

Acute Histoplasmosis with Pleural Effusion in an Immunocompetent Patient

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ABSTRACT

Histoplasmosis is a mycosis caused by the organism *Histoplasma capsulatum* and is transmitted to humans through aerosol and ingestion. It is endemic in several areas of the United States, Central and South America and has variants in Europe, Asia and Africa where the mycelial form grows in soil rich in nutrients. When the soil is disturbed, either spores or hyphal elements can spread populating nearby areas or they may be carried by mechanical vectors. It is also possible for infected vectors to spread the fungus. The following case study presents an atypical occurrence of acute severe histoplasmosis in an immunocompetent adult. Included is an overview of the diagnostic process, the organism's history, characteristics, pathogenicity, and host responses.

ABBREVIATIONS: BAL - bronchial alveolar lavage, EIA - enzyme immunoassay, ELISA - enzyme linked immunosorbent assay, NK - natural killer, PAMPS - pathogen associated molecular patterns, PRR- pattern recognition receptors, TLR2 - Toll-like receptors, MR - mannose receptor, T_H1 - T helper cell 1

INDEX TERMS: Mycosis, Histoplasmosis

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Case Presentation

A 29-year old African-American male without any significant personal or familial history of pulmonary disease presented to an urgent care facility complaining of fever, body aches and shortness of breath for the previous two days. Doxycycline was prescribed.

On day five of symptoms the patient was seen again at the same facility but was sent to an Emergency Department after a chest radiograph revealed questionable results (Figure 1A). Our patient was experiencing a fever of 103°F, cough, body aches, chills that fluctuated in intensity, shortness of breath, chest wall pain on the right side, and abdominal pain. After reviewing routine lab work, the patient was released with a preliminary diagnosis of acute bronchitis. He was treated with Rocephin®, azithromycin and over the counter medication as needed for fever.



Figure 1 A and B. Chest Radiographs. The radiograph on the left (Figure 1A) was taken on day five and on the right (Figure 1B), day eight. Note in Figure 1B, left lower, the large opaque area which is due to fluid filling the lower right pleural space. Figure 1A shows what might be enlarged lymph nodes medially on each side of the sternum

On the eighth day of illness, the patient returned to the Emergency Department with a temperature of 100.6°F, chills, cough, night sweats and difficulty breathing. He complained of pleuritic chest pain that worsened with deep breathing and radiated to his back on the

right side. On examination, he was found to have diminished breath sounds. Chest radiographs (Figure 1B) and a computed tomography (CT) scan (Figures 2A and 2B) indicated a pleural effusion, a mass in the subcarinal region believed to be enlarged lymph nodes and a slight pericardial effusion. The subcarinal region is immediately inferior to the primary bronchial branching in the mediastinum. Both histoplasmosis and mediastinal adenopathy were suspected at this point so a bronchoscopy and fine needle aspirate were ordered.

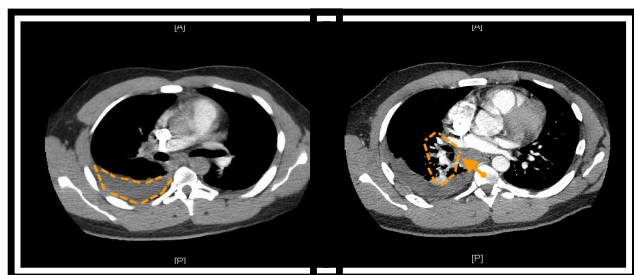


Figure 2A and 2B. Computed Tomography. On day eight, the CT confirmed findings observed on the radiographs. Figure 2A to the left shows pleurisy and Figure 2B demonstrates an area of necrotic tissue. In figure 2A, the two black areas immediately anterior to the vertebrate body are the right and left bronchi. The CT is read from the feet making the left portion of the picture the right lung. The left bronchi branches off laterally before the right does as you can see here. Figure 2B is a section more inferior to the previous. The right bronchus has branched. The area outlined shows enlarged nodes and necrotic tissue. Again, you can see the fluid accumulated below the right lung.

Clinical Laboratory Tests Performed

Both urinalysis and complete blood counts done on three different occasions showed only minor deviations from normal. A transiently elevated total protein was shown to be due to hypergammaglobulinemia and was attributed to a possible pneumonia. Testing for influenzae A and B were performed giving negative results but no respiratory bacterial or fungal cultures were ordered.

