

Disseminated histoplasmosis diagnosed on bone marrow aspiration in an immunocompetent patient

TO THE EDITOR: Histoplasmosis is caused by *Histoplasma capsulatum* variant *capsulatum* and *H. capsulatum* variant *duboisii*. It is a systemic fungal infection with a disease spectrum ranging from asymptomatic primary infection to disseminated disease. Disseminated histoplasmosis is usually seen in immunocompromised patients such as those with AIDS or hematological malignancies, or patients who have undergone a transplant or are receiving steroids [1]. Life threatening infections may be seen in infants [2]. We present a case of disseminated histoplasmosis, diagnosed incidentally on bone marrow aspiration, in a young, immunocompetent woman.

A 35-year-old woman presented with pallor, intermittent fever associated with chills, and body ache that had started 6 months earlier. There was no history of joint pain, vomiting, loose stools, cough, or dysuria. Respiratory and cardiovascular system examinations were normal. In the abdomen, non-tender hepatosplenomegaly was present, 5 cm and 1 cm below the costal margin. The patient had no history of steroid intake or diabetes, the results of the recombinant K39 and rapid malaria antigen tests were negative, and human immunodeficiency virus serology was non-reactive. However, a complete blood count revealed pancytopenia, and bone marrow aspirate smears showed many intracellular and extracellular yeast forms of *H. capsulatum*. These organisms were periodic acid Schiff-positive and gave a positive Prussian blue reaction with Perl's staining (Fig. 1). A diagnosis of disseminated histoplasmosis

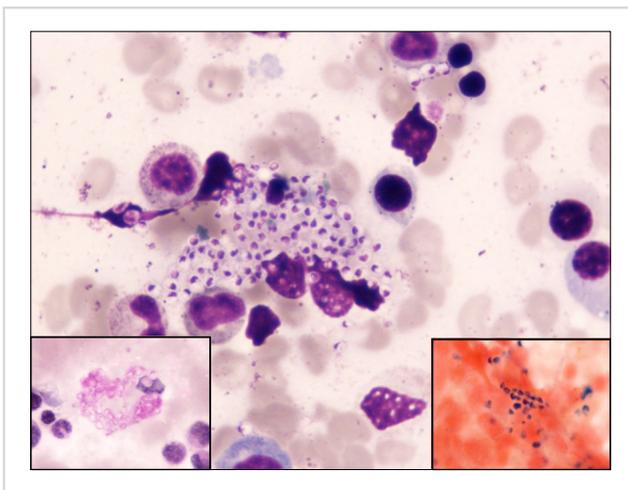


Fig. 1. A Wright stained-smear showing numerous extracellular and intracellular yeast forms of *Histoplasma capsulatum* ($\times 1,000$), which were PAS positive (inset on the lower left) and gave a positive Prussian blue reaction (inset on the lower right).

was made, and the patient started receiving amphotericin B, but was then lost in follow up.

Symptomatic infection is uncommon in immunocompetent patients exposed to *H. capsulatum*. The disease spectrum in these patients includes acute pulmonary histoplasmosis, chronic cavitary histoplasmosis, granulomatous mediastinitis, mediastinal fibrosis and, uncommonly, pericarditis, pleural disease, and broncholithiasis [2]. A high index of clinical suspicion is required to diagnose disseminated histoplasmosis in an immunocompetent patient, although timely diagnosis is important because it is associated with a high mortality.

Yeast forms of *H. capsulatum* are observed intracellularly and, rarely, in the extracellular space. On routine Romanowsky staining, the organisms are often overlooked because of the dense staining of hematopoietic cells [3]. The Grocott methenamine silver technique provides good staining of the yeast forms with minimal background staining. Perl's staining, which is routinely performed in all bone marrow aspirates, can aid in the diagnosis of histoplasmosis, avoiding the requirement for additional cytochemistry. Yeast cells give a positive Prussian blue reaction and are then highlighted against a red background [3]. The significance of this finding is unknown.

Most fungi proliferate in the presence of increased iron, and the monocyte phagocyte system of the bone marrow that is rich in iron stores may increase the growth rate of yeast cells. It is also possible that large stores of iron function as part of the host defense mechanism. Yeast cells might be inhibited by high iron concentrations within the histiocytes [3]. Further studies are required to determine the changes and deviations in iron metabolism in disseminated histoplasmosis.

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No potential conflicts of interest relevant to this article were reported.

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Unusual association of CD8+ T-cell lymphocytosis with invasive thymoma

TO THE EDITOR:Thymoma is known to be associated with autoimmune disorders such as myasthenia gravis, which occurs in approximately 30% of cases [1], and sometimes is also accompanied by T-cell lymphocytosis [2]. However, thymoma patients with concurrent myasthenia gravis rarely develop lymphocytosis [3]. In the majority of cases, thymoma-associated lymphocytosis consists of a polyclonal population of mature T cells expressing CD4, CD8, or neither marker [2]. We report the unusual case of a patient with a history of invasive thymoma and myasthenia gravis who was subsequently found to have peripheral blood (PB) and bone marrow (BM) CD8+ T-cell lymphocytosis and pure red cell aplasia.

