Diagnosis of Legionnaires' disease from transbronchial lung biopsy using the fibreoptic bronchoscope

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Legionnaires' disease is a recently described illness with findings similar to those of other pneumonias.[1-3] The diagnosis is usually made by demonstrating a fourfold rise in titres of antibodies to the gram-negative bacterium responsible for the disease — *Legionella pneumophila.* However, there are also elevated titres of antibodies to this agent in other diseases, namely tularemia, leptospirosis and plague.[4] Lung biopsy tissue has recently been used to diagnose this disease with the aid of direct fluorescent antibody staining,[5] but false-positive results have occurred with other bacteria found in the sputum, including strains of *Pseudomonas fluorescens.*[5] Direct fluorescent antibody staining has also been used to demonstrate *L. pneumophila* in the sputum.[6] There have been very few reports of the isolation of this organism from living persons.[7] Lung tissue may also be examined with the use of the Dieterle silver impregnation method or by the Wohlbach modification of the Giemsa stain.[8,9] Lattimer, McCrone and Galgon[10] have reported the isolation and identification of *L. pneumophila* from a transtracheal aspirate.

Fibreoptic bronchoscopy, since its introduction by Ikeda in 1966, has proved to be a safe method of obtaining lung tissue for both staining and culture.[11,12] It has been used in the diagnosis of slowly resolving pneumonias and pulmonary infiltrations of unknown cause.[13,14] In this paper we describe a case of progressive pneumonia in which transbronchial lung biopsy helped in the final diagnosis of Legionnaires' disease; to our knowledge this is the first report in which this procedure has helped to confirm the diagnosis of Legionnaires' disease in a patient with pneumonia of undetermined cause. We recommend that a transbronchial lung biopsy be done in cases of pneumonia that does not respond to therapy or early in the course of severe pneumonia when the cause is obscure.

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Case report

Clinical course

A 52-year-old alcoholic woman was admitted to hospital in August 1978 because of right lower lobe pneumonia. Increasing shortness of breath and a cough productive of green sputum had begun approximately 2 weeks earlier. A few days prior to admission she had first noted general malaise, weakness and muscle aches. There had been no diarrhea, and her health had previously been good.

She appeared to be acutely ill. Her pulse rate was 120 beats/min, blood pressure 130/80 mm Hg and temperature 39.5°C. The only significant physical findings were in the chest: dullness to percussion, increased tactile fremitus and crepitations over the right lower lobe of the lung.

A chest roentgenogram revealed right lower lobe consolidation. The hemoglobin level was 10.6 g/dl and the leukocyte count 9.1 × 10^9/l (83% neutrophils, 11% lymphocytes and 5% band cells, with evidence of toxic granulation of the neutrophils). Gram-staining of the sputum showed more than 10 epithelial cells and fewer than 25 pus cells per high power field and normal oral flora. Arterial blood gas values were as follows: pH 7.53, carbon dioxide tension 28 mm Hg and oxygen tension 50 mm Hg.

The patient was admitted, and therapy with oxygen and penicillin G, 1 million units given intravenously every 6 hours, was begun. Over the next 2 days there was no clinical improvement, and on the third day after admission the antibiotic therapy was changed to cephalothin, 1 g given intravenously every 4 hours. Two days later there was clinical deterioration, with worsening of the blood gas values and chest abnormalities, and radiologic evidence of bilateral pneumonia. She was transferred to the intensive care unit, where an endotracheal tube was inserted and ventilation begun. Gentamicin, 80 mg given intravenously every 8 hours, was added to the antibiotic regimen. On the seventh day after admission there was no evidence of improvement and the patient was referred to the respiratory service for fibreoptic bronchoscopy. Bronchial washings and transbronchial biopsies were done in the right lower lobe. Therapy with isoniazid, 300 mg/d, and streptomycin, 1 g/d, was started while the results of the bronchoscopy were awaited.

The following day the antituberculous medications and other antibiotics were discontinued, and therapy with erythromycin, 500 mg intravenously every 6 hours, was begun. Within 24 hours the patient became afebrile, and progressive clinical improvement along with radiologic clearing of the pneumonia occurred.