A flexible bronchoscopy, bronchial ultrasound and fine-needle aspiration of a mass off the right primary bronchi were performed. The examination revealed a large heterogeneous appearing lymph node cluster with areas of suspected necrosis. The aspirated material was

submitted for flow cytometry, a thin preparation, cell block and Gomori-Grocott Methenamine Silver stain (GMS).

The flow cytometric analysis and histological preps found no indication of malignancy but the GMS stain showed yeast forms consistent with histoplasma (Figure 3). In addition, material removed showed necrosis with some intact inflammatory cells.

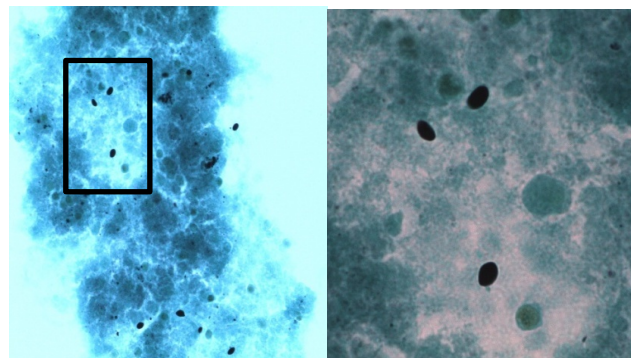


Figure 3. Gomori-Grocott Methenamine Silver of fine needle aspirate at both 400x and 1000x

Diagnosis and Treatment

On day ten of symptoms the patient was diagnosed with histoplasmosis and placed on Sporanox®, an itraconazole, and a steroid. The case presented may be considered acute severe pulmonary histoplasmosis (ASPH) though this designation is often reserved for serious cases following heavy exposure to material rich in *Histoplasma capsulatum* growth and which have diffuse rather than isolated opaque areas on the radiograph.¹ The patient's radiographs showed areas of tissue necrosis closer to the hilar region. He developed mild pericarditis and moderate pleurisy putting him in danger of developing acute respiratory distress syndrome. Necrotic tissue develops when inflammatory cells accumulate and then die releasing lytic granules in and around infected lymph nodes. The infection is usually located in lymph nodes because following phagocytosis, dendritic cells travel to regional lymph nodes in order to present the peptides to naïve T helper cells.

Neither effusion was sampled; however, they are on occasion positive for histoplasmosis.¹ A thoracentesis was deemed unnecessary at this visit but was performed

later because of an expansion of volume in the pleural space. At that time, 1000 milliliters were removed.

HISTOPLASMOSIS

Fungal spores are commonly inhaled and are usually trapped in the nasal mucus or engulfed by phagocytic immune cells along the respiratory tract. On occasion, these spores may act as allergens and trigger a hypersensitivity response in sensitized individuals; however, they rarely cause disease. Even among the primary pathogenic fungi, those that can infect immunocompetent individuals, most infections are asymptomatic or are mild and self-limited with a causative agent rarely identified. Such is the case with the dimorphic pathogenic fungi *Histoplasma capsulatum*. For many people living in one of the endemic areas in the U.S., exposure, infection and resolution are uneventful and for the majority, come and go unbeknownst to the patient thanks to a healthy cell mediated immune response. Serious and even fatal infections associated with compromised immune systems are seen among the very young, patients with prior pulmonary damage, and in individuals who have experienced a large inoculating dose. Of the dimorphic fungal infections requiring hospitalization in the US, over half are caused by histoplasmosis.² Our patient resides in a highly endemic area of the country.

Etiological Agent and Mode of Transmission

Histoplasma capsulatum is a thermally dimorphic fungus which has a mycelial form in the soil where it has hyaline septate hyphae and is accompanied by macro and microconidial spores. When the soil is disturbed, microconidia are inhaled by vertebrates. Under these warm moist conditions, a metamorphosis is triggered and a yeast form develops.³ The yeast is considered the pathogenic form and the spore the infective form.⁴ If not phagocytized prior to this transformation, killing in the phagolysosome is circumvented by factors displayed or excreted by the yeast. Growth and replication within the cell ensues.

