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Disseminated histoplasmosis: a rare clinical phenotype with difficult diagnosis

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Abstract

We describe a rare and interesting case of a 37-year-old man who presented with an intermittent fever, progressive cytopenia, and hepatosplenomegaly. Histopathological examination of a bone marrow smear revealed haemophagocytes and intracellular yeast-like *Histoplasma capsulatum* (*H. capsulatum*); thus, we prolonged the blood culture duration to detect fungi, and *H. capsulatum* was detected in the peripheral blood. After the diagnosis of disseminated histoplasmosis, the patient was successfully treated with amphotericin B and symptomatic therapy. This is the first case in southwest China for which *H. capsulatum* was cultivated in peripheral blood, illustrating that the duration of specimen culture should be lengthened if a specific pathogen infection is suspected. Moreover, this case enriches our understanding of clinical manifestations of disseminated histoplasmosis.

Introduction

Histoplasmosis is a systemic mycosis caused by the soil-based dimorphic fungus *Histoplasma capsulatum* (*H. capsulatum*). It occurs more frequently in the mid-western region of the United States, Latin America, and Central and Western Africa [1,2]. Only sporadic cases of autochthonous histoplasmosis have been reported in China, which is traditionally considered a non-endemic area for *H. capsulatum* infection [3–5]. Most *H. capsulatum* infections are asymptomatic or self-limiting. However, 0.05% of acute infections result in severe and progressive dissemination [6]. Disseminated histoplasmosis has been reported in patients with AIDS and, less frequently, in immunosuppressed patients without AIDS [5]. The rate of a positive blood culture for *H. capsulatum* is low. Here, we report a case of disseminated histoplasmosis in an HIV-negative patient. This is the first case in which *H. capsulatum* was cultivated in peripheral blood in southwest China.

Case Report

In early November 2014, a 37-year-old man was admitted to our department because of intermittent fever lasting >1 month, with fluctuating body temperatures of 37.6–39.6°C. The fever was more frequent in the afternoon and evening and dissipated without special treatment. He also complained of excessive perspiration, mild cough, and a 7-kg weight loss in two months. The patient first visited a local hospital in mid-October. A mycobacterial examination yielded negative results, and a bone marrow aspiration was performed after the onset of pancytopenia; the bone marrow smear revealed yeast-like bacteria, and a fungal infection was suspected. However, no fungus was cultivated in the initial culture; therefore, empirical treatment included moxifloxacin for 10 days and voriconazole for 3 days, without symptom improvement. He worked at a blood bank and had a long history of blood donation (plasma at 400 mL/time, 3–4 times a year through physical centrifugation) and contact with a soil-based environment

during farm work in the countryside within the previous year. The patient was from the Yangtze River region, which was not the endemic area of the human T-cell lymphotropic virus type 1 (HTLV-1); he was native to China and had never travelled abroad. His family history was non-contributory.

The physical examination showed the following: body temperature, 39.1°C; blood pressure, 107/62 mmHg; heart rate, 109 beats/min; and respiration rate, 20/min. He appeared ill without palpable lymph nodes or obvious heart or lung abnormalities. The liver could be palpated 2 cm below the ribs, with a hard texture and blunt edge. The spleen could be palpated 3 cm below the ribs.

