β-D-Glucan Testing Is Important for Diagnosis of Invasive Fungal Infections

Elitza S. Theel,[a] Christopher D. Doern[b]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA,[a] University of Texas Southwestern Medical Center, Dallas, Texas, USA[b]

Invasive fungal infections are a significant cause of morbidity and mortality in patients who receive immunosuppressive therapy, such as solid organ and hematopoietic stem cell transplant (HSCT) recipients. Many of the fungi associated with these infections are angioinvasive and are best diagnosed by visualizing the organism in or culturing the organism from deep tissue. However, obtaining such tissue often requires an invasive procedure. Many HSCT recipients are thrombocytopenic, making such procedure too risky because of potential bleeding complications. Additionally, positive blood cultures are rare for patients with angioinvasive fungal infections, making this diagnostic strategy of little value. Undiagnosed fungal infections in these patient populations are a significant cause of mortality. Prophylactic use of antifungal agents, such as the echinocandins, during periods of neutropenia or graft-versus-host disease may prevent some fungal infections but increase the risk for others. Detection of fungal antigens in body fluids, including cryptococcus capsular polysaccharide, histoplasma antigen, galactomannan, and β-D-glucan, is viewed as being clinically useful for at least the presumptive diagnosis of invasive fungal infections. β-D-Glucan is an attractive antigen in that it is found in a broad range of fungal agents, including the commonly encountered agents *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii*. Cross-reactions with certain hemodialysis filters, beta-lactam antimicrobials, and immunoglobulins, which raise concerns about false-positive tests, have also been described. As a result, the use of this testing must be closely monitored. In this point-counterpoint, we have asked Elitza Theel, who directs the Infectious Disease Serology Laboratory at the Mayo Clinic, to address why she believes that this test has value in the diagnosis of invasive fungal infections. We have asked Christopher Doern, Director of Clinical Microbiology at Children's Medical Center of Dallas, why he questions the clinical value of β-D-glucan testing.

POINT

An abundant cell wall polysaccharide, (1-3)-β-D-glucan (BDG) is found in most fungi, with the notable exception of the cryptococci, the zygomycetes, and *Blastomyces dermatitidis*, which either lack the glucan entirely or produce it at minimal levels. At least four BDG detection assays have been developed: Fungitell (Associates of Cape Code, Inc., East Falmouth, MA, USA), Wako (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), Fungitec-G (Seikagaku, Kogyo, Tokyo, Japan), and Maruha (Maruha-Nichiro, Foods Inc., Tokyo, Japan), of which only the Fungitell assay is FDA approved for use on serum in the United States. This assay is a chromogenic, quantitative enzyme immunoassay (EIA) designed to detect BDG (in ng/ml) by using purified, lysed horseshoe crab (*Limulus polyphemus*) amebocytes. These cells contain components of the *Limulus* clotting cascade, including factors C and G, which initiate coagulation in the presence of bacterial liposaccharide and BDG, respectively. By eliminating factor C from the lysate, the manufacturers limit activation of the cascade to BDG alone.

For the purposes of this discussion, the following are arguments in favor of BDG testing and BDG's role as a surrogate marker for invasive fungal infections (IFIs). The caveat remains, however, that the interpreting clinician must be cognizant of the associated assay limitations.

Readily available specimen source—serum. Currently, the gold standard methods for laboratory-based diagnosis of IFIs in patients presenting with pulmonary insufficiency require testing of invasively collected specimens, including culture of bronchoalveolar lavage (BAL) fluid or submission of biopsy material for histopathologic examination and fungal culture. The invasive

procedures may, however, be counterindicated due to profound neutropenia, hypoxia, or the overall critical state of the patient. Furthermore, even if specimens are acquired, supportive laboratory evidence of infection is not guaranteed. Fungal culture from lower respiratory tract sources is notoriously insensitive, with a positivity rate of only 45 to 60% for cases of invasive aspergillosis ([1]). Depending on the inoculum and fungal growth characteristics, culture requires at least 2 to 3 days of incubation and, for some species, days to weeks longer, which may further delay initiation of antifungal therapy. Positive cultures from nonsterile sources, including BAL fluid specimens, also require cautious interpretation in order to differentiate between fungal colonization and isolation of the true invasive agent. Finally, fungal blood cultures, while noninvasive and highly specific, require prolonged incubation and can likewise be insensitive, with only 50% of *Candida* spp. and <10% of *Aspergillus* spp. being detected ([1], [2]). Sole reliance on culture can therefore lead to delayed diagnosis, and so a need exists for additional testing methods.

