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MAJOR ARTICLE

Comparison of Indirect Fungal Diagnostic Tests in Patients With Proven Histoplasmosis

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Background. Histoplasmosis is a common cause of invasive fungal infection in endemic regions and accurate diagnosis is difficult without direct tissue culture or pathology. Indirect fungal antigen testing for various fungal pathogens are typically performed to assist with diagnostic workup, though cross-reaction can lead to difficulty in interpreting results. We aimed to compare indirect fungal diagnostic tests and evaluate prevalence of positive antigen testing for non-*Histoplasma* fungal pathogens in patients with proven histoplasmosis.

Methods. We performed a single-center retrospective review of adult patients with proven histoplasmosis diagnosed by fungal culture and/or cytology from January 2010 to March 2018. Patient demographics, clinical characteristics, and results of fungal antigen testing for *Histoplasma*, *Blastomyces*, *Aspergillus*, *Cryptococcus*, and (1→3)-β-D-glucan were evaluated. Two different urine *Histoplasma* antigen assays were used during the study period.

Results. Fifty-seven of 182 (31.3%) patients reviewed had proven histoplasmosis and presented with acute pulmonary (n = 10), chronic pulmonary (n = 7), and disseminated (n = 40) disease. Forty-one (72%) of these patients were immunosuppressed. Urine *Blastomyces* antigen (93%) and serum (1→3)-β-D-glucan (88%) were commonly positive in patients with histoplasmosis, whereas *Aspergillus* antigen was detected in 50% of patients and *Cryptococcus* antigenemia was rare (5%). In patients with disseminated disease, the MiraVista urine *Histoplasma* antigen assay had higher sensitivity than the Viracor urine *Histoplasma* antigen assay (86% vs 50%, respectively; $P = .019$).

Conclusions. Noninvasive fungal antigen assays are helpful diagnostic tools; however, given their low specificity, clinicians must be aware of the various clinical presentations of invasive fungal infections and be aware of the limitations of these tests.

Keywords. cross-reactivity; fungal diagnostics; histoplasmosis; urine *Histoplasma* antigen.

Histoplasmosis, infection with *Histoplasma capsulatum*, is an endemic fungal infection frequently seen in the central and eastern United States (US), especially around the Ohio and Mississippi river valleys [1]. Histoplasmosis has a wide spectrum of presentation ranging from isolated pulmonary disease to disseminated infection that can involve fungemia, central nervous system (CNS) infection, bone marrow infiltration, and other organ involvement [2, 3].

The gold standard method for diagnosis of proven histoplasmosis is by either isolation of *Histoplasma capsulatum* from fungal culture or identification of histopathological findings consistent with this organism from clinical specimens [4]. However, diagnosis is challenging as invasive sampling of appropriate tissue can be difficult and fungal culture results can take weeks [5]. Consequently, more rapid indirect markers are frequently used, such as *Histoplasma* antigen or antibody testing in urine, serum, cerebrospinal fluid (CSF), and other clinical specimens [5–7]. The first noninvasive antigen assay was developed in 1986, providing a novel, sensitive, and rapid alternative for *Histoplasma* testing [5]. Newer-generation enzyme immunoassays are now available [8–10]. The urine *Histoplasma* antigen test has proven to be quite sensitive in disseminated disease with various studies showing 89%–92%, but less sensitive in limited forms of infection [11–14]. However, cross-reaction with other invasive fungal pathogens has resulted in unreliable specificity [15–17]. Due to rapid turnaround time, decreased need for invasive sampling, and adequate sensitivity, antigen testing remains a preferred testing modality for histoplasmosis.

