MAJOR ARTICLE

Role of *Coccidioides* Antigen Testing in the Cerebrospinal Fluid for the Diagnosis of Coccidioidal Meningitis

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Background. Coccidioidal meningitis (CM), a common cause of chronic meningitis in endemic area, is usually diagnosed by detection of anti-*Coccidioides* antibodies in cerebrospinal fluid (CSF), and findings may be negative in up to one-third of cases. CSF cultures and cytology are infrequently positive. Antigen detection has been used for the diagnosis of other forms of coccidioidomycosis and meningitis caused by other mycoses. The purpose of this study was to assess the diagnostic utility of CSF *Coccidioides* antigen (CAg) detection for the diagnosis of CM.

Methods. The medical records of patients with clinically suspected meningitis, in whom CSF was tested for *Coccidioides* antibodies and CAg, were retrospectively reviewed, and CSF CAg testing was prospectively conducted in patients with CM. All specimens were submitted for CAg testing.

Results. Thirty-six patients with 42 episode of CM were studied. The sensitivity and specificity of CAg were 93% and 100%, respectively. Cultures of CSF were positive in 7%, antibodies were demonstrated by immunodiffusion in 67% and complement fixation in 70%, and immunoglobulin M and G antibodies were demonstrated by enzyme immunoassay in 8% and 85%, respectively.

Conclusions. Testing CSF for CAg is a useful addition to diagnostic methods in suspected CM and complements testing with CSF antibodies and culture.

Keywords. coccidioidal meninigits; *coccidioides* antibodies; coccidioides antigen; disseminated coccidoidomycosis; fungal meninigits.

Central nervous system (CNS) involvement occurs in <5% of patients with coccidioidomycosis [1, 2], and the diagnosis may be difficult [3, 4]. Wet mount demonstration of spherules in the cerebrospinal fluid (CSF) is possible in <10% of cases [3, 4], and cultures are positive in 20%–30% [1–3, 5]. In <10% of cases the diagnosis has been made by means of meningeal biopsy [1–5].

Coccidioidal meningitis (CM) is usually diagnosed by detecting anti-*Coccidioides* antibodies in the CSF [6–8]. Anti-*Coccidioides* antibodies can be detected in up to 80% of cases with immunodiffusion (ID), complement fixation (CF), or enzyme immunoassay (EIA) [1, 3, 5, 7]. CSF EIA, though more sensitive than CF (75% vs 59%, respectively) [4], can have false-positive results in nonmeningitic coccidioidomycosis, leading some experts to advise against its use as a sole laboratory test for the diagnosis of CM [6–10]. Galgiani et al [11] reported detection of antibodies to a 33-kDa antigen by EIA in the CSF in 72% of patients with CM, compared with 56% by CF. The detection of anti-*Coccidioides* CF antibodies in the CSF, even though less sensitive, with reported false-negative rates between 17% and 41% [3, 4], is highly specific for the diagnosis of CM [9, 10, 12], with rare false-positive results caused by transfer of antibody from the serum to the CSF in patients with high levels of CF antibodies in the serum [13].

Antigen detection in CSF is the most sensitive and rapid method for diagnosis of cryptococcal meningitis [12]. *Coccidioides* antigen (CAg) detection became

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available in 2007 and was shown to complement antibody detection and cytopathology for the diagnosis of coccidioidomycosis [14]. In the setting of moderate to severe coccidioidomycosis, predominantly occurring in immunocompromised patients, *Coccidioides* antigenuria was detected in 71% of patients [14]. Subsequently, in milder cases, *Coccidioides* antigenemia was detected in 73% of cases, complementing detection of antigenuria, present in 50% of cases [15]. Cross-reactivity was noted in 11% of patients with other endemic mycoses, but its specificity was 99% in those with no other fungal infection [16]. Although the detection of antigen in the CSF is used in the diagnosis of CM, sensitivity and specificity have not been reported. The objective of our study was to assess the accuracy of CSF CAg detection for the diagnosis of CM.

METHODS

Patient Selection

A retrospective review was performed, including records of all patients at Maricopa Integrated Medical System (MIHS) with suspected CM who underwent CSF CAg testing as part of clinical care between July 2008 and May 2014. Patients who did not meet study criteria for CM (see Criteria for Diagnosis) and had evidence of CSF abnormalities consistent with meningitis were used as controls. From all patients at St Joseph's Hospital and Medical Center (SJHMC) with established CM, between November 2012 and May 2014, CSF samples were prospectively collected, stored frozen, and periodically sent to MiraVista Diagnostics for CAg testing.

