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False-Positive DNA Probe Test for *Legionella* Species Associated with a Cluster of Respiratory Illnesses

SUZANNE LAUSSUCQ,[1]* DAVID SCHUSTER,[1] W. JAMES ALEXANDER,[2] W. LANIER THACKER,[1] HAZEL W. WILKINSON,[1] AND JOHN S. SPIKA[1]

Respiratory Diseases Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Bureau of Communicable Diseases, Jefferson County Department of Health, Birmingham, Alabama 35203[2]

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Between 11 November 1986 and 28 February 1987, legionellosis was diagnosed in 23 patients at one hospital with a recently marketed *Legionella*-specific DNA probe for respiratory secretions. Only 10 of the 23 probe-positive patients showed findings typical of *Legionella* pneumonia, including a temperature of ≥100.5°F (approximately 38.1°C) and radiographic evidence of pneumonia. No differences were found in the results of laboratory studies, demographic features, or underlying risk factors for these 10 probe-positive patients when compared with the 13 probe-positive patients with nonpneumonic illnesses. A case-control study comparing probe-positive and -negative patients failed to identify any different features of disease or epidemiologic characteristics. Probes of repeat specimens of sputum were still positive 2 to 13 weeks after the initial test in 5 (50%) of the 10 probe-positive patients. The clinical features in most patients were atypical for legionellosis, and the diagnosis could not be confirmed by traditional laboratory tests performed on duplicate specimens processed at the Centers for Disease Control. This report emphasizes the need for clinical microbiology laboratories to confirm test results from new procedures by accepted diagnostic methods.

Since recognition of the clinical entity of Legionnaires disease, the diagnosis has been defined by at least one of the following procedures: isolation of the etiologic agent, demonstration of the bacterium in body fluids or lung tissue by direct immunofluorescence assay (DFA), detection of antigenuria by enzyme-linked immunosorbent assay or radioimmunoassay, or detection of an antibody response to infection. In July 1986, a DNA probe (Gen-Probe, Inc., San Diego, Calif.) became available for detecting members of the *Legionella* genus in clinical specimens. Prerelease testing of the probe for confirmation of *Legionella* spp. in pure culture resulted in a specificity of 100% (3, 8). Use of the probe with respiratory secretions to confirm legionellosis was associated with a specificity of at least 99.1% and a sensitivity ranging from 56 to 81% (4).

The commercial test requires 2 h to perform and contains ^{125}I-labeled cDNA, which specifically hybridizes with the rRNA of all *Legionella* species. Radiolabeled cDNA hybridized with rRNA is quantified as counts per minute by using a gamma counter. A ratio of sample counts per minute to control counts per minute (with background counts removed) of ≥5 was considered positive for the presence of *Legionella* spp. However, for ratios between 3 and 5, counts for the sample, negative control, and background tubes were taken for 5 min, and then a ratio of ≥4 was positive. This ratio was selected to optimize specificity of the test (2).

Between 11 November 1986 and 28 February 1987, 23 patients at one hospital were diagnosed as having Legionnaires disease by use of the probe on respiratory secretions. Confirmatory testing by culture, DFA, or indirect immunofluorescence assay was not done. Fewer than half of these probe-positive patients were documented as having fever and pneumonia. The local health department first learned of this cluster of cases of respiratory illness from the hospital infection control personnel and inquiries from the families of ill patients. This report describes our investigation of these cases.

MATERIALS AND METHODS

Case definition. A case was defined as illness in a patient with respiratory secretions positive for *Legionella* spp. by the DNA probe test. To find cases, we reviewed clinical microbiology records.

Case-control study. We reviewed the charts of all 23 probe-positive patients. We looked at demographic features, preceding illnesses, results of laboratory studies, and risk factors for Legionnaires disease. A case-control study was performed to identify risk factors for a positive DNA probe test result, including characteristics of the preceding respiratory illness, laboratory test results, and risk factors for Legionnaires disease. The next patient with a negative probe result after each probe-positive patient was identified was chosen as a control.