A key advantage of evaluation for BDG is the required specimen source, serum. A readily available and easily accessible spec-

The views expressed in this feature do not necessarily represent the views of the journal or of ASM.

imen, regardless of patient status, serum allows for serial BDG analysis, which can significantly enhance the assays' clinical performance (discussed below). As with testing for other fungal antigens, detection of BDG in alternative, invasively collected specimens (i.e., BAL fluid and cerebrospinal fluid [CSF]) has the potential to further enhance the sensitivity of standard laboratory practices for IFI diagnosis. Detailed studies evaluating this testing option, however, are still needed.

Good performance using serial testing of high-risk patients. An initial, overarching review of the BDG literature may lead many readers to completely discount the utility of this biomarker due to inconsistent performance characteristics. Sensitivity and specificity values, regardless of the invasive organism, can range from 38% to 100% and 45% to 99%, respectively, with similar ranges observed for the positive predictive value (PPV; 30% to 89%) and negative predictive value (NPV; 73% to 97%) (3–11). These widely dispersed statistics can be attributed largely to heterogeneity both within and between evaluations, which differ with respect to which BDG assay was evaluated, what positive cutoff criteria was used, the patient and control population tested, and the number of BDG tests performed per individual. The vast majority of these studies appropriately applied the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria to stratify patients with proven/ probable/possible or no IFI as the comparator groups against which BDG results were evaluated. However, while these guidelines remain the sole standard, they can lead to overcalling cases of possible fungal pneumonia and completely miss autopsy-proven IFIs (12, 13). Despite these limitations, careful dissection of the data reveals certain scenarios where testing for BDG antigenemia is relevant and may lead to improved patient outcome.

Two populations that have been shown to consistently benefit from BDG testing, particularly during episodes of neutropenia, are patients with hematologic malignancies and those who have undergone allogeneic hematopoietic stem cell transplants (6, 7, 9, 14). Prospective, serial BDG antigenemia testing (at least biweekly) in these patients, starting at the onset of neutropenia (<500 cells/mm^3), has led to significantly higher specificities (76% to 99%) and NPVs (87% to 96%) for the presence of proven or probable IFI than single-time-point testing. Unfortunately, despite interval testing, the sensitivities and PPVs of the BDG assays remain unacceptably low. An intriguing meta-analysis by Lamoth and colleagues, which included six cohort studies, recently reported a diagnostic odds ratio of 111.8 versus 16.3 for the presence of IFIs in neutropenic hemato-oncological patients following two consecutively positive BDG assays compared to a single positive BDG assay. This meta-analysis also reported a pooled sensitivity of 49.6%, alongside a PPV and NPV of 83.5% and 94.6%, respectively (4).

A number of conclusions can be drawn from the aforementioned data. First, due to the consistently low sensitivity reported among studies, and despite the strong NPV, a negative BDG result should not be used to exclude the possibility of invasive fungal disease. The lower sensitivity of the BDG assay, however, is not unique among fungal biomarkers. A meta-analysis of 27 studies evaluating the performance of the galactomannan (GM) assay among patients with hematologic malignancies identified a pooled sensitivity of 61% for patients with proven or probable invasive aspergillosis (15). Therefore, as with other serologic tests for fungal antigens associated with IFIs, single negative results from BDG assays are

of limited value and need to be considered in light of available clinical and laboratory data. Secondly and perhaps more importantly, these studies indicate that among patients with prolonged neutropenia who present with symptoms consistent with an IFI, repeatedly positive BDG results may be used as supportive evidence for the presence of an IFI. This conclusion is further supported by the guidelines of the 3rd European Conference on Infections in Leukemia, which categorized BDG testing as "B II," indicating that there is "moderate evidence to support recommendation for use" in patients with leukemia (5, 16). The EORTC/MSG guidelines, while not used for clinical diagnosis, have also recently included a positive BDG result as meeting their criteria for mycological evidence of infection. Currently, however, neither the ECIL 3 nor the EORTC/MSG provides BDG timing or interval testing guidelines, and studies to better define serial BDG analysis are needed.

BDG positivity prior to alternative testing methods. A number of groups have now reported that among critically ill patients with proven or probable IFIs, many will develop detectable BDG antigenemia prior to the onset of clinical symptoms or radiologic signs or the return of positive culture results. The percentages of patients in whom this occurs vary between studies (64 to 87%), as do the numbers of days between BDG and culture (blood, biopsy, or BAL fluid) positivity (1 to 10 days) (6–9, 17). While these studies are limited by the number of enrolled patients, the findings argue that a single positive BDG result should not be haphazardly discounted. Instead, among patients with a high pretest probability of developing an IFI (which was hopefully the impetus for initial BDG evaluation), a single positive BDG test warrants close patient monitoring and further clinical and, if possible, laboratory-based evaluation.

