Histoplasmosis

Histoplasma capsulatum is a dimorphic fungus which is prevalent in certain areas of North America and Latin America. In the United States this fungus is found in fertile, humid river valleys such as the Missouri, Mississippi, and Ohio valleys (1). The organism apparently thrives in soil supplemented by bird and bat droppings. In its mycelial stage it forms micro- and macro-conidial spores which are infectious upon inhalation. Outdoor activities in which soil containing the fungus is disturbed carry a higher than usual risk for infection (2). Histoplasma enters the host usually via the respiratory tract by inhalation of spores and, depending on a variety of factors, may cause one of several clinical infectious outcomes. Most infections with Histoplasma are acute, self limited, sometimes asymptomatic, and resolve on their own without specific treatment. It is estimated that 95% of Histoplasma infections are of this type (1). The fungus can also result in a chronic pulmonary histoplasmosis which may or may not involve pulmonary cavity formation. The chronic pulmonary type of infection may or may not progress, with most non-cavitary cases resolving spontaneously. Similar to the chronic pulmonary infectious form of histoplasmosis is mediastinal fibrosis in which prior histoplasmal infection results in excessive mediastinal granuloma formation and scarring, causing a mediastinal mass effect (2). The most feared type of Histoplasma infection is the disseminated form. Disseminated histoplasmosis is a relatively uncommon form of the infection occurring in 0.02% to 0.5% of all acute cases. The organism has been reported in many body sites in disseminated disease especially lung, blood, bone marrow, lymph nodes, mucous membranes, and others (3).

Patients with AIDS as well as other immunosuppressed patients have an increased risk for developing disseminated histoplasmosis rather than the other self limited or chronic forms. However, there is also a subset of patients with disseminated histoplasmosis with no underlying immunosuppression. It is theorized that these patients have some sort of intrinsic defect in their cellular immune systems which predisposes them to develop the disseminated form of the disease (2). The clinical course of disseminated histoplasmosis can be especially rapid and morbid involving septicemia, respiratory difficulties, hypotension, renal failure, hepatic failure, coagulopathies, encephalopathy, meningitis, and death (4). There is obviously a need for quick and accurate diagnosis in patients with disseminated histoplasmosis so that appropriate anti-fungal treatment can be started early enough to prevent some of the morbidity and sequelae.

Laboratory Diagnosis

There are three basic approaches in the laboratory to diagnosing histoplasmosis. Culture of the organism on fungal media from various body sites is viewed as the gold standard of diagnosis. On Sabouraud's dextrose agar
at 25 degrees C Histoplasma will grow rope-like hyphae with spherical micro- and macro-conidia. The young macroconidia are at first smooth, but as they age they become "tuberculate" developing knob-like projections on their surface (5). Exoantigen testing on the colonies can be performed to confirm the diagnosis. In disseminated disease cultures taken from various body sites during the course of illness have been positive in 77% to 88% of patients with the highest yields reported from the bone marrow (77%-84%), tissue (lung, lymph nodes, etc.: 69.2%-100%), sputum (70%-79%), urine (76%), and blood (52%-71%) (6,7). However, the major drawback to culture is the long period it takes to grow Histoplasma. Histoplasma has a reported growth rate of 5 to 45 days (5) with most authors reporting 3 to 4 weeks to grow and isolate the organism using conventional culture techniques. In a patient with disseminated disease with a quickly worsening clinical condition the wait for culture results may be too long. This may be changed in light of recent reports of blood cultures for Histoplasma using the Dupont Isolator - lysis centrifugation blood culture system. In one report of blood cultures which actually grew out the organism the mean recovery time was 8 days versus 24 days for conventional biphasic blood culture method (8). Using the Isolator method one could theoretically grow out Histoplasma in about 8 days in a majority of disseminated patients.

The second major method in diagnosing disseminated Histoplasma infection is histopathological examination of biopsy specimens. Tissues or specimens examined for the organism include bone marrow, buffy coat of peripheral blood, lung, liver, oral mucosa, and other tissues. Specimen slides are usually stained by Gomori's Methenamine Silver (GMS) stain to show the organisms. Several authors have reported that biopsy and histopathological examination of specimens by silver stains was the means of intial diagnosis in 43% to 68% of patients (7,9,10). Of those culture positive patients reported by Paya et al 88% had Histoplasma organisms seen on pathological examination of tissue biopsies. Of course, the major drawbacks of this diagnostic technique is its dependency on the pathologist's experience in identifying Histoplasma in tissue sections and it may involve an invasive surgical procedure to obtain tissue in a critically ill patient.