The laboratory evaluation revealed a complete blood cell count indicating progressive pancytopenia. The white blood cell (WBC) count had decreased from 3.33 to 1.86×10^9/L, haemoglobin from 114 to 86 g/L, and platelet count from 114 to 2×10^9/L (Table 1). C-reactive protein (43 mg/L; normal value < 5 mg/L) and liver enzyme (aspartate aminotransferase, 70 IU/L; alanine aminotransferase, 82 IU/L; alkaline phosphatase, 222 IU/L; and lactate dehydrogenase, 352 IU/L) levels were abnormal. In the serological TORCH (*Toxoplasma gondii*, rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV)) test, the CMV IgM (22.7 AU/mL) and HSV antibody I/II type (2.8) levels were high. Immune indicators were low: complement C4, 0.49 g/L; CD3 cell subsets, 65.1%; CD4 cell subsets, 17.3%; actual CD4 positive T-cell count, 320 cells/μL (normal value, 471–1220 cells/μL), and CD4/CD8, 0.53. The serum immunoglobulin levels were as follows: IgG 15.3 g/L (normal value, 8–15.5 g/L), IgA 1010 mg/L (normal value, 836–2900 mg/L), and IgM 1640 mg/L (normal value, 700–2200 mg/L). In the arterial blood gas analysis (2-L oxygen inhalation at rest), the partial pressure of oxygen in arterial blood was 76 mmHg, and oxygen saturation was 95.8%. The results for renal function (serum creatinine 68 μmol/L), erythrocyte sedimentation rate, serum (1,3)-β-D-glucan, and serum immunofixation electrophoresis were within the normal levels. Serological results for HIV, the tuberculin purified protein derivative (PPD) test, and the T-cell spot test for tuberculosis infection (T-SPOT) were negative. Repeated blood and sputum cultures were negative.

Abdominal computed tomography (CT) confirmed hepatosplenomegaly and intrahepatic lymphatic stasis and revealed an abdominal cavity and retroperitoneal lymphadenopathy (Fig. 1A). Chest CT revealed a few chronic inflammations in the posterior segment of the left lung and the medial segment of the right middle lobe of the lung (Fig. 1B), which did not indicate the findings of active histoplasmosis. Positron emission tomography-CT (PET-CT) revealed bone marrow and splenomegaly with increased glucose metabolism, which were likely reactive changes. No evidence of malignant tumour formation was found.

The patient was treated with ganciclovir (5 mg/kg, every 12 h) for 10 days from the second day of admission; the results of the serological CMV and HSV tests were normal. However, persistent fever, liver and spleen growth, and a progressively diminishing blood count remained. We administered symptomatic and supportive therapies for

Table 1. Changes in the relevant haematological laboratory tests after admission.

Day of test	RBC ($\times 10^{12}$/L)	HGB (g/L)	HCT (L/L)	MCV (fL)	PLT ($\times 10^9$/L)	WBC ($\times 10^9$/L)	NEUT %
Reference intervals	4.3–5.8	130–175	0.4–0.5	82–100	100–300	3.5–9.5	40–75
Day 1*	3.84	114	0.34	81.2	114	3.33	66
Day 13	3.72	98	0.28	78.1	100	1.54	70
Day 16	3.71	98	0.28	77.9	9	1.86	66
Day 19	3.69	94	0.29	77.3	2	6.36	84
Day 20	3.72	97	0.3	79.7	4	6.67	83
Day 21	3.89	103	0.31	78.3	5	10.6	92
Day 26	3.76	91	0.29	77.1	14	3.82	57
Day 28	3.66	91	0.29	80.1	44	9.79	85.7
Day 30	3.93	93	0.3	75.3	108	4.27	85.5
Day 34	4.5	110	0.35	78.7	249	3.71	49.9
Day 42	4.7	121	0.39	83.4	262	8.06	68.8

***Admission day.**

RBC, red blood cell; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; PLT, platelet; WBC, white blood cell; NEUT%, neutrophil percentage.