Histoplasma capsulatum var. *capsulatum* is found in the soil in the Mississippi, Missouri and Ohio River valleys (Figure 4) in addition to areas in Central and South America. A second variant, *Histoplasma capsulatum* var. *duboisii* is found in Africa.⁵ These classical designations have largely been replaced with chemotypes based on cell wall composition or phylogenetic groups based on

gene sequencing. The organism thrives in soil enriched with nitrogen and phosphorus so it is often found where birds or bats roost.⁶ In addition, bats can develop chronic intestinal histoplasmosis and shed *Histoplasma* in the feces.⁷

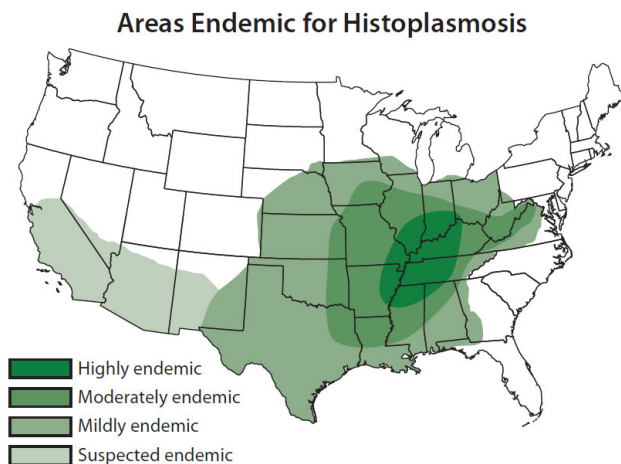


Figure 4. Areas Endemic for Histoplasmosis

Historical Overview

The first description of this agent in literature was made between 1903 and 1909 by Samuel T. Darling, an American physician and pathologist working to control malaria and yellow fever in Panama during the building of the canal. He described findings for three different patients. At autopsy, lesions from various organs were stained and viewed. At the time, he believed the causative agent to be protozoan, possibly a new form of visceral leishmaniasis (kala-azar) and named it based on its encapsulated appearance within histiocytes and the resemblance to plasmodia. Years later in 1934, two separate groups were able to show that specimens taken from similar lesions produced mycelium at room temperature and yeast at body temperature.⁸

In 1940, Normal Conant from Duke University published a review which summarized the work accomplished in the years since Samuel Darling's first description and the multiple name changes which ensued before finally returning to the original. An environmental source for the organism was sought because both man and animals were infected; in 1948 the organism was finally isolated from soil and linked directly back to infections.⁹

Through gene sequencing, this species has been divided into six primary phylogenetic groups that are associated with specific geographical locations, five of the six are in North, Central and South America. In addition, chemotypes are assigned based on the presence of cell wall polysaccharide.^{10, 11}

Most cases of histoplasmosis are due to inhalation of spores when the soil is disturbed during occupational or recreational activities. Many cases have been documented where immunocompetent individuals have received large doses. Some examples include workers that have cleaned debris from bridges, individuals restoring old abandoned buildings, groups that have visited caves and those returning from humanitarian trips planting crops in El Salvador.¹²

Histoplasmosis generally is regarded as a “granulomatous disease”. Though respiratory symptoms are the more likely seen, a spectrum of gross and microscopic lesions may be seen rarely in the gastrointestinal tract and liver. These may be seen even without pulmonary symptoms.¹³

Immunocompetent Patient’s Response to Acute Self-limited Pulmonary Infections

Normal initial host response to the presence of fungal spores involves phagocytosis and destruction by lysosomal granules and reactive oxygen species. Binding between fungal spore and phagocyte is generally a ligand/lectin bond that triggers endocytosis of the organism. Ligands on the fungal surface are sugar rich molecules which are referred to as pathogen associated molecular patterns (PAMPs). For many fungi, one of the major molecules is the cell wall polysaccharide, β -(1,3)-glucan which is found on both major chemotypes of *Histoplasma capsulatum*. This molecule is recognized by a lectin or carbohydrate binding protein on the phagocyte surface called Dectin-1, a pattern recognition receptor (PRR).¹⁴ Other PRR are the Toll-like receptors TLR2 and TLR4 and the mannose receptor (MR).¹⁵

Human phagocytes which generally respond to the spores that reach the alveoli are alveolar macrophages. In addition to triggering phagocytosis, the binding between β -glucan and Dectin-1 triggers production of nitric oxide synthase and up regulates cytokine production in the macrophage or dendritic cell, especially TNF- α and Macrophage Inflammatory

