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Diagnosing coccidioidomycosis outside an endemic area

A recent case in Montreal, Quebec, Canada

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Abstract

A 65-year-old female with a long-standing controlled Crohn's disease was admitted in August 1979 to a Montreal hospital for investigation of a non-calcified RUL lung nodule. After an extensive work-up (negative bronchoscopy and mediastinoscopy showing a granulomatous reaction) she underwent thoracotomy; the nodule was enucleated and showed caseous material within a fibrous capsule.

Cultures in mycology produced a heavy growth of *Coccidioides immitis,* a diphasic pathogenic fungus endemic in South Western USA, identified by its morphology in autoclaved cultures, reversion to the spherule phase in mice and a 1:80 immunofluorescent stain with both commercial and patient's serum.

The patient, who travelled to Arizona three times in recent years, was soon discharged and remains apparently well. Because of inherent risks from handling cultures of *C. immitis,* the authors urge that, when coccidioidomycosis is considered clinically and epidemiologically, the pertinent information be transmitted to the laboratory.

Résumé

Une patiente de 65 ans présentant une illéite régionale controlée depuis plusieures années, fut admise à un hôpital montréalais pour étude d'un nodule non-calcifié sans cavité, au lobe supérieur droit.

Après de nombreuses analyses, dont une bronchoscopie négative et une médiastinoscopie démontrant une réaction granulomateuse, la patiente subit une thoracotomie: le nodule énucléé était composé de matière caséeuse entourée d'une capsule fibreuse.

Les cultures pour champignons démontrèrent la présence de *Coccidioides immitis,* champignon pathogène dimorphique, endémique dans le sud-ouest américain, dont l'identification fut basée sur les critères morphologiques de la culture autoclavée, la réversion à la phase 'sphérules' sur souris, et par épreuve d'immunofluorescence directe avec un sérum commercial et sérum de la patiente.

Cette patiente qui avait voyagé 3 fois en Arizona depuis quelques années, reçut vite son congé et son état demeure bon.

Etant donné les risques propres à la manipulation du *Coccidioides immitis,* les auteurs recommandent fortement que le personnel des laboratoires, surtout hors des régions endémiques, soit avisé des possibilités d'exposition du malade à ce champignon.

Introduction

Coccidioidomycosis is a fungal infection contracted by inhalation of air-borne spores. In most instances, the disease is benign but can be fatal if disseminated. The causative agent is a soil-borne diphasic fungus, *Coccidioides immitis,* which grows as a mold in, typically, semi-desert regions producing highly infectious arthrospores. Upon inhalation the spores expand and in a few days turn into sporangia filled with endospores which spill into adjacent tissues to repeat the cycle. If spilling occurs near a blood vessel the spores disseminate giving rise to foci of infection elsewhere in the body. The fungus is geographically restricted to southwestern United States (Arizona, California, Texas, Nevada, New Mexico and Utah), Central America (Guatemala and Honduras) and South America (Venezuela, Paraguay and Argentina); isolated cases have been reported from El Salvador, Colombia and Bolivia (2). In these areas the disease is endemic and visitors, if infected, may develop symptoms after they return to their domiciles. The literature on coccidioidomycosis is voluminous and readers may consult the appropriate chapter in any textbook of medical mycology or an excellent comprehensive review on the subject which appeared recently (2).

Case report

The patient was a 62-year-old non-smoking white female retired school teacher referred to in July 1979 for an investigation of a lung nodule. She had a history of well controlled Crohn's disease diagnosed in 1969 involving predominantly the mid-portion of the ileum and was on Prednisone (5 mg P.O., Q.A.M.). She travelled to Phoenix, Arizona, in 1972, 1973, 1974 and 1977 staying a few weeks at each occasion. Her January 1977 chest X-ray was normal. She was asymptomatic except for a 9 lb. weight loss over the past six months, modest loss of appetite and easy fatiguability. She denied any febrile respiratory illness during or after any of her trips, with more emphasis on the last one. There was no articular or cutaneous manifestation. On physical examination, she appeared well nourished. Her pulse was regular, 72/min, blood pressure 130/80 and her temperature was 37 °C. There was no clubbing, palpable lymphoadenopathy, or skin lesions. Her eyes, ENT, neck and breasts were normal. Her chest was clear to IPPA. Her CVS revealed N S1, S2. There was no gallop, murmurs or edema. Her JVP was normal. Abdominal examination revealed no organomegaly, masses or tenderness. Her CNS was grossly normal. Her extremeties were normal. The July '79 chest X-ray showed a 1 cm × 1 cm well defined homogenous nodular density in the anterior segment of the right upper lobe. There was no cavitation, or calcification, no air bronchogram. Mediastinal or paratracheal adenopathy were not apparent. The tomos of the lesion confirmed the above findings (Fig. 1). The patient underwent a thoracotomy revealing a rubbery 1 cm × 1 cm nodule in the anterior segment of the right upper lobe.

