Review

BETA-D-GLUCAN TESTING IN PATIENTS WITH FUNGAL ENDOPHTHALMITIS

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Purpose: To review the literature on beta-D-glucan (BDG) testing in fungal endophthalmitis.

Methods: Review of primary literature using PubMed through April 2017 and presentation of an illustrative case report. A total of 231 articles were identified and 43 were ultimately chosen for review based on relevance and presence of ophthalmologic examination and objective data.

Results: Beta-D-glucan is a major component of fungal cell walls. It is quantified using a calorimetry-based Fungitell assay based on modification of the limulus amebocyte lysate. Serum BDG levels are commonly used clinically in conjunction with other tests for early surveillance and diagnosis of invasive fungal infections. In the ophthalmic literature, elevated levels of BDG have been detected in vitreous fluid of patients undergoing vitrectomy for fungal endophthalmitis, tear fluid of patients with mycotic keratitis, and serum of a patient with bilateral endogenous subretinal abscesses. Elevated serum BDG levels appear to be highly associated with fungal endophthalmitis. Potential uses and considerations with regards to test limitations are discussed.

Conclusion: Beta-D-glucan testing may be used as an adjunct to support a diagnosis, initiate pharmacologic therapy or surgical intervention, and optimize overall clinical management in patients diagnosed with or under clinical suspicion for invasive fungal infections, including endophthalmitis. Additional clinical studies are necessary to fully characterize the utility of BDG testing in patients with fungal endophthalmitis.

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Endogenous fungal endophthalmitis is a relatively rare but potentially devastating ophthalmic condition with visually detrimental outcomes.[1] Most patients who develop this disease are immunosuppressed and have significant systemic risk factors for invasive fungal infections such as hematologic malignancy, HIV/AIDS, recent hospitalization or critical illness, and history of solid organ or stem cell transplantation.[2,3] The most commonly reported organisms responsible for endogenous fungal endophthalmitis are *Candida* (primarily *Candida albicans*) and *Aspergillus* species. Diagnosis of endogenous fungal endophthalmitis can be challenging and is based primarily on a careful clinical examination and suspicion, consideration of potential risk factors, and supportive testing.[1] Definitive diagnosis necessitates a positive culture of vitreous fluid obtained either through a vitreous tap-and-inject procedure performed in the clinic or analysis of a vitreous sample obtained during vitrectomy. This aids in rapid diagnosis and initiation of most appropriate pharmacologic therapy either systemic (oral or intravenous) and/or intravitreal.

Although blood or tissue cultures and histology are the gold standard for diagnosing systemic invasive fungal infection, a number of ancillary tests are used to

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aid in the diagnosis.[4] These tests are important clinically given fungal organisms are often not grown in blood cultures despite evidence of disseminated or invasive infection. For example, *Aspergillus* is rarely grown from blood cultures, and rates of candidemia in patients with disseminated candidiasis are only around 50%.[5] Ancillary tests are also useful in clinical settings where tissue is unable to be obtained safely for pathology and culture, or diagnosis is delayed from time of fungemia. Clinical presentation and radiographic findings in patients with invasive fungal infections is often nonspecific, leading to challenges in diagnosis without culture or histologic data.[6]

One such ancillary test is the quantification of serum β-D-glucan (BDG) levels. Beta-D-glucan is a polysaccharide cell wall component found in many fungal species and is released into the bloodstream during an invasive fungal infection.[7] Serum BDG testing has begun to be used more routinely within the immunocompromised population as a means of early surveillance and diagnosis of invasive fungal infections in conjunction with clinical and radiographic data, and may be useful in the monitoring of clinical response to systemic antifungal therapy.[8–10] This test may especially be useful for species such as *Aspergillus* that tend not to grow out from blood cultures despite the organism's angioinvasive characteristic. In fact, serum BDG has been shown to have a higher sensitivity for detecting invasive fungal infection than blood cultures.[11] Several large-scale studies also have shown that serum BDG levels can be elevated up to 5 days to 10 days before detection of fungemia based on positive blood cultures, and that very high serum levels (>400 pg/mL) may be associated with severe sepsis and mortality in intensive care unit patients at risk for intra-abdominal candidiasis.[9,12] Negative BDG testing can also potentially help avoid the toxicities of empiric antifungal therapy or invasive diagnostic procedures in cases of low clinical suspicion. Although many studies within the infectious disease literature have assessed the utility and diagnostic accuracy of BDG testing primarily within immunocompromised populations at high risk of invasive fungal infection,[8–10] BDG testing is seldom mentioned in the ophthalmologic literature[1,2,13] and may, therefore, be clinically underused in cases suspicious for fungal endophthalmitis.

