Coccidioidomycosis

Adenosine Deaminase Levels, Serologic Parameters, Culture Results, and Polymerase Chain Reaction Testing in Pleural Fluid

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Background: In a patient with positive serum serology for coccidioidomycosis, the differential diagnosis of concurrent pleural effusions can be challenging. We, therefore, sought to clarify the performance characteristics of biochemical, serologic, and nucleic-acid-based testing in an attempt to avoid invasive procedures. The utility of adenosine deaminase (ADA), coccidioidal serology, and polymerase chain reaction (PCR) in the evaluation of pleuropulmonary coccidioidomycosis has not been previously reported.

Methods: Forty consecutive patients evaluated for pleuropulmonary coccidioidomycosis were included. Demographic data, pleural fluid values, culture results, and clinical diagnoses were obtained from patient chart review. ADA testing was performed by ARUP Laboratories, coccidioidal serologic testing was performed by the University of California-Davis coccidioidomycosis serology laboratory, and PCR testing was performed by the Translational Genomics Research Institute using a previously published methodology.

Results: Fifteen patients were diagnosed with pleuropulmonary coccidioidomycosis by European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria. Pleural fluid ADA concentrations were <40 IU/L in all patients (range, <1.0-28.6 IU/L; median, 4.7). The sensitivity and specificity of coccidioidal serologic testing was 100% in this study. The specificity of PCR testing was high (100%), although the overall sensitivity remained low, and was comparable to the experience of others in the clinical use of PCR for coccidioidal diagnostics.

Conclusion: Contrary to prior speculation, ADA levels in pleuropulmonary coccidioidomycosis were not elevated in this study. The sensitivity and specificity of coccidioidal serologic testing in nonserum samples remained high, but the clinical usefulness of PCR testing in pleural fluid was disappointing and was comparable to pleural fluid culture. *CHEST 2013; 143(3):776-781*

Abbreviations: ADA = adenosine deaminase; CF = complement fixation; PCR = polymerase chain reaction

Coccidioidomycosis refers to the spectrum of disease caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*. Clinical manifestations vary depending upon both the extent of infection and the immune status of the host.¹ Pulmonary infection is the most common clinical manifestation and primary coccidioidal pneumonia may account for 17% to 29% of all community-acquired pneumonia in endemic regions.^{2,3} Pleural effusions have been estimated to occur in 5% to 15% of all primary pulmonary coccidioidomycosis cases and are typically present with cough, pleuritic chest pain, and dyspnea.^{4,5}

The underlying diagnosis of coccidioidomycosis is not always immediately apparent, however. *Coccidioides*-specific serologic testing is not readily available at most institutions, frequently requires the assistance of a reference laboratory, recovery of the organism may require invasive diagnostic testing, and cultures represent a severe biohazard to laboratory personnel. Therefore, the etiology of pleural effusions may not be clear on initial evaluation and other diagnoses including malignancy, autoimmune diseases, and mycobacterial or fungal disease may be sought. Even after extensive examination, the cause of pleural effusions

is not always apparent. In prior series, no definitive cause was found in approximately 20% of all cases.⁶

Biochemical, serologic, and nucleic-acid-based testing have been studied in an attempt to avoid invasive procedures in patients without a definitive diagnosis. One of these tests, adenosine deaminase (ADA), has been shown to be a useful marker of tuberculous pleurisy when levels exceed 40 IU/L, and several reports have demonstrated that elevated ADA concentrations predict tuberculous pleurisy with a sensitivity of 90% to 100% and a specificity of 89% to 100%.⁷ It has been speculated that coccidioidomycosis also causes elevated ADA levels due to its similar pathophysiology to TB, although no data in this regard have been presented.⁸

Specific diagnostic methods including the sensitivity and specificity of serologic testing and nucleic-acid amplification have also not previously been evaluated in pleural effusions evaluated for coccidioidomycosis. Despite the common use of these tests in the diagnosis of pleuropulmonary coccidioidomycosis, the performance characteristics have not been studied. We sought to evaluate the characteristics of pleural fluid in a cohort of patients who underwent evaluation for coccidioidomycosis.

MATERIALS AND METHODS

Forty consecutive patients evaluated for pleuropulmonary coccidioidomycosis between May 1, 2010, through April 30, 2011, were included in this study. Clinical follow-up was available through February 2012. Chart abstraction was performed to identify demographic variables including age, sex, ethnicity, pleural fluid values, culture results, and clinical diagnoses. This study was approved by the institutional review board of the University of California-Davis (protocol number: 270449-1).

