# Two Non-invasive Diagnostic Tools for Invasive Aspergilosis: (1-3)-β-D-Glucan and the Galactomannan Assay

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Invasive aspergillosis (IA) is a serious cause of morbidity and mortality among immunocompromised patients. Prompt and non-invasive methods for diagnosing IA are needed to improve the management of this life-threatening infection in patients with hematological disorders. In summary, this retrospective review of studies performed on the two assays finds that both assays have high sensitivity and specificity but are more useful when used together as a diagnostic strategy for patients with invasive aspergillosis.

**ABBREVIATIONS:** BDG = beta-D-glucan; GM = galactomannan; IA = invasive aspergillosis; IFI = invasive fungal infections; ODI= optical density index.

**INDEX TERMS:** aspergillosis; galactomannan; glucan.

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Invasive aspergillosis (IA) is one of the most serious causes of morbidity and mortality among immunocompromised patients. Among several factors that contribute to the high mortality rate, difficulties in establishing a reliable diagnosis early enough for successful intervention have been reported.[1]

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The crude mortality rate of IA is very high despite appropriate antifungal treatment, since the difficulty in obtaining an early diagnosis results in a delay in establishing treatment. The diagnosis of IA is frequently established postmortem. The establishment of a prompt and optimal noninvasive method for diagnosing IA is needed to improve the management of this life-threatening infection in patients with hematological disorders.[2,3]

Current conventional diagnostic methods such as histological examination and cultures of deep tissues are not only insensitive, but require an aggressive approach. This often precludes their use due to profound thrombocytopenia, hypoxemia, and the critical condition of these patients. As a result, many physicians begin empiric or prophylactic amphotericin B therapy before making a definitive diagnosis. Initiation of empiric or prophylactic therapy with amphotericin B may lead to treatment failure of a full systemic infection or risk of nephrotoxicity.[4]

Over the past decade there have been many advances made to further the options of both diagnostics and therapeutics. Diagnostic options have widened with the addition of diagnostic imaging, histopathology, and several non-invasive laboratory tests for IA. These tests include a double-sandwich enzyme-linked immunosorbent assay (ELISA) for galactomannan (GM) antigen (Platellia *Aspergillus*), tests for (1→3)-β-D-glucan (BDG) (Glucatell or FungiTec G test), and a number of PCR-based assay systems for *Aspergillus* DNA.[5]

The GM and BDG test monitors *Aspergillus* GM by detecting the polysaccharide cell wall component based on the use of a rat monoclonal antibody (Mab), EB-A2, that recognizes the 1→5-β-galactofuranoside side chains of the GM molecule. GM is a polysaccharide that is attached to and released from *Aspergillus* hyphae during growth. As little as 0.5 ng to 1.0 ng of circulating GM per ml may be detected with this double-sandwich enzyme-linked immunosorbent assay.[6] The excellent sensitivity and specificity of this assay have been repeatedly demonstrated and validated in tests of patients with hematological disorders.[7]

The assay for (1→3)-β-D-glucan detects (BDG) glucans produced by fungi. BDG is a ubiquitous component of

diverse fungal species. The assay system is currently available for the sensitive detection of circulating BDG, based on the *Limulus* reaction, crab coagulation cascade through Factor G. The activation events result in clot formation when coagulogen is cleaved to coagulin by the clotting enzyme. The introduction of a chromogenic peptide substrate permits spectrophotometric quantitation of the activated proclotting enzyme.[8]

## RESULTS

In a study performed by Odabasi and others, the (1→3)-β-D-glucan detection kit by Glucatell was examined. The results of this study showed that the Glucatell and the Fungitec-G assays were found to be specific for polysaccharides composed of, or containing, BDG sequences. Both assays were non-reactive with two other types of polysaccharides, (1→4)-β-D-glucan and (1→6)-β-D-glucan, as well as non-glucans containing (1→3)-β-D-glucan linkages. The determined BDG cutoff for the Glucatell assay was 60 pg/mL and was chosen as the positive cut-off for the diagnosis of invasive fungal infections (IFI). Testing was performed with serial serum samples from 283 subjects with acute myeloid leukemia or myelodysplastic syndrome who were receiving antifungal prophylaxis. The absence of a positive BDG finding had a 100% negative predictive value, and the specificity of the test was 90% for a single positive test result and >96% for more than two sequential positive results. This study demonstrated that the Glucatell serum BDG detection assay is highly sensitive and specific as a diagnostic for IFI.[9]

A multi-center clinical evaluation of the (1→3)-β-D-glucan assay, Glucatell, was described in a study completed by Ostrosky-Zeichner and others as an aid to diagnose fungal infections in humans. In this study, patients at six clinical sites in the United States were enrolled as either fungal infection negative or with proven or probable IFI. Using a cutoff of 60 pg/mL, the sensitivity and specificity of the assay were 69.9% and 87.1%, respectively. The positive and negative predictive values were 83.8% and 75.1%. Additionally, a cutoff value of 80 pg/mL, the sensitivity and specificity were 64.4% and 92.4%, respectively, with a positive and negative predictive value of 89% and 73%. Of the ten patients with aspergillosis, 80% had positive results at cutoff values of 60 pg/mL and 80 pg/mL. The study concluded that the reproducible assay results with high specificity and high positive predictive values in a multi-center setting demonstrate that use of the assay to detect serum BDG levels is a useful diagnostic adjunct for IFI.[8]

