Diagnosis of Coccidioidomycosis by Antigen Detection Using Cross-Reaction with a *Histoplasma* Antigen

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**Background.** In 2005, patients with coccidioidomycosis were observed to have positive *Histoplasma* antigen test results.

**Methods.** We performed a review of the records of patients with coccidioidomycosis who were under our care who underwent testing for *Histoplasma* antigen to determine the value of this test in the diagnosis of coccidioidomycosis. Many of the patients were immunosuppressed and critically ill.

**Results.** The *Histoplasma* antigen test had positive results when urine samples from 11 (58%) of 19 patients who had acute or chronic coccidioidomycosis were tested. The sensitivity was highest for patients who had acute coccidioidomycosis, and antigenuria was detected in 11 (79%) of 14 patients. One patient who had chronic coccidioidomycosis but who had a negative result when a urine sample was tested had antigen detected in bronchoalveolar lavage fluid.

**Conclusions.** Physicians should be alerted that infections with *Coccidioides* species may cause positive *Histoplasma* antigen test results. There is potential for the use of this test in the diagnosis of coccidioidomycosis by taking advantage of this observed cross-reactivity. The greatest benefit appears to be in the population of seriously ill patients with acute pneumonia who live in areas that are endemic for *Coccidioides* infection.

In regions of endemicity, coccidioidomycosis is a common cause of pneumonia and systemic illness [1]. In a report of ambulatory patients from Arizona who had community-acquired pneumonia, 29% had coccidioidomycosis [2]. The diagnosis of coccidioidomycosis can sometimes be difficult to make, particularly in immunosuppressed patients who have acute, severe disease. Antibody testing results may be negative early in the course of illness [3] and in immunosuppressed patients [4]. In an analysis of cases of severe coccidioidomycosis, cultures were positive in only 6% of pulmonary cases and 39% of disseminated cases [5]. Furthermore, cultures for *Coccidioides* species can require >1 week before positive results can be reported.

Identification of *Coccidioides* species using histopathological methods can also be difficult and can frequently require an invasive procedure, and results can be false negative in up to one-third of cases [3, 6].

The *Histoplasma capsulatum* antigen test has proven to be very useful for the rapid diagnosis of histoplasmosis [7]. Cross-reactions with other endemic mycoses were noted with the original *Histoplasma* antigen enzyme immunoassay (EIA), but cross-reactions were not noted with coccidioidomycosis [8]. In 2005, positive results for a second-generation *Histoplasma* antigen test were observed in several patients who ultimately received a diagnosis of coccidioidomycosis [9]. To determine the potential value of this test in the diagnosis of coccidioidomycosis, we reviewed our experience with patients who had coccidioidomycosis and who underwent *Histoplasma* antigen testing.

**METHODS**

**Clinical specimens.** Records were reviewed from 19 patients with coccidioidomycosis for whom *Histo-
plasma antigen testing was performed at MiraVista Diagnostics (Indianapolis, IN). The patients were from 3 clinical practices: 2 in Phoenix, Arizona, and 1 in Los Angeles, California. All patients were treated by at least 1 of the authors of this article. Coccidioidomycosis was classified as acute if symptoms were present for <14 days and chronic if symptoms were present for >14 days.

H. capsulatum antigen detection. This assay is modified [9] from the original EIA [10]. In brief, the test is a sandwich EIA that uses microplates coated with polyclonal rabbit anti-Histoplasma antibodies (capture antibodies). Patient specimens and control samples are incubated in the precoated plates, thereby permitting binding of antigen to the capture antibody. Bound antigen is then detected with a biotinylated detector antibody, followed by streptavidin horseradish peroxidase and tetramethylbenzidine. The detector antibody has been modified to reduce false-positive results caused by antirabbit antibodies [11]. Results are calculated by comparison with a negative control. Results that have an optical density of greater than twice the optical density of the negative control are considered to be positive. Results are divided by the cutoff optical density and reported as antigen units, with results >1.0 U considered to be positive.