Laboratory investigations

Hematology

The patient's hemoglobin was 11.7 gm/dl, of normochromic and normocytic morphology. The white blood cell count was 5 600/mm³. The differential count was normal. ESR was 43 mm/H (Wintrobe). The platelet count was 384 000/mm³. Her P.T., P.T.T., and SMA 16 were normal. Protein

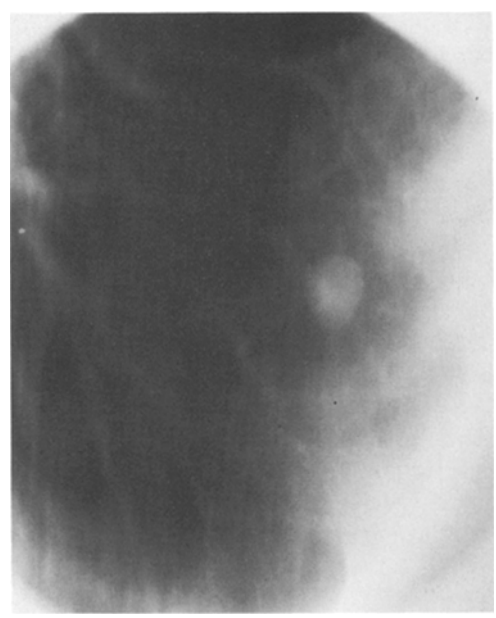

Fig. 1. The tomos of the nodule in RUL, July 1979.

electrophoresis and urine analysis were normal. Stool was negative for occult blood on 3 tests. The U.G.I. series with small bowel follow through and barium enema were unchanged from the previous ones. Her IVP and bilateral mammograms were normal. Sputa, mostly induced, were negative for cytology and for bacterial, mycobacterial and fungal stains and cultures. Pulmonary functions revealed Mild Airways Obstruction with FEVI/FVC ratio of 1.55/2.07 - 75%. Arterial blood gases (R.A.) pH 7.45, PO_{22} was 77 mmHg and PCO_2 was 37 mmHg. Bronchoscopy was normal. The washings and brushings were negative for both cytology and microbiology stains and cultures. A mediastinal lymph node was resected revealing a granulomatous inflammation or histopathology. Tuberculosis, fungal stains and cultures were negative.

Skin tests

5TU and histoplasmin were negative. The intradermal spherulin 'Usual skin strength' equivalent to 1:100 coccidioidin revealed a 4 mm erythematous reaction with a 2 mm induration. Histoplasma complement fixation test was negative (Coccidioidomycosis serology is presented below).

Histopathology revealed the presence of a soft cheesy material surrounded by a fibrous greyish capsule. Acid fast stain was negative. The Periodic Acid Schiff stain was positive revealing thinly scattered roundish structures with an indistinct cellular content. The smallest cells measured 4×4 to 4×5 μm, the largest 16 to 18 μm in diameter. The larger cells were often collapsed, irregular in outline and appeared empty. An occasional cell was thick-walled (Fig. 2). No budding was evident. The cultures of the nodule grew *Coccidioides immitis* (see Mycology below).

Final diagnosis

Coccidioidal nodule with microscopic mediastinal granulomatous involvement. On follow-up, the patient remained asymptomatic for the past 33 months, regained weight and, at this time, is in good health.