Other invasive fungal infections are routinely diagnosed with urine or serum antigen testing [4, 5, 18, 19]. Simultaneous *Histoplasma*, *Blastomyces*, *Cryptococcus*, *Aspergillus*, and serum (1→3)-β-D-glucan antigen testing is commonly performed to

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evaluate at-risk patients who present with a clinical syndrome suspicious for invasive fungal disease. Interpretation of results can be difficult when multiple antigens are detected concurrently, in geographic locations with multiple endemic fungi [20, 21], and when invasive testing to obtain tissue cannot be safely performed or is delayed. There are many studies evaluating the performance of *Histoplasma* antigen and antibody tests [5, 11–14, 17]; however, there are limited published data on positive rates of non-*Histoplasma* fungal antigen tests in proven histoplasmosis.

The primary aim of this study was to determine the rate of positivity of various non-*Histoplasma* fungal antigen tests in patients with proven histoplasmosis to better understand cross-reaction of these other antigen assays. Sensitivity of urine *Histoplasma* antigen testing in our center's experience was also assessed.

METHODS

Study Design

We performed a single-center retrospective chart review of adult patients from January 2010 to March 2018 diagnosed with proven histoplasmosis at the University of Arkansas for Medical Sciences (UAMS), a tertiary care academic center with a wide catchment area. Patients were considered proven cases if they had evidence of histoplasmosis by positive culture for *Histoplasma* or cytological examination revealing an organism consistent with *Histoplasma*. *International Classification of Diseases, Ninth Revision* codes and microbiological culture data were used to identify potential patients. Patients with a positive *Histoplasma* antigen test without evidence of histoplasmosis on culture or tissue cytology were considered probable cases and not included in further analysis. Patients with an invasive fungal infection other than histoplasmosis were planned to be excluded; however, there were no patients in this cohort with a proven invasive fungal infection other than histoplasmosis. Patients were also excluded if they had a previous diagnosis of histoplasmosis or if they were only diagnosed with presumed ocular histoplasmosis syndrome.

Data Collection

Data were collected on patient demographics and comorbid medical conditions including diabetes mellitus, hepatic cirrhosis, human immunodeficiency virus (HIV), AIDS, end-stage renal disease, active malignancy, and immunosuppression. Stages of histoplasmosis (acute pulmonary, chronic pulmonary, and disseminated) were determined based on chart review of discharge diagnoses set by the treating infectious diseases physician. Chart review was performed by medical trainees (G. K. and M. P.) and an infectious diseases attending physician (R. D.). Culture results, pathology results, and fungal laboratory data including urine *Histoplasma* antigen, serum *Histoplasma* antigen, CSF *Histoplasma* antigen, respiratory *Histoplasma* antigen, serum *Histoplasma* antibodies, CSF *Histoplasma* antibodies, urine *Blastomyces* antigen, respiratory *Blastomyces* antigen, serum (1→3)-β-D-glucan, serum cryptococcal antigen, CSF cryptococcal antigen, and serum *Aspergillus* antigen (galactomannan) were collected.

Fungal Antigen Testing

During the study period, *Blastomyces* antigen testing was performed by MiraVista Laboratories (Indianapolis, Indiana), *Aspergillus* antigen immunoenzymatic sandwich microplate assay was performed by Bio-Rad (Hercules, California), serum (1→3)-β-D-glucan assay was performed by Viracor Laboratory (Lenexa, Kansas), and *Histoplasma* antigen testing was performed by Viracor Laboratory (Lenexa, Kansas) during January 2010–February 2015 and MiraVista Laboratory (Indianapolis, Indiana) during March 2015–March 2018. The *Cryptococcus* antigen testing was performed at UAMS using the lateral flow assay produced by Immy Corporation (Norman, Oklahoma). Fungal cultures were performed by plating specimen on both blood heart infusion agar and potato dextrose agar. Identification of fungal isolates was performed using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry using VITEK MS (bioMérieux). Direct examination of tissue for identification by pathology includes both hematoxylin and eosin stain and Grocott methenamine silver stain.