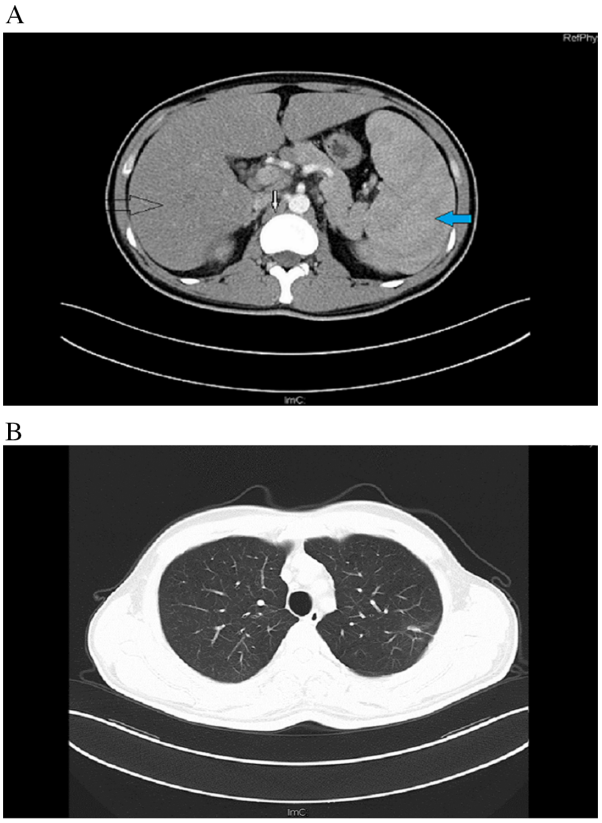

Figure 1. (A) Abdominal computed tomography (CT) image confirming hepatosplenomegaly, abdominal cavity, and retroperitoneal lymphadenopathy. (B) Chest CT image showing a few chronic inflammations in the posterior segment of the left lung and the medial segment of the right middle lobe of the lung.

leukopenia, thrombocytopenia, and hypoimmunity (intravenous immunoglobulin (IVIG) 25 g/day for 5 days and subcutaneous thymalfasin injection 1.6 mg/day for 10 days). The serum ferritin level (543.3 ng/mL) increased on the 13th day. Therefore, the bone marrow examination was repeated on the 17th day. Unexpectedly, Gomori methenamine silver staining showed sparse fungal spore distribution (Fig. 2A). On the 25th day, to identify the fungal species, a third bone marrow examination was conducted, and this smear showed visible haemophagocytes and intracellular yeast such as *H. capsulatum* (Fig. 2B). Therefore, we diagnosed progressive disseminated histoplasmosis. Peripheral blood was cultured for fungi in Sabouraud dextrose agar at 28°C. *H. capsulatum* began to grow on day 15. Upon microscopic examination, hyaline hyphae produced tuberculated macroconidia on day 28, confirming the presence of *H. capsulatum* (Fig. 2C,D).

After the diagnosis of progressive disseminated histoplasmosis, on 3 December, the patient was administered intravenous amphotericin B (1 mg/kg per day) and symptomatic therapy. His general condition improved one month post-treatment. Because of economic problems, the patient sequentially refused treatment with itraconazole. Follow-up bone marrow aspirate denied the presence of *H. capsulatum*. Routine blood tests and chest–abdominal CT revealed no abnormalities after three months. In the subsequent one-year follow-up, there was no relapse.

Discussion

Diagnosing this complex case was painstaking. Our patient showed clinical manifestations of fever, pancytopenia, and hepatosplenomegaly. Both infectious and non-infectious causes were responsible for the persistent fever. The non-infectious causes might have been lymphoma, but the PET-CT revealed no malignant tumour, excluding lymphoma. For the infectious sources, we considered tuberculosis, fungal agents, viral agents, or kala-azar. However, no pathogenic evidence of tuberculosis was found, and the PPD and T-SPOT results were negative. For viral infections, although the levels of CMV IgM and HSV antibody I/II type were high, suggesting a possible viral cause, the patient's condition worsened after 10 days of antiviral therapy. Kala-azar was considered because the patient had travelled to Jiuzhaigou Valley. However, Leishman-Donovan bodies were not found in the bone marrow, and the RK39 test result was negative; this test involves a rapid immunochromatographic assay to qualitatively detect Leishmania donovani antibodies in human serum. As the patient had used voriconazole as antifungal therapy without clinical improvement, mycosis was not initially suspected until the first bone marrow smear showed a suspicious fungus with a normal bone marrow biopsy result. To confirm the results, two more bone marrow biopsies were performed. Finally, a few fungal spores were detected, and the bone marrow smear showed visible haemophagocytes and some phagocytes that were suspected to devour a fungus, which was likely *H. capsulatum*. Thus, we prolonged the blood culture duration for fungi, and *H. capsulatum* was detected in the blood culture 28 days later.