RESULTS

Clinical and laboratory findings. The pertinent clinical and laboratory findings for the 19 patients are summarized in table 1. All cases of coccidioidomycosis were supported by clinical, radiological, serological, and microbiological evidence. Underlying conditions in 17 of the patients included HIV infection in 10 patients, solid-organ transplantation in 2 patients, diabetes in 2 patients, liver disease with cirrhosis in 2 patients, and pregnancy in 1 patient. Four patients received corticosteroids, 2 for organ transplantation and 2 for other reasons. Two patients had no underlying conditions. Fourteen patients were classified as having acute coccidioidomycosis. In the 5 chronic cases of coccidioidomycosis, the duration of illness prior to antigen testing ranged from 31 to 252 days (median duration, 46 days). Pulmonary coccidioidomycosis was diagnosed in all 19 patients, and 8 (42%) had disseminated disease. Six (32%) of the 19 patients died. Laboratory evidence for coccidioidomycosis included positive histopathological results that exhibited characteristic Coccidioides spherules for 4 patients and positive culture results for 14, including 4 who had positive blood culture results. Serological testing results were positive in 11 patients and were the sole laboratory basis for diagnosis in 5. Serological test results were positive in 7 (50%) of 14 patients with acute coccidioidomycosis and in all 5 patients who had chronic disease. Seven (42%) of 12 patients who were immunosuppressed and who had acute coccidioidomycosis had positive serological test results, and an eighth patient had a positive result 1 month later, 5 days before death (patient 19).

Histoplasma antigen test results. Antigen was detected in urine samples from 11 (58%) of 19 patients. Positive results ranged from 1.2 to 21.8 U. Antigen was detected in urine samples from 11 (79%) of the 14 patients with acute cases and in a twelfth patient following 10-fold concentration of the urine sample (which had a positive result of 5.3 U), increasing the antigen detection rate to 86%. Antigenuria was not detected in the 5 patients with chronic cases, but antigen was detected in the bronchoalveolar lavage (BAL) fluid of 1. All patients with positive antigen results had cultures positive for Coccidioides species, and none had cultures positive for H. capsulatum.

Case descriptions illustrating the potential usefulness of antigen detection. Patient 2, a 27-year-old Hispanic woman, presented to the hospital with fever, oral candidiasis, and bilateral miliary pulmonary infiltrates. She was found to be HIV positive. Tuberculosis was suspected, in part because of serologic test results negative for coccidioidomycosis. On the basis of blood cultures that were positive for Coccidioides species, treatment was initiated with amphotericin B followed by fluconazolae. The Histoplasma urine antigen test had initial positive results of 12.4 U that decreased to 1.3 U after 6 weeks of treatment.

Patient 17 was a 50-year-old HIV-infected man who presented with a 3-week history of fever, cough, malaise, dyspnea, and a 13.5-kg body weight loss. Examination revealed a cachectic, ill-appearing individual who had oral candidiasis. CT of the chest revealed multiple bilateral, patchy infiltrates and a pleural-based mass. Routine analysis of BAL fluid was negative for pathogenic organisms. A percutaneous biopsy of the pleural-based mass revealed Coccidioides spherules; cultures of the BAL fluid and the pleural-based mass grew Coccidioides species. The Coccidioides complement fixation antibody titer was 1:64. The Histoplasma urinary antigen test had positive results of 2.5 U. The patient received treatment with amphotericin B, and his condition improved.

