Legionnaire's Disease in Johannesburg

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SUMMARY

Legionnaires' disease (LD) was first identified as an epidemic pneumonia occurring in the USA in 1976. Sporadic cases have since been identified in many countries. This report describes the first 2 cases in South Africa of LD with pneumonia in patients in whom the diagnosis was established by the finding in early acute and follow-up convalescent sera of a greater than fourfold rise in titre with the immunofluorescent antibody test. The microbiological investigations are discussed in some detail and the clinical, radiological and laboratory features of LD are briefly reviewed.


Legionnaires' disease (LD) was first identified as an epidemic pneumonia which occurred in July and August of 1976 among persons attending an American Legion convention at a hotel in Philadelphia. Since then more than 600 epidemic-associated cases (involving 12 separate epidemics) and more than 270 sporadic cases have been confirmed by laboratory methods.

The illness is caused by an aerobic, Gram-negative bacillus which has been named Legionella pneumophila. The organism has unique cultural and staining properties which are probably the main factors responsible for its late discovery as an important human pathogen. In this article, 2 cases of LD diagnosed on the basis of serological conversion are presented.

CASE REPORTS

Case 1

A 52-year-old businessman was admitted to the Johannesburg General Hospital on 24 June 1979 with a 1-day history of severe myalgia and lethargy. There were no symptoms of a previous upper respiratory tract infection.

On the day of admission he experienced chills, felt feverish and became confused. He had had maturity-onset diabetes for the past 10 years which was well controlled on chlorpropamide 200 mg daily. He smoked 30 cigarettes per day and had a moderate alcohol intake. On examination the patient was confused and disoriented. His temperature was 39.5°C, pulse rate 80/min and blood pressure 140/80 mmHg. The respiratory rate was 36/min, but there was no cyanosis, pallor or jaundice. Coarse crepitations were heard at the right base of the lungs. The heart and abdomen were both normal. The chest radiograph showed dense consolidation of the right lower lobe. The haemoglobin value was 12.5 g/dl, white cell count 16 300/μl with a differential cell count of 96% neutrophils, 3% lymphocytes and 1% eosinophils, prothrombin index 97%, blood urea 5.0 mmol/l, serum creatinine 117 μmol/l, sodium 113 mmol/l, potassium 3.0 mmol/l, serum proteins 57 g/l, serum albumin 31 g/l, serum calcium 1.97 mmol/l, serum inorganic phosphate 0.66 mmol/l, serum cholesterol 2.95 mmol/l, serum glucose 7.1 mmol/l, serum urate 0.19 mmol/l. The total serum bilirubin level was 14 μmol/l, direct bilirubin 10 μmol/l, alkaline phosphatase 187 U/l, alanine transaminase (SGPT) 141 U/l, aspartate transaminase (SGOT) 251 U/l. Arterial blood gas levels were PO2 10.4 kPa, PCO2 4.71 kPa, standard bicarbonate 23.1 mmol/l, base excess -0.5 mmol/l and pH 7.42. Serum osmolality was 270 mmol/kg and urine osmolality 570 mmol/kg. Microscopic examination and culture of urine were negative. Blood cultures were all negative. A Mycoplasma complement fixation test (CFT) yielded a titre of < 1/8. The Coombs and cold agglutinin tests and CFTs for Q fever, psittacosis and leptospirosis were all negative.

Penicillin and gentamicin were administered parenterally for 7 days. The inappropriate antidiuretic hormone (ADH) secretion was treated by discontinuing the chlorpropamide (which may have been a contributing factor), and instituting fluid restriction and oral diphenylhydantoin. The diagnosis of LD was considered when the investigations revealed the inadequate ADH secretion, hypophosphataemia and raised serum alkaline phosphatase and alanine and aspartate transaminase levels. Blood was drawn to test this possibility on 4 July 1979. The temperature settled and the patient became fully orientated on the 4th day after admission. He was discharged on 5 July, and advised to take erythromycin 500 mg every 6 hours for 2 weeks to prevent relapse and a prolonged convalescence. Follow-up was uneventful and 2 months later the chest radiograph had returned to normal.