Criteria for Diagnosis

The diagnosis of meningitis was established by the presence of symptoms and/or signs of meningitis accompanied by CSF pleocytosis (CSF white blood cell [WBC] count, >5 cells/μL), hypoglycorrhachia (CSF glucose, <45 mg/dL), or elevated protein (CSF protein, >50 mg/dL). Definite diagnosis of CM required presence of meningitis and CSF evidence of *Coccidioides* infection by fungal culture, visualization of spherule at cytopathology, detection of anti-*Coccidioides* antibodies with ID/CF, or a confirmed medical history of definite CM [3–5, 17, 18]. The latter cases were considered relapses.

Patients with meningitis and either non-CNS coccidioidomycosis or no proof of coccidioidomycosis were classified as having probable CM if no alternative diagnosis was established and the patient responded to therapy for CM [3–5, 17, 18]. Medical records were reviewed to ensure absence of alternative diagnoses for 2 years after the initial diagnosis.

Antigen Detection

Antigen testing was performed at MiraVista Diagnostics, Indianapolis, Indiana. The *Coccidioides* quantitative antigen assay was performed as described elsewhere, using microplates coated with anti-*Coccidioides* antibodies [11, 12]. The assay was validated for testing CSF according to Clinical and Laboratory Science Institute guidelines for laboratory developed tests. CSF was first treated by adding 200 μL of 4% ethylenediaminetetraacetic acid (EDTA) (pH 4.6) to 600 μL of CSF, vortexing the mixture, and placing it in a heat block at 104°C for 6 minutes, as described for serum [11]. The samples were centrifuged and the supernatants were tested in the antigen assay. Results above a standard containing 0.07 ng/mL were classified as positive. Results above the highest standard, 8.2 ng/mL, were designated as above the limit for quantification, and were arbitrarily assigned a value of 8.2 ng/mL for calculation of mean and/or median concentration.

Anti-*Coccidioides* Antibody Detection

Antibody testing was performed at Associated Regional and University Pathologists laboratories for MIHS and Mayo Clinic laboratories for SJHMC. Criteria for a positive result for ID and CF included presence of precipitin bands of identity with CAg and CF titers of ≥1:2. Positivity for immunoglobulin (Ig) M or IgG by EIA was determined according to instructions from the manufacturers.

Statistical Analysis

Two-tailed *t* tests were performed on continuous variables, and 2-tailed *Z* tests were performed to compare proportions between case patients and controls for all categorical variables. Differences were considered significant at $P < .05$.

Ethical Considerations

The study was approved by the institutional review boards of the participating institutions. Written informed consent was obtained for the patients enrolled in the prospective study at SJHMC.

RESULTS

Twenty-two MIHS patients were identified with CM and were combined with 14 patients prospectively identified with confirmed CM at SJHMC, for a total of 36 patients with CM. Four patients had probable CM, as defined previously, and 32 had definite CM. Ten patients were identified with an initial episode of CM, and 26 had a relapse of previously diagnosed CM. Five different patients had more than one independent relapse, separated by periods of ≥3 months, for a total of 42 episodes of CM. Of the 10 patients with initial episode, 6 had evidence of non-CNS *Coccidioides* infection upon diagnoses of CM (3 had pulmonary coccidioidomycosis and another and 3 had disseminated coccidioidomycosis without CNS infection). A total of 88 patients, all at MIHS, had CSF abnormalities consistent with meningitis owing to other causes and constituted the control group. Eight of these 88 control patients had evidence of prior coccidioidomycosis, 7 with pulmonary and 1 with disseminated

non-CNS disease. Their charts were reviewed, and evidence of any alternative cause of meningitis was documented.

Demographic findings are presented in Table 1. There were no significant differences in age, sex, ethnicity distribution, or underlying immunosuppressive conditions between the control group and patients with CM. All patients had symptoms of CNS involvement, such as headaches, altered mental status, seizure, or neurologic deficit. Fever was present in 38% of CM episodes (16 of 42) .

