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The diagnosis of coccidioidomycosis

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Abstract

Until recently, culture, microscopy, and serology have been the available methods for the diagnosis of coccidioidomycosis. While *Coccidioides* is frequently isolated by culture, special precautions must be taken because of the risk of laboratory infection and because *Coccidioides* is on the Select Agent list. Serology is useful but the sensitivity remains lower than desired. A commercially available test for coccidioidal galactomannan antigenuria now exists and appears useful for immunocompromised hosts with severe disease. Polymerase chain reaction assays targeting specific coccidioidal genes have demonstrated utility but are not commercially available. Moreover, their sensitivity and the best sample type remain unestablished.

Introduction and context

The endemic region for coccidioidomycosis is restricted to the Western Hemisphere. Almost all coccidioidal infections are due to inhalation of arthroconidia, 2-5 μm barrel-shaped spores that dislodge from hyphae and become airborne [1]. A full 60% of all coccidioidal infections are completely asymptomatic, with only the expression of specific delayed-type hypersensitivity as a marker for infection [2]. Unfortunately, coccidioidal skin tests are not currently available in the US.

The other 40% of individuals with acute pulmonary coccidioidomycosis present with a syndrome similar to a bacterial community-acquired pneumonia, with cough, pleuritic chest pain, and fever. In one recent study performed in the coccidioidal endemic region, coccidioidomycosis was misidentified in approximately 29% of individuals as a bacterial process [3]. In fewer than 1% of all individuals infected, disseminated clinical infection occurs outside of the thoracic cavity. This most commonly presents as a skin or soft tissue lesion, osteomyelitis, or meningitis.

Coccidioidomycosis is now recognized to be caused by two distinct species, *Coccidioides immitis* and *C. posadasii*. The former appears to be geographically limited to the southern portion of California and Northern Mexico while the latter occurs in all geographic areas [4]. At this time, the presentation of disease and diagnosis do not appear to differ between the two species.

Available diagnostic tests

For the past several decades three modalities – culture, microscopy, and serology – have been the mainstays of specific diagnosis of coccidioidomycosis.

Culture

Unlike other endemic fungi, *Coccidioides* grows readily on a variety of culture media at 35°C and is usually visually apparent in 2-7 days. It can be presumptively identified based on its early lack of pigmentation and the later presence of septate hyphae with barrel-shaped arthroconidia on microscopic examination [5]. A proprietary nucleic acid chemiluminescent probe (AccuProbe, Gen-Probe, Inc., San Diego, CA, USA) allows rapid, specific identification once visible growth has occurred [6]. A major drawback of culture is the extreme danger of *Coccidioides* in the laboratory. Several documented accidental exposures have occurred and Stevens and colleagues [7] have recently outlined their approach to managing such events. Moreover, *Coccidioides* is listed as a 'Select Agent' of bioterrorism [8]. Because of these

issues, suspected and established cultures of *Coccidioides* should be handled using Biosafety Level 3 containment.

Microscopy

Coccidioides can be identified directly in fresh respiratory secretions using potassium hydroxide, calcifluor, and Papanicolaou. However, the sensitivity of these methods is relatively low [9,10]. The diagnosis can also be established in fixed tissue using a variety of stains, including hematoxylin-eosin and Gomori methenamine-silver. The pathognomonic structure is the spherule.

Serology

Serologic tests for the diagnosis of coccidioidomycosis are time honored and perhaps the most frequently employed assays for the diagnosis of coccidioidomycosis [1,11]. Smith and colleagues established the modern use of coccidioidal serology with the development of the tube precipitin (TP) and complement fixation (CF) assays [12]. Each relies on a specific antigen. For TP, this is now known to be a heat-stable 120 kDa β-glucosidase [13], while for CF it is a heat-labile chitinase [14]. The IDTP and IDCF are variants of these assays that employ immunodiffusion (ID) in agar. All are very specific and, at least from early studies, relatively sensitive [12]. The TP and IDTP assays conform to an immunoglobulin (Ig)M reaction and are positive early in illness and, at times, during recurrence. The CF and IDCF assays become positive usually after 2-3 weeks of illness, conform to an IgG reaction, and their titer has prognostic implications, with high titers portending severe or disseminated disease. In persons who resolve their clinical illness, both reactions become negative over time.

Recent advances

Serology

Since the 1990s, a proprietary enzyme-linked immunosorbant assay (Premier EIA, Meridian Diagnostics, Inc., Cincinnati, OH, USA) has been widely employed in the endemic region. Martins and colleagues [15] initially compared this assay to standard TP and CF assays as well as the less specific latex agglutination assay and, after combining the IgM and IgG enzyme immunoassay (EIA) results, found that the specificity was 98.5% and the sensitivity was 95.5%. Kaufman and coworkers [16] examined 47 specimens from persons with confirmed coccidioidomycosis and a positive IDCF and found all were also positive for either IgM or IgG using the EIA; however, 12 of 345 samples from patients with other pulmonary infections were also EIA positive. In another study, among 41 patients with culture-confirmed coccidioidomycosis who had three serologic tests performed, the CF was positive in 23 (56%), the IDCF in 29 (71%), and the EIA in 34 (81%) [17]. In six (15%) patients, all serologic tests were negative. Blair and colleagues [18] examined immunocompromised patients and found, as expected, that serologic tests were less likely to be positive than among normal hosts. They subsequently examined patients with an isolated IgM EIA reaction and were able to confirm a diagnosis of coccidioidomycosis by other means in all cases [20]. Hence, while there is lingering concern regarding the specificity of the Premier EIA, particularly the IgM (D Pappagianis, personal communication), published reports suggest that it is sensitive. Another proprietary EIA (*Coccidioides* DxSelect™, Focus Diagnostics, Inc., Cypress, CA, USA) became available in 2006. There are no published studies on its performance parameters.