Bacteriologic findings

Cultures of sputum and transbronchial washings were negative for bacterial pathogens, including *L. pneumophila*, which was sought on chocolate agar and Thayer–Martin medium. Smears and cultures for fungi and mycobacteria were also negative. Serologic testing for influenza viruses, adenoviruses and *Mycoplasma* showed no significant rise in antibody titres, but titres of antibodies to *L. pneumophila*, measured by the indirect fluorescent anti-

body technique at the Ontario Provincial Laboratory, were 1:128 the day the patient became afebrile and 1:4096 3 weeks later.

Biopsy results

The transbronchial biopsy specimen showed changes of pneumonia. The alveolar spaces were filled with a fibrinous, proteinaceous exudate containing many macrophages and a few neutrophils. There was some sloughing of alveolar lining cells. The alveolar walls were widened by edema, slight fibrosis and a mild infiltrate of mononuclear inflammatory cells (Figs. 1 and 2). The use of special stains for microorganisms gave negative results. Because the clinical and biopsy findings conformed to those described for Legionnaires' disease, Dieterle staining was done, but its results were read as negative.[15,16] However, the paraffin block was sent to the Center for Disease Control in Atlanta, Georgia, where indirect immunofluorescent staining of sections gave positive results for the Knoxville strain of *L. pneumophila*.

Discussion

Legionnaires' disease is a recently described cause of pneumonia that is usually diagnosed retrospectively.[1] Some features may help in differentiating it from other types of pneumonia; these include a prodromal viral illness, a dry cough, confusion, diarrhea, lymphopenia, neutrophilia and hyponatremia.[3,17,18] The organism is a gram-negative bacterium that is difficult to grow. As a result, serologic methods and immunofluorescent staining of autopsy material have been used in the diagnosis of the disease.[5,8]

Lattimer and colleagues[10] have described the isolation of *L. pneumophila* from a transtracheal aspirate. Fibreoptic bronchoscopy, an alternative to transtracheal aspiration, yields tissue as well as bronchial secretions, and has been used to diagnose other types of pneumonias, such as that caused by *Pneumocystis carinii*. The morbidity of the procedure is low, and open lung biopsy is avoided.[19] In our patient transbronchial biopsy yielded enough tissue for staining by the Dieterle method and by indirect immunofluorescence. Even though there was a successful outcome in this case, earlier transbronchial biopsy might have been used to make the diagnosis.

The light microscopic appearance of the biopsy specimen, while not specific for Legionnaires' disease, was consistent with it, and this plus the clinical findings led to its further examination for specific evidence of Legionnaires' disease. The negative results of Dieterle staining were probably due to poor technique, since even in retrospect we could not make a positive diagnosis from this one section. Negative results of Dieterle staining have been found in biopsy specimens from other patients with serologically proven disease, but usually the direct immunofluorescent test has also given negative results. Recently a transbronchial biopsy specimen from a patient with serologically proven Legionnaires' disease gave negative results with direct immunofluorescent staining.[6]

In summary, transbronchial biopsy may be useful in cases of Legionnaires' disease in making an early diagnosis if bronchial secretions and lung tissue are examined by immunofluorescent staining.

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References

1. FRASER DW, TSAI TR, ORENSTEIN W, et al: Legionnaires' disease: description of an epidemic of pneumonia. *N Engl J Med* 297: 1189, 1977
2. LATTIMER GL, RHODES LV III: Legionnaires' disease. Clinical findings and one-year follow-up. *JAMA* 240: 1169, 1978
3. SWARTZ MN: Clinical aspects of Legionnaires' disease. *Ann Intern Med* 90: 492, 1979
4. TSAI TF, FRASER DW: The diagnosis of Legionnaires' disease (E). *Ann Intern Med* 89: 413, 1978

