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Original contribution

Visibility of *Histoplasma* within histiocytes on hematoxylin and eosin distinguishes disseminated histoplasmosis from other forms of pulmonary histoplasmosis☆

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Summary The visibility of *Histoplasma* within histiocytes on hematoxylin and eosin is a well-known feature of disseminated histoplasmosis. However, it is unclear whether this finding can be used to differentiate disseminated histoplasmosis involving the lung from other forms of pulmonary histoplasmosis. The aim of this study was to determine whether the visibility of *Histoplasma* within histiocytes on hematoxylin and eosin in lung biopsies suggests disseminated disease. Lung biopsies in which *Histoplasma* was identified were re-examined to determine whether organisms were visible within histiocytes on hematoxylin and eosin. Clinical findings were reviewed retrospectively to determine the type of histoplasmosis. *Histoplasma* was visible within histiocytes on hematoxylin and eosin in lung biopsies from 4 patients (2 men, 2 women, 50-74 years) who presented with pulmonary manifestations without definite evidence of disseminated disease at the time of biopsy. Subsequently, all 4 manifested clinical and/or microbiologic features of disseminated disease (positive extrapulmonary cultures and fatal outcome in 2, positive extrapulmonary cultures in 1, and multiorgan failure and fatal outcome in 1). In contrast, organisms were identified on silver stains but could not be visualized on hematoxylin and eosin in 42 patients, none of whom showed clinical or microbiologic evidence of disseminated disease (pulmonary histoplasmoma, 38; acute pulmonary histoplasmosis, 4). In lung biopsies, the visibility of *Histoplasma* within histiocytes on hematoxylin and eosin suggests disseminated disease. Recognition of the significance of this finding is helpful in diagnosing disseminated disease in patients who present primarily with pulmonary manifestations without definite clinical evidence of dissemination at the time of biopsy.

1. Introduction

Histoplasmosis involves the lungs in several distinctive forms [1-13]. Of these, the form requiring the most aggressive antifungal therapy is disseminated histoplasmosis, a progressive, potentially fatal illness in which impaired cell-mediated immunity allows hematogenous spread of organisms from the lungs to extrapulmonary tissues. Disseminated histoplasmosis is usually diagnosed by demonstrating *Histoplasma* by cultures or histology in individuals with clinical, radiologic, microbiologic, or histologic evidence of extrapulmonary disease [1,14-16]. However, not all patients with disseminated histoplasmosis

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we have evidence of extrapulmonary disease at presentation. Instead, some present with symptoms and radiologic findings suggestive of pulmonary disease, prompting biopsies of the lung rather than extrapulmonary tissues [14,17].

In such cases, in which clinical evidence of extrapulmonary disease is absent at presentation and at the time of lung biopsy, can histologic findings differentiate pulmonary involvement in disseminated histoplasmosis from other forms of pulmonary histoplasmosis? Although it has been suggested that the visibility of *Histoplasma* within histiocytes on hematoxylin and eosin (H&E) is a distinctive feature of disseminated disease, the validity of this suggestion has not been systematically examined [18-22]. The aim of our study was to determine whether the visibility of *Histoplasma* within histiocytes on H&E in lung biopsies is suggestive of the disseminated form of histoplasmosis.

2. Materials and methods

Lung biopsies in which *Histoplasma* organisms were identified over a 10-year period (2002-2012) were retrieved from the surgical pathology archives of the State University of New York Upstate Medical University. Each case had been previously stained with H&E, Ziehl-Neelsen, and Grocott methenamine silver (GMS). All slides were reviewed to confirm the presence of *Histoplasma* and then carefully re-examined to determine whether organisms were visible within histiocytes on H&E-stained sections or whether they were only identifiable by GMS staining. The peak *Histoplasma* yeast count was derived by counting the number of yeasts in the high-power field (objective ×40) with the highest number of organisms. Clinical, radiographic, microbiologic, and serologic findings were retrieved from available medical records and reviewed retrospectively to determine whether the clinical findings supported a diagnosis of disseminated histoplasmosis or a form of primary pulmonary histoplasmosis. Our laboratory holds fungal cultures for 4 weeks and offers lysis centrifugation for fungal cultures requested on blood and bone marrow samples. The State University of New York Upstate Medical University Institutional Review Board for Protection of Human Subjects approved the study.

3. Results

Of 46 lung biopsies in which *Histoplasma* was identified, organisms were visible within histiocytes on H&E in 4. In the other 42, organisms could not be seen on H&E, although they were present on corresponding GMS-stained sections in all cases. In the 4 patients whose biopsies showed *Histoplasma* within histiocytes on H&E, subsequent clinical and microbiologic findings confirmed a diagnosis of disseminated histoplasmosis (see below). The 42 patients whose biopsies did not show organisms on H&E had clinical and radiologic features either of pulmonary histoplasmoma (38) or acute pulmonary histoplasmosis (4). The latter cases have been previously reported [11].

3.1. *Histoplasma* visible within histiocytes on H&E

3.1.1. Clinical findings

Patients 1 to 3 were residents of towns close to Syracuse, New York. Patient 4 resided in a histoplasmosis-endemic state (Indiana).

3.1.1.1. Case 1. A 53-year-old male renal transplant recipient on prednisone, mycophenolate, and cyclosporine presented with fever, chills, night sweats, and dyspnea for 2 weeks and a 12-lb weight loss over 3 months. He was found to have a left upper lobe infiltrate, received antibiotics for presumed bacterial pneumonia, and defervesced. Subsequently, his fever relapsed and was associated with acute renal failure, thrombocytopenia, and elevated hepatic transaminases. Chest computed tomography (CT) showed bilateral airspace opacities accompanied by scattered centrilobular nodules and bilateral hilar and mediastinal lymphadenopathy. With a clinical differential diagnosis of cytomegalovirus (CMV) infection and lymphoma, transbronchial lung biopsy was performed, resulting in a diagnosis of histoplasmosis and administration of amphotericin B. *Histoplasma* was subsequently cultured from lung biopsy tissue and bronchoalveolar lavage fluid 13 days later. *Histoplasma* urine antigen was positive. A single blood culture was negative. Despite aggressive antifungal therapy, the patient developed *Clostridium difficile* colitis and urosepsis and died of multiorgan (hepatic, renal, and respiratory) failure 5 weeks after onset of symptoms.

3.1.1.2. Case 2. A 74-year-old woman presented with weakness, dry cough, and dyspnea for 2 weeks. She had been diagnosed with sarcoidosis 8 years previously based on nonnecrotizing granulomas in a bone marrow biopsy (special stains for organisms were negative) and was being treated with infliximab (Remicade; Janssen, Titusville, NJ, USA) for 6 years with several remissions; she had recently also received prednisone. Chest CT showed diffuse bilateral reticulonodular infiltrates. Pertinent laboratory findings included thrombocytopenia and elevated hepatic enzymes (alanine transaminase). Transbronchial biopsy showed an organizing lymphohistiocytic infiltrate but no organisms. Three days later, wedge biopsies of the lung were performed, and histoplasmosis was diagnosed. Amphotericin B was started, but she worsened and died 5 days later. Cultures from the transbronchial biopsy and blood subsequently yielded *Histoplasma* (transbronchial, 14 days; blood, 31 days). Serology for *Histoplasma* antibodies and urine *Histoplasma* antigen were negative.

3.1.1.3. Case 3. A 66-year-old man with a remote history of thymectomy for myasthenia gravis presented with persistent fever, chills, night sweats, and weakness for 2 weeks. Five months previously, he had received a renal transplant from a CMV-positive donor and was receiving