Trending of BDG levels may be used to monitor responses to therapy. In addition to monitoring qualitative BDG results during interval testing, tracking quantitative values following initiation of antifungal therapy may be used as a prognostic marker for patient response. Consistently decreasing BDG levels during treatment have been shown by multiple groups to result in a favorable therapeutic responses among patients with proven or probable IFIs (6, 7, 17, 18). Perhaps among the most alluring of these studies is that of Jaijakul and colleagues, who plotted serial BDG levels collected over time from 203 patients with proven invasive candidemia during anidulafungin treatment. Using this charting method, the authors correlated a negative slope in BDG levels from patients with a favorable treatment outcome (PPV of 90%) and a positive slope following treatment failure (NPV of 90%) (18). Interestingly, among those who responded to treatment and showed a negative BDG slope, only 16% had a negative BDG result upon endpoint testing. This is not entirely surprising, as the precise kinetics of release and the route of BDG elimination remain unclear. What needs to be underscored, however, is that while monitoring trending of BDG values over time can be a useful prognostic marker for response to treatment, the presence or absence of BDG should not be used to guide cessation of therapy or as a "test of cure."

BDG detection as an aid for diagnosis of *Pneumocystis jirovecii* pneumonia. Immunosuppressed populations are at risk for infection with *Pneumocystis jirovecii*, in addition to invasive disease with *Aspergillus* or *Candida.* Pneumocystis pneumonia (PCP) classically presents with dry cough, dyspnea, and fever in the setting of diffuse ground glass opacities on chest X ray, and while characteristic, these symptoms remain broad and can be induced by a diverse range of microbial pathogens. As with the

diagnostic challenges of IFIs, the preferred specimens for detection of *P. jirovecii* are BAL fluid or biopsy material obtained by video-assisted thoracoscopic surgery (VATS), which may be unattainable at presentation due to concerns for patient safety. Furthermore, diagnostic procedures, including microscopy of stained specimens, can be insensitive, while molecular methods may detect low-level, noncontributory colonization. As with other fungal pathogens, BDG is a major component of the *P. jirovecii* surface structure and has been considered a potential marker for PCP. Recently, a meta-analysis evaluating 11 retrospective studies of patients with laboratory-confirmed PCP and at-risk patient controls found a pooled sensitivity and specificity of 94.8% and 86.3%, respectively, for detection of BDG in cases of proven PCP (19). Additionally, this group reported a diagnostic odds ratio of 113.7 for the presence of PCP in the setting of a positive BDG result. In light of the lower specificity and despite multiple reports of significantly elevated, quantitative BDG values among patients with PCP compared to values for patients with other IFIs, BDG remains a pan-fungal biomarker and positive results require clinical correlation for a PCP diagnosis to be made. However, the high sensitivity coupled with a strong NPV (>95%) identified in individual studies (20) collectively indicate that a negative BDG result may be used to downgrade *P. jirovecii* as a likely cause of infection.

Serial BDG testing may be cost-effective. A natural concern that arises when any assay is recommended to be performed at multiple intervals is cost. As with most serologic assays, testing of multiple samples (i.e., acute- and convalescent-phase sera) is preferred, and detection of BDG should not be considered any differently. Cost per BDG assay can vary (depending on contracts, the performing laboratory, etc.) but typically ranges between $100 and $200. While not inexpensive, considering the economic burden of prolonged hospitalization in intensive care units, which can quickly mount into the tens of thousands of dollars, serial BDG testing has the potential to significantly decrease patient cost. When used in the appropriate setting, repeatedly positive BDG results and/or increasing BDG levels may prompt sooner initiation of broad antifungal therapy and result in quicker resolution or even prevention of severe disease. Detailed studies evaluating the potential cost savings for BDG testing are needed, however, for this to be conclusively established.

Conclusions. Detection of BDG antigenemia can be a useful diagnostic tool if used in the proper clinical setting (i.e., immunosuppressed, neutropenic patients) by a provider knowledgeable of both the advantages and limitations of the assay as applied to each individual patient. BDG detection will not replace current laboratory methods for IFI diagnosis, and questions remain regarding appropriate clinical use (i.e., timing of specimen collection, duration of testing, meaning of quantitative values, etc.). However, the ease of specimen collection and the potential information that can be garnered from serial BDG evaluations argue for consideration of this assay as a diagnostic screen in many diagnostic protocols.

