Subscription Information for: CHICAGO JOURNALS
Clinical Infectious Diseases

MAJOR ARTICLE

Diagnosis of Acute Pulmonary Histoplasmosis by Antigen Detection

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Background. Antigen detection, which has proven useful in diagnosis of disseminated histoplasmosis, has not been studied in acute pulmonary histoplasmosis (APH). Because treatment is indicated in most patients with moderately severe or severe APH, antigen detection for rapid diagnosis could be helpful.

Methods. *Histoplasma* antigen detection was evaluated in 130 patients with APH.

Results. Antigenuria was detected in 64.6%, antigenemia in 68.6%, and antibody in 64.3%. If both urine and serum specimens were tested, antigen was detected in 82.8%, of which 45.8% had antigenemia only; and if both antigen and antibody were measured, results were positive in 93.3%, of which antigen only was positive in 35.7%.

Conclusions. Testing for antigenemia, antigenuria, and antibodies using the complement fixation test offers a sensitive, noninvasive method for diagnosis of APH.

Following heavy exposure to *Histoplasma capsulatum,* patients usually present within one to four weeks with respiratory symptoms, referred to as the "epidemic type" of acute pulmonary histoplasmosis (APH) [1]. Chest radiographs or computed tomographs typically show diffuse reticulonodular or miliary infiltrates.

Although APH usually resolves without treatment [1], many patients require hospitalization [2–6], which might be prolonged for management of respiratory failure [7–11]. Even in those who do not require hospitalization, recovery might be slow [12, 13], causing prolonged absences from work [2] or school [10].

Untreated APH can be fatal. In one report, a young woman died of respiratory failure 6 weeks after cleaning a basement containing bat guano [7]. In an outbreak involving 28 patients that was attributed to the bulldozing of a bird roost site, the bulldozer operator died of progressive respiratory failure 2 months after exposure and a 34 year old woman died of progressive disseminated histoplasmosis 6 months after exposure [14]. Self-limited disseminated histoplasmosis is thought to occur in most individuals following initial infection with *H. capsulatum* [15] , and has been demonstrated by pathology and/or culture in ~40% of cases presenting with APH [16–22].

Rapid diagnosis is important in patients with moderately severe or severe manifestations of APH because early treatment might shorten the clinical course and severity [13, 23–25]. Accordingly, treatment is recommended [26]. Two recent and 7 prior outbreaks provided the opportunity to evaluate the sensitivity of *Histoplasma* antigen and antibody detection for diagnosis of APH.

METHODS

El Salvador church renovation. Histoplasmosis was diagnosed in 15 mission group members who renovated a church in El Salvador in January 2008 [27] and who had *Histoplasma* antigen testing performed. A case was defined as (1) a laboratory-confirmed *H. capsulatum* infection or (2) illness characterized by self-reported fever and 2 additional symptoms (ie, headache, cough, chest pain, or difficulty breathing) beginning at least 24 h after arrival in El Salvador.

Louisville bridge painting. Histoplasmosis was diagnosed in 5 men who removed bird guano in preparation for painting a bridge in Louisville, Kentucky,

Received 11 June 2009; accepted 27 July 2009; electronically published 13 November 2009.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Clinical Infectious Diseases 2009; 49:1878–82
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1058-4838/2009/4912-0016$15.00
DOI: 10.1086/648421

Table 1. Clinical Findings, Antigen Concentration, and Antibody Response in the El Salvador and Louisville, Kentucky, Outbreaks of Acute Pulmonary Histoplasmosis

