Point-Counterpoint: Should Serum β -D-Glucan Testing Be Used for the Diagnosis of *Pneumocystis jirovecii* Pneumonia?

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INTRODUCTION Despite the widespread use of prophylactic antibiotics in high-risk individuals, *Pneumocystis jirovecii* remains an important cause of pneumonia in immunocompromised patients. During the peak of the AIDS epidemic, many hospitals and outpatient clinics were very proficient at collecting induced sputum specimens for the diagnosis of *Pneumocystis jirovecii* pneumonia (PJP). With the dramatic reduction in the occurrence PJP in the current era of highly effective antiretroviral therapy, many centers no longer collect induced sputum samples. Thus, the diagnosis of PJP requires bronchoalveolar lavage (BAL) specimens or a decision to treat the patient empirically without a definitive diagnosis. Sputum or BAL specimens are tested for *P. jirovecii* using special stains or molecular assays, which require highly trained staff that may not be available with a rapid turnaround time. Given the invasive nature of collecting BAL specimens and the expertise needed for interpreting PJP test results, there is interest in using serum 1,3- β -D-glucan (BDG) testing for the diagnosis of PJP. In this point-counterpoint, Luis Ostrosky-Zeichner and Gabriela Corsi-Vasquez discuss the pro view of using BDG testing for the diagnosis of PJP, while Paul E. Sax and Edward F. Pilkington III present the con view of using BDG testing for the diagnosis of PJP.

KEYWORDS β -D-glucan, PJP

POINT

β -Glucans are a class of polysaccharides consisting of D-glucose units that are polymerized primarily via β -1,3 glycosidic bonds, in addition to β -1,4 and/or β -1,6 bonds. They are present in various food products, such as cereals, mushrooms, and seaweeds, and are known for their numerous effects on the human body (1). 1,3- β -D-Glucan (BDG) is also the common cell wall constituent of most pathogenic fungi, including *Pneumocystis jirovecii*, and as such, it has become a useful biomarker for some invasive fungal diseases (IFDs). Detection of serum levels of BDG has become a useful tool in the diagnosis and therapeutic monitoring of invasive fungal infections due to select yeasts, some molds, and dimorphic fungi, although most of the data have focused on pulmonary aspergillosis and candidiasis (2, 3). The first assay to detect BDG was developed in Japan in 1985, and currently several commercial kits from different countries are available. The FDA has approved the Fungitell assay (Associates of Cape Cod, USA), which is widely used in the United States, as well as in Canada and in Europe. The utility of the BDG assay in the diagnosis of IFD has been extensively reviewed in general populations, with a pooled sensitivity of 76.8% and a specificity of 85.3%; however, its use for diagnosing IFD in HIV patients may require further study (4).

The genus *Pneumocystis* comprises highly diversified and ubiquitous fungal species that cause severe pneumonia (5). *Pneumocystis jirovecii* pneumonia (PJP) classically develops in immunocompromised hosts with AIDS, prolonged corticosteroid exposure, or other disorders of T-cell function (3). Standard PJP diagnosis requires visualization of *P. jirovecii* cysts by microscopic examination of respiratory secretions, such as bron-

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choalveolar lavage (BAL) fluid or lung biopsy samples. For a definitive diagnosis, both techniques require invasive procedures that are not feasible for all patients (4).

Clinical trials of BDG assay performance for the diagnosis of PJP are limited, but various meta-analyses of clinical evaluations exist. Overall, sensitivity is high, and BDG negativity can also be used to exclude PJP, although false-negative results have been noted (6). Fujii et al. in 2007 first evaluated the use of testing for BDG in serum for PJP diagnosis in 32 patients with HIV/AIDS. The authors found a sensitivity of 97% for the use of the BDG assay to diagnose PJP and suggested that increasing the cutoff value for the diagnosis of PJP might improve the specificity of the test (7). Watanabe et al. also reviewed 111 patients with HIV and confirmed PJP infection by testing BAL fluid specimens and evaluated the BDG level. The median serum level of BDG among all patients with PJP (174.8 pg/ml) was significantly higher than that among the control patients (8.2 pg/ml). The authors found a sensitivity of 96.4% and a specificity of 87.8% in the diagnosis of PJP (8).