Protein-2. Lysosomes in the cytosol combine with the phagosome containing the spore and it is destroyed by degrading enzymes and reactive oxygen species produced during the cells respiratory burst.¹⁴

Unfortunately, when a *Histoplasma capsulatum* yeast or a spore transforming into a yeast is engulfed, excreted and cell bound factors produced by the yeast are able to neutralize the acidified environment and the organism escapes destruction.¹⁶ Not only does it escape, it is able to reproduce inside the phagocyte and eventually kill the cell.⁴ Dissemination via lymphatic and hematological routes likely occurs due to the migratory nature of the macrophage/dendritic cell type.¹ This is a setback and many times the initial battle is lost; however, the war is ultimately won.

Many people, in endemic regions of the country, are infected as children and for the vast majority, development of cell mediated immunity follows. A T_H1 pro-inflammatory response destroys the yeast and prevents further spread of the fungus. The T helper 1 subset is more effective when fighting intracellular organisms. Resolution of the infection occurs because of initial Natural Killer (NK) cell involvement and later T cytotoxic cells triggering apoptosis of the infected macrophages. Phagocytosis and antigen presentation to T helper cells trigger the production of more specific cytokines. These cytokines encourage macrophage and neutrophil migration to the site by altering their trafficking patterns, enhance NK activity and involve T cytotoxic cells. They enhance the killing power of both infected and newly arriving phagocytes. Triggering apoptosis in infected cells and lysis of infected cells releases pathogen which can be phagocytized and presented for eventual humoral involvement and long term immunity.¹⁷ Essential pro-inflammatory cytokines involved include interleukin-12 (IL-12), interferon gamma (IFN- γ) and tumor necrosis factor alpha (TNF- α).

Evidence of this infection can be found serologically for years to come. It is essential for the host to mount a T_H1 immune response to activate macrophages, NK cells, cytotoxic T cells and resolve the infection. CD4+T cells and IFN-gamma must be present, TNF- α and GM-CSF are also required.¹⁸ A key event is the activation of phagocytic cells which includes increased production of reactive oxygen species and nitric oxide.

Phagocytic cells which are not activated are more likely to fall victim to the yeast phase of the fungus.

Acute self-limited pulmonary histoplasmosis may include fever, malaise, headache and weakness. If investigated, it may appear to be an acute pneumonia with radiographs showing a patchy pneumonia and possibly enlarged hilar and mediastinal lymph nodes. This generally goes undiagnosed and resolves without treatment.

Possible Virulence Factors

Five of the six major phylogenetic strains of *H. capsulatum* belong to chemotype II which has two cell wall constituents of interest, β -(1,3)-glucan and α -(1,3)-glucan.¹⁹ Edwards et al outlines what we now know about the cell walls relationship to organism virulence; β -(1,3)-glucan is the more immunostimulatory molecule and its binding with the lectin Dectin-1 on the macrophage triggers the formation of reactive oxygen species, nitric oxide synthetase, TNF- α , Macrophage Inflammatory Protein 2 and the secretion of pro-inflammatory cytokines.²⁰ In the yeast phase, the alpha forms a layer which masks the beta blocking innate immune recognition by the Dectin-1 receptor thus delaying phagocytosis. Mutants of chemotype II which lack α -(1,3)-glucan are avirulent.²¹

Other organism/macrophage binding combinations occur including the integrin CR3 receptor (CD11/CD18) on the macrophage surface and various other ligands including the heat shock protein Hsp60 on the organism. This binding only triggers a mild host immune reaction within the phagocyte and no respiratory burst. It may also hamper the release of metabolites into the phagosome. This decrease in toxic oxygen metabolites 'allows' the organism to live in the phagosome rather being killed thereby acting as a safe portal for entry into the cell.^{15,22} Binding of the CR3 receptor to the spore triggers down regulation of IL-12 which is needed for inflammatory cell recruitment.^{23,24}

Other heat shock proteins, in particular Hsp82, help the spore survive the metamorphoses into yeast. Under temperature stress, these proteins help the phagocytized yeast survive the oxidative stress in the phagolysosome and they help protect essential pathogen molecules survive the additional elevated temperatures associated with fever in the host. Heat shock proteins chaperone

and protect many transcriptional products in the yeast and prevent their denaturation during this period.²⁰