Detection of antigenuria

During the 1980s, assays were developed to detect coccidioidal circulating antigen [20-22]. Unfortunately, these never achieved the sensitivity required for clinical use. However, Kuberski and coworkers [23] recently reported several instances where a commercially available assay for detecting *Histoplasma capsulatum* antigenuria was found to be positive in samples from patients with coccidioidomycosis. This led to the development of a specific assay for *Coccidioides* [24]. To develop this test, antibody was raised in rabbits immunized to galactomannan isolated from the mycelial stage of clinical isolates of *Coccidioides* and used in an enzyme-linked immunosorbent assay. The assay detected as little as 0.03 ng/ml of *Coccidioides* galactomannan. Among 24 patients with coccidioidomycosis, most with underlying immunosuppression and severe disease, 17 (71%) had a positive test. The *Histoplasma* antigenuria assay was positive in 14 of these patients while coccidioidal antigenuria was detected in 3 of 28 (11%) samples from patients with other endemic mycoses, including two with histoplasmosis, confirming the problem of cross-reactivity.

Genomic analysis

Tests based on detection of the genetic fingerprint of the fungus offer the promise of rapidity and specificity without the requirement of growing the organism or relying on the response of the host. While several investigators have demonstrated the feasibility of such approaches, no such tests are currently commercially available.

Because of high copy number and variability among fungal species, one region of particular interest in such analyses has been the 18S ribosomal rRNA genomic sequence (rDNA). Johnson and coworkers [25] used primers for this region in a polymerase chain reaction (PCR) to determine if the coccidioidal rDNA genome

could be detected in human sera obtained from patients with suspected coccidioidomycosis. Among 94 samples tested, all originally submitted for coccidioidal serologic testing, 6 demonstrated an appropriately sized 239 base-pair DNA band. Using these same primers, Japanese investigators were recently able to detect this band in DNA extracted from formalin-fixed paraffin-embedded lung tissue from four patients with coccidioidomycosis [26].

Binnicker and colleagues [27] employed real-time PCR with primers amplifying a 170 base-pair region in the ITS2 (internal transcribed spacer 2) region of the rDNA genome to examine 266 respiratory specimens submitted for fungal culture. Most of these specimens were submitted from within the coccidioidal endemic region. Among 20 samples that were culture positive for *Coccidioides*, 16 were positive by real-time PCR, compared to none of the culture negative samples, yielding a sensitivity of 98% and a specificity of 100%. In addition, real-time PCR was positive in all 47 samples found to demonstrate *Coccidioides* by histopathological examination, whereas it was positive in 17 cases among 101 where histopathology was negative.

Bialek and colleagues [28] have performed several studies using PCR to detect the unique coccidioidal gene, antigen-2/proline rich antigen (*Ag2/PRA*) [29,30], which exists as only one copy in the genome. They initially correctly identified 120 isolates of *C. posadasii* from Mexico using both conventional nested and real-time PCR and found no false positives [28]. Since then, Brazilian investigators were able to apply nested PCR to a respiratory sample from a patient with pulmonary coccidioidomycosis and detected the appropriate 349 base-pair product of Ag2/PRA in the sample [31].

Implications for clinical practice

The concerns regarding the specificity of Premier EIA compared to the older serologic assays are likely to persist. For the present, a TP or IDTP should be obtained for all positive IgM EIA tests, and a CF or IDCF assay should be obtained and titrated in all instances of a positive EIA IgG. However, experts recommend that the EIA is very sensitive and other assays need not be obtained if it is negative [32]. It is also clear that a serologic test may be negative during active coccidioidomycosis and serology should not be wholly relied upon in such instances.

A critical question regarding coccidioidal antigenuria is its sensitivity for different syndromes of coccidioidomycosis. While preliminary data suggest it is helpful for those who are highly immunocompromised and for those with widely disseminated disease, it is not clear if it will be useful for those with less severe illness and for those with disease confined to the thoracic cavity. In addition, there is documented cross-reactivity, particularly in patients with histoplasmosis, which may reduce its diagnostic utility.

Finally, while genomic detection of *Coccidioides* is promising, much more work on sensitivity and sample type needs to be done. Moreover, PCR has been notoriously difficult to perform in clinical laboratories because of the risk of contamination. These issues will need to be resolved prior to these assays becoming clinically available.

Abbreviations

Ag2/PRA, antigen-2/proline rich antigen; CF, complement fixation; EIA, enzyme immunoassay; ID, immunodiffusion; Ig, immunoglobulin; ITS2, internal transcribed spacer 2; PCR, polymerase chain reaction; rDNA, ribosomal DNA; TP, tube precipitin.

Competing interests

The author declares that he has no competing interests.

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