Bacteriologic examination. Ten sputum and 15 serum specimens were collected from patients who had previously had positive probe test results. The sputum specimens were tested with the DNA probe in the hospital laboratory. Duplicate sputum specimens were tested at the Centers for Disease Control with DFA conjugates to *Legionella pneumophila* serogroups 1 to 6, *Legionella longbeachae* serogroups 1 and 2, *Legionella micdadei*, *Legionella dumoffii*, *Legionella bozemanii* serogroup 1, and *Legionella gormanii* and were cultured for *Legionella* organisms as follows (7). Half of each specimen was treated with acid for 15 min and then neutralized. Acid-treated and untreated portions were added to buffered charcoal-yeast extract agar and semiselective buffered charcoal-yeast extract agar (containing 0.5 μg of vancomycin, 80 μg of anisomycin, and 40 U of polymyxin B per ml) plates. Heavy (large drop) and light (small drop) inocula were used on duplicate plates of each formulation. One set of plates was incubated at 35°C in ambient air, and the other set was incubated at 35°C with 5% CO_2 added.

* Corresponding author.

Plates were examined microscopically for *Legionella*-like colonies for 2 weeks. The convalescent-phase sera were tested at the Centers for Disease Control against the *L. pneumophila* serogroup 1 indirect immunofluorescence assay antigen (2, 6, 7).

RESULTS

Laboratory investigation. Within 5 weeks of initiating genetic probe tests, four of five sputum specimens examined during a one-day run gave positive probe test results. The positive specimens were sent to the manufacturer for confirmation. The company reported that all four specimens were probe positive and that *Legionella* species were isolated from two of the four specimens suitable for culture. The cultures were unavailable for further tests. Between 11 November 1986 and 28 February 1987, 24 (22%) of 109 sputum specimens processed were positive by DNA probe (from 23 inpatients and 1 outpatient). An additional 12 specimens of other body fluids (9 bronchial washings, 2 pleural fluids, and 1 tissue) were probe negative. The percentage of samples shown positive by probe varied from month to month: 8 of 17 (47%) in November, 11 of 30 (37%) in December, 3 of 35 (9%) in January, and 2 of 27 (7%) in February. Of 109 sputum specimens, 22 (20%) had ratios of 3.0 to 3.9, 12 (11%) had ratios of 4.0 to 5.0, 6 (6%) had ratios of 5.1 to 6.0, 3 (3%) had ratios of 6.1 to 7.0, and 3 (3%) had ratios of over 7.1 (highest ratio, 13.6). Thus, 21 (88%) of the 24 positive specimens had ratios between 4 and 7; 34 (31%) of the total 109 sputum specimens had an initial ratio of 3 to 5, requiring a repeat determination of the probe score.

The DNA probe test was performed on specimens according to the guidelines of the manufacturer on a daily basis during the first 2 weeks after the laboratory began to use the test; however, this schedule was changed to a Monday, Wednesday, and Friday schedule. Specimens were stored at 4°C until they could be tested, except for those collected on Saturday, which were discarded and recollected on Sunday or Monday. Positive results were not associated with a particular laboratory worker, the day of the week the test was performed, or the lot number of the probe. Gram stains were not performed on these specimens; however, all but two patients had specimens submitted for Gram stain and routine culture within 3 to 5 days of the probe test.

Epidemiologic and clinical investigation. We reviewed the charts of all 23 hospitalized probe-positive patients. The median age of the patients was 60 years (range, 29 to 86 years); 13 (57%) were male. Ten patients (43%) had chronic obstructive lung disease, four (17%) had cancer, four (17%) had diabetes mellitus, and one (4%) had ischemic heart disease. When sputum samples were obtained for the probe test, 22 patients (96%) had a cough, 12 (52%) had a fever, 10 (43%) had influenzalike symptoms, and 9 (39%) had chest pain. Ten (43%) showed radiographic evidence of pneumonia and a temperature of at least 100.5°F (approximately 38.1°C) documented during hospitalization. The 10 patients with elevated temperatures and roentgenographic confirmation of pneumonia were compared with the 13 probe-positive patients with milder illnesses. No difference was detected between the two groups in demographic characteristics, antecedent illnesses, risk factors for Legionnaires disease, results of laboratory studies, or number of days of hospitalization before a specimen was obtained for testing.