Some other factors which may be important in fungal pathogenicity include iron acquisition, calcium binding protein (CBP) 1, glycanases, enzymes related to defense against oxidative stress, dehydrogenase enzymes, melanized cell walls and YPS3, an extracellular yeast product.²⁵

Testing for Histoplasmosis

Fungal Culture

Cultures can be attempted using sputum, bronchial alveolar lavage (BAL), tissue biopsy or blood. On a Sabouraud's dextrose agar plate incubated at room temperature, the organism will start to grow after 2 and up to 6 weeks following inoculation. The mycelial colony may appear white to tan. When the colony develops, presumptive identification can be made by seeing the typical tuberculate macroconidia. These distinct thick walled conidia are generally 8 to 15 μ m in diameter. Confirmation is needed because other opportunistic fungi may display the same projections from the spore surface. Microconidia that measure 2 to 4 μ m are also seen and may predominate. These small smooth structures are the infectious form. Conversion from mold to yeast can be accomplished after transferring to a blood heart infusion slant (BHI) and incubating at 37 degrees Celsius. In pathology samples, the yeast appears encapsulated, thus the name, but lacks a true capsule.⁵

To increase yield, the clinical specimens can undergo a technique called lysis-centrifugation. Phagocytic cells which may contain intracellular organisms are lysed and the sample is concentrated. Another technique used to aid in recovery of the organism from sputum is to add ammonium hydroxide to the surface of the agar. This change in pH makes the media more selective for fungus by inhibiting commensal organisms.

After a visual identification is made on the culture, confirmation can be obtained by performing an exoantigen test on the growth. Definitive identification though is more likely to be done using a chemiluminescent DNA probe.²⁶ In the past, conversion of the mold to a yeast was a requirement for identification.⁵ Patients were put on therapy when the typical macroconidia were seen on growth from the clinical sample and the laboratory proceeded to set up

the confirmatory yeast conversion step.

Serological Testing

For culture negative patients both antigen and antibody tests are available. In the past, tests detecting antibody were generally complement fixation assays using yeast and mycelial antigens as targets. These were replaced by methods using immunodiffusion or counterimmunoelectrophoresis. The presence of precipitin bands which indicate antibodies to M(catalase) and H(β -glucosidase) were significant. The M band develops with acute infection and the H band with chronic, severe acute, mediastinal lymphadenopathy or disseminated disease.⁴ The newest versions utilize ELISA and EIA to detect soluble antigen or serum antibodies.

Antigen testing using serum, urine or BAL fluid has proven helpful. Shed antigen can be detected using an EIA procedure with the best sensitivity seen using urine samples. The antigen detected is a cell-wall polysaccharide.^{5, 27}

Histology on Fine Needle Aspirate or Tissue

Samples are stained with GMS stain or the Periodic Acid-Schiff stains (PAS) and a tentative diagnosis can be made if narrow based budding yeasts are seen. The yeast are generally 2 – 4 μ m and oval in shape. Yeast are often clustered within phagocytes; although, they can be seen free as they are in this case.

Case Conclusion and Comment

As was stated by Goodwin et al in 1981, "The diagnosis of histoplasmosis begins with thinking of it." Even though the patient lived in an endemic area, histoplasmosis is not one of the first things a physician thinks of when presented with early symptoms.¹

The patient received a preliminary diagnosis approximately 9 days after symptoms at his third visit, probably not too atypical for this organism. The development of painful pleurisy prompted additional radiographs and a CT scan. The infection may have resolved in a few weeks despite not being on antifungal medication but the effusion could have also progressed leading to pulmonary insufficiency and possible collapse of the lung. Administration of itraconazole, the recommended systemic antifungal drug for histoplasmosis, may have shortened the episode or it

may decrease the chance of reoccurrence in the future if the patient experiences an event that weakens his immune system.

After reviewing phone and banking records for the previous month, the patient could only identify two possible instances which may have offered the exposure. One was a stop at a state park while traveling and the other, recent yard work. It is smart to have a healthy respect for this fungal pathogen despite the fact that so many infections are asymptomatic and go unnoticed. It also serves as a reminder that those potential virulence factors expressed by organisms are able to turn avirulent microbes into potential pathogens. This organism has many tricks up its sleeve, any one of which could find a way around the many intricacies of our immune system.

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