Light's criteria were used to characterize pleural fluid samples as transudates or exudates.⁹ Pleural fluid was kept frozen at -80°C ,

and ADA testing was performed by a centralized laboratory (ARUP Laboratories). Serologic testing for the presence of IgM and IgG antibodies by immunodiffusion was performed as previously described using heated antigen to detect IgM antibody reactivity and unheated antigen to detect the antigen (chitinase *Cts1*) reactive with IgG.¹⁰ Real-time polymerase chain reaction (PCR) was performed using a PCR assay (CQ34) composed of primers and probe targeting a genus-specific, internal transcribed spacer region.¹¹ The CQ34 assay was previously shown to have 100% sensitivity against a panel of 450 *C immitis* and *C posadasii* isolates, with an established limit of detection of $<10^1$ colony-forming units/mL. DNA and RNA were extracted from pleural material using the RNA/DNA Purification kit (Norgen Biotek Corp). Extracted RNA was reverse transcribed using standard procedures, and cDNA and genomic DNA were run against CQ34 PCR assay and the CocciQuant Assay¹¹ (Translational Genomics Research Institute) using standard, real-time, and PCR procedures at the Translational Genomics Research Institute.

RESULTS

Forty patients with pleural fluid undergoing evaluation for coccidioidomycosis were included in this analysis. No patient in this cohort had HIV/AIDS.

Twenty-seven male and 13 female patients ranging in age from 12 to 95 years (median, 55 years) made up this cohort (Table 1). Pleural fluid analysis of patients with proven or probable coccidioidomycosis ($n = 15$) revealed a large range of values. The median total pleural-fluid WBC count was 764 cells/mm³ (range, 90-4,380 cells/mm³); lymphocyte count, 56 cells/mm³ (range, 71-1,841 cells/mm³); neutrophils, 16 cells/mm³ (range, 4-4,161 cells/mm³); monocytes, 5 cells/mm³ (range, 9-113 cells/mm³); and eosinophils, 1.5 cells/mm³ (range, 1-338 cells/mm³). The median lactate dehydrogenase level was 232 IU/L (range, 44-7,746 IU/L), and the total protein median was 4.35 g/dL (range, 2.7-6.5 g/dL).

Fifteen patients were diagnosed with coccidioidomycosis using previously established European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria (five with proven disease, 10 with probable disease) (Table 2).¹² Twenty-five patients were given an alternative diagnosis (eight, bacterial pneumonia; four, malignancy; three, heart failure; two, pulmonary embolus; two were attributed to preceding trauma; one, idiopathic; and five, spontaneous resolution without specific diagnoses). No patient given an alternative diagnosis by their primary physician had a positive serologic test for coccidioidal IgM or IgG antibody.

Patients with coccidioidomycosis were most commonly diagnosed from positive serum samples (13 of 15 patients); however, two patients with pleural fluid positive for coccidioidal antibody did not undergo serologic testing of their sera. Serologic testing of pleural fluid and sera were in agreement in all 13 patients who had both samples tested. Complement fixation

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Table 1—Pleural Fluid Characteristics of Patients Evaluated for *Coccidioidomycosis*

Characteristics	Noncoccidioidal Diagnosis (n = 25)	Coccidioidomycosis (n = 15)
Demographics		
Age, mean $\pm$ SD, y	60 $\pm$ 19	46 $\pm$ 21
Sex, male (%)	14 (56)	13 (87)
Pleural fluid values, median (range), cells/mm ³		
WBC	860 (16-4,380)	764 (90-4,380)
Neutrophils	17 (5-95)	16 (4-4,161)
Lymphocytes	61 (5-1,882)	53 (71-1,841)
Monocytes	4 (1-42)	5 (9-113)
Eosinophils	2 (1-16)	1.5 (1-338)
LDH, IU/L	280 (90-7,746)	232 (44-7,746)
Protein, g/dL	3.60 (2.7-6.5)	4.35 (2.7-6.5)

LDH = lactate dehydrogenase.

(CF) titers were generally in agreement with the exception of three patients.

Pleural fluid cultures were positive for *Coccidioides* species in two of 15 patients, and pleural biopsy specimen was positive in an additional two patients. All patients undergoing pleural biopsy had a definitive diagnosis (two with coccidioidomycosis and two with malignancy).

Transudative pleural effusions were seen in nine of 15 patients, suggesting noninfectious diagnoses, while the other six were exudative. All four patients with recovery of *Coccidioides* from pleural fluid cultures or pleural biopsy specimen exhibited exudative-type pleural effusions; the other two patients with exudative effusions had evidence of widely disseminated coccidioidal infection.

ADA levels were <40 IU/L in all patients with proven or probable coccidioidomycosis (range <1.0 -28.0 IU/L; mean, 7.67 IU/L). In fact, the only patient with an ADA pleural fluid concentration exceeding this value was a patient with culture-confirmed pneumococcal empyema (ADA concentration, 80.8 IU/L). ADA values did not significantly differ between patients with proven or probable coccidioidomycosis, nor with patients given a noncoccidioidal diagnosis (Fig 1). PCR testing of pleural fluid provided evidence of infection in three of 14 confirmed or probable case patients and provided no evidence of infection in any of the established noncase patients.