Definitions

Comorbidities were determined by their presence in medical record. AIDS was defined as having CD4 <200 cells/μL in the presence of HIV diagnosis. Immunosuppressive medications included chemotherapy, immunotherapy, being on a corticosteroid equivalent of oral prednisone ≥20 mg for >2 weeks, or being on other known immunosuppressive medications. Stage of histoplasmosis infection was determined by either documentation of stage by the treating infectious diseases physician in the medical record or by chart review.

Acute pulmonary histoplasmosis was defined as a pulmonary infection with acute onset of symptoms associated with heavy inhalation exposure to airborne *Histoplasma* spores. Chronic pulmonary histoplasmosis was defined as a chronic pulmonary disease, which can follow acute pulmonary histoplasmosis and is characterized by low-grade chronic pulmonary symptoms, lung cavitation, pulmonary fibrosis, and progressive pulmonary insufficiency. Progressive disseminated histoplasmosis or disseminated histoplasmosis was defined as a progressive extrapulmonary infection characterized most commonly by fever, fatigue, and weight loss, among other symptoms, depending on the organ system affected [2, 3]. Proven histoplasmosis was diagnosed in a host with illness consistent with histoplasmosis and (1) recovery in culture from specimen

obtained from affected site or from blood or (2) histopathologic or direct microscopic demonstration of morphologic forms consistent with *Histoplasma* [4].

Statistical Analyses

The χ^2 and Fisher exact tests were used for comparative analysis were appropriate. All statistical analyses were performed using Stata software version 14.1 (StataCorp, College Station, Texas).

RESULTS

Fifty-seven of 182 (31.3%) patients diagnosed with histoplasmosis during the study period had definitive proof of histoplasmosis with either positive fungal cultures (30 [52.6%]), characteristic findings on histopathologic examination (12 [21.1%]), or both (15 [26.3%]) and met inclusion criteria for proven histoplasmosis. The majority of remaining cases were diagnosed with antigen testing alone and thus not included in further analysis.

Cases were predominantly male (56.1%), with a mean age of 50.1 ± 18.6 years. White race (61.4%) was more common than other races. Seventy-two percent of this patient population was considered immunosuppressed. Patient demographics are listed in Table 1.

Of the 57 patients with proven histoplasmosis, 10 (17.5%) had acute pulmonary, 7 (12.3%) had chronic pulmonary, and 40 (70.2%) had disseminated disease. One patient with disseminated histoplasmosis had evidence of CNS infection. There were 40 patients with disseminated histoplasmosis, of which 36 (90%) had an obvious immunodeficiency. Out of the 36, 19 patients had HIV/AIDS, 15 were on immunosuppressive medicines (4 with history of solid organ transplant; 3 with malignancy; others for autoimmune diseases), 1 had malignancy not on immunosuppressive medicines, and 1 had cirrhosis. Four others had no obvious or known immunodeficiency.

Table 2 shows results of indirect fungal testing in the included patients. Urine *Histoplasma* antigen was positive in 30 of 46 (65%) patients tested though positivity rate differed between Viracor (8/18 [44%]) and MiraVista (22/28 [79%]) assays ($P = .017$). MiraVista assay was positive in 18 of 21 (86%) patients with disseminated disease, 4 of 5 (80%) with acute pulmonary disease, and 0 of 2 (0%) with chronic pulmonary disease. Serum *Histoplasma* antigen was tested in 3 patients, and all were negative. CSF *Histoplasma* antigen was tested in 1 patient, and it was positive. Respiratory or bronchoalveolar lavage *Histoplasma* antigen was not tested in any patients. Serum *Histoplasma* antibody was tested in 8 patients, of which 5 were positive and 3 were negative. CSF *Histoplasma* antibodies were tested in 2 patients and 1 was positive. The patient with positive CSF *Histoplasma* antigen and CSF *Histoplasma* antibody had evidence of CNS disease along with disseminated disease and was classified under disseminated histoplasmosis.