Case-control study. The mean age of the 23 case patients (60 years) was less than that of the 20 controls (mean age, 65 years); 57% of the case patients were male, compared with 43% of the controls. We detected no difference between the case patients and the controls in the pattern of illness, laboratory studies done, underlying risk factors, or responses to treatment. No difference in location within the hospital was noted between the case patients and the controls. Locations of residence for the case patients and the controls were similar; the two groups were clustered primarily in the area of the county corresponding to the catchment area of the hospital. Infection control personnel from local hospitals were surveyed for knowledge of Legionnaires disease in the community during this time. Only one other hospital reported cases of Legionnaires disease during the 4-month period. Four patients had at least a fourfold rise in titer to $\geq$128 in appropriately timed, paired serum-confirmatory evidence of Legionnaires disease. All four of these patients showed radiographic evidence of pneumonia, documented temperatures of at least 101°F (approximately 38.8°C), and underlying immunosuppression.

Bacteriologic investigation. Ten sputum specimens from probe-positive patients who agreed to a repeat probe were examined; five (50%) remained positive on repeat examination in the hospital laboratory 2 to 13 weeks after the original test. One of the three patients with initial probe results of over 7 was available for a repeat probe test. The probe result for this patient was 2.8 on repeat examination 11 weeks after the initial test.

We compared the histories of repeat probe-positive case patients with those who showed negative results on repeat testing and could find no differences in their antecedent respiratory illnesses, treatment with erythromycin, existence of underlying lung disease, or duration of symptoms before hospitalization. Legionellae were not detected by culture or DFA in any of the 10 duplicate specimens examined at the Centers for Disease Control. Fifteen serum samples from probe-positive patients were tested by indirect immunofluorescence assay; in specimens collected 10 to 11 weeks after the initial probe results, two had titers of 128, seven had titers of 64, and six had titers of less than 64. These titers do not support a diagnosis of *Legionella* infection in these patients.

DISCUSSION

Our investigation of a cluster of putative cases of Legionnaires disease detected with a DNA probe for legionellae suggests that this new diagnostic method may not be as specific as expected from premarket testing. Only half of the probe-positive patients had an illness characterized by fever and pneumonia. Legionellosis could not be confirmed by indirect immunofluorescence assays of convalescent-phase sera, isolation of *Legionella* spp., or DFA staining of *Legionella* cells in convalescent-phase sputum samples that were persistently positive for the probe when tested 2 to 13 weeks after the acute illness. There could be several explanations for these discrepant results, including binding of the probe to a non-*Legionella* organism that was not included in previous evaluations, the existence of a previously unrecognized *Legionella* species that causes nonpneumonic respiratory illness, respiratory tract colonization by *Legionella* species, or a ratio for positive test interpretation that was set too low for optimal specificity.

Premarket testing of the probe for confirming cultures of *Legionella* spp. showed a broad range of values for *L. pneumophila* and other *Legionella* species; however, none of these values overlapped with values found for non-*Legionella* species (3, 6). This suggested that the probe was

specific for *Legionella* spp. in isolated cultures. A premarket evaluation of the direct-test probe also suggested high specificity. Only 2 of 230 clinical specimens that were culture- and DFA-negative for *Legionella* spp. gave positive probe results (2).

It is possible that previously unrecognized species of *Legionella* spp. caused the milder forms of respiratory illness seen in this investigation; however, we were unable to detect infection by culture, serology, or DFA. Our inability to confirm the probe-positive samples by culture strongly suggests that *Legionella* spp. were not involved in the illnesses of the case patients.