Desmet et al. studied the BDG assay as a diagnostic tool of PJP in 16 HIV patients. The mean BDG concentration was 1,496 pg/ml in HIV-infected patients with PJP. When the proposed cutoff value of 80 pg/ml was used, the sensitivity was 100% and the specificity was 89.3%. Specificity increased to 96.4% when a BDG cutoff value of 100 pg/ml was used (9).

According to a meta-analysis published by Karageorgopoulos et al. in 2013, the average sensitivity of BDG testing for the diagnosis of PJP was reported as 94.8%, and the average specificity was 86.3%. This modest specificity in a population with low prevalence of the disease will incur an increased rate of false-positive results, for which the authors recommend careful interpretation of BDG test results in the presence of factors likely to produce a false-positive result, such as the use of cellulose membranes for hemodialysis, application of glucan-containing gauzes on mucosal or serosal surfaces, administration of blood products produced through cellulose filters, presence of Gram-negative endotoxemia, and use of certain antimicrobials, such as cephalosporins and β -lactam- β -lactamase inhibitor combinations (10).

A study by Son et al. in 2017, which evaluated the performance of BDG testing in the diagnosis of PJP pneumonia compared to other IFDs in 136 patients (including 50 patients with PJP), showed that at a cutoff value of >80 pg/ml, as recommended by the manufacturer, the assay had a sensitivity of 74% and a specificity of 75% in the diagnosis of PJP pneumonia (11).

These studies show that BDG testing may be used as a diagnostic adjunct for PJP in HIV-infected individuals but that adjustments of the cutoff values depending on the geographic areas and degree of immunodeficiency may be needed to optimize sensitivity and specificity (4). As a greater cutoff value is favored, those individuals with higher fungal burdens and presumably more clinically significant disease will be detected and the rate of false-positive results will decrease. Beyond relying on the BDG assay's sensitivity, one can rely on the negative predictive value (NPV), which often exceeds $>99\%$, to rule out the disease and de-escalate empirical therapy (12).

Among the limitations of the BDG assay, the rate of false-positive results at the manufacturer-recommended cutoff value for positivity of 80 pg/ml can be observed with the use of cellulose-containing hemodialysis filters, therapy with immunoglobulins or albumin, and glucan-containing surgical gauzes. After exposure to glucan-containing surgical gauze or sponges, BDG levels are expected to return to normal within 3 to 4 days. In this setting, repeating the BDG test may give some insight if a decreasing value is observed (13). The approach to the interpretation of the test will be influenced based on the patient's presentation and whether the previously mentioned conditions were present. In such cases, choosing a higher cutoff value can help identify those patients with clinically significant disease.

Colonization of the airways by other fungi is another cause for elevated BDG concentrations, as this is a component common to multiple fungal species, such as *Candida* spp., *Fusarium* spp., and *Aspergillus* spp., in addition to *Pneumocystis*. This can be misleading in symptomatic patients who are colonized; however, the clinical context

and the presence or absence of risk factors more specific to other types of fungal infections need to be considered (6).

The possibility of infection with other fungal species is a common problem for AIDS patients, as it may yield a positive BDG test result when the patient does not have PJP but instead a different underlying fungal infection, such as histoplasmosis or candidiasis (10). However, we postulate that in the right clinical context (i.e., a patient with AIDS with typical pulmonary and laboratory findings) and in patients for whom a reasonable effort has been made to rule out other fungal infections, BDG testing can be used as an adjunct to diagnose PJP.