The patient population who will benefit from early and accurate diagnosis of invasive fungal infections, including fungal endophthalmitis, is growing. These infections are a major cause of morbidity and mortality among solid organ transplant recipients, with systemic infections most often caused by *Candida*, *Aspergillus*, and *Cryptococcus*.[14] Based on examination of recent solid organ transplant literature, fungemia may account for up to 10% of all bloodstream infections within this population.[15] Liver transplant recipients are at the highest risk for developing invasive fungal infection (5%–42%), which is associated with a mortality rate of 25% to 71%.[16] Invasive fungal infections are also of significant concern and high mortality within the allogeneic stem cell transplant and hematologic malignancy population, where chemotherapeutic agents, prolonged neutropenia, steroid use, and graft versus host disease all contribute to increased risk.[17,18] The number of allogeneic stem cell and solid organ transplants within the United States continues to increase (https://optn.transplant.hrsa.gov/news/more-than-30-000-transplants-performed-annually-for-first-time-in-united-states/. Accessed September 2, 2017). A growing number of patients are also receiving biologic and immunosuppressive medications for rheumatologic diseases that can predispose them to certain fungal infections.[19] In addition, a recent outbreak of fungal endophthalmitis in intravenous drug users in New England highlights the diversity of conditions, which may predispose to fungal infections.[20] Given that earlier diagnosis and treatment initiation of fungal endophthalmitis results in better long-term visual outcomes,[21] the incorporation of nonculture-based methods to facilitate early diagnosis of invasive fungal infections should be a goal of ophthalmologic practice.

In the following, we review the literature on the use of BDG testing in ophthalmology. Furthermore, we discuss testing recommendations, limitations, and potential future applications. As an illustrative example, we describe a case of a 19-year-old man who presented with a panuveitis of the left eye in whom we used serum BDG testing to aid in the diagnosis of fungal endophthalmitis.

Case Presentation

A 19-year-old white man presented with several weeks of left eye redness, tearing, photophobia, and decreased vision. His medical history included intravenous drug abuse with last exposure 7 months prior, hepatitis C, and left forearm cellulitis 7 months to 8 months prior. The patient appeared well, and there was no history of immunosuppression, autoimmune disease, or animal exposure. Review of systems was otherwise negative.

On presentation, visual acuity and intraocular pressures were 20/20 and 14 mmHg in the right eye and 20/400 and 8 mmHg in the left eye, respectively. There was no afferent pupillary defect, and extraocular motility and visual field testing were normal. Examination of the right eye was normal, whereas that of the

left eye revealed 3+ diffuse conjunctival injection, 3+ anterior chamber cell/flare without hypopyon, 3+ vitreous cell/haze with scant vitreous opacities, and a peripheral hyperpigmented scar without active retinitis or associated hemorrhages. Left eye B-scan ultrasonography revealed dense membranous vitreous opacities overlying the macula (Figure 1A).

Considering infectious versus inflammatory panuveitis of the left eye, the patient was started on topical prednisolone acetate 1% every hour and atropine 1% twice per day. Hematologic, infectious, inflammatory, and radiologic work-up was negative. Based on serial clinical examinations, there was a clinical suspicion for fungal endophthalmitis, but there was no history of immune suppression, no known systemic fungal infection, no recent hospitalization, and no reported history of recent intravenous drug abuse. Blood cultures were drawn, and serum BDG as well as serum histoplasmosis and cryptococcus antigen testing was performed. All were negative except for elevated serum BDG levels (428 pg/mL; normal, 0–79 pg/mL) strongly suggesting fungal endophthalmitis. At this time, the patient's left eye visual acuity decreased to counting fingers. After additional questioning, he reported using crack cocaine 4 months before presentation.

