A traveller in time: Phylogenetic analysis to identify a disseminated infection acquired 30 years earlier

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A 79-year-old East Indian man and retired office manager, originally from Tanzania (Africa) presented with hypotension and respiratory distress requiring intensive care unit admission. His background included a medical history of type 2 diabetes, hypertension, hypercholesterolemia and chronic obstructive pulmonary disease (40 packs/year smoker). A reticulonodular chest radiograph pattern (Figure 1A) had been identified three months earlier because of new constitutional symptoms (fatigue, weight loss, anorexia and fever) and dyspnea. He had not travelled since immigrating to Canada 30 years prior. His vital signs included an oral temperature of 35.3°C, blood pressure of 73/49 mmHg, heart rate of 113 beats/min, respiratory rate of 33 breaths/min and an oxygen saturation of 99% (inspired mixture of 31% oxygen). Pertinent physical examination findings showed a cachectic individual with splenomegaly and expiratory wheezes but no rash, clubbing or palpable lymphadenopathy. Tazobactam-piperacillin, intravenous hydrocortisone and noradrenaline infusions were commenced. His blood test results were as follows: white blood count $4.0 \times 10^9/L$; hemoglobin level 109 g/L (normocytic); platelet count $139 \times 10^9/L$; hyponatremia with a sodium level of 125 mmol/L; normal potassium, bicarbonate and creatinine

levels; and glucose level 7.5 mmol/L. Liver function and enzyme testing showed cholestasis (alkaline phosphatase 339 U/L, gamma-glutamyl transferase 647 U/L, alanine aminotransferase 14 U/L, total bilirubin 33 $\mu\text{mol/L}$, conjugated bilirubin 19 $\mu\text{mol/L}$ and albumin 27 g/L). Computed tomography scan identified bilateral emphysematous lung changes, multiple precarinal/paratracheal lymph nodes (smaller than 1 cm), bilateral adrenal masses, hepatosplenomegaly, colitis, a splenic lesion and mild ascites (Figure 1B). Functional testing of the adrenal glands (1 μg and 250 μg adrenocorticotrophic hormone stimulation test) indicated adrenal insufficiency.

Review of the patient's records from three months previously revealed similar computed tomography abnormalities. His nuclear bone scan was normal, and upper endoscopy revealed two small antral ulcers. The T cell subset ratio was normal (CD4/CD8 ratio 2.4, CD4 $0.31 \times 10^9/L$ and CD8 $0.13 \times 10^9/L$), and serology for HIV by enzyme immunoassay was negative. Tumour markers (carcinoembryonic antigen, alpha-fetoprotein and prostate-specific antigen) and serum protein electrophoresis were within normal ranges.

Bronchoscopy and standard microbiological analyses were performed. What is the diagnosis?

Figure 1) Chest x-ray and computed tomography scan of patient at presentation to the intensive care unit. **A** Reticulonodular pattern. **B** Splenomegaly with polar lesion (white arrow) and bilateral adrenal hyperplasia (white arrowheads)

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Figure 2) Maximum parsimonious tree depicting the relationships among strains of *Histoplasma capsulatum* from representative regions of the world. The DNA sequences from our strain were analyzed in the context of the data from Kasuga et al (4). The arrow indicates the phylogenetic affiliation of our strain (HcH) to representatives of *H. capsulatum* var. *duboisii*. In total, 1585 base pairs were analyzed from fragments of four genes (ADP-ribosylation factor, *H* antigen precursor, delta-9 fatty acid desaturase and alpha-tubulin), following the protocols described in Kasuga et al (4). Reference strains in the African cluster are H87, H88, H137, H147 *H. capsulatum* var. *duboisii*; H143 *H. capsulatum* var. *capsulatum*; H189 *H. capsulatum* var. *farciminosum*

DIAGNOSIS

The patient's blood, urine and sputum cultures were negative for routine pathogens (including *Mycobacterium* species, cytomegalovirus, herpes simplex virus, Enterovirus species, *Legionella* species and *Mycoplasma* species). Bronchoalveolar lavage grew *Histoplasma capsulatum*, which was confirmed by DNA probe, and the patient's serology was reactive for *Histoplasma* M and H bands by immunodiffusion.