Elitza S. Theel

REFERENCES

1. **Singh N, Paterson DL.** 2005. Aspergillus infections in transplant recipients. Clin. Microbiol. Rev. **18:**44–69.
2. **Ellepola AN, Morrison CJ.** 2005. Laboratory diagnosis of invasive candidiasis. J. Microbiol. **43:**65–84.
3. **Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME.** 2011. β-D-Glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin. Infect. Dis. **52:**750–770.
4. **Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, Marchetti O.** 2012. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin. Infect. Dis. **54:**633–643.
5. **Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S.** 2012. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. Bone Marrow Transplant. **47:**846–854.
6. **Senn L, Robinson JO, Schmidt S, Knaup M, Asahi N, Satomura S, Matsuura S, Duvoisin B, Bille J, Calandra T, Marchetti O.** 2008. 1,3-β-D-Glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. Clin. Infect. Dis. **46:**878–885.
7. **Ellis M, Al-Ramadi B, Finkelman M, Hedstrom U, Kristensen J, Ali-Zadeh H, Klingspor L.** 2008. Assessment of the clinical utility of serial beta-D-glucan concentrations in patients with persistent neutropenic fever. J. Med. Microbiol. **57:**287–295.
8. **Del Bono V, Delfino E, Furfaro E, Mikulska M, Nicco E, Bruzzi P, Mularoni A, Bassetti M, Viscoli C.** 2011. Clinical performance of the (1,3)-beta-D-glucan assay in early diagnosis of nosocomial Candida bloodstream infections. Clin. Vaccine Immunol. **18:**2113–2117.
9. **Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, Ketchum PA, Finkelman MA, Rex JH, Ostrosky-Zeichner L.** 2004. β-D-Glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin. Infect. Dis. **39:**199–205.
10. **Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL.** 2005. Evaluation of a (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J. Clin. Microbiol. **43:**5957–5962.
11. **Mohr JF, Sims C, Paetznick V, Rodriguez J, Finkelman MA, Rex JH, Ostrosky-Zeichner L.** 2011. Prospective survey of (1→3)-beta-D-glucan and its relationship to invasive candidiasis in the surgical intensive care unit setting. J. Clin. Microbiol. **49:**58–61.
12. **Subira M, Martino R, Rovira M, Vazquez L, Serrano D, De La Camara R.** 2003. Clinical applicability of the new EORTC/MSG classification for invasive pulmonary aspergillosis in patients with hematological malignancies and autopsy-confirmed invasive aspergillosis. Ann. Hematol. **82:**80–82.
13. **Obayashi T, Negishi K, Suzuki T, Funata N.** 2008. Reappraisal of the serum (1→3)-beta-D-glucan assay for the diagnosis of invasive fungal infections—a study based on autopsy cases from 6 years. Clin. Infect. Dis. **46:**1864–1870.
14. **Kawazu M, Kanda Y, Nannya Y, Aoki K, Kurokawa M, Chiba S, Motokura T, Hirai H, Ogawa S.** 2004. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. J. Clin. Microbiol. **42:**2733–2741.
15. **Pfeiffer CD, Fine JP, Safdar N.** 2006. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin. Infect. Dis. **42:**1417–1427.
16. **Metan G, Koc AN, Atalay A, Kaynar LG, Ozturk A, Alp E, Eser B.** 2012. What should be the optimal cut-off of serum 1,3-beta-D-glucan for the detection of invasive pulmonary aspergillosis in patients with haematological malignancies? Scand. J. Infect. Dis. **44:**330–336.
17. **Pazos C, Ponton J, Del Palacio A.** 2005. Contribution of (1->3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. J. Clin. Microbiol. **43:**299–305.
18. **Jaijakul S, Vazquez JA, Swanson RN, Ostrosky-Zeichner L.** 2012. (1,3)-β-D-Glucan as a prognostic marker of treatment response in invasive candidiasis. Clin. Infect. Dis. **55:**521–526.
19. **Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME.** 2013. Accuracy of beta-D-glucan for the diagnosis of Pneumocystis jirovecii pneumonia: a meta-analysis. Clin. Microbiol. Infect. **19:**39–49.
20. **Held J, Koch MS, Reischl U, Danner T, Serr A.** 2011. Serum (1→3)-beta-D-glucan measurement as an early indicator of Pneumocystis jirovecii pneumonia and evaluation of its prognostic value. Clin. Microbiol. Infect. **17:**595–602.

