid washing procedure described by Buesching *et al.*⁷ Plates were incubated at 35° with CO₂ 5–10% and examined daily for up to 10 days.

Fluorescent antibody test

DFA testing was performed with polyclonal reagents prepared by the Michigan Department of Public Health. The result was considered to be positive when at least five typical rod-shaped or coccobacillary fluorescent organisms smear were identified.

DNA probe assay

The kits used for the DNA probe test were provided by Gen-probe, Inc., San Diego, CA, USA. The assay was performed according to the manufacturer's directions and as previously described.⁵ A ratio > 7.0 was considered to be a positive result for the presence of *Legionella* spp.

Serology

Paired sera were submitted to the Michigan Department of Public Health for determination of antibody titres against 13 different *Legionella* spp. by the legionella haemagglutination test.⁸ Serology was considered to be positive if there was a four-fold rise in titre to at least 128 or a convalescent titre of at least 512.

Statistical analysis for the evaluation of diagnostic efficacy was performed with standard definitions published previously.^{9,10} Results were analysed according to four different criteria for legionella pneumonia which are summarised in table I.

Results

A total of 167 consecutive patients with clinical and radiographic evidence of pneumonia were evaluated

between November 1987 and March 1988; 47 patients had at least one positive laboratory test supportive of a diagnosis of legionella pneumonia. The remaining 120 patients were considered to have a pneumonia of other aetiology and were classified as a negative control group. Among the 47 patients with a possible diagnosis of legionella pneumonia, cultures were positive in six, the DNA probe test was also positive in six, the serological tests in 13 and the DFA test in 37 (table II).

All four test results were positive in only one patient. Four patients had three positive results: the probe assay was positive in all of them, culture and serology were positive in two, culture and DFA in one and serology and DFA in one. Four patients had two positive results: serology and DFA were positive in two, culture and DFA in one and culture and serology in one. Thirty-eight patients had only one positive result: the DNA probe was positive in one, serology was positive in six and the DFA test was positive in the remaining 31. In 23 of these patients the DFA test was positive with a polyvalent conjugate against *L. pneumophila* serogroups 1–4. *L. pneumophila* was the only species isolated in cultures (serogroup 1 in three patients, serogroup 2 in two patients and serogroup 8

Table II. Distribution of positive tests among the 47 patients with possible legionella pneumonia

Number of positive tests	Type of test	Number of patients
4	Culture, serology, DFA and DNA probe	1
3	Culture, DFA and DNA probe	1
	Culture, serology and DNA probe	2
	Serology, DFA and DNA probe	1
2	Culture and DFA	1
	DFA and serology	2
	Culture and serology	1
1	DFA	31
	DNA probe	1
	Serology	6

Table III. Diagnostic efficacy (%) of the DNA probe assay in the diagnosis of legionella pneumonia

Determinant of efficacy	Efficacy values (%) with diagnostic criteria			
	A (15/152)*	B (6/152)	C (6/161)	D (13/152)
Sensitivity	33	67	67	31
Specificity	99	99	99	99
Predictive value of positive test	83	80	67	80
Predictive value of negative test	94	99	99	94
False positive rate	17	20	33	20
False negative rate	6	1	1	6

* (Number of patients diagnosed on legionella pneumonia/number of controls).

Table IV. Diagnostic efficacy (%) of the DFA test in the diagnosis of legionella pneumonia

Determinant of efficacy	Efficacy values (%) with diagnostic criteria			
	A (15/152)*	B (6/152)	C (6/161)	D (13/152)
Sensitivity	40	50	50	31
Specificity	80	80	79	80
Predictive value of positive test	16	9	8	11
Predictive value of negative test	93	98	98	93
False positive rate	84	91	92	89
False negative rate	7	2	2	7

*(Number of patients diagnosed on legionella pneumonia/number of controls).

in one patient). The probe assay result was positive in four of the six culture-positive patients. Three of these patients also had positive serology. The DNA probe assay result was also positive in another patient who had positive serological tests and negative cultures. The DFA test result was positive in three patients with positive cultures; one of them also had positive serological results. In one additional patient with a positive DFA result, serology was also positive.