Imaging studies were abnormal in the vast majority of CM episodes (62% of brain computed tomography [CT] and 91% of brain magnetic resonance [MR] imaging studies). Hydrocephalus was the most common abnormality noted with both modalities (seen in 51% with brain CT and 65% with brain MR imaging). MR imaging was more sensitive than CT in detecting meningeal enhancement (48% with MR imaging and 5% with CT; $P < .001$). Mass lesions were uncommon (3% with CT and 4% on MR imaging). Results are presented in Table 2.

CSF WBC counts were similarly elevated in patients with CM and controls (mean [standard deviation (SD)], 124 [161] and 100 [419] cells/μL, respectively; $P = .72$). CSF protein levels were higher in patients with CM than in controls (mean [SD], 246 [283] and 129 [206] mg/dL, respectively; $P = .008$), and CSF glucose levels were lower in patients with CM (44 [25] and 59 [24] mg/dL, respectively; $P = .001$). CSF fungal cultures for *Coccidioides* were positive in only 7% (3 of 42 patients). None of the patients with CM had *Coccidioides* spherules seen on CSF wet mount preparations. Results are presented in Table 3.

The sensitivity and specificity of the diagnostic CSF laboratory tests were determined for the 42 episodes of CM and 88 episodes of meningitis in the controls group (Table 4). The sensitivity of CAg detection was 93%; specificity, 100%; positive predictive value (PPV), 100%; and negative predictive value (NPV), 97%. Antigen detection identified 5 cases with negative antibody results by ID and CF. The sensitivities for ID and CF antibody detection were 67% and 70%, respectively, and the specificities were 99% and 100%, respectively. The sensitivity and specificity of combined antibody detection with ID and CF were 85% and 99%, respectively; the PPV, 97%; and the NPV, 93%. Combining CAg testing results with ID and CF results, the sensitivity was 98% and the specificity 99%, with a PPV of 98% and a NPV of 99%. The sensitivity for detection of IgG antibody with EIA was 85%, and the specificity was 99%. One patient in the control group had disseminated histoplasmosis and had a positive CSF Coccidioides IgG result at 3.7 (IV). The sensitivity for detection of IgM antibody with EIA was 8%, and the specificity was 100%.

The CSF CAg concentrations were similar in initial episodes (n = 10) and relapses (n = 32) (mean [SD], 1.42 [2.51] and 3.12 [3.14] ng/mL, respectively; $P = .13$). During the study, 5 patients had 6 episodes of CM relapses, allowing comparison of CSF parameters at relapse with those at the initial testing. The most consistent finding was that CSF CAg concentrations were higher during subsequent episodes than during the baseline episodes.

Table 1. Demographic Data and Risk Factors

Demographics and Risk Factors	Control Patients (n = 88)	Patients With CM (n = 36)	*P* Value[a]
Age, mean (SD), y	43.5 (11.3)	39.9 (11.3)	.10
Male sex, No. (%)	67 (76)	30 (83)	.18
Ethnicity, No. (%)			.11
African American	17 (19)	7 (19)	. . .
Hispanic	43 (49)	14 (39)	. . .
Asian/Filipino	1 (1)	2 (5.5)	. . .
White	27 (31)	11 (31)	. . .
Native American	0	2 (5.5)	. . .
Immunosuppressive condition, No. (%)[b]			
None	24 (27)	10 (28)	.95
Diabetes mellitus	12 (14)	7 (19)	.41
HIV/AIDS	48 (55)	20 (56)	.92
Other	15 (17)	4 (11)	.41

Abbreviations: CM, coccidioidal meningitis; HIV, human immunodeficiency virus; SD, standard deviation.

[a] No significant differences.

[b] The totals are >100% because some patients had multiple immunosuppressive conditions.

Table 2. Imaging Findings in Patients With Coccidioidal Meningitis

Imaging Finding	Patients, No. (%)		
	Brain CT (n = 37)	Brain MRI[a] (n = 23)	*P* Value
Normal	14 (38)	2 (9)	.01
Meningeal enhancement	2 (5)	11 (48)	<.001
Hydrocephalus	19 (51)	15 (65)	.29
Mass	1 (3)	1 (4)	.73
Other	1 (3)	1 (4)	.73

Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging.

[a] The total is >100% because brain MRI showed multiple type of abnormalities in individual cases.