COUNTERPOINT

When the error rate of a test exceeds the prevalence of the disease it is designed to detect, then you don't have much of a test.

—Gary V. Doern (personal communication)

The concept of the (1,3)-β-D-glucan (BDG) test is highly desirable; it provides a noninvasive test method which is designed to diagnose invasive fungal infections (IFIs). As more patients experience prolonged immunocompromised periods, IFIs have become increasingly common. BDG as a marker for infection holds great appeal because making the definitive diagnosis of IFI often requires tissue biopsy or is made at autopsy. In contrast to galactomannan testing, which can be used to diagnose only invasive *Aspergillus* (IA) infection, BDG testing is promising because it is capable of detecting infections caused by many fungi, excluding *Cryptococcus* and the zygomycetes (1).

This counterpoint will review the limitations of the BDG assay and argue that while, in principal, the test is promising, in practice, its poor performance renders it of limited use in establishing a diagnosis of invasive fungal infections. What follows is an objective assessment of performance data as well as a discussion of the utility of BDG testing in certain clinical situations, such as monitoring response to treatment.

Sensitivity and specificity of β-D-glucan testing. The value of any laboratory test can be judged by whether the result(s) it provides can be trusted and acted upon. The utility of a given test result is primarily dependent on the test's sensitivity and specificity, but also on the prevalence of disease and the resulting positive and negative predictive values. Ideally, a test will have both high sensitivity and high specificity.

The literature is now replete with studies evaluating the clinical performance of the BDG assay in a wide variety of patient populations. Although significant heterogeneity exists in these studies, one common finding is that the sensitivity of this assay is between 50 and 80% and its specificity ranges between 50 and 90% (1–5). One meta-analysis concluded that the average sensitivity and specificity were approximately 76% and 85%, respectively (2). Considering these performance characteristics, the error rate of BDG testing far exceeds the prevalence of invasive fungal infections in most settings in which BDG testing is applied. As a result, BDG testing is often of limited diagnostic value.

Limitations of BDG assay sensitivity. BDG testing is commonly utilized in patients that have underlying conditions, such as organ transplantation or malignancy, and therefore have highly compromised immune systems. It is in these patients that a test for IFI with a high sensitivity and, therefore, good negative predictive value would be of great value. Unfortunately, a literature review reveals an average sensitivity in the mid-70s and a correspondingly poor NPV (2, 6). Given this poor sensitivity, negative test results do not allow caregivers to confidently exclude invasive fungal disease.

Some subanalyses stratified by underlying condition suggest that BDG testing may function better for patients with hematological malignancy than for solid organ transplant patients or intensive care unit (ICU) patients (2). However, the data in this regard are mixed, as Hachem et al. have shown poor performance in patients with hematologic malignancy (7). To illustrate the heterogeneity in the literature, Odabasi and colleagues also evaluated

patients with hematologic malignancy and found the sensitivity and specificity of the assay to be 100 and 90%, respectively (5). Regardless of patient population, a significant limitation of the negative predictive value of BDG testing is its inability to diagnose *Cryptococcus* and zygomycete disease. Even in those patients for whom sensitivity could be maximized, a negative result does not exclude the possibility of disease by these important pathogens.

The issue of poor sensitivity is further complicated by the uncertainty surrounding what value truly defines a positive result. The three primary providers of BDG testing all interpret their assays differently, so it is particularly important that physicians ordering these tests be aware of the specific method being used. One group reported that the Fungitell BDG assay had greater sensitivity than both the Fungitec and the Wako tests (8). One meta-analysis supported these findings (2). When the tests were stratified by manufacturer, the Wako test was found to have the lowest sensitivity but did have higher specificity than its competitors. Differences in assay performance are not surprising considering that they differ in β-glucan standards used, specimen pretreatment methods, and kit lysates (5, 9). In addition, the literature evaluates a wide range of performance cutoff values (3 pg/ml to >500 pg/ml) outside those recommended by the manufacturer. Not surprisingly, when the threshold for positivity is lowered, specificity decreases (10). De Vlieger and colleagues evaluated various Fungitell positivity cutoffs in an autopsy-based study evaluating the BDG assay performance for IA (10). They found that in ICU patients with confirmed IA, a cutoff value of 80 pg/ml yielded a sensitivity of 85.7% but a specificity of only 36.4%. In a subgroup analysis that divided patients into hematology and nonhematology patients, a cutoff of 140 pg/ml improved the specificity to 77.8% but at the expense of sensitivity, which then dropped to 72.7% in hematology patients. Contrary to the findings of the meta-analysis discussed above, BDG testing functioned better for nonhematology patients, with a cutoff of 140 pg/ml and a sensitivity and specificity of 100 and 69.6%, respectively (10). In their final analysis, they state that although patients with IA had higher BDG levels than those who did not, performance characteristics did not justify the test's use as a diagnostic tool for IA. Due to the sliding scale of interpretive criteria and the heterogeneity in the literature, health care providers are left with the difficult task of deciding what positive cutoff to use and how to know when a negative result really indicates the absence of disease. If individual institutions are inclined to pursue BDG testing in spite of its relatively low sensitivity, they would be well served in conducting investigations aimed at defining its true utility in their own patient populations.