The third approach to diagnosing disseminated Histoplasma is serology. Serologic tests for Histoplasma involve complement fixation and immunodiffusion. In complement fixation the patient's serum is tested separately for antibodies against the yeast antigen and mycelial antigen (histoplasmin). Antibodies against the yeast antigen occur earlier in the course of the disease than those against the mycelial antigen and reach a higher titer (11). The cutoff titers used most often in the literature for positivity are >= 1:32 for strong presumptive evidence of infection and = 1:8 or 1:16 for presumptive evidence of infection (6). Immunodiffusion involves testing the patient's serum for the H and M antibodies against histoplasmin on a double diffusion agar plate and looking for lines of identity with reference samples. Any lines of identity seen for the H and M bands are viewed as a positive result (12). Both these serological tests are used together
in testing and a positive result by either CF or ID is viewed as a overall positive test. By using these serological tests in this way, sensitivities of 81% to 90% have been reported for disseminated Histoplasmosis. The specificity for disseminated histoplasmosis is much harder to figure, however. There have been reports of background seropositivity (false positives) for Histoplasma of 15% (13). Skin testing with histoplasmin, past infections with Histoplasma, and cross reactions with other fungal antibodies have all been reported to cause false positive results, thereby lowering specificity (11).

Radioimmunoassay for Histoplasma capsulatum antigen

In light of the limitations of culture, histopathological examination, and serology in the diagnosis of disseminated histoplasmosis there is much interest in developing a noninvasive, accurate, and quick test for diagnosis. Just such an assay was reported by Wheat et al in 1986 (14) in which they reported measuring an antigen of Histoplasma in the urine and serum of patients with various forms of histoplasmosis. Patients were identified as having histoplasmosis by positive cultures and/or positive serologies and of those patients with the diagnosis urine and serum specimens were obtained. The assay was a double antibody "sandwich" radioimmunoassay. Polyclonal antibodies were prepared by injecting Histoplasma yeast cells into rabbits and then the IgG fraction purified out. Unlabeled IgG antibody was then coated onto polystyrene test tubes and the serum or urine aliquot (undiluted) was added. After a wash step, the second radiolabelled IgG antibody fraction was then added to the tubes and the amount of histoplasma antigen present was quantified by scintillation counting (see schematic diagram). These raw counts were converted to "radioimmunassay units" (RU) by dividing the count by 1.5*mean value of normals. This meant that the cutoff for positivity was a count 50% higher than the mean count for normals and any values above 1.0 RU were positive. The normal values were obtained from two laboratory employees.
The assay appeared to work much better for patients with disseminated disease rather than the acute self-limited or chronic forms. In 22 episodes of disseminated disease (16 patients; 10 initial presentations, 6 relapsing patients) antigen was detected in the urine of 20 cases giving a sensitivity of 91%. Of 295 controls with various fungal and bacterial infections in the lungs and urinary tract none of the urines were positive for the histoplasma antigen giving a specificity approaching 100%. The antigen was only present in the serum in half of the disseminated cases, giving a sensitivity of 50% for serum. The assay did not perform well for other forms of histoplasmosis giving sensitivities of 19% for acute self-limited disease, 6% for pulmonary cavitary disease, and 50% for the granulomatous sarcoid-like histoplasmosis. Of note, antigenuria did appear to fall after treatment in 89% of cases and rise in relapse in 89% of cases. As for reproducibility, 21 positive urine specimens were retested and 18 (86%) continued to be positive with a correlation of initial test and retest values of R=0.946. 20 initially negative specimens were retested and all continued to be negative.

Wheat et al have also reported on the use of the antigen in patients with Histoplasma meningitis (15). In evaluating Histoplasma meningitis, CSF of patients were assayed for the Histoplasma antigen and found in 4 of 12 patients giving a sensitivity of 33% (15). Of interest also was a false positive result in a patient with coccidiodal meningitis indicating a possible cross reacting antigen between C. immitis and H. capsulatum.

Using the urinary Histoplasma antigen assay in patients with AIDS and disseminated histoplasmosis Wheat et al have reported similar values to their initial report (16). In 61 cases of AIDS and disseminated histoplasmosis 59 patients had elevated levels of urinary Histoplasma antigen giving a sensitivity of 96.7%, slightly higher than the initial report. The antigen levels also fell during treatment in 90% of patients and increased in all relapsed cases. In the report involving the AIDS patients the authors did note an unpublished observation of 4 false positive results in the urine of 25 patients with blastomycosis.

Realizing that an assay which uses a radioimmunometric approach would only be able to be performed by a limited number of laboratories Wheat et al developed and tested two ELISA based assays which would be more practical for clinical commercial laboratories (17). The assays used alkaline phosphatase (AP) or horseradish peroxidase (HRP) as the enzymes linked to the antibodies against histoplasma antigen and the end reaction was measured photometrically. Apart from the replacement of the radioactive Iodide molecule with AP or HRP the assays were exactly the same as the previously published RIA assay. In disseminated histoplasmosis these urinary ELISAs performed similar to the urinary RIA. The HRP ELISA had sensitivity of 94.7% (18/19 patients) and specificity of 97.6% (40/41 controls). The AP ELISA had sensitivity of 89.5% (17/19 patients) and specificity of 92.7% (38/41 controls). In testing these assays false positive results were noted in 1 control with blastomycosis, 1 control with candidemia, and 1 control with no known coexisting infections.
Problems with assay