Table 3. CSF Laboratory Findings in Patients With CM Compared With Controls

CSF Finding	Mean Value (SD)		*P* Value
	Controls (n = 88)	Patients With CM (n = 42)	
WBC count, cells/μL	100 (419)	124 (161)	.72
Protein, mg/dL	129 (206)	246 (283)	.008
Glucose, mg/dL	59 (24)	44 (25)	.001

Abbreviations: CM, coccidioidal meningitis; CSF, cerebrospinal fluid; SD, standard deviation; WBC, white blood cell.

Table 4. Results of Diagnostic Studies for Coccidioidomycosis in the CSF

CSF Parameter	Sensitivity, %	Specificity, %	PPV, %	NPV, %
CAg	93	100	100	97
ID	67	99	96	87
CF	70	100	100	88
ID and CF	85	99	97	93
CAg, ID, and CF	98	99	98	99
CSF IgM EIA	8	100	100	70
CSF IgG EIA	85	99	97	93

Abbreviations: CAg, *Coccidioides* antigen; CF, complement fixation; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; ID, immunodiffusion; IgG, immunoglobulin G; IgM, immunoglobulin M; NPV, negative predictive value; PPV, positive predictive value.

Changes were variable for other CSF parameters. Of note, CSF specimens were not available between relapses dates to assess the effect of treatment on CSF CAg concentrations.

CSF CAg concentrations were higher in patients with human immunodeficiency virus (HIV) infection (n = 16) than in those without HIV infection (n = 26) (mean [SD], 3.46 [3.22] and 1.51 [2.39] ng/mL, respectively; $P = .04$), probably reflecting a higher burden of CSF *Coccidioides* organisms. There was no correlation between the CSF CAg concentrations and HIV viral loads or serum CD4 cell counts (Figure 1). Comparing HIV-infected/immunocompromised patients with non–HIV-infected patients, there was no difference in the performance of CSF and serum *Coccidioides* antibodies or CSF and serum CAg in the diagnosis of CM. Meanwhile, urine CAg detection outshines antibody detection in HIV-infected/immunocompromised patients compared with non–HIV-infected patients; 17 of 18 patients with HIV infection or immunocompromising conditions had positive urine CAg results, compared with 3 of 11 patients without HIV infection ($P = .0002$). Of note, only 2 patients with immunocompromising conditions were not HIV infected.

There was no correlation between CSF CAg concentrations and other CSF parameters, such as CSF WBC counts, CSF glucose level, CSF protein concentration or CSF CF antibody titers (Figure 2). Serum and urine CAg concentrations were positive in 16 of 19 (84%) and 20 of 28 (71%) of the CM episodes, respectively. Serum CF antibody results were positive in 30 of 36 CM episodes (83%), and titers were ≥1:32 in 7 of 36 (19%). There was no correlation between CSF CAg concentration and serum CF antibody titers, serum CAg concentrations, or urine CAg concentrations in patients with CM (Figure 2).

