Serum (1→3)-β-D-Glucan Measurement in Coccidioidomycosis

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Serum (1→3)-β-D-glucan has emerged as an important diagnostic test for invasive fungal disease. The utility of this assay in coccidioidomycosis has not been previously studied. Using a cut-off value of $\geq 80$ pg/mL, we found the sensitivity (43.9%), specificity (91.1%), positive (81.8%) and negative predictive values (64.1%) similar to that of other invasive mycoses.
The incidence of invasive fungal disease (IFDs) has increased in recent years, primarily due to the expanding immunosuppressed population (8, 15). IFDs are associated with significant morbidity and mortality and are often not readily diagnosed, leading to delays in treatment. Blood cultures are frequently unhelpful in the diagnosis of IFDs and histopathologic diagnosis is not always feasible in those at highest risk. For these reasons interest in non-invasive diagnostic testing has increased. Among the newer diagnostic techniques is the assay for the serum (1→3)-β-D-glucan (BG) derived from fungal cell walls. This assay has exhibited a high specificity and positive predictive value (PPV) in studies evaluating its use in the diagnosis of invasive candidiasis or aspergillosis (9, 11-13, 17), however its utility in the diagnosis of coccidioidomycosis, has not been previously examined. We evaluated the performance characteristics of BG testing in a diverse cohort of patients with coccidioidomycosis.

Subjects evaluated for serologic evidence of coccidioidomycosis by the University of California Davis Coccidioidomycosis Serology Laboratory were included in this analysis. Patient samples and medical information arrive from requesting physicians across California and Arizona. Samples were included if sufficient clinical information was available for chart abstraction between September and December of 2010 and subsequently deidentified. Patients with hematologic malignancy, receiving dialysis, receiving current care within an intensive care unit, or receiving medications known to cause false-positive BG values were excluded as these conditions were more likely to cause false-positive BG testing, or were at significantly higher risk for an alternative IFD (10).

All samples were tested for coccidioidal antibodies by both immunodiffusion and complement fixation at the University of California – Davis Coccidioidomycosis Serology Laboratory using previously described methods (14). BG testing was performed in blinded
fashion by Beacon Diagnostics® Laboratory using the Fungitell (Associates of Cape Cod) assay. All samples were stored serum aliquots kept frozen (-80°C) and shipped in bulk for testing. This study was approved by the UC-Davis Medical Center IRB.

Two-hundred and twenty-eight patients met criteria for inclusion in this study. Of these, 40 patients were excluded secondary to underlying diagnoses outlined above. The remaining 188 patients included: 47 with acute coccidioidomycosis (positive coccidioidal precipitin (IgM) antibody and pulmonary symptoms); 52 with past coccidioidal infection (positive coccidioidal CF (IgG) antibody and without symptoms of ongoing infection and off antifungals for one year); 45 with confirmed meningeal or disseminated coccidioidomycosis (positive CSF coccidioidal antibody titer or recovery of Coccidioides spp. from extrapulmonary site) who were receiving triazole antifungal therapy; 44 uninfected controls (no evidence of coccidioidomycosis clinically, serologically and the patient was given an alternative diagnosis by their treating physician).

Of the 47 patients with acute coccidioidomycosis 25 (53.2%) had BG values > 31 pg/mL (median 31 interquartile range 61). Three patients had a positive BG test prior to detectable IgM antibody (detected on subsequent samples). Nine patients (19.0%) with acute coccidioidomycosis had BG values > 80 pg/ml (Table and Figure 1). In the group with past coccidioidomycosis 26/52 (50%) exhibited BG values greater than 31 pg/mL (median <31; interquartile range 47pg/mL). Of note, 7/52 (13.5%) exhibited values exceeding 80 pg/mL. All seven patients with BG values > 80 pg/mL were positive by both complement fixation and immunodiffusion testing, whereas patients with past infection and an undetectable BG were more commonly positive only by immunodiffusion (18/26).

In the group with disseminated/meningeal coccidioidomycosis, 34/41 (83%) had BG values > 31 pg/mL (median 85, interquartile range 175). BG values were also significantly
higher in the group with disseminated coccidioidomycosis than in the other three groups (P<0.001 by Kruskal-Wallis and Dunn’s Multiple comparison test). However BG values correlated poorly with serum coccidioidal CF (IgG) antibody titers ($R^2$ value= 0.096). Among uninfected controls only 8/44 (18.2%) had BG values exceeding 31 pg/mL (median <31, interquartile range <31), while only four patients had values ≥ 80 pg/mL.

A pooled analysis of these four groups consisting solely of hospitalized patients was performed to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of BG testing for coccidioidomycosis in this setting (86 patients) (Table 1 and Figure 1). This group was examined to determine the utility of BG testing in patients with disease severity warranting hospitalization and thus potentially benefiting from earlier diagnosis and antifungal therapy. Using the higher cut-off value of ≥ 80 pg/mL, these values were 43.9%, 91.1%, 81.8% and 64.1% respectively (Table 1). The receiver operating characteristic curves (ROC) of BG in the evaluation of patients with acute coccidioidomycosis and hospitalized with coccidioidomycosis are included in figures 1A and 1B.