Patient 18 was a 35-year-old Hispanic woman who underwent orthotopic heart transplantation for idiopathic dilated cardiomyopathy. Two months after transplantation, she was admitted to the hospital with a fever (temperature, 39.5°C), headache, and right knee pain. Right knee radiographs and CT and MRI of the brain had normal findings. CT of the chest revealed multiple bilateral micronodular and macronodular densities and lower lobe consolidation. On day 6 after admission to the hospital, culture of blood samples that were obtained at admission was positive for fungus that was subsequently identified as Coccidioides species. Voriconazole therapy that was initiated at patient admission was switched to amphotericin B lipid complex. On day 7 after admission, the patient developed respiratory failure and died, despite ventilatory support. His-
Table 1. Summary of key findings and *Histoplasma* antigen results.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Underlying condition</th>
<th>Presentation</th>
<th>Laboratory evidence of coccidioidomycosis</th>
<th>Histoplasma urinary antigen assay results, U&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cirrhosis; CCS use</td>
<td>Chronic</td>
<td>Positive BAL culture result; IgM-positive and IgG-negative EIA result</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Death</td>
</tr>
<tr>
<td>2</td>
<td>HIV infection</td>
<td>Acute&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive blood, BAL, and urine culture results; IgM-negative and IgG-negative EIA result</td>
<td>12.4</td>
<td>Survival</td>
</tr>
<tr>
<td>3</td>
<td>HIV infection</td>
<td>Acute</td>
<td>Positive BAL culture result; IgM-negative and IgG-positive EIA result</td>
<td>2.4</td>
<td>Death</td>
</tr>
<tr>
<td>4</td>
<td>Diabetes</td>
<td>Chronic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>IgM-positive and IgG-positive EIA result</td>
<td>0.5</td>
<td>Survival</td>
</tr>
<tr>
<td>5</td>
<td>Diabetes; CCS use</td>
<td>Acute</td>
<td>Positive sputum culture result; IgM-positive and IgG-positive EIA result</td>
<td>2.0</td>
<td>Survival</td>
</tr>
<tr>
<td>6</td>
<td>Pregnant</td>
<td>Chronic</td>
<td>IgM-negative and IgG-positive EIA result; CF, 1:4</td>
<td>0.4</td>
<td>Survival</td>
</tr>
<tr>
<td>7</td>
<td>...</td>
<td>Chronic</td>
<td>IgM-negative and IgG-positive EIA result</td>
<td>0.2</td>
<td>Survival</td>
</tr>
<tr>
<td>8</td>
<td>HIV infection</td>
<td>Acute</td>
<td>Positive sputum culture result; IgM-negative and IgG-negative EIA result</td>
<td>12.8</td>
<td>Survival</td>
</tr>
<tr>
<td>9</td>
<td>...</td>
<td>Chronic</td>
<td>IgM-positive and IgG-positive EIA result</td>
<td>0.3</td>
<td>Survival</td>
</tr>
<tr>
<td>10</td>
<td>HIV infection</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Positive BAL, sputum, and blood culture results; IgM-positive and IgG-positive EIA result</td>
<td>3.4</td>
<td>Death</td>
</tr>
<tr>
<td>11</td>
<td>Cirrhosis</td>
<td>Acute</td>
<td>Positive sputum culture result; IgM-positive and IgG-positive EIA result</td>
<td>9.9</td>
<td>Death</td>
</tr>
<tr>
<td>12</td>
<td>HIV infection</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HP in BAL; positive BAL and blood culture result; IgM-negative and IgG-negative ID result; negative CF result</td>
<td>4.8</td>
<td>Survival</td>
</tr>
<tr>
<td>13</td>
<td>HIV infection</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HP in BAL; positive BAL culture result; IgM-positive and IgG-negative ID result</td>
<td>0.7</td>
<td>Survival</td>
</tr>
<tr>
<td>14</td>
<td>HIV infection</td>
<td>Acute</td>
<td>IgM-positive and IgG-positive ID result</td>
<td>0.4</td>
<td>Survival</td>
</tr>
<tr>
<td>15</td>
<td>HIV infection</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HP in BAL; positive BAL culture result; IgM-negative and IgG-negative ID result; negative CF result</td>
<td>0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Survival</td>
</tr>
<tr>
<td>16</td>
<td>HIV infection</td>
<td>Acute</td>
<td>Positive BAL culture result; IgM-positive and IgG-negative ID result</td>
<td>1.2</td>
<td>Survival</td>
</tr>
<tr>
<td>17</td>
<td>HIV infection</td>
<td>Acute</td>
<td>Positive BAL and pleural fluid culture results; IgM-negative and IgG-positive ID result; CF, 1: 64</td>
<td>2.5</td>
<td>Survival</td>
</tr>
<tr>
<td>18</td>
<td>Heart transplantation</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Positive blood culture result; IgM-negative and IgG-negative EIA result; IgM-negative and IgG-negative ID result; negative CF result</td>
<td>21.8</td>
<td>Death</td>
</tr>
<tr>
<td>19</td>
<td>Kidney transplantation</td>
<td>Acute&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Positive blood, BM, and BAL culture results; IgM-negative and IgG-negative EIA result; negative CF result</td>
<td>4.3</td>
<td>Death</td>
</tr>
</tbody>
</table>

