Short Communication

Positive (1-3) B-d-Glucan and cross reactivity of fungal assays in coccidioidomycosis


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Abstract

Fungal antigen testing in immunosuppressed patients has emerged as a powerful diagnostic tool. Some assays are relatively nonspecific, and misinterpretation can have severe clinical consequences. Additionally, when new assays become commercially available it is important to evaluate the potential for cross reactivity. We recently observed several immunosuppressed patients with positive (1→3)-β-D-glucan (BG) who were eventually diagnosed with coccidioidomycosis in the endemic area of Tucson, Arizona. Although the BG assay is known to detect glucans of many fungal pathogens, reports of cross-reactivity with Coccidioides remain sparsely reported. To test the cross-reactivity of fungal antigens in detection assays, serum samples from patients with coccidioidomycosis testing positive for Coccidioides antigen were evaluated for BG. Of 12 samples positive for Coccidioides antigen (>0.07 ng/ml), 11 (92%) were positive by BG (>80 pg/ml), and of 11 positive for Aspergillus galactomannan, 10 (91%) were positive by BG (>80 pg/ml). We conclude that the BG assay is nonspecific, detecting glucans from many fungal pathogens, including Coccidioides. In the endemic area, a positive BG warrants further specific testing.

Key words: Coccidioidomycosis, immunoassay, glucan, galactomannan.

Introduction

Among immunosuppressed patients, including solid-organ transplant recipients, disseminated coccidioidomycosis is associated with a high mortality. [1–3] Serological antibody testing for coccidioidomycosis is useful in the endemic regions, but tests provide false negative results in immunosuppressed patients. Blair and colleagues reported the positivity for any single serologic test ranged from 21% to 36% in these populations [2]. Using a newly developed Coccidioides galactomannan detection assay, Coccidioides
antigenuria was detected in 70.8% of patients with likely disseminated coccidioidomycosis. Cross-reactions with other endemic mycoses were observed in 10.7% of patients. [3]

BG, that is, (1–3)-β-D-glucan is a component of the cell wall of many fungal pathogens, including Candida, Aspergillus, Pneumocystis, and Histoplasma and is increasingly being used in the critically ill for early diagnosis of invasive fungal diseases (IFDs). The BG test may be positive in a variety of IFDs, highlighting the ubiquity of the antigen among fungi and the nonspecificity of the assay [4].

Another important fungal test is the Aspergillus galactomannan immunoassay, which detects galactofuranose-containing side chains of galactomannan released from Aspergillus hyphae during growth in the host [5]. Cummings et al. reported Aspergillus species cross-reactivity occurring in this assay with a variety of fungal organisms [6], while other studies have described “false-positive” results in the Aspergillus assay among patients with histoplasmosis [7–8].

The clinical implications of misinterpreting a broadly cross-reactive test can be catastrophic, particularly when treatments vastly differ. The aim of this clinical laboratory based study was to analyze the cross-reactivity of Coccidioides galactomannan antigen, BG, and Aspergillus GM. We hypothesized that the BG assay would detect Coccidioides glucans, uncovering a newly recognized cross-reactivity of importance in clinical laboratory testing and patient care.

Methods

Serum samples positive for Coccidioides antigen by MVista Coccidioides Quantitative antigen EIA were used in the investigation. Due to the laboratory-based nature of this study, the samples were from patients considered to have probable or definite coccidioidomycosis based on demographics and available clinical information. Those samples positive for Aspergillus GM did not have a secure diagnosis of Aspergillus given the limited clinical information available. As cross-reactivity was the main concern, samples testing highly positive were selected and sent to a single reference laboratory for clinical testing. Only a small number of the total samples sent to the lab had residual volumes adequate for further testing; hence advanced testing characteristics could not be performed. Coccidioides positive samples were tested for Aspergillus GM with Platelia Aspergillus EIA and (1–3)-β-glucan levels with the Fungitell Assay as previously described [3,7,9]. Serum samples positive for Aspergillus GM were tested for Coccidioides antigen, Histoplasma antigen, and (1–3)-β-D-glucan levels.

Four residual Aspergillus GM positive samples from bronchoalveolar lavage (BAL) fluid was also available in adequate volume, but tested only for Coccidioides and Histoplasma antigen by the aforementioned assays were conducted as Fungitell is not available for BAL fluid testing. Due to limited sample availability Histoplasma testing was not repeated on positive Coccidioides samples as our previous study suggests a 10% cross-reactivity [3]. All assays were performed at MiraVista Diagnostic Laboratory (Indianapolis, Indiana), and specimens were blinded with equal quantities of sample and control. All assays were performed following manufacturer instructions.