The patient promptly underwent a diagnostic and therapeutic pars plana vitrectomy (PPV) and received an intraoperative intravitreal injection of amphotericin B 5 μg/0.1 mL. Postoperatively, systemic voriconazole therapy was initiated. An additional intravitreal injection of amphotericin B 5 μg/0.1 mL was administered 2 days after the PPV as vitreous cultures grew out *Candida albicans* thereby confirming a diagnosis of fungal endophthalmitis. Despite a systemic work-up by the infectious disease consult service, a primary source of fungal infection was never identified.

One week after the initial PPV, repeat B-scan showed dense vitreous opacities and focal peripheral temporal elevation consistent with probable retinal detachment (Figure 1B) for which the patient underwent a PPV, membrane peel, endolaser, C_3F_8 gas, and intravitreal injection of voriconazole 50 μg/0.1 mL because of concern for possible resistance to amphotericin B. Intraoperatively, the retinal detachment was confirmed to be traction-induced from a fibrotic fungal lesion in the temporal periphery. Voriconazole was injected into the fluid-filled eye, the concentration was allowed to equilibrate, and then the fluid–gas exchange was performed, leaving 20% fluid in the vitreous cavity. This previously described method allows the injectable to attain appropriate concentration within the fluid phase.[22] One month after the tractional retinal detachment repair, the vision remained at counting fingers and the patient was found to have a complex, total retinal detachment. The inferior retina contracted from proliferative vitreoretinopathy with complete disinsertion of the retina from the pars plana for the inferior 6 clock hours (Figure 2A). The patient underwent PPV, membrane peel, endolaser, perfluorocarbon, and 5,000 centistoke silicone oil instillation (Figure 2B). Four months later (approximately 6 months after initial presentation), the retina remained attached and the patient's visual acuity improved to 20/200 with a 2 to 3+ posterior subcapsular cataract.

Methods of Literature Search

PubMed literature search was conducted for all years through April 2017 using the following combination of terms: "(1,3)-β-D-glucan," "β-D-glucan," or "BDG" AND "sepsis," "fungus," "fungal," "fungemia," "eye," "vision," "retina," "retinal," "vitreous," "endophthalmitis," or "ophthalmology." Additional pertinent articles were identified using the "related citations" link in PubMed and reference lists of selected articles. A total of 231 articles were identified and 43 were ultimately chosen for review based on relevance and presence of ophthalmologic examination and objective data. English abstracts were used for non-English articles when available.

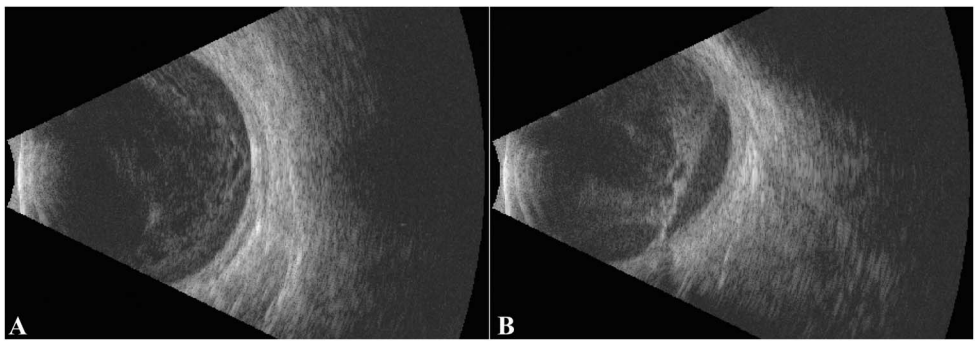

Fig. 1. B-scan ultrasonography. **A.** Dense membranous vitreous opacities overlying the macula were evident at presentation. **B.** Dense vitreous opacities and focal peripheral temporal elevation consistent with probable retinal detachment were found 1 week after the initial PPV.