In summary, in the correct clinical context, BDG testing is a helpful diagnostic adjunct to diagnose and to rule out PJP in AIDS patients. It is not an absolute diagnostic tool, but when faced with the inability to perform invasive procedures for a definitive diagnosis of PJP, the use of the BDG assay can help identify those patients more likely to have PJP. As most studies have shown, certain adjustments to the test's cutoff value might be needed, as raising the cutoff value resulted in an increased specificity of the test in these studies, depending on the degree of immunosuppression and expected fungal burden of the host.

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COUNTERPOINT

Consider the following clinical scenario. A 27-year-old female patient with a history of Crohn's disease who had recently been treated with a prolonged course of high-dose corticosteroids is admitted to the hospital with acute onset of fevers and severe abdominal pain. A computed tomography (CT) scan of her abdomen and pelvis reveals a perforation in her ascending colon with associated intra-abdominal fluid collection. She is started on broad-spectrum antibiotic therapy with intravenous cefepime and metronidazole and is taken to the operating room for abdominal washout and colonic resection with primary anastomosis. The patient's fever persists postoperatively, and on

hospital day 5, she develops recurrent emesis and acute-onset hypoxia. A CT of her chest, abdomen, and pelvis reveals bilateral ground-glass pulmonary opacities, findings consistent with a small bowel obstruction and multiple intra-abdominal fluid collections. A serum β -D-glucan (BDG) assay is performed as part of the workup for the patient's fevers, ground-glass opacities, and hypoxia and returns with a positive result at 120 pg/ml.

The case noted above—which describes a patient with a number of potential causes for an elevated serum BDG level, including recent serosal exposure to surgical gauze, the likely presence of *Candida* spp. within her intra-abdominal fluid collections, and potential *Pneumocystis jirovecii* pneumonia (PJP) due to prolonged high-dose corticosteroid use—highlights the primary limitation of the serum BDG assay as a diagnostic test for PJP: its lack of specificity. As we will demonstrate in this counterpoint, the test too often yields a false-positive result. This is especially common in patients with a low pretest probability for PJP, a scenario commonly encountered in many institutions (1). We will also illustrate some specific clinical settings where use of this test may be helpful when considering a diagnosis of PJP.

Serum enzyme immunoassay kits that assess for the presence of (1,3) β -D-glucan, a polymer of glucose found in the outer cell wall of many pathogenic fungi (2), have been lauded as an ideal method for diagnosing PJP as they do not require patient participation or invasive procedures for sample collection. Although elevated levels of serum BDG strongly correlate with the presence of PJP in both HIV-infected and non-HIV-infected patients (3, 4), the currently available BDG assays lack the specificity needed to establish a diagnosis of PJP with this test alone.

Three meta-analyses have now evaluated the clinical performance of the serum BDG assay in the diagnosis of PJP (5–7). These studies demonstrated that serum BDG assays reliably detected the presence of PJP, with a range of pooled sensitivities of 91 to 96% between the three meta-analyses. These studies also revealed the greatest weakness of the serum BDG assay: its low specificity. The pooled specificities between the three meta-analyses ranged between 75 and 86%. Importantly, two of these meta-analyses excluded patients with known invasive fungal infection from the analysis, increasing the specificity for PJP. One of the meta-analyses also found that specificity declined to 73% when only non-HIV-infected patients were included (7).

Studies not included in these meta-analyses further expose the low specificity of BDG testing for a diagnosis of PJP. A study from a tertiary-care center in South Korea tested available patient and volunteer blood samples for the presence of BDG, including samples from 50 patients with documented PJP, and demonstrated a specificity of 74% using the recommended upper limit of the normal cutoff value of 80 pg/ml (8). An AIDS Clinical Trials Group study of patients presenting with respiratory symptoms found that the specificity of this test for diagnosing PJP was 75% (9).

These consistently low diagnostic specificities should certainly raise concerns about the diagnostic value of the serum BDG test for diagnosing PJP. Use of this test alone undoubtedly leads to many false-positive results and has been shown to trigger inappropriate antibiotic use (1). Ascribing an elevated BDG level to PJP may also lead to further progression of a misdiagnosed and untreated process, often in patients who are immunocompromised and/or have critical illness.