Case 2

A 48-year-old woman was admitted to the Johannesburg Hospital on 2 February 1980 with a 1-day history of...
confusion, fever and a cough productive of thick, yellow sputum. A week previously she had developed persistent right-sided pleuritic pain associated with general malaise and lethargy after a grand mal seizure. She was a known epileptic, well controlled on diphenylhydantoin 100 mg and phenobarbitone 60 mg every 8 hours. She had smoked 10 cigarettes per day for many years.

On examination the patient was confused, cyanosed and tachypnoeic. Her temperature was 39.5°C, the pulse rate was 96/min and blood pressure was 115/75 mmHg. The respiratory rate was 40/min. Dullness, decreased breath sounds and crepitations were present at the right lung base. The heart and abdomen were both normal. The chest radiograph showed consolidation of the right lower lobe.

Investigations revealed a haemoglobin concentration of 14,3 g/dl and a white blood cell count of 16 700/µl with a differential count of 90% neutrophils, 6% lymphocytes and 2% monocytes. The prothrombin index was 74%, blood urea 4,6 mmol/l, serum creatinine 88 µmol/l, sodium 127 mmol/l, potassium 3,5 mmol/l, serum proteins 54 g/l, serum albumin 27 g/l, serum calcium 1,87 mmol/l, serum inorganic phosphate 0,63 mmol/l, serum cholesterol 3,14 mmol/l, serum glucose 7,5 mmol/l, serum urate 0,24 mmol/l. The total bilirubin level was 26 µmol/l, direct bilirubin level 19 µmol/l, alkaline phosphatase 61 µ/l, alanine transaminase 40 U/l, aspartate transaminase 36 U/l. Arterial blood gas levels were Po, 6,55 kPa, Pco, 3,5 kPa, standard bicarbonate 23,8 mmol/l, base excess -0,4 mmol/l and pH 7,51. Microscopic examination of urine showed leucocytes 60 000/ml, but culture was negative. A bone marrow aspirate showed depressed, normoblastic erythropoiesis and active and left-shifted granulopoiesis with marked toxic granulation; there was also erythrophagocytosis. Blood cultures were negative. CFTs for the adenovirus group and Mycobacterium pneumoniae were negative; that for Q fever was weakly positive at a titre of 10 and that for Leptospiraicterohaemorrhagiae was positive at a titre of 15. The latter two results suggested past infections.

**Microbiological Investigations**

Procedures for the isolation and demonstration of *L. pneumophila* and the indirect fluorescent antibody test for LD were performed according to the methods used by the Center for Disease Control, Atlanta, Georgia (CDC). The sputum specimen was plated onto Mueller-Hinton agar supplemented with Isovitalex (BBL) and haemoglobin powder, agar containing casein hydrolysate, L-cysteine, ferric pyrophosphate and starch, and on cysteine-yeast extract agar containing activated charcoal. The plates were incubated in 5% CO₂ at 36°C for a fortnight and were examined for growth at 2-day intervals. Four male guinea-pigs were inoculated intraperitoneally with 1 ml aliquots of sputum and were examined daily for signs of illness. For the direct fluorescent antibody test fluorescein-conjugated *L. pneumophila* serum was obtained from the CDC, as were the reagents for the indirect fluorescent antibody test. The heat-killed Phila-delphia group I antigen (lot No. 78/0296) was suspended in normal yolk-sac diluent (NYS). Initially the serum samples were diluted in NYS according to the original directions of the CDC. In later tests, after unacceptably high titres were obtained, the NYS was replaced with a fresh batch and sera were only diluted in NYS at the 1:16 dilution. For the higher dilutions phosphate-buffered saline was used according to the more recent CDC directives. Titres were expressed as the reciprocal of the highest serum dilution showing the required fluorescent LD bacilli. A positive control serum at a titre of 512 was used throughout.

The sputum specimen obtained from the first patient on 4 July 1979 showed neutrophils and Gram-positive cocci but no LD bacilli on direct fluorescent antibody testing and the biological test in guinea-pigs was negative. No attempt was made to demonstrate *L. pneumophila* in the sputum of the second patient.