Case	Hospitalization	Chest radiograph finding(s)	Antigen concentration, ng/mL		ID result	CF result
			Urine	Serum		
1	No	NI	ND[a]	None	None	None
2	No	MN, A	ND[b]	ND[c]	None	None
3	No	NI	ND[b]	None	None	None
4	No	None	ND[a]	None	None	None
5	No	NI	1.06	ND[d]	None	None
6	No	NI	ND[b]	ND[d]	ND	ND
7	No	NI	ND[a]	<0.6[d]	ND	ND
8	No	MN, A	9.3	<0.6[d]	ND	ND
9	No	RN	9.09	3.2[d]	ND	1:32
10	No	RN	8.49	1.8[d]	ND	1:8
11	No	MN	2.73	1.9[d]	M	1:8
12	No[e]	Unknown	9.59	None	None	None
13	Yes[e]	RN	<0.6	ND[c]	None	1:32
14	Yes	NI	<0.6	<0.6[d]	ND	ND
15	Yes	MN	0.94	None	None	None
16	Yes	MN	5.4	<0.6[d]	ND	ND
17	Yes	MN, A	3.63	ND[c]	None	None
18	Yes	NI	<0.6[a]	None	None	None
19	Yes	MN	4.78	None	None	None
20	Yes	MN, A	ND[b]	ND[c]	None	None

NOTE. A, adenopathy; ID, immunodiffusion; M, M precipitin band; MN, multiple nodules; ND, none detected; NI, no infiltrate; none, no radiograph or antigen test performed; RN, reticulonodular.

[a] Urine concentrated 10-fold.

[b] Urine negative unconcentrated, but no specimen was available for concentration.

[c] ND untreated, but no specimen was available for EDTA heat treatment.

[d] EDTA heat–treated result.

[e] Culture of bronchoalveolar lavage fluid yielded positive results.

in October 2007 and who had *Histoplasma* antigen testing performed. A case was defined as illness in a person with respiratory symptoms with either (1) nodular disease on chest radiographs or (2) positive*Histoplasma* antigen or antibody test results.

Earlier outbreaks. Specimens from seven earlier outbreaks [28–34] that had been stored at −20°C or −70° C were also tested. Clinical and radiographic information was not available for these cases, except that all patients in the Virginia correctional facility outbreak had been hospitalized [28].

Histoplasma *antigen assay.* This assay was performed as previously described, using a cutoff for positivity of three times the optical density of the negative control [35]. Methods that have been shown to improve the detection of antigenuria at concentrations that are below the threshold of the assay were employed if the untreated specimen was negative and sufficient volume was available for additional testing. The urine was concentrated 10-fold by ultra filtration using 5000MWCO Amicon Ultra Centrifugal Filter tubes if at least 2 mL of urine was available [36]. Serum was heated at 100°C in EDTA for 4 minutes to dissociate immune complexes and denature the freed antibody if at least 0.5 mL was available [37].

Histoplasma *antibody test.* The *Histoplasma* immunodiffusion test was performed according to the manufacturer's instructions (Meridian Bioscience) and the *Histoplasma* complement fixation test at the Clarian Pathology Laboratory (Indianapolis, IN) if at least 0.1 mL of serum was available.

Statistical analysis. The respective proportion of patients with positive results was compared using the Fisher exact test.

RESULTS

Clinical and laboratory findings in patients with APH acquired during the El Salvador church renovation or Louisville bridge project are listed in Table 1. Respiratory symptoms were present in 17 of the 20 patients, including four who reported shortness of breath. Chest radiographs were performed on 18 patients, and were abnormal in 11, showing diffuse nodules <1 cm in diameter or reticulonodular infiltrates in all 11, and intrathoracic lymphadenopathy in 4. In 1 patient, the initial chest radiograph was normal but a follow-up radiograph 6 days later showed diffuse nodules. Follow-up radiographs were not performed in the other 7 patients who did not have infiltrates or adenopathy. Cultures of bronchoalveolar lavage fluid were positive in two patients. One (12.5%) of 8 patients had a positive immunodiffusion test, and 4 (44.4%) of 9 had positive complement fixation tests.

Antigenuria was detected in 12 (60.0%) of 20 patient specimens, and in 1 (5.0%) additional specimen, it was detected following ultrafiltration [36]. Antigen was not detected in any of the 13 untreated serum specimens tested. However, 9 (69.2%) of these 13 serum specimens had sufficient serum for EDTA-heat treatment and 7 (77.8%) of these 9 specimens had detectable antigen present following treatment.