Limitations of BDG assay specificity. The greatest limitation of BDG testing is its poor specificity. The list of factors which can generate false-positive results is extensive and includes albumin, intravenous immune globulin, gauze packing, intravenous amoxicillin-clavulanic acid, and use of cellulose depth filters (11, 12). It has also been suggested that bacteremia due to Gram-positive organisms and *Alcaligenes faecalis* can result in false-positive BDG tests. In addition, *in vitro* studies have demonstrated that colistin, ertapenem, cefazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam can all yield positive BDG test results (13). Marty and colleagues examined these antimicrobial agents at reconstituted vial concentrations and found BDG reactivity. However, when these agents were diluted to what would be considered maximum plasma concentrations, they

found no reactivity, suggesting that these compounds might not yield false-positive results in patients receiving these agents therapeutically (13). There may still be cause for concern though, as at least one study found elevated BDG values in 37 of 117 serum samples from patients being treated with ampicillin-sulbactam and no other explanation for the false-positive result (11).

The limitations of BDG cross-reactivity have been well documented in the literature. What are less well described are the adverse events that almost certainly result from these false positives. Of primary concern is the misdiagnosis of IFIs, leading to unnecessary antifungal treatment and distraction from diagnosing the actual cause of a patient's disease. The poor specificity is particularly problematic in immunocompromised patients, in whom it is most commonly used. With the high morbidity and mortality associated with IFIs, it is very difficult to justify withholding antifungal treatment for a test result that may suggest invasive disease. This leads to excessive use of antifungal agents and all of the negative consequences associated with inappropriate usage. Indeed, resistance among fungal pathogens is becoming more common in the face of increased exposure (14). Some studies have now shown that the MICs for *Aspergillus* isolates increase as the isolates are exposed to increasing concentrations of triazoles (15). What is particularly concerning is that exposure to one triazole correlated positively with increased MICs of different triazoles (15).

Clearly, the less-than-optimal specificity of BDG assays creates a dilemma for health care providers who have to make treatment decisions for patients who are critically ill. The negative outcomes associated with BDG testing have yet to be quantified. However, one ICU-based study prospectively evaluated the use of BDG testing for deciding when to preemptively treat patients for IFIs. In that study, patients in the intervention arm were preemptively treated with anidulafungin if BDG testing was positive. Of relevance to the specificity concerns, the positive predictive value of a positive BDG test in that study was only 30%. In other words, 70% of the time, BDG testing yielded a positive result and it was incorrect. The study was different than other retrospective studies, which may inflate prevalence due to preselection and therefore exaggerate the positive predictive value of BDG positivity. One other study of patients with hematological malignancies undergoing treatment had specimens prospectively collected but retrospectively tested. In this study, the prevalence of IFI was found to be 8.7%, resulting in a positive predictive value of only 12% (16).

One could make the case that if you were aware of all the different conditions which may lead to BDG false positivity, you could simply avoid ordering the test for those patients. The problem with this approach is that the full extent of BDG cross-reactivity has not been determined. Indeed, when Koo et al. evaluated BDG performance in stem cell transplant patients, they found that excluding those patients with risk factors for BDG false positivity did not significantly improve its specificity (3). Another study by Bellanger et al. (18) found that 4 of 11 hematology control patients had false-positive BDG tests despite the preselection of patients with no known risk factor for BDG cross-reaction. Lastly, in the Racil et al. study mentioned above, a thorough analysis of the patients was conducted in an attempt to explain a false-positivity rate of >50% (16). They were unable to identify known risk factors for false positivity. The erroneous results appeared to happen at random and without explanation. This may suggest that there are other yet-to-be-identified factors which may lead to BDG assay false positivity. In addition, when evaluating the literature that

addresses the use of the BDG assay in clinical practice, it is clear that laboratories cannot assume that their physicians are aware of their own patients' risk factors for a falsely positive BDG result. A number of retrospective studies looking at BDG test use show that it is quite common for this test to be ordered for those with risk factors for false positivity (3).