The diagnosis of subacute disseminated histoplasmosis presenting with adrenal insufficiency in an immunocompetent host was made. Liposomal amphotericin B therapy (3 mg/kg/day) was initiated. After six weeks of successful therapy, attempts to switch to oral itraconazole (200 mg/day) were hampered by acute cholecystitis, and liposomal amphotericin B was continued. Unfortunately, the patient died from complications related to a fall approximately two months after the diagnosis and while on therapy.

DISCUSSION

H. capsulatum is a soil-borne dimorphic fungus that is endemic in some regions of the world. The clinical disease spectrum varies both in temporal course and severity. Immunocompetent people have asymptomatic or self-limited pulmonary infections with resolution due to cell-mediated immunity. In contrast, subacute disseminated histoplasmosis occurs primarily in immunocompromised individuals and is a rare disease entity among immunocompetent hosts (less than 0.1% of cases) (1,2). The above time course, disease extent and presenting complication of adrenal insufficiency are compatible with the diagnosis of subacute disseminated histoplasmosis. The clinical picture can mimic other multisystem diseases and includes fever, weight loss and fatigue (1,2). Extrapulmonary organ involvement includes hepatosplenomegaly, gastrointestinal mucosal ulcerations (especially cecal), bone marrow infiltration and adrenal masses (1-3). Adrenal glands are vulnerable to infection (unilaterally or bilaterally involved), and complicated by adrenal insufficiency in less than 10% of disseminated cases (2). It is thought that disseminated histoplasmosis in immunocompetent patients results from progressive primary disease, reactivation of quiescent yeast in the reticuloendothelial system or reinfection (1,2). Temporal dissociation between the primary infection and reactivation can cause it to be overlooked as a cause of a multisystem illness (2,3).

Given the patient's discrete time period in Tanzania and no subsequent travel, the hypothesis of reactivation versus reinfection was tested. Our isolate underwent polymerase chain reaction amplification and sequence analysis of four gene fragments based on a protocol and reference strains used by Kasuga et al (4) to determine its affiliation to potentially geographic-specific populations of this species, using phylogenetic analysis. Our isolate fell within the clade constructed primarily from *H. capsulatum* var. *duboisii* reference strains, the etiological agent of African histoplasmosis (Figure 2).

Classic African histoplasmosis is hallmarked by chronic cutaneous and bony abnormalities, with disseminated disease reported only in HIV-positive patients (3,5-7). Its presumed geographical distribution is tropical Africa (between 15° north and 10° south latitude, and from Senegal to Uganda) and Madagascar (8,9). It primarily has been reported to infect bats, nonhuman primates and humans (9-11). However, 75% of the approximately 250 cases reported have originated from six countries (Nigeria, Niger, Senegal, Congo, Zaire and Uganda), with cases in many other endemic countries presumed, but not formally recognized (6,7). To our knowledge, human cases of *H. capsulatum* var. *duboisii* from Tanzania have not been reported. Identification in clinical samples has traditionally relied on the observance of large yeast forms (12 µm to 15 µm) to differentiate it from *H. capsulatum* var. *capsulatum* (2 µm to 4 µm) (5). However, small-sized yeast cells occur early in an infection and are gradually replaced by large yeast forms (7). Given that our isolate is phylogenetically of African ancestry, the results suggest latent reactivation of a histoplasmosis infection acquired 30 years earlier. If such is the case, we would add Tanzania to the list of countries where human infections with *H. capsulatum* var. *duboisii* can occur. Also, it highlights potential limitations of yeast cell morphology for classification in clinical and epidemiological studies (4).

SUMMARY

The present article reports a case of subacute disseminated *H capsulatum* in an immunocompetent man, which seems to have manifested from a *H capsulatum* var. *duboisii* strain acquired in Tanzania 30 years earlier.

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