A 38-year-old woman presented to the emergency department with abdominal pain. Nine years earlier, she had been diagnosed as having invasive thymoma and myasthenia gravis. The thymoma had been treated surgically, followed by chemotherapy and radiation therapy until one year prior to the present admission. A computed tomography scan of her abdomen and chest revealed multiple thymoma metastases involving the lung, pleura, pericardium, liver, both

ovaries, and peritoneal seeding. Percutaneous needle biopsy of the pleural mass was consistent with thymoma. The patient's complete blood count showed the following results: white blood cell count: $44.4 \times 10^9/L$ (58.9% neutrophils, 34.9% lymphocytes); hemoglobin: 9.5 g/dL; platelet count: $170 \times 10^9/L$. In the workup for anemia, progressive anemia (6.7 g/dL) and persistent lymphocytosis ($7.8\text{--}31.0 \times 10^9/L$) were noted over 3 months. Analysis of a PB smear showed an increased number of mature lymphocytes with dense chromatin, no nucleoli, and scanty agranular cytoplasm (Fig. 1). Flow cytometry of the PB revealed that 78.4% of lymphocytes were CD3+ and CD8+ T cells. The ratio of CD4+/CD8+ cells was 0.13. A BM study revealed mild hypocellular marrow with erythroid aplasia and diffuse scattered infiltration of small lymphocytes (26.3% lymphocytes on BM aspirate) (Fig. 1). Immunophenotyping by flow cytometry showed proliferation of T cells with expression of CD2, CD3, CD5, CD7, and CD8, which was consistent with the pattern expressed by the T cells in the PB. The result of TCR gene rearrangement in the BM demonstrated no clonal rearrangement. The patient underwent one cycle of chemotherapy with cisplatin, etoposide, and ifosfamide to treat thymoma. After the first cycle, chemotherapy was put on hold because the patient was in poor clinical condition. PB analysis showed somewhat reduced T-cell lymphocytosis ($5.8 \times 10^9/L$) over four months of observation (Fig. 2).

To the best of our knowledge, this is the first case report of PB and BM CD8+ T-cell lymphocytosis occurring concurrently with invasive thymoma. In most reported cases of T-cell lymphocytosis associated with thymoma, lymphocytes consist of a mixed mature population of T cells with a normal or slightly abnormal CD4:CD8 ratio [2, 4-6]. In the present case, besides peripheral T-cell lymphocytosis, a predominance of mature lymphocytes was found in the

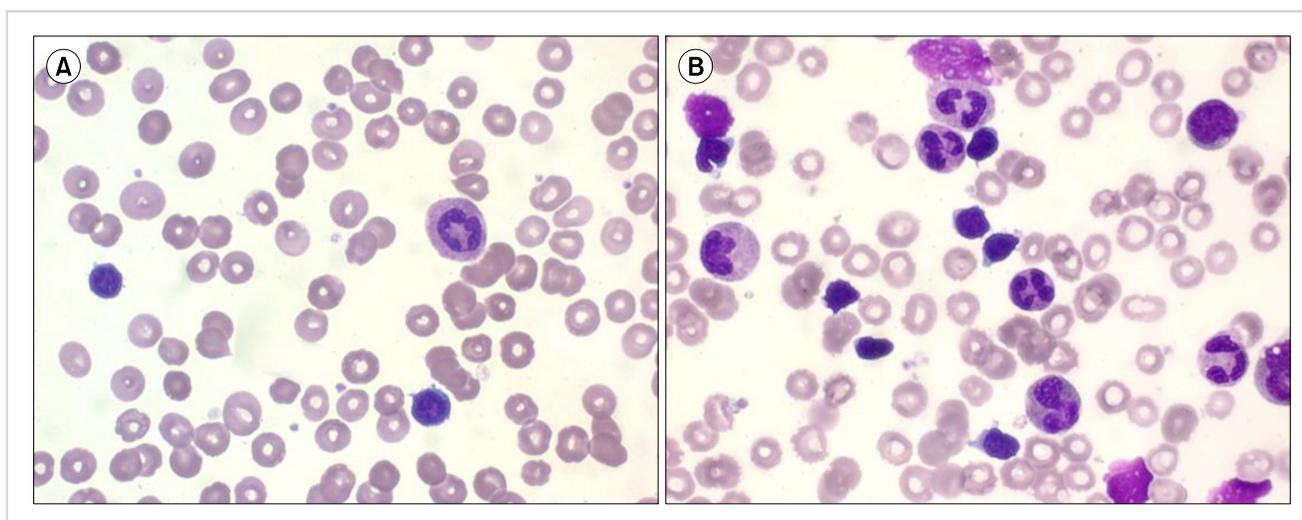


Fig. 1. (A) Peripheral blood smear showing mature lymphocytes with dense chromatin, no nucleoli, and scanty agranular cytoplasm (Wright-Giemsa stain, $\times 1,000$). (B) Bone marrow lymphocytosis on aspirate (Wright-Giemsa stain, $\times 1,000$).