The value of multiple positive tests. Some studies and reviews suggest that the BDG assay has a high negative predictive value when multiple negative tests are considered. It is difficult to determine a consensus negative predictive value because the literature is very heterogeneous and prevalence rates vary widely and are greatly influenced by study design. However, a meta-analysis concluded that the sensitivity of two consecutive positive tests was 65%, with a 95% confidence interval of 52 to 78% (2). Although the sensitivity of this approach is poor, the authors did find that two consecutive positives yielded a specificity of 93%. Senn et al. published an evaluation of serial BDG testing and various cutoff values using the Wako test (17). In this study, when the lowest cutoff values were applied, two consecutive positives generated a sensitivity of 97% but a specificity of 51%. Conversely, when the highest cutoff values were used, two consecutive positives generated a specificity of 99% but with only a 40% sensitivity. Neither of these scenarios is acceptable for clinical care. When a middle cutoff value was applied to maximize overall diagnostic efficiency, the sensitivity was 63% and the specificity was 96%. The authors did go on to calculate the positive and negative predictive values, but the prevalence in this study was high, which makes it difficult to draw strong conclusions from these calculations. It is interesting, though, that even in the face of a 31% prevalence, the positive predictive value of two consecutive tests was still only 79%. If we extract their sensitivity and specificity calculations and insert the prevalence obtained by other prospective studies of 10%, the positive predictive value drops to 62% (4, 17).

Conclusions. Although the BDG assay fills a void in diagnostic testing for invasive fungal infection in the immunocompromised, it does so with poor sensitivity and specificity. The sensitivity is not sufficient to confidently rule out fungal infection, and the poor specificity makes it very difficult to interpret positive results. The frequently generated false-positive results are particularly concerning, as they may be difficult to ignore even in patients with known risk factors for false positivity. In worse-case scenarios, patients awaiting transplants may be committed to long periods of antifungal treatment until a presumed fungal infection has been completely treated, thus delaying a potentially life-saving transplant. There is a growing body of evidence that BDG performs with high sensitivity for patients with *Pneumocystis jirovecii* pneumonia (PJP); however, all of the same limitations in specificity still apply. That said, the test may serve as a suitable screen for PJP infection, but providers should be aware that it is only a good rule-out test and that positive results should be confirmed with a *Pneumocystis*-specific assay.

In conclusion, despite the desperate need for improved diagnostics of invasive fungal infections, detection of BDG does not appear to be a test with sufficient sensitivity or specificity to be recommended for routine use.

Christopher D. Doern

REFERENCES

1. Ostrosky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, Ketchum PA, Wingard J, Schiff R, Tamura H, Finkelman MA,

Rex JH. 2005. Multicenter clinical evaluation of the (1→3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clin. Infect. Dis. **41**:654–659.

2. **Lu Y, Chen YQ, Guo YL, Qin SM, Wu C, Wang K.** 2011. Diagnosis of invasive fungal disease using serum (1→3)-beta-D-glucan: a bivariate meta-analysis. Intern. Med. **50**:2783–2791.

3. **Koo S, Bryar JM, Page JH, Baden LR, Marty FM.** 2009. Diagnostic performance of the (1→3)-beta-D-glucan assay for invasive fungal disease. Clin. Infect. Dis. **49**:1650–1659.

4. **Hanson KE, Pfeiffer CD, Lease ED, Balch AH, Zaas AK, Perfect JR, Alexander BD.** 2012. β-D-Glucan surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. PLoS One 7(8):e42282. doi:10.1371/journal.pone.0042282.

5. **Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, Ketchum PA, Finkelman MA, Rex JH, Ostrosky-Zeichner L.** 2004. β-D-Glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin. Infect. Dis. **39**:199–205.

6. **Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, Morinobu A, Nishimura K, Kumagai S.** 2012. Diagnostic accuracy of serum 1,3-beta-D-glucan for *Pneumocystis jiroveci* [*sic*] pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J. Clin. Microbiol. **50**:7–15.