Many infectious and noninfectious processes increase BDG levels. Systemic infections by almost all clinically relevant fungal organisms, except *Zygomycetes* and, in most cases, *Blastomyces* and *Cryptococcus*, are associated with elevated levels of BDG, as it is a component of the cell wall of many fungi (10, 11). It is for this reason that BDG is colloquially referred to as a “pan-fungal marker.”

Interestingly, elevated BDG levels are not exclusively seen in infections due to fungal organisms; they have also been demonstrated in patients with *Streptococcus pneumoniae*, *Alcaligenes faecalis*, and *Pseudomonas* bacteremia who were without other known causes of BDG assay reactivity (3, 12).

Several products and procedures may also increase serum BDG levels without any underlying infection. Examples include exposure of serosal surfaces to certain types of

surgical gauze, hemodialysis performed with cellulose membranes, and the administration of intravenous immunoglobulin, albumin, and other blood products exposed to cellulose (3, 13–17). Since many of these exposures occur in patients who are at risk for both PJP and other systemic fungal infections, they are the source of considerable diagnostic uncertainty for clinicians.

Ironically, certain antibiotics test positive for BDG when diluted or solubilized to reconstituted-vial concentrations. Examples include colistin, ertapenem, cefazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam. This may not be clinically relevant, as no reactivity of the BDG assay was demonstrated when these antibiotics were diluted to the usual maximum plasma concentrations (18). Findings from a subsequent prospective study do, however, suggest that false-positive serum BDG assay results may be produced in patients receiving standard therapeutic doses of ampicillin-sulbactam (19). Thirty-seven of 117 serum samples from 15 patients receiving ampicillin-sulbactam in this study had a positive serum BDG assay. None of the 15 patients had invasive fungal infection, recent hemodialysis using cellulose membranes, or administration of intravenous albumin, immunoglobulin, or other products prepared using cellulose depth filters. Five of the 15 patients had undergone surgical debridement of a diabetic foot infection, though only 6 of 40 serum samples from these patients had serum BDG levels of >80 pg/ml (19).

Given the multitude of causes beyond PJP that can lead to elevations in serum BDG, it is not surprising that a single center cross-sectional study of 46 intensive care unit (ICU) patients revealed no significant difference in serum BDG levels between ICU patients with confirmed fungal infections and those with bacterial infections (20). A positive BDG test result in a critically ill patient with potential infection by, or exposure to, any of the above-named organisms or factors should therefore be interpreted with caution.

It has, however, been our experience that it is in these types of clinical scenarios, namely, immunocompromised patients with critical illness, in which serum BDG testing is commonly done: the patient with a recent solid organ transplant and a complicated postoperative course who develops acute respiratory distress syndrome, the patient receiving treatment for a hematologic malignancy who develops septic physiology and shows abnormalities on a CT scan of the chest, the critically ill patient with hepato-renal syndrome who is dialysis dependent and develops fever and hypoxia and is found after imaging to have bilateral pulmonary opacities, and the patient receiving immunosuppressive therapy for connective tissue disease who develops flu-like symptoms and altered mental status and is found to have imaging consistent with atypical pneumonia. Each of these clinical scenarios has a broad differential diagnosis which includes PJP. Each scenario also lends itself to the possibility that the patient may have recently received treatments or acquired an infection by other organisms which contain BDG. It is therefore inappropriate to rely solely on a positive serum BDG test result to make the diagnosis of PJP.