Serum samples obtained from patient 1 on 4, 6 and 10 July and 1 August initially gave titres of >10 000 with ill-defined end-points, and it was obvious that a technical problem existed, in spite of the fact that the positive control serum gave the expected titre of 512. The serum samples were sent to the CDC and the titres of the four sera were reported to be 256, 1 024, 8 192 and 8 192 respectively. In the meantime the technique used in Johannesburg was carefully reviewed and the tests were repeated using freshly prepared reagents. The use of saline diluent instead of NYS for the dilutions of less than 16 and the changing of micropipettes from the capillary transfer variety to a microvolume delivery type were the only other modifications introduced. Using the revised procedure the titres obtained at the CDC were consistently reproduced in Johannesburg.

Indirect fluorescent antibody titres of serum samples from patient 2 were 32, 4096 and 8 192 on 6, 12 and 20 February 1980 respectively. Seroconversion with slightly higher titres was also demonstrated in this case by the CDC.

**DISCUSSION**

At present the indirect fluorescent antibody test is the most convenient and most widely used procedure for the diagnosis of LD, the criteria being a fourfold or greater rise in titre to a level of >256. Our 2 cases are the first reported of LD with pneumonia in South Africa in which the diagnosis was established by the availability of early acute and follow-up convalescent phase sera showing a greater than fourfold rise in titre with this test. The final titres of 8 192 were well above the diagnostic level.

The titre of 256 obtained on the 12th day in patient 1 is unusually high but not unique for that early stage of the illness. Although the serological diagnosis of LD is usually retrospective, titres below the diagnostic level in patients with suspicious clinical manifestations can be a useful adjunct for an early provisional diagnosis. This was recently stressed by Taylor and Harrington from the Central Public Health Laboratory, Colindale, London, who, using formalized yolk-sac antigen, found titres between 16 and 128 within 8 days of onset of illness in 8
out of 14 patients. The initial titre of 32 early in the course of patient 2 further illustrates this. Most patients show seroconversion at the end of 3 weeks\textsuperscript{11,12} but this may not occur for 6 weeks.\textsuperscript{12}

The degree of specificity of the indirect fluorescent antibody test for LD is not yet fully established and cross-reactions with mycoplasmal pneumonia, psittacosis, leptospirosis, plague and tularemia have been described.\textsuperscript{8,13-15} At the SAIMR approximately 28% of 289 serum samples submitted for LD serological testing have titres of 32 - 128, suggesting either a high prevalence of LD or significant cross-reactions with antigens from the environment or pathogenic micro-organisms. Workers at the CDC have recently shown that antibodies against non-specific antigens common to Legionella and many other Gram-negative bacteria can be blocked from reacting in the indirect fluorescent antibody test if the serum is first diluted with a heat-stable soluble substance extracted from E. coli.\textsuperscript{16} It has been claimed, furthermore, that the formalized L. pneumophila antigen is more specific than the heat-killed antigen supplied by the CDC.\textsuperscript{17} Several sera which gave low titres with the heat-killed antigen gave negative results with the formalized antigen when tested at the SAIMR. The significance of this finding is not clear. It is possible that the formalized antigen is more specific, but it is not known whether it lacks sensitivity and whether some cases, especially mild ones, may be missed when this antigen is used. Our patients had initial titres of 32 and 128 respectively, both rising to 4 096 with the formolized antigen, which adds further credence to the diagnosis.

Diagnosis is possible, but thus far very difficult, by direct culture of L. pneumophila from lung tissue, pleural fluid, transtracheal aspirates and blood cultures.\textsuperscript{18-21} The culture medium generally used in diagnostic laboratories has been Mueller-Hinton agar supplemented with ferric phosphate and L-cysteine. Lung tissue obtained by biopsy may possibly be a source of diagnosis by direct fluorescent antibody staining or by use of the Dieterle silver impregnation stain. Lung imprints made from fresh tissue may show the organism on Brown-Brenn or even Gram staining.