Antigenuria or antigenemia was present in 7 (87.5%) of 8 hospitalized patients compared to 7 (58.3%) of 12 nonhospitalized patients ($P = .085$), and in 9 (81.8%) of 11 patients with radiographic abnormalities compared to 4 (64.6%) of 7 patients without radiographic abnormalities ($P = .326$). In 1 patient the initial chest radiograph and antigen tests were negative, but after 6 days, the radiograph showed diffuse infiltrates and the urine antigen was 9.3 ng/mL.

Results of antigen testing during all 9 outbreaks are summarized in Table 2. Antigenuria was detected in 84 (64.6%; 95% confidence interval [CI], 56.068%–72.294%) of 130 patients and antigenemia in 24 (68.6%; 95% CI, 52.049%–81.472%) of 35 patients. EDTA-heat treatment performed on 22 serum specimens from patients identified in the earlier outbreaks and 9 from recent outbreaks resulted in 24 of the 31

Table 2. Results of Antigen and Antibody Tests from 9 Histoplasmosis Outbreaks

Outbreak	No. of weeks after exposure	Antigenuria	Antigenemia	Antibody-CF	Year and reference
Costa Rica	3–5	4/15 (26.7)[a]	11/14 (78.6)[a]	8/13 (61.5)[a]	1988 [31]
Virginia	2–3	21/24 (87.5)	None	None	1994 [28]
Belize	4–10	5/6 (83.3)	0/1 (0)	None	2000 [29]
Acapulco	4–10	24/34 (70.6)	1/1 (100)	None	2001 [32]
Nicaragua	2–3	9/13 (69.2)	1/1 (100)	1/1 (100)	2001 [33]
Illinois	3–4	3/4 (75)	None	None	2003 [34]
Nebraska	1–10	5/14 (35.7)	4/5 (80)	5/5 (100)	2003, 2004 [30][b]
Louisville, Kentucky	3–7	5/5 (100)	3/4 (75)	4/4 (100)	2007 (unpublished)
El Salvador	3–4	8/15 (53.3)	4/9 (44.4)	0/5 (0)	2008 [27]
Total	1–10	84/130 (64.6)	24/35 (68.6)	18/28 (64.3)	…
95% confidence interval, %	…	56.068–72.294	2.049–81.472	45.844–79.306	…

NOTE. Data are proportion of persons with positive results (%), unless otherwise indicated. CF, complement fixation; none, no specimen available for testing.

[a] Urine specimens were obtained 5 weeks after exposure, and serum specimens were obtained 3–5 weeks after exposure.

[b] Specimens from patients identified in 2 outbreaks.

(77.4%; 95% CI, 53.440%–83.930%) specimens testing positive. Results for the Virginia, El Salvador, and Louisville outbreaks combined showed that antigen was detected in 28 (87.5%; 95% CI, 71.321%–95.030%) of 32 hospitalized patients compared to 7 (58.3%; 95% CI, 31.923%–80.651%) of 12 nonhospitalized patients ($P = .087$). Results from individual patients are shown in Figure 1. The median concentration of antigenuria was 2.53 ng/mL (range, <0.6 to >39.0 ng/mL) and of antigenemia was 1.25 ng/mL (range, <0.6 to 9.58 ng/mL).

Of the 29 patient who were tested for antigenemia and antigenuria, both tests were positive in 12 (41.4%; 95% CI, 25.530%–59.282%), antigenuria alone was present in 1 (3.4%; 95% CI, 0.597%–17.105%), antigenemia alone in 11 (37.9%; 95% CI, 22.663%–55.968%), either antigenuria or antigenemia in 24 (82.8%; 95% CI, 65.498%–92.478%), and neither in 5 (17.2%; 95% CI, 7.572%–34.502%). Of 24 specimens with either antigenemia or antigenuria, antigenemia alone was present in 11 (45.8%; 95% CI, 27.863%–64.896%). Insufficient urine volume prevented ultrafiltration in specimens from the earlier outbreaks.