Results

Among the 12 samples that had a positive Coccidioides antigen (≥0.07 ng/ml), 11 (92%) were positive by BG (≥80 pg/ml), and 1 (8%) was positive for the Aspergillus GM assay (GMI ≥ 0.5) (Fig. S1). Values for Coccidioides antigen range from 2.3 ng/ml to >8.2 ng/ml. The one instance in which there was no cross reactivity with BG the value was 3.05 ng/ml of Coccidioides galactomannan. The one sample that exhibited cross-reactivity with Aspergillus GM had >8.2 ng/ml Coccidioides antigen levels. Among the 11 samples that had a positive Aspergillus GM, 10 (91%) were also positive by BG and one was weakly positive for the Coccidioides assay (Fig. S2). The weakly positive sample had a very high Aspergillus GM value (8.53 GMI). The sample that did not cross react with BG testing had an Aspergillus GM value of 5.53 GMI. Similarly, all four BAL samples testing highly positive in the Aspergillus assay (GMI 9.09, 6.09, 5.45, and 5.95, respectively) demonstrated no detectable antigen by either the Histoplasma or Coccidioides antigen assays.

Discussion

Thompson et al. recently reported their experience with BG testing in the setting of known cases of coccidioidomycosis. BG testing had a sensitivity of 43.9%, specificity of 91.1% and positive predictive value of 81.8% for patients with coccidioidomycosis. The authors suggested BG testing may be a useful marker in those with severe coccidioidomycosis, requiring hospitalization and in the window before serologic antibodies develop [10]. Our smaller, laboratory based study supports their findings as samples highly positive for Coccidioides antigens were almost always (>90%) positive in the BG assay. To our knowledge this is the first report showing laboratory based cross-reactivity of BG with Coccidioides antigen. The additional cross-reactivity seen between BG and the Aspergillus GM assays further
highlights the nonspecificity of the test. While culture is considered the “gold standard” for mycological diagnosis, reliance on this method alone is imperfect and impractical and contributes to delays in diagnosis and treatment. Serology, which is the most widely used test to support a diagnosis of coccidioidomycosis, is often negative in immunosuppressed patients and early in the course of disease before antibodies develop [2,11]. Hence, the emerging niche for antigen testing is its increasing availability at most centers with a rapid turnaround time early in the course of active disease.

BG testing is readily available and routinely used in immunocompromised hosts in which invasive candida infections, endemic fungal infections, Aspergillus and Pneumocystis pneumonia are all viable considerations. Antigen testing is gaining a more prominent role in clinical practice, particularly among critically ill patients, who require rapid diagnosis and appropriate antimicrobial therapy [12]. Treatment of fungal diseases is vastly different (Pneumocystis being the main outlier), and it is not uncommon to have patients with more than one invasive fungal infection. With increasing use of the BG test, the clinician must remain vigilant and place this test in clinical and demographic context. The importance of knowing the limitations of BG testing and cross-reactivity in these scenarios is highly important.

Our data support that Aspergillus galactomannan and Coccidioides antigen tests are more specific for their respective diseases than BG. A limitation of the current study is the sparse clinical information associated with the samples analyzed. Nevertheless, cross reactivity was readily seen in the laboratory. In the case of the one sample in which there was not cross-reactivity observed between BG and the positive Coccidioides galactomannan, one could speculate the possibility of a false positive specific test causing this; however, we believe that this is unlikely for our single sample. The sample with a negative BG had a modest Coccidioides specific antigen level (3.05) and therefore may simply not have been detected by the BG assay due to its performance characteristics. Serial confirmatory tests from blood and urine and clinical context would be useful to further examine the testing characteristics in future studies. We did not specifically identify negative controls, however, the Aspergillus positive samples do essentially provide a surrogate negative control. Surprisingly, we noted that despite a large burden of Coccidioidomycosis in the southwest, Coccidioides antigen is not widely used, limiting the number of samples with adequate residua to test in other assays. Further studies are needed to more rigorously evaluate the sensitivity and specificity of these tests across the spectrum of fungal diseases and patient immune status. In conclusion, in areas of endemic fungal diseases, a positive BG warrants further definitive testing.

**Declaration of interest**

Dr. L. J. Wheat is president of MiraVista Diagnostics and performs Coccidioides antigen testing as a laboratory service. Remaining authors: No disclosures.

**References**