Direct isolation of the organism from sputum or transtracheal aspirates is rarely successful, possibly because of the intra-alveolar site of growth of L. pneumophila, the competition by the more rapidly growing normal components of the respiratory tract flora and the usually sparse quantity of sputum. There is still a need for a good selective medium and further evaluation of the enzyme-linked immunosorbent assay and other techniques for the demonstration of L. pneumophila antigens in tissue fluids.\textsuperscript{20}

**Clinical Findings.**

With regard to the clinical manifestations of LD, an incubation period of 2 - 10 days is estimated for epidemic-related LD, based on findings during the epidemic at the American Legion Convention in Philadelphia in 1976.\textsuperscript{21} In the explosive outbreak of non-pneumonic, acute febrile illness, designated 'Pontiac fever', and caused by L. pneumophila, the mean incubation period was considerably shorter, being 36 hours.\textsuperscript{22} The incubation period for sporadic cases is unknown. Typically, LD occurs in middle-aged or older persons. The age range is 10 months - 84 years, with a median age for men of 54 years and for women of 56 years.\textsuperscript{23,24} Males predominate over females, in a ratio of 3 : 1. The disease afflicts both Whites and Blacks.\textsuperscript{25} Although cases occur throughout the year, both sporadic cases and epidemics appear to be more common in the summer and autumn.\textsuperscript{26}

An enhanced susceptibility to infection and an increased mortality rate appear to occur in immunocompromised patients. A third of the patients with LD at the Wadsworth Veterans' Hospital had been receiving corticosteroid therapy at the onset of the pulmonary infection.\textsuperscript{27} Of the patients with LD in the Vermont epidemic, 43% were immunosuppressed (underlying cancer, chronic renal failure necessitating haemodialysis, or treatment with corticosteroid or cytotoxic drugs).\textsuperscript{28} In the latter epidemic some underlying disease (diabetes mellitus, alcoholism, chronic obstructive pulmonary disease, cardiovascular disease) was present in 86% of the patients. In the Philadelphia\textsuperscript{29} and Wadsworth Veterans Hospital epidemics\textsuperscript{30} 50 - 70% of the patients were cigarette smokers. The diabetes mellitus in patient 1 and the cigarette smoking in both cases may have predisposed to LD. Secondary cases do not as a rule occur after exposure to patients with LD, although there is indirect evidence that this may happen on rare occasions.\textsuperscript{31}

The initial prodromal symptoms usually consist of malaise, diffuse myalgias and headaches, followed within 12 - 48 hours by the sudden onset of a high, non-remittent fever (up to 40.5°C), recurrent shaking chills, and severe prostration. In some patients the onset is more gradual. The illness is usually not associated with a preceding upper respiratory tract infection.\textsuperscript{22,32} Gastro-intestinal symptoms may appear in this early stage. Nausea and vomiting were noted in about 20% of patients in the Vermont series.\textsuperscript{33} Watery diarrhoea and abdominal pain may also occur.

- On the 2nd or 3rd day of illness a dry cough begins, occasionally producing small amounts of mucoid sputum. Minor haemoptysis (blood-streaked sputum) occurs in 20 - 40% of patients. Chest pain, usually pleuritic in nature, is present in 30 - 40%. Dyspnoea is common as the pulmonary involvement rapidly progresses.

High, unremitting fever (39.4° - 40.5°C) continues until the institution of appropriate antimicrobial therapy or until spontaneous resolution of the infection begins, usually on the 8th - 10th day of the illness.\textsuperscript{29}

During the first few days the patient is acutely ill, appears toxic, sweats, and is tachypnoeic. Confusion and disorientation are sometimes present and seem disproportionate to the height of the fever or the degree of hypoxaemia. Their presence may indicate some degree of toxic encephalopathy. Localizing neurological findings have rarely been observed; in the few instances where cerebrospinal fluid examinations have been done, no abnormalities have been noted. A relative bradycardia was present in both cases, and occurs in about one-half of the
patients early in the clinical course before extensive lung consolidation has developed. Physical findings in the chest during the first few days consist only of fine inspiratory crepitations. After the pulmonary process has progressed over the next few days, findings of frank consolidation become evident.

Radiological Findings

In about 70% of patients only one lung is involved at first. The most common initial radiological patterns reported are poorly marginated, round opacities, either in the centre or on the periphery (often abutting on the pleural surface), and diffuse patchy bronchopneumonia. As the disease progresses, peripheral shadows enlarge and characteristically become lobar in extent with a 'ground glass' appearance or dense consolidation. Multilobar involvement is found in 65% of cases during the peak of radiological changes. Total opacification of an entire lung is sometimes observed in fulminant cases. Pleural effusions are not uncommon and may precede pulmonary infiltration. Cavitation is usually not a feature but a few cases have been reported.