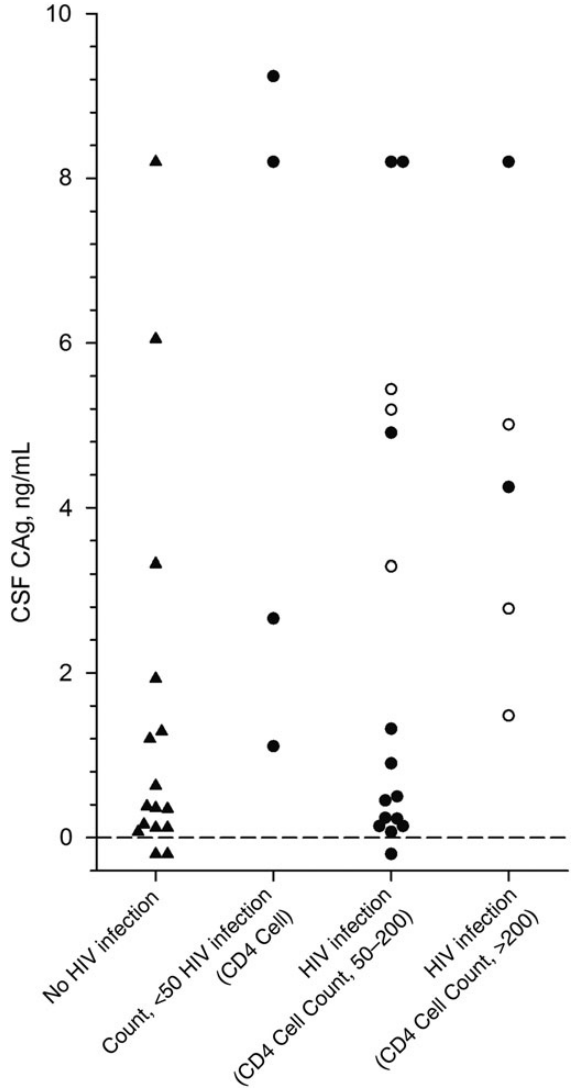

Figure 1. Cerebrospinal fluid (CSF) *Coccidioides* antigen (CAg) concentration in patients with coccidioidal meningitis by human immunodeficiency virus (HIV) infection status and CD4 cell count (cells/μL).

DISCUSSION

This is the first large study evaluating the comparative performance of CSF CAg and CSF *Coccidioides* antibody detection for the diagnosis of CM. The sensitivity of CSF antibody testing with ID or CF was suboptimal at 67%–70%, similar to previous reports [3–5, 7]. In our case series, the sensitivity of CSF CAg antigen was higher at 93%. The sensitivity of combined antibody testing with ID and CF together was comparable to that of antigen testing. Combining all modalities (CSF CAg, CSF ID, and CSF CF) further increased the diagnostic yield to 98%. No correlation was observed between CSF CAg concentration and CSF CF antibody titers. Five patients who had negative CSF CF and ID antibody results had positive CSF CAg results, and 2 patients with negative CSF CAg results had positive CSF CF and/or ID antibody results, supporting a complementary role of both antigen and antibody detection for the diagnosis of CM. This study also confirmed previous reports indicating