**NOTE.** BAL, bronchoalveolar lavage fluid; BM, bone marrow; CCS, corticosteroid; CF, complement fixation; HP, spherules detected by histopathology; ID, immunodiffusion.

<sup>a</sup> Results >1.0 U (bolded) are considered to be positive.
<sup>b</sup> When the assay was performed using a sample of BAL, the results were positive (2.8 U).
<sup>c</sup> Concurrent disseminated disease.
<sup>d</sup> When the assay was performed using a 10-fold concentrated urine sample, results were positive (5.3 U).

toplasma* urine antigen from samples obtained on day 6 after admission had a positive result of 21.8 U. *Coccidioides* species were also isolated from BAL fluid. Serum *Coccidioides* IgM and IgG antibodies were absent from samples obtained at the time of transplantation and on days 1 and 5 after admission to the hospital.

Patient 19 was a 56-year-old Hispanic man who was admitted to the hospital with fever, diarrhea, and pancytopenia 2 months after receiving his third cadaveric renal transplantation. On hospital day 2, the patient developed hypotension and respiratory failure that required the use of vasopressors and mechanical ventilation, which prompted the administration of broad-spectrum antimicrobial therapy. On hospital day 4, cultures of blood samples that had been obtained at admission grew *Coccidioides* species. Treatment was initiated with amphotericin B lipid complex. *Histoplasma* urine antigen was re-
ported to have positive results of 4.3 U. *Coccidioides* IgM and IgG antibodies were initially absent from serum samples. On hospital day 13, chest CT demonstrated diffuse, patchy, ground-glass opacities and focal consolidation. On hospital day 29, the *Histoplasma* urine antigen results increased to 8.8 U, and anti-*Coccidioides* IgG antibodies were now detected in serum samples. Respiratory sample cultures grew *Coccidioides* species on multiple occasions. Examination of a bone marrow biopsy specimen on hospital day 30 demonstrated noncaseating granuloma with spherules that were typical for *Coccidioides* species. Despite treatment, the patient died on hospital day 34.

**DISCUSSION**

The diagnosis of coccidioidomycosis can be difficult in patients who have acute, fulminate disease, particularly if they are immunosuppressed. Although the tests used for the diagnosis of coccidioidomycosis are reliable, testing can be time-consuming or may only be performed a few times a week, resulting in delays in the receipt of diagnosis. A test to define the etiology as coccidioidomycosis early in the course of illness of an acutely ill patient would be helpful in making what might be lifesaving therapeutic decisions.

The usefulness of the *Histoplasma* antigen test in the diagnosis of histoplasmosis is well documented [7]. *Coccidioides* antigen detection has been reported in coccidioidomycosis [12], but this technology has not been refined for clinical use. Cross-reactivity with the other endemic mycoses in the original *Histoplasma* antigen EIA has been observed, but cross-reactivity with coccidioidomycosis has not been observed [8]. Our results suggest that the current *Histoplasma* antigen assay may be useful in the diagnosis of coccidioidomycosis because of the cross-reactivity between the 2 fungi. The *Histoplasma* urinary antigen test had positive results in 11 (58%) of the 19 patients overall and 11 (79%) of 14 who had acute coccidioidomycosis. An additional patient’s unconcentrated urine sample had a negative result, but a positive result was obtained after the urine sample was concentrated. If the latter patient is counted as having a positive result, the sensitivity of the assay increases to 86%. That the positive antigen test results were caused by coccidioidomycosis and not histoplasmosis in our patients is supported by the isolation of *Coccidioides* species from each patient and *H. capsulatum* from none. Cross-reactivity observed in these patients presumably results from recent modifications made to the *Histoplasma* antigen assay [9], but the exact reason has not been determined.