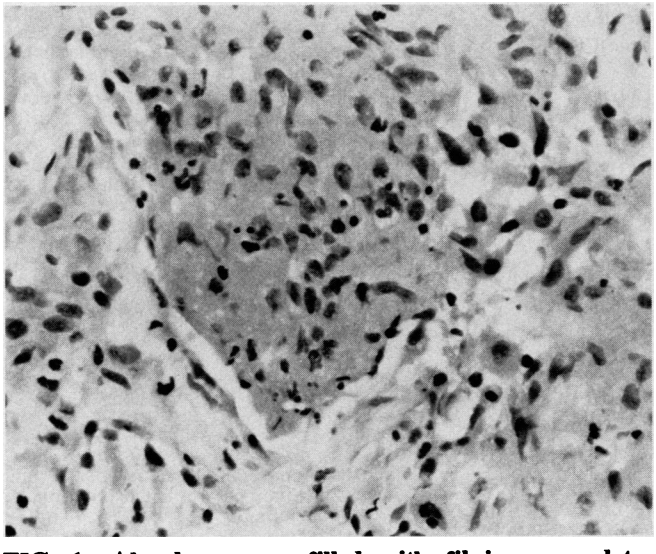

FIG. 1—Alveolar spaces filled with fibrinous exudate, many macrophages and few neutrophils (hematoxylin–eosin [H–E]; ×400, reduced 40%).

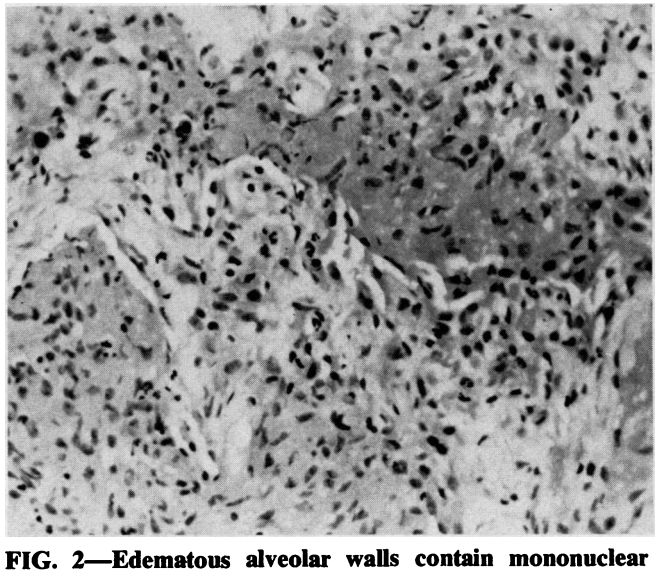

FIG. 2—Edematous alveolar walls contain mononuclear inflammatory cells and occasional neutrophils (H–E; ×250, reduced 40%).

5. CHERRY WB, PITTMAN B, HARRIS PP, et al: Detection of Legionnaires disease bacteria by direct immunofluorescent staining. *J Clin Microbiol* 8: 329, 1978
6. BROOME CV, CHERRY WB, WINN WC JR, et al: Rapid diagnosis of Legionnaires' disease by direct immunofluorescent staining. *Ann Intern Med* 90: 1, 1979
7. DUMOFF M: Direct in-vitro isolation of Legionnaires' disease bacteria in two fatal cases. Cultural and staining characteristics. Ibid, p 694
8. CHANDLER FW, HICKLIN MD, BLACKMON JA: Demonstration of the agent of Legionnaires' disease in tissue. *N Engl J Med* 297: 1218, 1977
9. FRENKEL JK, BAKER LH, CHONKO AM: Autopsy diagnosis of Legionnaires' disease in immunosuppressed patients. A paleodiagnosis using Giemsa stain (Wohlbach modification). *Ann Intern Med* 90: 559, 1979
10. LATTIMER GL, MCCRONE C, GALGON J: Diagnosis of Legionnaires' disease from transtracheal aspirate by direct fluorescent-antibody staining and isolation of the bacterium. *N Engl J Med* 299: 1172, 1978
11. IKEDA S: *Atlas of Flexible Bronchofiberscopy*, Univ Park, Baltimore, 1974
12. WANNER A, AMIKAM B, ROBINSON MJ, et al: Comparison between the bacteriologic flora of different segments of airways. Examination by bedside bronchofiberscopy. *Respiration* 30: 561, 1973
13. CHENG WK, THOMAS P: Fiberoptic bronchoscopy in the diagnosis of diffuse lung disease and slowly resolving pneumonia, in *Abstracts of Scientific Sessions, Annual Meeting of Canadian Tuberculosis and Respiratory Disease Association, Moncton, New Brunswick, June 13–15, 1977*
14. CLARK RA, GREY PB, TOWNSEND RM, et al: Transbronchial lung biopsy: a review of 85 cases. *Thorax* 32: 546, 1977
15. BLACKMON JA, HICKLIN MD, CHANDLER FW: Legionnaires' disease — pathological and historical aspects of a new disease. *Arch Pathol Lab Med* 102: 337, 1978
16. SCULLY RE, GALDABINI JJ, MCNEELY BU: Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 32–1978. *N Engl J Med* 299: 347, 1978
17. MILLER AC: Early clinical differentiation between Legionnaires' disease and other sporadic pneumonias. *Ann Intern Med* 90: 526, 1979
18. CLAYTON AJ: Legionnaires' disease: an illness whose time has come (E). *Can Med Assoc J* 120: 1482, 1979
19. HODGKIN JE, ANDERSEN HA, ROSENOW EE III: Diagnosis of Pneumocystis carinii pneumonia by transbronchoscopic lung biopsy. *Chest* 64: 551, 1973