DISCUSSION

The evaluation of lymphocytic pleural effusions remains a difficult clinical problem. The use of established serologic and biochemical markers in unstudied patient populations has increased despite the lack of established performance characteristics in these

groups. Although ADA testing of pleural fluid is well established for the diagnosis of tuberculous pleurisy and prior reports have speculated ADA may be positive in coccidioidal pleural effusions as well,⁸ we found no positive tests in our cohort of patients with effusions undergoing evaluation for coccidioidomycosis.

These results are somewhat surprising given that ADA is found in most cells, particularly lymphocytes, which are the predominant cell type in coccidioidal pleural effusions.⁴ However, prior studies have shown that ADA levels tend to be higher in tuberculous pleural effusions compared with those found in other exudates,^{13,14} and evidence of elevated ADA levels even in neutrophil-predominant tuberculous pleural effusions provides further evidence of test specificity.¹⁵ No patient in our study was infected with HIV (CD4⁺ cells are responsible for the majority of pleural-fluid ADA), and thus it is unlikely that ADA levels in our cohort were biased by patient factors.

Our results further support the use of ADA testing to differentiate tuberculous pleurisy from coccidioidomycosis-associated pleural effusions. The incidence of TB in the United States per 100,000 people is highest in Texas and California,¹⁶ and even higher rates of infection are seen in Mexico, and Central and South America.¹⁷ These geographic regions are known endemic areas for *Coccidioides* species. The similar epidemiology and clinical presentation of TB and coccidioidomycosis may, thus, cause diagnostic confusion as the results of serologic testing or more invasive procedures such as pleural biopsy are awaited. We believe our results further reveal the performance characteristics of ADA in a cohort without evidence of TB and, therefore, assist the clinician in prompt and appropriate treatment.

Serologic testing of body fluids for the presence of coccidioidal antibody is frequently requested although limited data are available and the performance characteristics in non-serum samples have not been previously evaluated.¹⁸ Our results suggest the presence of coccidioidal precipitin or CF antibodies in pleural fluid is diagnostic of coccidioidomycosis and is of comparable sensitivity and specificity to serologic testing of patient sera. In most cases, titers do not substantially differ between serum and pleural fluid.

It remains unclear if coccidioidal antibody is made locally within the pleural space or passively diffuses from patient sera. Prior reports comparing concurrent serum and pleural fluid TB antibody titers have shown nearly equivalent results, suggesting that antibody passively diffuses into the pleural space.¹⁹ Similarly, in our study, coccidioidal CF antibody titers were equivalent or lower in the pleural fluid of patients with transudative pleural effusions, suggesting passive diffusion under noninflammatory circumstances.

Table 2—Pleural Fluid Characteristics of Patients With *Coccidioidomycosis* (n = 15)

Case	Age, y	Sex	Serum Results			Pleural Fluid Results			Pleural Fluid		ADA Concentration, IU/L	PCR Result
			ID	CF Titer	Transudate/Exudate	ID	CF Titer	Culture Results	Pleural Biopsy	Result		
1	27	M	+ IgG	1:2	Exudate	+ IgG	Negative	Lung Biopsy ^a	NP		1.4	Negative
2	59	M	+ IgM/IgG	Negative	Exudate	+ IgG	Negative	Positive	NP		4.3	Positive
3	39	M	+ IgM/IgG	1:8	Exudate	+ IgM/IgG	Negative	Negative	NP		4.1	Negative
4	65	M	+ IgM/IgG	1:512	Exudate	+ IgM/IgG	1:512	Negative	Positive		13.3	Negative
5	24	F	+ IgM/IgG	1:4	Exudate	+ IgM	Negative	Negative	Positive		4.7	Positive
6	50	M	+ IgG	1:16	Exudate	+ IgG	1:128	Positive	NP		19.0	N/A ^b
7	57	M	+ IgG	1:2	Transudate	+ IgG	1:2	Negative	NP		<1.0	Negative
8	60	M	+ IgG	1:1024	Transudate	+ IgG	1:32	Negative	NP		1.0	Negative
9	87	F	NP	...	Transudate	+ IgM	Negative	Negative	NP		19.1	Negative
10	80	M	+ IgG	Negative	Transudate	+ IgG	Negative	Negative	NP		<1.0	Negative
11 ^c	12	M	+ IgG	1:32	Transudate	+ IgM/IgG	1:16	Negative	NP		28.6	Negative
12	30	M	NP	...	Transudate	+ IgM	Negative	Negative	NP		8.9	Negative
13	36	M	+ IgM	Negative	Transudate	+ IgM	Negative	Negative	NP		1.5	Positive
14	39	M	+ IgM	Negative	Transudate	+ IgM	Negative	Negative	NP		5.0	Negative
15	32	M	+ IgM	Negative	Transudate	+ IgM	Negative	Negative	NP		2.4	Negative

ADA = adenosine deaminase; CF = complement fixation; F = female; ID = immunodiffusion; M = male; N/A = not available; NP = not performed; PCR = polymerase-chain reaction.

