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Original Research

CHEST INFECTIONS

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 Assay[11] (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/mm^3 (range, 90-4,380 cells/mm^3); lymphocyte count, 56 cells/mm^3 (range, 71-1,841 cells/mm^3); neutrophils, 16 cells/mm^3 (range, 4-4,161 cells/mm^3); monocytes, 5 cells/mm^3 (range, 9-113 cells/mm^3); and eosinophils, 1.5 cells/mm^3 (range, 1-338 cells/mm^3). 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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