Human histoplasmosis is caused by var. capsulatum and var. duboisii of the dimorphic fungus *H. capsulatum*. These varieties are endemic to central and western Africa and the mid-western United States and most of Latin America [1,2]. This organism possibly grows naturally in some areas of China. Since 1990, >300 cases of histoplasmosis have been reported in China, of which only 17 cases were possibly imported. Most patients were from the Yangtze River region [5], which includes the province where our patient resided. He had never travelled abroad. He was an employee at a blood bank and had a home in the countryside, where he could do some farm work during vacation. Therefore, he had a nearly one-year history of contact with a soil-based environment. Soil containing *H. capsulatum* spores could be inhaled during work.

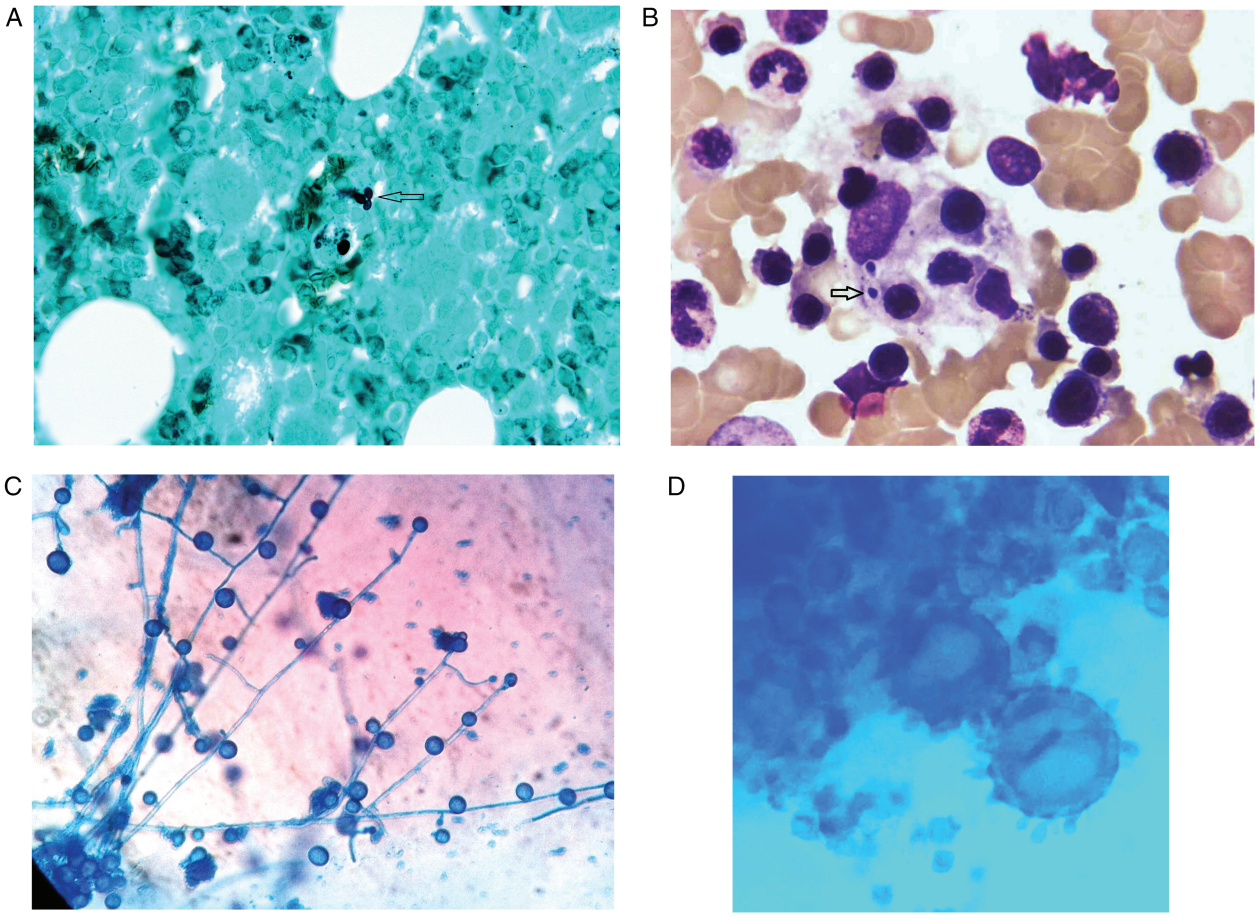

Figure 2. *Histoplasma capsulatum* (*H. capsulatum*) identified in bone marrow and blood. (A) Gomori methenamine silver staining of the bone marrow showing a few fungal spores (400×). (B) Bone marrow aspirate with the Wright-Giemsa stain showing small oval spores with refractive edges simulating a capsule in the visible monocytes (1000×). (C) Mycelial phase of *H. capsulatum* with the lactophenol cotton blue stain showing tuberculate microconidia (400×). (D) Mycelial phase of *H. capsulatum* with the lactophenol cotton blue stain showing tuberculate macroconidia, which are thick-walled and display characteristic surface tubercles or projections (1000×).

Moreover, the patient displayed decreased cellular immune competency. All of these risk factors increased his vulnerability to histoplasmosis. We attempted to perform a phylogenetic analysis for *H. capsulatum*, which was discontinued because of test tube contamination. Therefore, we could not identify if *H. capsulatum* was imported or native to China.

Based on the clinical characteristics, there are four types of histoplasmosis: asymptomatic (95%), acute pulmonary, chronic pulmonary, and disseminated (rarest) [7]. Disseminated histoplasmosis is a systemic mycosis that specifically affects the reticulohistiocytic system [8]. It usually invades visceral organs (liver, spleen, pancreas, and intestines). Therefore, patients