^aPleural fluid was negative; however, the patient underwent a lung biopsy and that specimen grew *Coccidioides* species.

^bDue to insufficient sample quantity remaining.

^cPatient 11: pleural fluid was submitted 15 d prior to serum.

FIGURE 1. Pleural fluid ADA levels among different groups. ADA = adenosine deaminase.

In contrast, one patient with a culture-positive exudative pleural effusion had markedly higher coccidioidal CF antibody titers in the pleural fluid than serum, suggesting possible local antibody production. Likewise, pleural disease in the setting of autoimmune phenomena may exhibit local production of antibody due to site-specific differences between tissue-surface antigens.²⁰ Furthermore, antibody production within the cerebrospinal fluid in cases of coccidioidal meningitis has been previously reported, lending further credibility to possible local antibody production in pleuropulmonary disease.²¹

In the absence of an exudative-type effusion, pleural fluid cultures were unhelpful, and should not routinely be obtained in attempts to diagnose pleural coccidioidomycosis. It is interesting to note that pleural biopsy specimens were obtained in two patients despite the presence of serum coccidioidal antibodies. In one patient, the coccidioidal CF antibody titer was 1:512, strongly suggesting disseminated disease.¹⁸ Others have also recognized the high diagnostic yield of pleural biopsy specimens in the diagnosis of coccidioidomycosis, and this site is considered the most rewarding culture source if culture positivity is desired.⁵

The interest in PCR for coccidioidal diagnostics has increased in past years given the potential ability for testing to be performed on-site and for quantitative results to be obtained.²² There are no currently published data that demonstrate the utility of PCR for determining the presence of *Coccidioides* in pleural fluid. Our results, using three different PCR assays, did not improve case finding over culture. *Coccidioides* was detected in the single case tested with a

positive pleural fluid culture, in one of two cases later diagnosed from pleural biopsy specimen, and in one patient who was otherwise only positive by IgM. All noncases were negative by PCR. Others have reported similar results with PCR testing for *Coccidioides* species directly from various clinical specimens.¹¹ While various PCR assays have proven extremely sensitive and specific on isolate material¹¹ and from sputum in active cases (D. M. Engelthaler, BS, unpublished data, 2011), direct detection from other clinical samples has not been as successful to date. Vucicevic et al¹¹ found positive PCR results in five of nine (56%) proven or probable pulmonary coccidioidomycosis cases (whereas four of nine were positive on fungal culture), and in neither of two patients with coccidioidal meningitis, despite the recovery of *Coccidioides* on cerebrospinal fluid culture in one of these patients. Their group considers PCR in clinical samples to have a sensitivity similar to culture.

CONCLUSIONS

In summary, we evaluated the performance characteristics of ADA, coccidioidal serology, PCR, and culture results in a cohort of patients with pleuropulmonary coccidioidomycosis. Our results dispel previous notions that ADA levels are elevated in coccidioidal pleural effusions and provide further evidence for the high sensitivity and specificity of serologic testing of pleural fluid. The clinical usefulness of PCR and pleural fluid cultures were disappointing in our analysis. Further refinement of PCR methodologies for these sample types will be necessary to make this technology useful.

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Dr Thompson: contributed to the original idea, concept, and design of the study; writing and revision of the manuscript; and served as the principal author.

Dr Sharma: contributed to the conception and design of the study, patient recruitment, and writing of the manuscript.

Mr Bays: contributed to the design of the study, data acquisition analysis, laboratory work, and writing of the manuscript.

Ms Pruitt: contributed to the design of the study, data acquisition and analysis, laboratory work, and writing of the manuscript.

Mr Engelthaler: contributed to the conception and design of the study, designed the primers and probes used for PCR testing, and writing and revision of the manuscript.

Dr Bowers: contributed to the conception and design of the study, designed the primers and probes used for PCR testing, and writing of the manuscript.

Dr Driebe: contributed to the conception and design of the study, designed the primers and probes used for PCR testing, and writing of the manuscript.

Dr Davis: contributed to the conception and design of the study, patient recruitment, and writing of the manuscript.

Dr Libke: contributed to the conception and design of the study, patient recruitment, and writing of the manuscript.

Dr Cohen: contributed to the design of the study and the writing and revision of the manuscript.

Dr Pappagianis: contributed to the design of the study and the writing and revision of the manuscript.

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