Table 1. Demographic Details of the Patients With Proven Histoplasmosis

Characteristics	Proven Histoplasmosis (n = 57)
Sex, female	25 (43.9)
Age, y, mean ± SD	50.1 ± 18.6
Race	
White	35 (61.4)
African American	19 (33.3)
Asian	1 (1.8)
Other	2 (3.5)
Comorbidity	
Diabetes mellitus	9 (15.7)
Congestive heart failure	4 (7)
ESRD	5 (8.7)
Hepatic cirrhosis	4 (7)
Hepatitis B	2 (3.5)
Hepatitis C	2 (3.5)
HIV	20 (35)
Immunosuppressed[a]	41 (72)
AIDS	18 (31.5)
Malignancy	6 (10.5)
History of solid organ transplant	5 (8.7)
Immunosuppressive medications[b]	21 (36.8)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ESRD, end-stage renal disease; HIV, human immunodeficiency virus; SD, standard deviation.

[a]Total number of immunosuppressed patients. Some patients may have >1 reason to be considered immunosuppressed.

[b]Immunosuppressive medications included chemotherapy, immunotherapy, being on a corticosteroid equivalent of oral prednisone ≥20 mg for >2 weeks, or being on other known immunosuppressive medications.

Urine *Blastomyces* antigen was positive in 26 of 33 (79%) patients tested with proven histoplasmosis showing high cross-reactivity with urine *Histoplasma* antigen. This cross-reaction was most profound in disseminated disease, with 25 of 27 (93%) testing positive (Table 2). Serum *Blastomyces* antigen, CSF *Blastomyces* antigen, and respiratory *Blastomyces* antigen were not tested in any patients. Serum (1→3)-β-D-glucan, serum *Aspergillus* antigen, and serum *Cryptococcus* antigen were positive in 21 of 28 (75%), 13 of 26 (50%), and 1 of 22 (5%) patients with proven histoplasmosis, respectively (Table 2). The 1 patient with positive serum cryptococcal antigen had AIDS (CD4 1 cell/mL) with multiple positive fungal blood cultures for *Histoplasma* and tested positive for urine *Histoplasma* antigen. Fungal blood and CSF cultures never revealed *Cryptococcus*, and CSF cryptococcal antigen was negative on this patient.

Specificity in the setting of proven histoplasmosis is very low for urine *Blastomyces* antigen (21%), serum *Aspergillus* antigen (50%), and serum (1→3)-β-D-glucan (25%). However, specificity of serum *Cryptococcus* antigen in the setting of proven histoplasmosis was 96%.

DISCUSSION

This is the largest single-center retrospective study in the US looking at various fungal antigen assays in the setting of proven

Table 2. Analysis of Various Fungal Antigen Testing in the Setting of Proven Histoplasmosis

Proven Histoplasmosis (n = 57)	Urine *Histoplasma* Antigen[a]	Urine *Blastomyces* Antigen	Serum *Cryptococcus* Antigen	Serum *Aspergillus* Antigen	Serum (1→3)-β-D-Glucan
Acute pulmonary (n = 10)	4/5 (80)	1/2 (50)	0/1 (0)	1/4 (25)	0/2 (0)
Viracor	0/0 (…)				
MiraVista	4/5 (80)				
Chronic pulmonary (n = 7)	0/4 (0)	0/4 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Viracor	0/2 (0)				
MiraVista	0/2 (0)				
Disseminated (n = 40)	26/37 (70)	25/27 (93)	1/19 (5)	12/20 (60)	21/24 (88)
Viracor	8/16 (50)				
MiraVista ($P = .019$)[b]	18/21 (86)				
Total	30/46 (65)	26/33 (79)	1/22 (5)	13/26 (50)	21/28 (75)
Viracor	8/18 (44)				
MiraVista ($P = .017$)[b]	22/28 (79)				

Data are presented as No. (%) unless otherwise indicated.

[a]During the study period, reference testing for urine *Histoplasma* antigen testing transitioned from Viracor to MiraVista assay in March 2015.