Mycology

The excised nodule, cut into small fragments, was cultured on 4 slopes of mycological media, which included 2 brain heart infusion glucose blood agars, and incubated at 25 °C. In about 6 days sparse white hyphae appeared on most fragments which suggested none of the common opportunistic pathogens. Subcultures were therefore prepared on 2 Kurung egg agar slopes for incubation at 37 °C and 4 Sabouraud agar modified slopes for incubation at 25 °C, to provide for possible diphasic pathogenic fungi, *Histoplasma capsulatum* or *Blastomyces dermatitidis*. Following the preparation of subcultures, a careful microscopic examination of one good primary culture showed fine septate hyphae and no conidia. However, occasional broad and closely septate branches contrasted markedly with the rest of the mycelium and *Coccidioides immitis* was considered. Until proven otherwise, the cultures were considered highly hazardous. When, a few days later, the cultures at 37 °C pro-

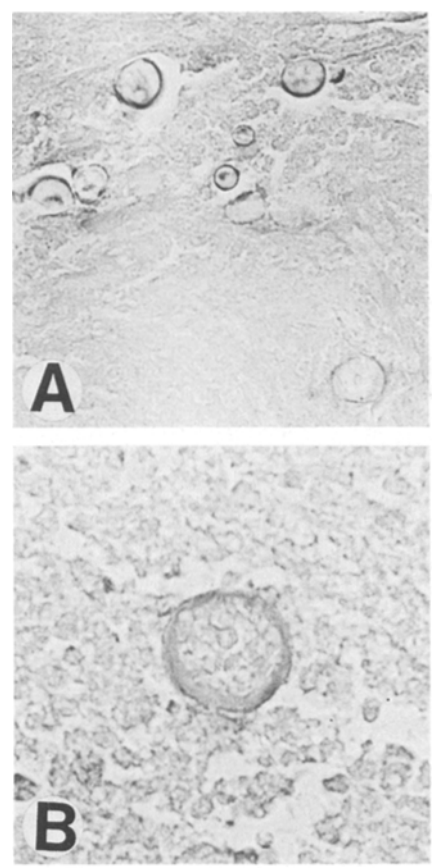

Fig. 2. C. immitis spherules in the patient's nodule. PAS stain.
A. Young spherules. × 400
B. An older spherule with immature endospores. × 640

duced a thin, fluffy and fast spreading mycelial growth, it was decided that an examination of live cultures would not be made any more. To avoid accidental mix-ups, the cultures were set apart, away from the bulk of routine isolations. The Sabouraud cultures at 25 °C became, in 10 days, chamois and granular in their older parts, with the surrounding hyphae forming a fine canopy over those areas. At 18 days one culture was autoclaved and examined. Broad, fertile, arthrosporic hyphae branching at nearly right angle showed clearly the characteristic, for *C. immitis,* barrel-shaped spores alternating with thin-walled cells (Fig. 3). At 27 days large numbers of loose spores were seen, each showing the wall remnants of the empty adjacent cells. Examination of the 4 primary cultures showed similar spores, their abundance varying on different media. To complete the identification, the reversion to the spherule phase had to be performed and, to accomplish this a Sabouraud culture was sent by a messenger to the Quebec Provincial Laboratory where proper equipment allows for safe handling of hazardous organisms.

Reversion to spherule phase

With maximum precautions and under a laminar flow class 3 inoculating cabinet, the spores were washed off with normal saline with a syringe. The suspension contained about 50 spores per ml as determined by a hemacytometer count of an aliquot killed with 10% formaldehyde. Three mice, of CB type, white males, about 4 week old, were injected intraperitoneally each with 1 ml of the spore suspension and the infection allowed to progress under the hood. On the 6th day one mouse was sacrificed and the autopsy revealed a small, 1 mm, nodule on the diaphragm and spherules in the peritoneal fluid. By the 10th day the 2 remaining mice, both torpid, were sacrificed and revealed numerous nodules in the lungs and liver (Fig. 4). Histologic sections showed spherules in the lungs, liver, spleen and kidneys which stained deeply with periodic acid Schiff and silver methanamine stains. The spherules stained very well with the patient's serum and with the commercial coccidioidin antiserum (Bol-

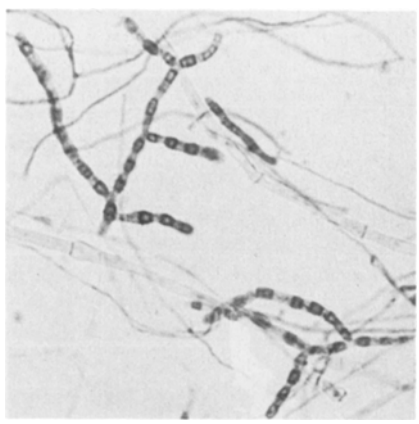

Fig. 3. Arthrospore-forming hyphae of *C. immitis* cultured from the nodule (Sabouraud agar, 25 °C). × 400