Mortality due to coccidioidomycosis is high in immunosuppressed patients, ranging from 40% to 50% in some reports [13, 14]. Six (32%) of our 19 patients died of coccidioidomycosis, including 2 of 10 patients with HIV infection and both patients who underwent solid-organ transplantation. Results of this report suggest that this test will be of value in the diagnosis of acute coccidioidomycosis before other tests have positive results. A test that assists in the diagnosis of coccidioidomycosis early in the course of disease and that has a quick turnaround time would be very useful to the clinician who must make critical empirical decisions when a diagnosis is not known. An example of a common diagnostic dilemma in regions of endemicity is the use of corticosteroids for presumed pneumocystis pneumonia in a patient with HIV infection who has pulmonary infiltrates. A early diagnostic test to suggest the presence or absence of coccidioidomycosis would be clinically useful.

There is a window of time in the course of severe *Coccidioides* infection when *Coccidioides* antigen is present in the blood and urine, as previously described by Galgiani et al. [12]. The duration of its presence may be influenced by the presence or absence of anti-*Coccidioides* antibody. A normal immune response may clear antigen from the blood and, subsequently, from the urine. This is supported by our observations of detecting antigen in 79% of the acutely ill patients with coccidioidomycosis, 6 of whom had undetectable IgM or IgG antibody by EIA. In the patients with chronic cases of coccidioidomycosis, IgM and/or IgG antibody was present in all 5, and no antigen was detected in urine samples. Meaningful comparison of serological and antigen test results was not possible, however, because serological testing was performed in multiple laboratories using different methods, and convoluted testing to identify seroconversion was performed for only a few patients.

Testing body fluids other than urine may potentially be useful. This is illustrated by patient 1. He experienced acute pulmonary coccidioidomycosis several months prior to admission to the hospital and was not compliant with the antifungal therapy regimen that was prescribed. This resulted in readmission to the hospital with a progression to pneumonia. Upon readmission, a urinary antigen assay had negative results, but antigen was detected in BAL fluid. BAL fluid subsequently grew *Coccidioides* species. This was the only patient for whom the assay was performed using BAL fluid.

Several factors should be considered when interpreting the findings of this report. First, patients in this study generally had more-severe coccidioidomycosis. The majority of patients were significantly immunosuppressed, and 32% died. The sensitivity of this test is likely to be lower in patients who have less-severe coccidioidomycosis. Second, many patients received antifungal therapy prior to antigen testing, which could potentially reduce antigen burden and decrease sensitivity. Third, patients may occasionally have histoplasmosis in an area where the disease is not endemic. This test does not have the ability to distinguish between histoplasmosis and coccidioidomycosis but suggests the presence of either infection. Fourth, the assay used antibodies to *H. capsulatum*; therefore, the sensitivity and specificity of the test could be improved using antibodies to...
Coccidioides species. Research is in progress to produce such antibodies. Prospective studies are needed to assess the usefulness of antigen detection in patients who have different manifestations of coccidioidomycosis, including immunocompromised and immunocompetent hosts.

In summary, an antigen of Coccidioides species that cross-reacts in the Histoplasma antigen assay is present in the urine of patients who have coccidioidomycosis. These findings support the potential for use of the Histoplasma antigen test for the diagnosis of coccidioidomycosis in areas of endemcity for Coccidioides species. We anticipate that the sensitivity and specificity of antigen detection in coccidioidomycosis can be improved using anti-Coccidioides species antibodies; however, until that time, the current Histoplasma antigen test appears to be diagnostically useful.

Acknowledgments

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Reference