Previous attempts to document respiratory tract colonization with *Legionella* spp. suggest that if colonization does occur, it occurs infrequently (1). Furthermore, the reported sensitivity of the probe on respiratory secretions from patients with culture-confirmed isolation was 56 to 81%, a level of sensitivity which may be too low to detect colonization in the absence of classic Legionnaires disease.

The probe has been evaluated only with patients with pneumonia and a clinical history suggestive of Legionnaires disease (mental-status changes, gastrointestinal symptoms, or pneumonia with normal sputum culture results). If the test specificity is lower than previously reported or if the incidence of disease is low, the probability that a person with a positive test has Legionnaires disease (predictive value positive) decreases. A low ratio interpreted as positive would result in a decreased ability to predict disease with a positive test.

Although the investigation was limited by the retrospective design and the small number of patients available for convalescent-phase serologic testing, the results suggest a specificity far lower than that reported in premarket testing. The two highest titers (128) were for sera collected 11 and 13 weeks after the original illness. These results seem inconsistent with a resolving infection as studied by other investigators (5).

After learning of this investigation, Gen-Probe modified the package insert section on Interpretation of Results to recommend that probe ratios of 4.0 to 7.0 be treated as presumptive for the presence of *Legionella* RNA, ratios of 3.9 or less be considered negative, and ratios of 7.1 or greater be considered positive. The manufacturer now suggests that a probe of a specimen resulting in a ratio of 4.0 to 7.0 be followed by probes of additional specimens or by other diagnostic methods to assist in the final diagnosis.

With its purported high specificity and ease of interpretation, the probe has been recommended by the manufacturer for use in high-volume and small laboratories with limited capability for immunofluorescence assays (9). The results of this investigation suggest that hospitals and clinics using the probe should confirm their results by culture and DFA until a correlation of results within the laboratory is adequate to ensure reproducibility of results by both methods. When a definitive diagnosis of Legionnaires disease is desirable, positive probe results should be followed with serologic or cultural confirmation.

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LITERATURE CITED

1. **Bridge, J. A., and P. H. Edelstein.** 1983. Oropharyngeal colonization with *Legionella pneumophila*. J. Clin. Microbiol. **18**:1108–1112.
2. **Edelstein, P. H.** 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. **14**:298–303.
3. **Edelstein, P. H.** 1986. Evaluation of the Gen-Probe DNA probe for the detection of legionellae in culture. J. Clin. Microbiol. **23**:481–484.
4. **Edelstein, P. H., R. N. Bryan, R. K. Enns, D. E. Kohne, and D. L. Kacian.** 1987. Retrospective study of Gen-Probe rapid diagnostic system for detection of legionellae in frozen clinical respiratory tract samples. J. Clin. Microbiol. **25**:1022–1026.
5. **Kirby, B. D., K. M. Snyder, R. D. Meyer, and S. M. Finegold.** 1980. Legionnaires' disease: report of 65 nosocomially acquired cases and review of the literature. Medicine (Baltimore) **59**:188–205.
6. **Pasculle, A. W., J. C. Feeley, R. C. Gibson, L. G. Cordes, R. L. Myerowitz, C. M. Patton, G. W. Gorman, C. L. Carmack, J. W. Ezzell, and J. N. Dowling.** 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. J. Infect. Dis. **191**:727–732.
7. **Wilkinson, H. W.** 1987. Hospital-laboratory diagnosis of Legionella infections, p. 22–25. Centers for Disease Control, Atlanta.
8. **Wilkinson, H. W., J. S. Sampson, and B. B. Plikaytis.** 1986. Evaluation of a commercial gene probe for identification of *Legionella* cultures. J. Clin. Microbiol. **23**:217–220.
9. **Young, F. E.** 1987. DNA probes: fruits of the new biotechnology. J. Am. Med. Assoc. **258**:2404–2406.