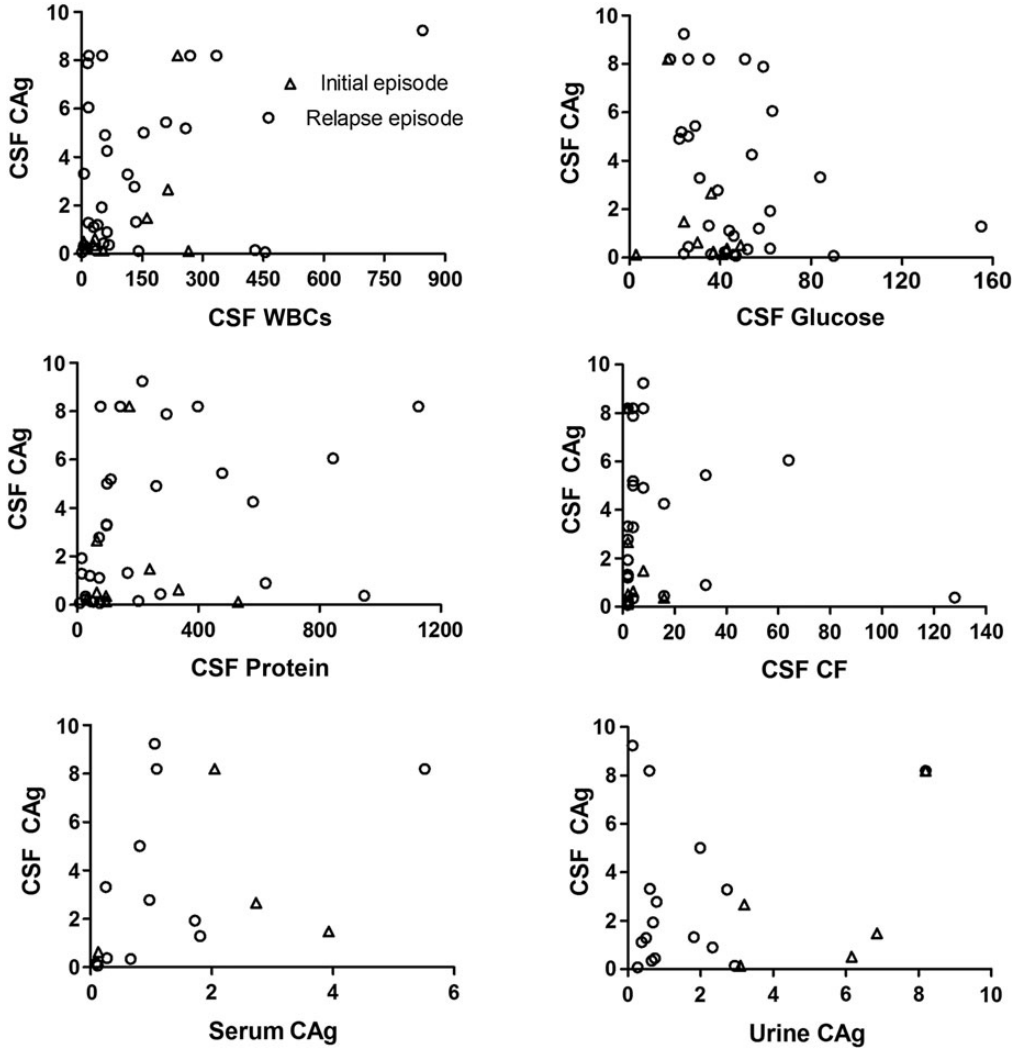

Figure 2. Correlation of cerebrospinal fluid (CSF) *Coccidioides* antigen (CAg) concentrations (ng/ml) with CSF parameters; CSF WBC (cells/μL), CSF glucose (mg/dl), CSF protein (mg/dl) and serum and urine CAg concentrations (ng/ml). Abbreviations: CF, complement fixation; WBCs, white blood cells.

that findings of cytopathology and/or CSF fungal culture were rarely positive, in 0% and 7% of patients, respectively [3, 4]

A potential advantage of antigen testing over antibody testing is a more rapid turnaround time. For example, the antigen test can provide results the same day the specimen is received at the reference laboratory. Development of a Food and Drug Administration–cleared CAg assay would permit the test to be performed locally on the same day the specimen was obtained. ID requires up to 3 days to identify positive specimens (precipitin bands) and an additional 3 days to provide a titer. CF is primarily offered at national reference laboratories, requiring 1 or 2 days for shipment and an overnight incubation. Most reference laboratories do not perform the CF tests daily, contributing to the delay. If the antibody EIA is performed at a reference laboratory, similar delays for shipment and testing are incurred. An additional advantage of antigen testing is that the results are expressed quantitatively, supporting a potential use for assessing fungal burden and response to treatment, hypotheses requiring further study.

This is the largest study of anti-*Coccidioides* antibody testing in CSF using EIA methods. The sensitivity for detection of IgM antibodies was 8%, and the specificity was 100%, whereas the sensitivity for detection of IgG antibodies was 85%, and the specificity 99%. Analysis of the antibody EIA was limited by performance of the testing at 2 reference laboratories using assays developed by different manufacturers, neither of which had been validated for CSF. Another study [2] reported sensitivities of 75% for IgG and 35% for IgM antibody. Although antibody testing of CSF with EIA seems promising, larger studies using assays that have been validated for CSF specimens are needed.

This study also contributes to the understanding of the role of antigen detection in urine and serum for diagnosis of

coccidioidomycosis. In other studies, antigenuria was detected in 71% of patients with severe disease requiring hospitalization [14] and in 50% of those with mild disease not requiring hospitalization [15]. The combined performance of antigenemia and antigenuria testing increased the yield to 71% in mild disease [15]. In our study, the sensitivities of antigenemia and antigenuria were higher, at 84% and 71%, respectively. Combined testing of serum and urine for CAg was positive in 87% of cases.

Several limitations of the study should be recognized. First, most of the patients were identified by retrospective review of medical and laboratory results of patients at MIHS who underwent testing as part of evaluation for meningitis. However, results were supported by those in the SJHMC prospective observational cohort. Second, 76% of the cases represented relapse of previously diagnosed CM. However, the performance of CSF CAg, was similar in initial and relapse episodes, with sensitivities of 90% and 94% respectively. Third, 56% of the patients had HIV infection. The sensitivity of the CSF CAg antigen test was similar in HIV-infected and non–HIV-infected patients, but the antigen concentration was higher in HIV-infected patients. More experience is needed to further delineate the role of CSF CAg in these patients.

In conclusion, these findings support a useful role for combined antigen and antibody in CSF for diagnosis of CM. They define the relationship of CSF CAg to other serum and CSF parameters and contribute to our knowledge of the sensitivity of antigen detection in the urine and serum of patients with disseminated coccidioidomycosis.

Note

Potential conflicts of interest. L. J. W. is owner and president of MiraVista Diagnostics. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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