In 2005 Pazos and others completed a study on the contribution of (1→3)-β-D-glucan for diagnosis and therapeutic monitoring of IA in neutropenic adults in comparison with serial screening for circulating galactomannan. The two tests (Glucatell, and Platelia *Aspergillus*) were used retrospectively in a twice-weekly screening for IA in 40 neutropenic adult patients. The cutoff used for GM assay and BDG assay were optical density index (ODI) of 1.0 and 60 pg/mL, respectively. Out of 11 cases there were five proven cases of invasive aspergillosis, three probable cases, and three possible cases. In both assays BDG and GM were detected in 100% of patients with proven IA and in 66% of patients with probable IA. The sensitivity, specificity, and the positive and negative predictive values for GM and BDG were identical: 87.5%, 89.6%, 70%, and 96.3%, respectively. False/positive reactions did occur at a rate of 10.3% in both tests. Although both tests anticipated clinical diagnosis, initiation of antifungal therapy, and computed tomography abnormalities, BDG showed positive results earlier than GM. This study concluded that joint use of both tests is very useful to identify false-positive reactions by each. Joint use improves each individual test's specificity and positive predictive value to 100%, without affecting the sensitivity and negative predictive values.[10]

In a study performed by Kawazu and others, 149 treatment episodes in 96 consecutive patients, including nine proven IA, two probable IA, 13 possible invasive fungal infections (IFI), and 125 no-IA episodes were studied. Overall, 1,233 samples were analyzed using the ELISA for GM detection and 1,243 samples were examined using the BDG test. The GM and BDG levels in a cohort of patients at high risk for IA were measured weekly. The two different tests were examined using receiver-operating characteristic analysis. The area under the receiver-operating characteristic curve was the greatest for ELISA, using two consecutive positive results (0.97; $p$ = 0.055 for ELISA versus BDG). The cutoff for ELISA could be reduced to an optical density index (ODI) of 0.6. With the use of this cutoff for ELISA and the cutoff for BDG, 60 pg/mL, that give a comparable level of specificity, the sensitivity, specificity, positive predictive value, and negative predictive value of the ELISA and BDG tests were 1.00/0.93/0.55/1.00 and 0.55/0.93/0.40/0.96, respectively. The conclusion of this study was that the double sandwich ELISA test was the most sensitive at predicting the diagnosis of IA in high-risk patients with hematological disorders, using a reduced cutoff of 0.6 ODI.[11]

A series of allogeneic stem cell transplant recipients were examined in a paper written by Maertens and others. The

study analyzed the relationship between antigenemia and other diagnostic triggers for initiation of antifungal therapy. The sensitivity and specificity of GM detection were 94.4%, and 98.8%, respectively. The positive and negative predictive values were also 94.4% and 98.8%. This method of detection, with a cutoff value of 1.0 ODI, was found to be better statistically than other triggers, such as unexplained fever, new pulmonary infiltrates, isolation of *Aspergillus* species, and computed tomography imaging. The results of the study were that antigenemia preceded diagnosis on the basis of radiologic examination or *Aspergillus* isolation by eight and nine days in 80% and 88.8% of patients. Antigenemia detection preceded therapy in 83.3% of patients. The conclusion of the study was that detection of GM allows earlier diagnosis of aspergillosis than conventional diagnostic criteria.[12]

## DISCUSSION

Diagnosis of invasive pulmonary aspergillosis still remains a challenge, mainly because of atypical clinical presentations, coexistence with other infectious and noninfectious diseases, and a relative inability to culture these organisms by standard microbiological techniques. Non-culture based techniques for diagnosing IA have improved steadily in recent years but improvements are still needed.

The development of GM antigen ELISA (Platelia Aspergillus, Bio-Rad Laboratories) tests significantly improved the quality of non-invasive diagnostics. The Bio-Rad GM assay is one such test that may be applied for these purposes; however, reported diagnostic performance has been controversial. Although specificity of the test has been high (i.e., >90%) in most studies performed, reported sensitivities have varied.

The reviewed articles discussed above also demonstrate that the (1→3)-β-D-glucan assay may be useful to measure serum BDG in clinical specimens with a high specificity and positive predictive values for patients with proven or probable IFI. A cutoff value of 60 pg/mL or 80 pg/mL appears to be appropriate for this test. Although the performance of this assay does not appear to be affected by the presence of antifungal therapy, it is very sensitive to glucans that naturally reside in the environment.

In summary, this large retrospective review of studies performed on both assays has demonstrated that GM and β-glucan detection assays do have high sensitivity and specificity, but have variable cutoffs that affect the use of these assays as tools for clinical diagnosis of invasive aspergillosis. Although tissue culture and radiologic examination remain the "gold standard" indicators of invasive disease, the low cost, rapid results, and non-invasive methods of the two assays make them more appealing to physicians and patients. The two assays used in combination may provide physicians and patients with a stronger diagnostic strategy for diagnosing invasive aspergillosis.

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