There are, however, situations in which a serum BDG test may be useful when considering a diagnosis of PJP. The high sensitivity that has previously been consistently demonstrated for this assay renders it an effective means of screening for PJP in the appropriate clinical setting, i.e., with patients who are not critically ill and in whom there is low-to-moderate suspicion for PJP. A negative serum BDG test in this setting would strongly support a diagnosis other than PJP. It should, however, be noted, that a recently published retrospective analysis of the serum BDG assay's performance with over 400 hospitalized patients with malignancy and unexplained lung infiltrates revealed a sensitivity of only 69.8% when the manufacturer's threshold of >80 pg/ml was used and when PJP PCR performed on bronchoalveolar lavage fluid, bronchial washings, or lung tissue was employed as the reference method (21). These findings suggest that the assay should not be used as a stand-alone means for ruling out PJP in immunocompromised patients with pneumonia.

Prior studies have demonstrated that many patients with PJP have a serum BDG level that is well above the standard cutoff of the commonly used assays. Koo et al.

found that 71% of 14 patients with probable PJP had a serum BDG level of >500 pg/ml (3). Sax et al. found that 45% of 173 study participants with HIV and PJP had a serum BDG level of >500 pg/ml (4). An argument may therefore be made for utilizing serum BDG assays with higher cutoff values for diagnosis in select patients when there is both strong clinical suspicion and an appropriate level of pretest probability for PJP. It should, however, be noted that between 14 and 36% of patients in these two studies with serum BDG levels of >500 pg/ml did not have strong evidence of PJP, which suggests that the assay continues to lack the appropriate level of specificity even when the cutoff level for diagnosis is significantly increased. It has, however, recently been demonstrated that the specificity of the serum BDG test can be optimized to 100% in patients with a clinical picture consistent with PJP when the BDG level is >200 pg/ml and is followed up with a positive PJP PCR test (21).

Finally, the serum BDG assay itself may also have an important use as an adjunct test when used in tandem with *P. jirovecii* qualitative PCR (qPCR). Damiani et al. found that concurrent use of both low and high *P. jirovecii* qPCR cutoff values for bronchoalveolar lavage (BAL) samples resulted in this method achieving both 100% sensitivity and 100% specificity in the diagnosis of PJP. They also found that the serum BDG level was useful in determining whether indeterminate levels of *P. jirovecii* qPCR—those that fell between the high and low cutoff levels—in BAL fluid samples represented airway colonization versus true PJP (22).

In conclusion, the serum BDG assay lacks the specificity needed to make a diagnosis of PJP with this test alone. Positive serum BDG assays should be interpreted with caution, especially for critically ill patients with possible exposure to, or infection by, the many known additional causes of elevated BDG levels. Except in settings with a high pretest probability of PJP and a BDG level of >500 pg/ml, clinicians should not rely on serum BDG to make the diagnosis of PJP.

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SUMMARY

Points of agreement

1. BDG is a sensitive screening test for PJP when an invasive procedure is not possible. A positive result is most useful when the BDG value is >500 pg/ml in a patient with a high pretest probability of PJP.
2. A negative BDG result strongly supports a diagnosis other than PJP in patients who are not critically ill and for whom there is low-to-moderate suspicion of PJP.
3. The major limitation of the test is a low specificity. Causes of false-positive results include cellulose-containing hemodialysis filters, therapy with immunoglobulins, albumin, or other blood products exposed to cellulose, exposure of serosal surfaces to certain types of surgical gauze, and therapy with multiple antimicrobials, including cephalosporins and β-lactam–β-lactamase inhibitor combinations.
4. Increasing the cutoff value of the BDG test (e.g., from 80 pg/ml to 100 pg/ml) increases the specificity of the test, with little impact on the sensitivity.
5. BDG results are positive in patients with systemic infections due to most fungi, except for *Zygomycetes*, *Cryptococcus*, and *Blastomyces dermatitidis*.

Issues requiring further consideration

1. Determine the optimal cutoff of the BDG test to maximize specificity without compromising sensitivity.
2. Assess how the presence of other fungal infections or colonization with other fungi (e.g., *Candida* species or *Aspergillus* species) impacts the performance characteristics of BDG testing in the diagnosis of PJP.
3. Better define the role of the BDG assay as an adjunct test when used in combination with PJP PCR testing.

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