7. **Hachem RY, Kontoyiannis DP, Chemaly RF, Jiang Y, Reitzel R, Raad I.** 2009. Utility of galactomannan enzyme immunoassay and (1,3) beta-D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. J. Clin. Microbiol. **47**:129–133.

8. **Yoshida K, Shoji H, Takuma T, Niki Y.** 2011. Clinical viability of Fungitell, a new (1→3)-β-D-glucan measurement kit, for diagnosis of invasive fungal infection, and comparison with other kits available in Japan. J. Infect. Chemother. **17**:473–477.

9. **Obayashi T, Negishi K, Suzuki T, Funata N.** 2008. Reappraisal of the serum (1→3)-beta-D-glucan assay for the diagnosis of invasive fungal infections—a study based on autopsy cases from 6 years. Clin. Infect. Dis. **46**:1864–1870.

10. **De Vlieger G, Lagrou K, Maertens J, Verbeken E, Meersseman W, Van Wijngaerden E.** 2011. β-D-Glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. J. Clin. Microbiol. **49**:3783–3787.

11. **Metan G, Agkus C, Nedret Koc A, Elmali F, Finkelman MA.** 2012. Does ampicillin-sulbactam cause false positivity of (1,3)-beta-D-glucan assay? A prospective evaluation of 15 patients without invasive fungal infections. Mycoses **55**:366–371.

12. **Marty FM, Koo S.** 2009. Role of (1→3)-beta-D-glucan in the diagnosis of invasive aspergillosis. Med. Mycol. **47**(Suppl 1):S233–S240.

13. **Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR.** 2006. Reactivity of (1→3)-β-D-glucan assay with commonly used intravenous antimicrobials. Antimicrob. Agents Chemother. **50**:3450–3453.

14. **Pfaller MA.** 2012. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. Am. J. Med. **125**(Suppl 1):S3–S13.

15. **Tashiro M, Izumikawa K, Hirano K, Ide S, Mihara T, Hosogaya N, Takazono T, Morinaga Y, Nakamura S, Kurihara S, Imamura Y, Miyazaki T, Nishino T, Tsukamoto M, Kakeya H, Yamamoto Y, Yanagihara K, Yasuoka A, Tashiro T, Kohno S.** 2012. Correlation between triazole treatment history and susceptibility in clinically isolated Aspergillus fumigatus. Antimicrob. Agents Chemother. **56**:4870–4875.

16. **Racil Z, Kocmanova I, Lengerova M, Weinbergerova B, Buresova L, Toskova M, Winterova J, Timilsina S, Rodriguez I, Mayer J.** 2010. Difficulties in using 1,3-β-D-glucan as the screening test for the early diagnosis of invasive fungal infections in patients with haematological malignancies—high frequency of false-positive results and their analysis. J. Med. Microbiol. **59**(Part 9):1016–1022.

17. **Senn L, Robinson JO, Schmidt S, Knaup M, Asahi N, Satomura S, Matsuura S, Duvoisin B, Bille J, Calandra T, Marchetti O.** 2008. 1,3-β-D-Glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. Clin. Infect. Dis. **46**:878–885.

18. **Bellanger A, Grenouillet F, Henon T, Skana F, Legrand F, Deconinck E, Millon L.** 2011. Retrospective assessment of β-D-(1,3)-glucan for presumptive diagnosis of fungal infections. APMIS **119**:280–286.

SUMMARY

Points of agreement

- This test is likely of value only for select patient populations (HSCT patients) or in the diagnosis of select infections, such as with *P. jirovecii* pneumonia.

- Multiple positive results are best at predicting the presence of invasive fungal infections.

- Because of many potential sources of false positives, a single positive test is of limited value.

- The test has poor sensitivity, so it has no value as a screening test; a negative test cannot exclude the diagnosis of invasive fungal infection.

- Monitoring β-D-glucan quantitative levels in patients being treated for invasive *Candida* infections has value.

Issues to be resolved

- Further studies are needed to determine the patient populations and clinical scenarios in which this test is most likely to be of value.

- The frequency with which this test should be performed for diagnosis or therapeutic monitoring is not well understood.

- Detailed studies of the performance of β-D-glucan testing with other specimen types, such as cerebrospinal fluid and bronchoalveolar lavage (BAL) fluid, are needed. Performance of this test with BAL fluids may be of particular value for detecting *P. jirovecii* pneumonia.

- Improvements in the assay to eliminate the high rates of false positives due to commonly encountered materials and therapeutics would greatly increase its clinical value.

Peter H. Gilligan, Point-Counterpoint Editor, *Journal of Clinical Microbiology*