Laboratory Findings

A mild-to-moderate leucocytosis (10 000-20 000/μl) with a shift to the left is commonly present and lymphopenia is also common. The erythrocyte sedimentation rate is elevated, but no consistent abnormalities in haematoctrit that can be attributed to LD have been observed. Thrombocytopenia and disseminated intravascular coagulation have been reported. Mild abnormalities in liver function have been noted; these consist of mild elevations of SGOT, lactic dehydrogenase, alkaline phosphatase, and serum bilirubin levels.

Microscopic haematuria of minor degree has been reported in up to 10% of cases. Proteinuria, acute tubulo-interstitial nephritis and renal failure have also been reported.

Hyponatraemia, probably a result of the inadequate ADH secretion secondary to pulmonary involvement, occurs in the majority of cases. Hypophosphataemia was present in both the present patients and occurs within the first 72 hours in 50% of cases of LD. Its significance is unknown. Hypocalcaemia proportional to the hypo-albuminaemia was noted in both our cases.

Differential Diagnosis

In the setting of an epidemic the clinical features of the disease are sufficiently characteristic to allow an early tentative diagnosis in the absence of laboratory confirmation. A sporadic case of LD may be more difficult to diagnose initially because many of its features are also seen in mycoplasmal pneumonia, psittacosis, Q fever, influenza, viral (e.g. adenovirus) pneumonia, tularaemia and plague.

Miller has suggested that a clinical diagnosis may be made early if any three of the following four features are present: (i) prodromal 'viral' illness; (ii) dry cough or confusion or diarrhoea; (iii) lymphopenia without marked neutrophilia; (iv) hyponatraemia. In the next few days the diagnosis is very likely if microbiological tests are negative and if there is radiological extension, an abnormal liver function test result or hypo-albuminaemia.

Treatment

Erythromycin is the drug of choice and 2-4 g/d is recommended. It should be administered for as long as 3 weeks, even though most patients respond dramatically, with subsidence of fever beginning within 24-48 hours. Relapse or prolonged convalescence may occur if treatment is discontinued after less than 2 weeks. Tetracycline appears to be less effective than erythromycin both in vitro and in vivo. Among the antibiotics, rifampicin shows the greatest activity in vitro and in vivo. Experience of its clinical efficacy is limited. It should not be used in treatment of sporadic, unconfirmed cases, because of the propensity for resistance to develop among many bacterial species. At present, it should be held in reserve for treatment of the patient who is not responding satisfactorily to erythromycin.

Supportive measures are of major importance in severely ill patients with the adult respiratory distress syndrome, often complicated by shock and acute renal failure. Mechanical ventilation and dialysis may be required.

Course and Prognosis

The course of LD may vary from a mild pneumonitis not requiring hospitalization to multilobar pneumonia with a fatal outcome resulting from respiratory failure.

A case fatality rate of 15% has been cited, but the correct figure is probably considerably lower now that the clinical diagnosis is more readily made, milder cases are detected, and the role of erythromycin in treatment is recognized.

CONCLUSION

Because of the time required for seroconversion and the difficulty in isolation or demonstration of the organism, clinical recognition is imperative for the diagnosis of the acute disease.

No single aspect of the disease is unique, but the overall clinical presentation is distinctive enough to permit the diagnosis of LD.

We wish to thank Dr David W. Fraser and Dr Hazel W. Wilkinson of the CDC for the confirmation of the serological tests.

REFERENCES

Eight adult patients from Port Elizabeth with significantly raised indirect fluorescent antibody titres of $\geq 256$ against the Legionnaires' disease bacterium are described. One of these patients had in addition elevated antibody levels against Mycoplasma pneumoniae. The clinical manifestations of the patients ranged from an 'influenza-like' illness in 1 patient to pneumonia of varying severity in 6. One patient had a severe illness with fever, cough and encephalopathy, together with the uncharacteristic features of lymphadenopathy and a petechial rash. This patient did not have pneumonia. Attention is drawn to unusual clinical aspects in some of the patients and the need for improved definitive diagnostic procedures is emphasized.