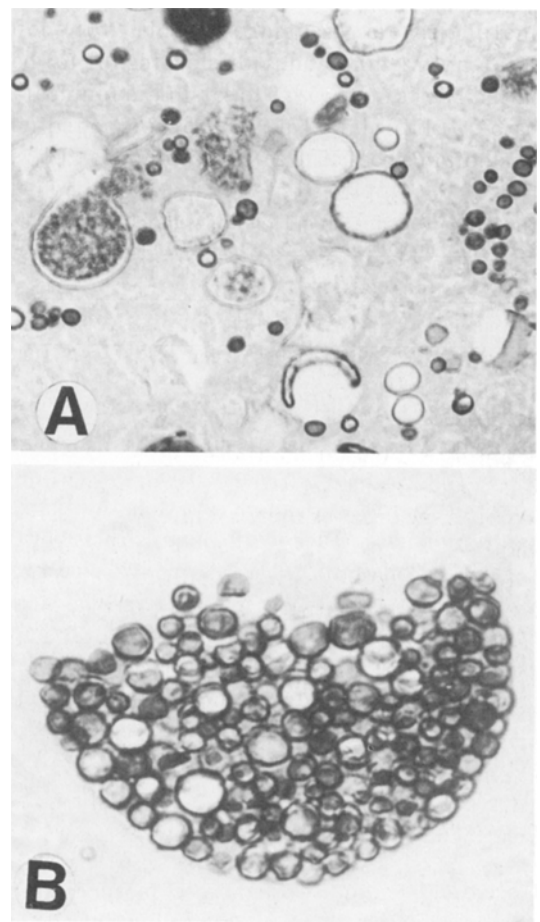

Fig. 4. The patient's *C. immitis* in mouse viscera. Silver methanamine stain.
A. Spherules and endospores. × 640
B. A large cluster of mature endospores released from a spherule. The degenerating wall of the spherule barely stained. × 1 365

lin, Hyland) in a fluorescent antibody stain. The above reversion to the spherule phase and the positive IFA stain proved that the isolated fungus was indeed *C. immitis*.

Serology

A direct IFA staining of the patient's lung biopsy was only faintly positive. On the other hand, the indirect IFA stain with the patient's serum used against her own isolated fungus in the mycelial phase and also with a control strain of *C. immitis* gave a titer of 1:80. A double radial immunodiffusion (Ouchterlony) test with a coccidioidin antigen failed to show coccidioidal antibodies in the patient's serum. A micromethod complement fixation for coccidioidomycosis in the patient's serum gave a titer of 1:2, too low to be significant.

Concluding remarks

In the endemic areas coccidioidomycosis is frequent enough for both the physician and the laboratories to be prepared for yet another case. By contrast, in non-endemic areas characterized by a low incidence of infections of, perhaps, one case every few years in a particular hospital, one cannot escape the all-too human pitfall of 'out of sight – out of mind' and, when a case does present itself, it may catch both the diagnositicians and the laboratories by suprise.

Considering the seriousness of disseminated coccidioidomycosis and the high infectivity of the arthrospores, it is desirable that when a granulomatous pulmonary disease is considered, the case history include information about the patient's visits to the endemic areas of coccidioidomycosis. The time lapse between exposure and symptoms may be as short as 2 to 3 weeks, or as long, as in this case, 2 years. Such information should be conveyed to the laboratory as a hint and a warning. Most laboratories, particularly in smaller hospitals, are not equipped to deal with air-borne pathogens of class 3 of hazardous organisms and would appreciate pertinent information in order to handle the cultures as safely as possible (6). When one considers that the lethal dose for a mouse is 50 spores, the incredibly large numbers of spores picked up at the tip of a needle for microscopic examination or subculture may constitute a massive dose for an unsuspecting technician and any person in the laboratory (3, 4, 5).

The fact that, in this case, the fungus was recognized grossly and microscopically (the patient's visits to Arizona and her histology having come to the attention of the laboratory later) was made possible because of the co-operation, some time ago, of two Montreal hospitals which had notified and invited the McGill Laboratory of Mycology to participate, from the ailes, in their cases. Above all, in one of these two cases, a positive specimen was allowed to be used in our own laboratory to observe the fungus in all its stages of colony development under routine handling.

Acknowledgements

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