with disseminated histoplasmosis can present with various symptoms such as fever, weight loss, anorexia, cough, vomiting, diarrhoea, and abdominal pain [9]. The most common symptoms of disseminated histoplasmosis are fever (89.1%), respiratory symptoms (38.1%), and weight loss (37.4%). The most common physical findings include splenomegaly (72.0%), hepatomegaly (68.1%), and lymphadenopathy (41.2%) [5]. Our patient presented with fever, weight loss, cough, pancytopenia, hepatosplenomegaly, and lymphadenopathy, which supports the diagnosis of disseminated histoplasmosis. The diagnosis of histoplasmosis is difficult. Culture tests have a high false negative rate [5], which might be due to the slow growth (up to six weeks) of *H. capsulatum* [2]. We prolonged the blood culture duration for fungi to 28 days, which contributed to the eventual success. Moreover, as histoplasmosis and penicilliosis have similar clinical presentations, laboratory findings, and chest radiographic abnormalities [10], differential diagnosis was challenging. Some reported cases of penicilliosis caused by *Penicillium marneffei* were histologically confused with histoplasmosis because of its intracellular nature and oval shape [4,11,12]. Thus, these two kinds of microorganisms should be microbiologically distinguished.

In conclusion, we present a rare and interesting case of disseminated histoplasmosis in a patient without AIDS. To the best of our knowledge, this is the first case in which *H. capsulatum* was cultivated in blood in southwest China, from which we learned many new facts. First, the specimen culture duration should be prolonged if a specific pathogen infection is suspected. Furthermore, awareness of the available serology, antigen, and polymerase chain reaction methods to help confirm the diagnosis of histoplasmosis is needed. Finally, this case enriches our understanding of the clinical manifestations of disseminated histoplasmosis and emphasizes the importance of collaboration among multiple specialties.

Disclosure Statements

No conflicts of interest declared.
Appropriate written informed consent was obtained for the publication of this case report and accompanying images.

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