The enzyme responsible for the production of (1→3)-β-D-glucan, (1→3)-β-glucan synthase, has been found essential in *Coccidioides* spp (6). Yet prior reports of BG testing in the endemic mycoses have been limited. Serum BG positivity has been previously reported in patients with histoplasmosis (4, 16) and in a limited number of patients with blastomycosis (5); however analysis of BG in *Coccidioides* infected patients has not previously been performed. In fact, only two prior reports have described a positive BG level suggesting coccidioidomycosis as a possible diagnosis (2, 9). In the first report, Baden et al describe a 60 year-old renal transplant patient cared for in the non-endemic area of Massachusetts and the patient was ultimately diagnosed with disseminated coccidioidomycosis. This case underscores previous reports that up
to 10% of all cases of coccidioidomycosis are seen outside of the typical area of endemicity and undoubtedly diagnostic and treatment delays exist under these circumstances (1). Furthermore, Baden’s report shows the potential impact that BG “screening” of patients may have – pointing to a possible fungal etiology of their illness while awaiting more specific diagnostic testing. The second report by Koo et al provides evidence of BG positivity in a patient with coccidioidomycosis, however no patient data or the BG value was presented.

It is noteworthy that a positive BG was seen in 3 patients prior to positive *Coccidioides* antibody testing. Coccidioidal precipitin (IgM) antibodies develop 1-3 weeks following exposure, suggesting a possible role for antigen testing for those with “hyperacute” infection during this window period. Our results suggest the sensitivity of BG testing in those with acute disease is disappointing and receiver operating characteristic analysis fails to identify an appropriate “cutoff” value that may be useful in clinical care. These findings underscore the elusive nature of a sensitive and specific coccidioidal antigen test and others have similarly noted poor sensitivity (range from 3.5 - 71.4%) and specificity (cross-reaction with other endemic mycoses) of coccidioidal antigen testing (3, 7).

The importance of the *Coccidioides* life cycle in the evaluation of BG is also of importance as *Coccidioides* spherules are roughly 60% β-glucan by dry weight, while arthroconidia contain only 20% BG (19). Kellner et al have previously shown decreasing levels of FKS1 gene expression (the gene encoding the glucan synthase enzyme) in mature spherules compared to immature spherules (6) and these stage-specific differences may play an important role on the performance of BG testing during different clinical forms of infection and have a significant impact on the kinetics of BG in patients with coccidioidomycosis.
Patients with disseminated/meningeal disease frequently exhibited positive BG values, likely from the high burden of infection. However seven patients were negative for BG despite evidence of ongoing clinical infection. Compartmentalization of BG in suspected CNS mycosis patients has been observed, with elevated CSF levels coincident with low or negative serum BG levels (M. Finkelman, unpublished data). All seven patients were on triazole antifungals at the time of laboratory draw.

There are several important limitations to this study including the lack of longitudinal follow-up for the included patients and the possibility that patients with a positive BG test may have been diagnosed with an alternative fungal infection at a later date. Additionally, the concurrent use of antifungal therapy may have altered the sensitivity and specificity of BG testing in those with disseminated disease as has been shown in animal models (20). Antifungals are also known to exhibit indirect effects on secondary targets (18) and thus the fungal expression of BG, in coccidioidomycosis, may have been altered although this remains speculative in this study.

In conclusion we have evaluated the characteristics of BG testing in a diverse group of coccidioidomycosis patients and controls. The findings suggest serum BG may be a useful diagnostic test in the initial evaluation of coccidioidomycosis when epidemiologic factors suggest the disease and more specific laboratory testing is not immediately available. The sensitivity, specificity, PPV and NPV are comparable to these characteristics in other fungal infections and BG testing may additionally be a useful marker in patients with hyperacute coccidioidomycosis. Clearly, further work on the kinetics of BG in coccidioidomycosis is needed, including an analysis of expression during its unique life cycle, and its performance in the setting of antifungal therapy.
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G.R.T has served as a consultant for Basilea, and has received research support from Pfizer. G.R.T is the assistant director of the Coccidioidomycosis Serology Laboratory, D.P. is the director of the Coccidioidomycosis Serology Laboratory. M.A.F. is an employee of Associates of Cape Cod, Inc.
FIGURE 1. (A) Receiver-operator characteristic (ROC) curve for β-D-glucan as a diagnostic test in acute coccidioidomycosis. (B) ROC curve for β-D-glucan as a diagnostic test for coccidioidomycosis in hospitalized patients.
TABLE 1. Test Performance of β-glucan in coccidioidomycosis patients using >80 pg/mL as the cutoff for a positive result.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acute Coccidioidomycosis</th>
<th>Hospitalized with Coccidioidomycosis</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>19.1%</td>
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<tr>
<td>Specificity</td>
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<td>91.1%</td>
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<tr>
<td>Positive Predictive Value</td>
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<td>Negative Predictive Value</td>
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<td>Negative Likelihood Ratio</td>
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REFERENCES