BOOKS

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PRINCIPLES AND MANAGEMENT OF UROLOGIC CANCER. Edited by Nasser Javadpour. 533 pp. Illust. The Williams & Wilkins Company, Baltimore; the Macmillan Company of Canada Limited, Toronto, 1979. $64.95. ISBN 0-683-04463-X

PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES. Gerald L. Mandell, R. Gordon Douglas, Jr. and John E. Bennett. 2350 pp. Illust. John Wiley & Sons, Inc., New York, 1979. $72. ISBN 0-471-03489-4

PROGRESS IN DRUG RESEARCH. PROGRES DES RECHERCHES PHARMACEUTIQUES. Vol. 23. Edited by Ernst Jucker. Authors: J. Kubinyi, N.W. Preston, L.H. Sarett and others. 320 pp. Illust. Birkhauser Verlag, Basel, 1979. Price not stated. ISBN 3-7654-1070-7

PSYCHIATRIC CONSULTATION IN MENTAL RETARDATION. Formulated by the Committee on Mental Retardation. Volume X, Publication No. 104, November, 1979. 100 pp. Group for the Advancement of Psychiatry, New York, 1979. $4, paperbound. ISBN 0-87318-142-5

A RESIDENT'S GUIDE TO PSYCHIATRIC EDUCATION. Edited by Michael G. Thompson. 230 pp. Plenum Publishing Corp., New York, 1978. $14.95. ISBN 0-306-31130-5

RHEUMATOLOGY. New Directions in Therapy. Edward E. Rosenbaum. 291 pp. Medical Examination Publishing Co., Inc., Garden City, New York, 1979. Price not stated, paperbound. ISBN 0-87488-683-X

SCREENING IN CANCER. A Report of a UICC International Workshop. Toronto, Canada, April 24–27, 1978. Edited by A.B. Miller. UICC Technical Report Series — Volume 40. 338 pp. Illust. International Union Against Cancer, Geneva, 1978. $14.80, paperbound. ISBN 92-9018-040-4

SHOCK TRAUMA MANUAL. William Gill and William Broughton Long III. 283 pp. Illust. The Williams & Wilkins Company, Baltimore; the Macmillan Company of Canada Limited, Toronto, 1979. $23.50, paperbound. ISBN 0-0683-3527-4

SURGICAL SEPSIS. Edited by Colin J.L. Strachan and Richard Wise. 153 pp. Illust. Academic Press Inc. (London) Ltd., London, 1979. $25. ISBN 0-12-794378-1

SYMPOSIUM ON THE NEUROLOGIC ASPECTS OF PLASTIC SURGERY. Volume seventeen. Proceedings of the Symposium on the Educational Foundation of the American Society of Plastic and Reconstructive Surgeons, Inc., held at Houston, Texas, January 27–30, 1977. Edited by Simon Fredricks and Garry S. Brody. 328 pp. Illust. The C.V. Mosby Company, Saint Louis, 1978. $65.50. ISBN 0-8016-1679-4