[b]χ^2 analysis performed comparing Viracor to MiraVista assays.

histoplasmosis and comparing sensitivity of urine *Histoplasma* antigen by different laboratories over a period of 9 years. Per the Centers for Disease Control and Prevention, >60% of people in the US who live in areas surrounding the Ohio and Mississippi river valleys have evidence of exposure to *Histoplasma* during their lifetime [1]. Both histoplasmosis and blastomycosis are endemic to Arkansas, while coccidioidomycosis is not [22]. However, progressive disseminated histoplasmosis is most common among immunosuppressed individuals, especially patients with HIV/AIDS [23, 24]. Immunosuppressed patients are prone to other fungal infections; thus, sensitive and specific tests are crucial to identify and distinguish these infections in at-risk populations [25]. Other conventional diagnostic tests such as radiological imaging also fail to specifically identify fungal pathogens at the organism level, and fungal cultures can take long time to result [4, 18]. Invasive diagnostic methods cannot always be safely performed in this population to get tissue diagnosis due to risk of complications including bleeding, risk of respiratory failure, high risk with anesthesia, etc [19]. Thus, use of noninvasive fungal antigen testing can assist with diagnosis of fungal infections.

Herein, we showed frequent cross-reaction of non-*Histoplasma* fungal antigen tests in patients with proven histoplasmosis. There was a high rate of cross-reaction with urine *Blastomyces* antigen, which can be confusing in regions where both pathogens coexist, such as the Ohio and Mississippi river valleys in the US [26]. Our study also showed that urine *Blastomyces* antigen test was more sensitive for diagnosis of proven histoplasmosis than the Viracor urine *Histoplasma* antigen test designed for this pathogen. This study shows high false-positive rates of other fungal antigen tests in the setting of proven histoplasmosis including urine *Blastomyces* antigen (79%) and serum *Aspergillus* antigen (50%) as these patients did not have proven coexisting fungal infections. Serum (1→3)-β-D-glucan, a common nonspecific fungal biomarker, was also frequently found to be positive (75%) in patients with proven histoplasmosis. There was even 1 patient with proven histoplasmosis who had a positive serum *Cryptococcus* antigen. While active concomitant cryptococcal infection cannot be completely ruled out, blood and CSF fungal cultures did not identify *Cryptococcus* and CSF cryptococcal antigen was negative. Although from a diagnostic perspective this adds to the provider's dilemma, recommended guidelines for management are very similar for histoplasmosis and blastomycosis, making less clinical impact. But this cannot be said for infections caused by organisms such as *Aspergillus* and *Cryptococcus*, where treatment guidelines are different and distinctive diagnosis is important. While noninvasive fungal antigen tests are helpful in diagnosis of these infrequent infections, clinicians must still maintain knowledge of the clinical differences between various fungal pathogens and be aware of the limitations of these tests and make the diagnosis based on the right clinical setting and exposure.

This study highlights the performance variability between commercial *Histoplasma* urine antigen testing as the sensitivities of the 2 assays utilized during this study period were quite disparate (79% vs 44%). Although fungal antigen assays have evolved over the past decades, more work needs to be done to develop sensitive and specific tests. The lack of specificity and cross-reactivity have been considered to be a consequence of the tests recognizing common polysaccharide moieties in these fungal antigens [15]. While there is significant paucity of previous literature to compare performance of non-*Histoplasma* antigen tests in the setting of proven histoplasmosis, smaller studies and case reports have reported similar cross-reactivity [27, 28]. In 1 study, serum *Aspergillus* galactomannan assay was positive in 73% patients with disseminated histoplasmosis [27, 28], while another study showed urine *Blastomyces* antigen cross-reactivity in 95.6% of patients

with proven disseminated histoplasmosis [29]. Other testing modalities, such as nucleic acid amplification testing may have improved specificity though clinical utility has not been proven in fungal pathogens due to their ubiquitous nature, and in many cases continue to require invasive testing modalities like bronchoalveolar lavage for appropriate sampling [30, 31]. In resource-limited settings, access to accurate and timely diagnostic methods continue to be a major barrier, although recent developments like MiraVista's lateral flow-based immunoassay which can be performed bedside, shows promise [32].