Serologic tests were performed in 30 patients. Immunodiffusion was positive in 5 (17.2%; 95% CI, 7.572%–34.502%) of 29 specimens and the complement fixation test was positive in 18 (64.3%; 95% CI, 45.844%–79.306%) of 28 specimens, with titers ranging from 1:8 to 1:256 (median, 1:32). The immunodiffusion test was not performed in 1 patient because of insufficient volume. Complement fixation test results were not available in 2 patients, because of insufficient volume in one and anti-complementary activity in the other. In no case was the immunodiffusion test result positive and complement fixation test result negative, but in 12 specimens, the complement fixation test result was positive while the immunodiffusion test result was negative. The results of both the serologic test and the antigen test were positive in 14 (46.7%; 95% CI, 30.261%–63.888%) of 30, antigen only in 10 (33.3%; 95% CI, 19.204%–51.187%), antibody only in 4 (13.3%; 95% CI, 5.290%–29.642%), and either in 28 (93.3%; 95% CI, 78.632%–98.138%), neither in 2 (6.7%; 95% CI, 1.862%–21.368%). Of

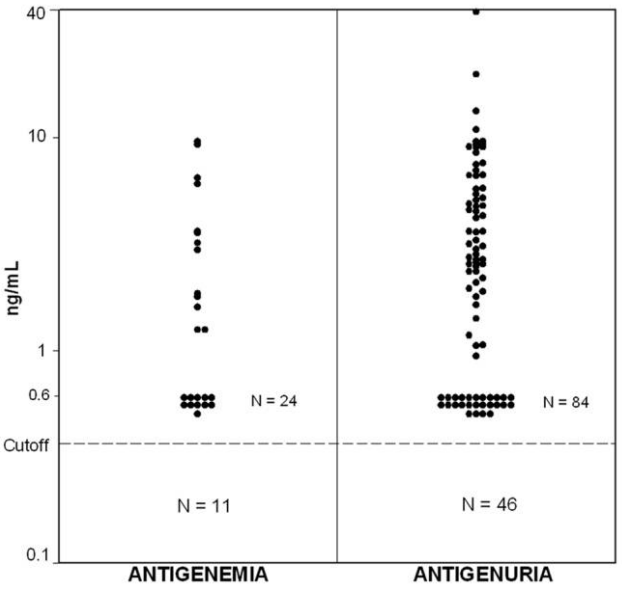

Figure 1. Antigenuria and antigenemia in acute pulmonary histoplasmosis. The broken horizontal line labeled cutoff represents the cutoff for positivity, at an optical density of 3 times that of the negative control. For the purpose of the figure, the cutoff is placed below the 0.6 ng/mL calibrator, which is the lowest calibrator included in the calibration curve and which does not represent a specific ng/mL designation. The number below the line represents the number of patients with negative results, and the number above the line represents the number of patients with positive results, each which is designated by a single point.

the 28 patients whose specimens were positive for antibody or antigen, the antigen test result only was positive in 10 (35.7%; 95% CI, 20.694%–54.156%).

DISCUSSION

Antigenuria and antigenemia were detected in 64.6% and 68.6% of the APH patients, respectively. In a subset in which both serum and urine were tested, antigen was detected in 82.8%, 45.8% of which demonstrated antigenemia only. Not surprisingly, antigen was detected more frequently among specimens from patients who required hospitalization. These findings support a useful role for antigen testing in the diagnosis of APH.