The diagnostic efficacy of the DNA probe assay and the DFA test are summarised in tables III and IV, respectively. The diagnostic efficacy of the probe assay was similar for all criteria used in the study, except when positive serological results were included in the definition of disease (criteria A and D). The sensitivity of the test when criteria A and D were used was lower than that observed with the other criteria (table III). The specificity of the test was 99%, regardless of the criteria and the positive predictive value of the test was c. 80%, except when culture was considered to be the only criterion for defining legionella pneumonia (criterion C). In this case, the positive predictive value was only 67%. The DFA test showed a high rate of false positive results (84–92%) and consequently low rates of accuracy for positive prediction (8–16%) (table IV).

Discussion

Although epidemics of pneumonia caused by *Legionella* spp. have been well described, the true incidence of sporadic community-acquired infection is unknown. In a retrospective review of studies published from 1976 to 1987, Reingold found only two studies that permitted calculation of the incidence of community-acquired legionella pneumonia.¹¹ Among patients in a pre-paid health plan in Seattle, Foy *et al.* found an incidence of 12 cases/100 000 persons/year;¹² 84% of these pneumonia cases were treated as outpatients. A second series by Woodhead *et al.* from Nottingham found a single case of legionella pneumonia in a population of c. 53 000, giving an incidence of 1.9 cases/100 000 persons/year.¹³ Reingold re-

viewed seven series of patients hospitalised with community-acquired pneumonia and found that *Legionella* spp. were the causative organisms in < 1–30% of cases.¹¹

The studies from which the prevalence of legionella pneumonia could be calculated relied entirely or predominantly on IFA testing, which has a sensitivity of 75–85% and an unknown specificity.¹¹ In the hospital-based series, studies in which culture or direct examination of respiratory tract secretions for *Legionella* spp., or both, were used tended to find a higher proportion of cases due to *Legionella* spp. Culture of legionellae from respiratory tract secretions remains the definitive method of diagnosis for legionella pneumonia. More rapid diagnosis can be accomplished by the detection of antigen in respiratory tract secretions or urine. The urinary antigen test is now commercially available and has a reported sensitivity of 75–90% and a specificity of 100%.¹⁴ The test can be used only for detection of infection with serogroup 1, but this serogroup is believed to be responsible for 70% of *L. pneumophila* infections.¹⁵ The direct immunofluorescence assay for detection of antigen in respiratory secretions has been considered specific for *L. pneumophila* serogroups 1–4, but the interpretation of a positive result depends upon the prevalence of disease in the population.^{16,17} Disadvantages of the DFA test include the need for multiple reagents for different species and serogroups, the need for technical expertise in test performance and interpretation, dependence on reference laboratories, and the possibility of cross reactions with other organisms.^{18,19} In our study, DFA examination of respiratory secretions had a sensitivity of 31–50% and a specificity of c. 80%. These results confirmed our clinical impression that the DFA test may give more false positive results than has been previously reported. Whether this is due to local technical problems or represents a true low rate of diagnostic accuracy is not certain.

In our prospective study, the DNA probe assay had a specificity of 99% regardless of whether culture, serology or both were used to define a case of legionella pneumonia. It also had high rates of accuracy for positive prediction (67–83%), particularly when compared with the DFA test. The sensitivity of the probe assay was in the range 31–67%. These results are consistent with those previously reported,^{5,6,17} but positive and negative predictive accuracies depend upon the prevalence of disease and may not be applicable to other medical centres. Since legionella pneumonia appears to have a generally low prevalence, only a positive probe result should be accepted without further tests and cultures should be performed on all negative samples. Because *L. pneumophila* was the only species isolated from our patients, the efficacy of the probe assay for the detection of other *Legionella* spp. could not be determined in this study. However, with isolated cultures, the DNA probe appears to hybridise better with *L. pneumophila* than with other species.⁴

The results of this study suggest that the Gen-Probe kit is a suitable screening diagnostic test for the rapid detection of legionellae in respiratory tract secretions.

Substitution of the probe assay for the polyclonal DFA test would result in improved specificity as well as a marked reduction in the false-positive test rate.

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