There are limitations to our study. This is a single-center study with only 57 cases; however, this is the largest published cohort of proven cases to date in the US. Retrospective study design is prone to bias and incomplete ascertainment of data. Additionally, our study was limited due to the number of cases that could be included based on gold standard of diagnosis. While concurrent invasive fungal infections were possible in this cohort of patients with proven histoplasmosis, cultures and/or histology did not identify other invasive fungal infections. Coccidioidomycosis is not endemic to Arkansas and hence was not tested for during this study, limiting evaluation of *Coccidioides* antigen test cross-reactivity in this population. Another limitation was the change in the reference laboratory testing for *Histoplasma* antigen in the middle of the study, although this helped us compare the performance between the laboratories. Ideally, every patient with proven histoplasmosis would have had comprehensive fungal antigen testing performed, though this would require a prospective study. A prospective study is needed to better define differences between individual *Histoplasma* tests and use this for making treatment decisions. The emerging antifungal treatment options with more specific antifungal effects also underscores the importance of diagnosing different endemic mycoses appropriately [33]. Not every third-generation *Histoplasma* antigen test is the same and in our experience with culture and histopathological proven cases, we saw a difference in the test results, but numbers here are small and larger prospective studies could better answer this question.

Our study underscores important take-home messages for practicing clinicians. For trainees and providers in less specialized settings, awareness of the common and uncommon clinical presentations of fungal infections would be helpful to guide diagnostic evaluation. While there are a lot of common clinical presentations between invasive fungal infections, there are notable differences too. Disseminated histoplasmosis often presents with chronic progressive symptoms of fever, fatigue, weight loss, and night sweats. Physical examination is dependent on organs affected and could manifest as hepatosplenomegaly, lymphadenopathy, pallor, mucous membrane ulcerations, skin nodules, etc [2, 3]. Screening laboratory workup should include urine *Histoplasma* antigen test, serum *Histoplasma* antibodies, complete blood count to look for cytopenias, and liver function tests. Interestingly, patients with disseminated histoplasmosis can have elevated aspartate aminotransferase to alanine aminotransferase ratio [34]. Histopathology of the affected tissue, or fungal blood culture, is the gold standard and can often isolate small, budding, intracellular yeast forms in macrophages [4]. The clinical presentations of disseminated blastomycosis are highly variable. While lung is the primary focus of infection, blastomycosis can present as cutaneous lesions ranging from nodules and verrucous lesions to ulcers, osseous lesions in the form of osteomyelitis, genitourinary infections like prostatitis, and even CNS infections [35]. Workup should include urine *Blastomyces* antigen and serum *Blastomyces* antibodies. Pathology and culture are, again, gold standard and typical broad-based budding yeast forms are characteristic [4]. Invasive pulmonary aspergillosis, on the other hand, is the most common invasive fungal infection of the lung in immunocompromised hosts, especially stem cell transplant recipients. Hematogenous dissemination is often fatal with thrombosis, hemorrhagic infarction, end organ damage and vascular invasion [36]. Cryptococcosis, on the other hand, can present as acute, subacute, or chronic pulmonary infection; meningitis (most common in immunocompromised hosts, especially patients with advanced HIV/AIDS); and cutaneous manifestation, which is usually in the form of raised nodules with umbilicated centers [37]. Given the wide range of presentations as mentioned above, the clinical scenario should dictate which diagnostic tests are ordered in a patient, and interpretation of results should be based on the pretest probability gleaned from clinical presentation. Providers should avoid ordering multiple fungal diagnostic tests in order to avoid confusion and to reduce cost. Lastly, providers should be aware of areas of epidemiological overlap with histoplasmosis and blastomycosis, and differences in their clinical presentation. While these noninvasive assays are helpful diagnostic tools, clinicians should be aware of their low specificity.

Notes

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