Tests for antibody to *Histoplasma* were positive by immunodiffusion in 17.2% of sera specimens and by complement fixation in 64.3%. In another outbreak, antibodies were not detected at three weeks after exposure but were detected in three-quarters of patients by six weeks after exposure [38]. In this study, the highest sensitivity for diagnosis of APH was achieved by testing for both antigen, positive in 82.8% of cases, and antibody, positive in 64.3% of cases, which when combined achieved a sensitivity of 93.3% . The immunodiffusion test was too insensitive to recommend as a screening test.

Rapid diagnosis of APH can also be made by direct examination of respiratory secretions or lung tissue obtained via bronchoscopy, showing structures resembling yeast. Bronchoscopy was performed in 2 patients in the Louisville bridge worker outbreak, and fungal culture was positive but yeast were not observed by direct examination. Bronchoscopy was not performed among any patients in the El Salvador outbreak, and information was not available for the patients in any of the earlier outbreaks. However, among reported cases of APH in which bronchoscopy was performed, direct examination was positive in 2 (20%) of 10 patients [7, 8, 13, 23, 39–42] and culture in 8 (42%) of 19 patients [2, 7, 13, 18, 23, 39–46]. Whether the sensitivity could be improved further over that achieved by histopathology or culture by testing bronchoalveolar lavage specimens for antigen [47] requires investigation. In patients with severe manifestations, where a delay in diagnosis while waiting for results of antigen tests or serology may be harmful, thorascopic or open lung biopsy may be required to establish a rapid diagnosis by histopathologic staining.

Several caveats should be considered when interpreting the findings of this study. First, the antigen test is not specific for*Histoplasma* and cross- reactions can occur with other mycoses [35]. However, there is little doubt that these patients had histoplasmosis rather than another endemic mycosis, as the findings in 8 of the outbreaks have been previously reported and the ninth (Louisville bridge outbreak) was confirmed by isolation by culture in 2 patients. Second, a positive antigen test or a compatible clinical syndrome was the criteria for meeting the histoplasmosis case definition in the El Salvador outbreak, potentially biasing the study for antigen detection. However, the sensitivity of the antigen test in the El Salvador outbreak was similar to that observed in several of the other outbreaks in which antigen positivity was not a criterion for diagnosis in the case definition. Third, sequential testing was performed in only a few cases, including one in which the chest radiograph and urinary antigen tests were negative initially but positive 6 days later. Conversely, delay in obtaining specimens might have contributed to false-negative antigen test results in some patients. Also, failure to obtain follow-up specimens might have contributed to false-negative antibody results. We believe that antigenemia and/or antigenuria are likely to be more sensitive than antibody detection during the first month of infection, before seroconversion occurs, and that antibody tests might be more sensitive later in patients who have improved spontaneously or in response to treatment. A study collecting specimens at multiple time points during at least the first three months of infection would be required to thoroughly compare the role of antigen and antibody testing for diagnosis of APH. The most likely explanation for the variation in sensitivity of antigen and antibody detection among the different outbreaks in this report is variation in interval between infection and collection of specimens. The highest sensitivity for diagnosis by antigen or antibody detection might have been achieved by obtaining serum and urine specimens for antigen and serum for antibody at the time the patient first presented, at the time of clinical worsening in patients with progressive illness, and 6–8 weeks later. Fourth, these findings should be applied only to patients with APH. Patients with subacute or chronic pulmonary histoplasmosis [48] have not been studied and were noted to exhibit lower rates of antigenuria (37%) in the original assay [49]. Finally, these findings should be applied only to testing performed at MiraVista Diagnostics.

In summary, testing for *Histoplasma* antigenemia, antigenuria, and antibodies using the complement fixation test offers a sensitive, noninvasive method for diagnosis of APH, especially in the more severe cases were treatment is recommended. If initial tests are negative and histoplasmosis is still suspected clinically, follow-up testing is recommended.

Acknowledgments

Financial support. MiraBella Technologies.

Potential conflicts of interest. L.J.W. is an owner of MiraVista Diagnostics. S.S., L.J.W., and P.C. are employees of MiraVista Diagnostics and MiraBella Technology. All other authors: no conflicts of interest.

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