The urinary Histoplasma antigen as described by Wheat et al in the articles mentioned appears to be a sensitive and specific assay for disseminated histoplasmosis which can give an answer faster than any of the more conventional methods. However, there are some problems in currently using this RIA test for diagnosis. In their study designs the authors screened for patients with disseminated histoplasmosis by looking at all patients with positive cultures for Histoplasma or positive serologies and then obtained urine and serum from these patients and ran the RIA Histoplasma test on them. This is in a sense a retrospective evaluation of their assay since these patients already had the diagnosis of disseminated histoplasmosis and by the time the serum/urine samples were obtained they were much farther along in their disease course. A prospective study needs to be done in which the serum/urine samples are drawn during the initial workup for disseminated histoplasmosis. Also, in their reports the authors don't adequately describe the clinical criteria for dictating which patients had disseminated disease. It is possible that what another group would call disseminated disease the Wheat group would call chronic or self-limited. There is therefore the risk that the disseminated patients in the studies could have only far-gone widespread dissemination and this possible preselection of patients would raise the sensitivity of the assay. Another problem which was raised in the accompanying editorial (18) to the original paper is that there are at least five serotypes of Histoplasma capsulatum which differ geographically (19) and the assay developed in Indiana by Wheat et al may have different sensitivities to different antigenic serotypes across the USA. In addition the total number of disseminated histoplasmosis patients tested by urinary RIA in these reports is low and it would be more convincing if a higher number of patients were tested. Unfortunately this ideal situation will be hard to attain as disseminated histoplasmosis is a relatively rare disease. The false positives reported for the urinary RIA are relatively low in number with the specificity for the assay probably in the 98% to 100% range. However, more attention probably needs to be paid to the number of false positives and their association. False positive reactions have been reported in patients with blastomycosis, coccidioidomycosis, and candidemia (AP ELISA), all of which are probably due to cross reactions of the anti-Histoplasma capsulatum with a common fungal cell wall antigen. The false positive that occurred in a patient with no known concurrent infections is also of concern even though it occurred with the AP ELISA test rather than the RIA.

Another difficulty with using this assay which was mentioned briefly is that it is radioisotope based assay which requires specialized facilities, including difficult waste disposal, which most clinical laboratories would like to avoid. As mentioned, the Wheat group have reported on an ELISA (HRP) for the urinary histoplasma antigen which appears promising with similar sensitivities and specificities to the RIA, yet it is not yet available commercially. The urinary Histoplasma antigen assay is as of now only
available through one reference laboratory (Wheat). This brings up many
issues involving quality assurance in that there is no other laboratory
(including the CDC) with which you could compare results with.

To examine the San Francisco Veterans Administration Medical
Center’s experience with this test we searched the SFVAMC hospital
laboratory computer for the total number of histoplasma antigen tests ever
sent to the Indiana reference laboratory. Of 16 samples (14 urines, 2 serums)
from 12 prospective patients who had clinical criteria suspicious for
disseminated histoplasmosis, none were positive. None of the patients had a
subsequent diagnosis of disseminated histoplasmosis; only one had
concurrent positive serologies (CF 1:64) with negative cultures and no firm
diagnosis as of this writing. Turnaround time was also investigated as this is
the only laboratory in the country performing this test and this involves
shipping specimens and reports long distances. The average turnaround time
from when the specimen was sent to when the report was received back at
SFVAMC was 32.15 days with the longest result taking 63 days and the
shortest 11 days. It is unlikely that vagaries in the mail system account for
these long and variable turnaround times, rather, this is the result of the
reference laboratory running this test in batches. Running the assay in batches
would be the most economical, however it may also result in long periods
between test runs as seen in the SFVAMC turnaround time. It should be
noted, however, that of the most recent tests sent, there have been shorter
turnaround times (see chart).

Turnaround time (Days)

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Summary

The urinary Histoplasma antigen assay for disseminated histoplasmosis is a promising test. There is the capability for a quick and accurate result for critically ill patients with possible disseminated histoplasmosis and this would significantly lower the morbidity and mortality associated with the disease. However, there are some serious limitations in currently using this test. There has of yet been no report of prospective evaluation of the assay which is essential to remove the biased patient selection in the previous articles. The fact that different antigenic serotypes exist across different geographical areas needs to be addressed; would this assay recognize all serotypes? Quality assurance is a problem in that since this is the only laboratory performing the assay there is no way of checking the results as is done for every other sendout test. Finally, the long turnaround time (32 days avg.) because of batch running makes this test no better than culture which takes 2 to 4 weeks. The report on the ELISAs developed by Wheat et al is promising (especially the HRP ELISA) since among other attributes it is non-radioimmunometric. If the ELISA could be developed and commercially available many of the problems mentioned with the RIA could be resolved and there would be a potentially excellent test for the diagnosis of disseminated histoplasmosis.
BIBLIOGRAPHY