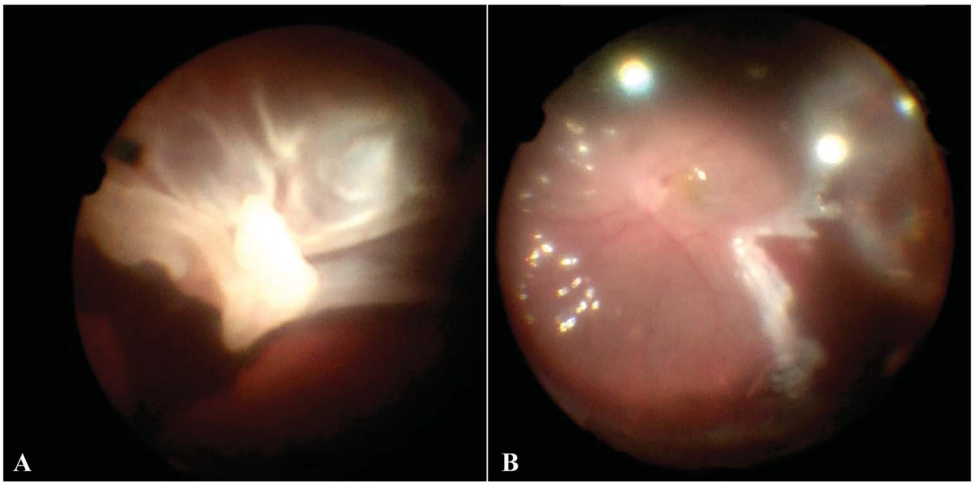

Fig. 2. A. Intraoperative view of recurrent, complex total retinal detachment. The inferior retina contracted from proliferative vitreoretinopathy with complete disinsertion of the retina from the pars plana for the inferior 6 clock hours. **B.** Intraoperative view at the end of surgery requiring PPV, membrane peel, endolaser, perfluoron, and 5,000 centistoke silicone oil instillation.

Beta-D-Glucan: Background and Testing Details

(1,3)-BDG is a major polysaccharide component of fungal cell walls.[7] There are three assays approved for clinical use, with the Fungitell assay (Associates of Cape Cod, East Falmouth, MA) as the only test approved by the US Food and Drug Administration. This assay quantifies serum BDG levels for a host of fungal species such as *Candida*, *Aspergillus*, *Fusarium*, and others (http://www.acciusa.com/pdfs/accProduct/Fungitell_multilang_pisheets/Fungitell%20Insert%20 EN.pdf. Accessed September 10, 2016). However, not all pathogenic fungal organisms produce BDG (or produce only low levels), and consequently the assay cannot be used to reliably diagnose certain infections including those caused by *Cryptococcus*, *Zygomycetes* such as *Mucor* and *Rhizopus*, and the endemic fungi *Blastomyces dermatitidis*.

The Fungitell assay is an enzymatic-based colorimetric assay that takes advantage of a modification of the limulus amebocyte lysate for serum quantification of BDG (Figure 3). The serum samples are first treated with an alkaline reagent to convert BDG into a single stranded form. Beta-D-glucan binds to the alpha subunit of factor G, a horseshoe crab coagulation factor, and activates the serine protease zymogen beta subunit, which in turn activates a clotting enzyme that converts coagulogen to coagulin.[7] Then, Boc-Leu-Gly-Arg-p-nitroanilide is cleaved by the clotting enzyme, which releases para-nitroaniline, a chromogenic substance that is measured calorimetrically with absorbance at 405 nm.

Beta-D-glucan quantification can be performed in any setting that can use the following equipment. In addition to the Fungitell kit, which includes all the necessary reagents and buffer solutions, required materials and equipment include pipette tips and pipettors, test tubes, storage tubes, an incubating plate reader capable of dual wavelength monitoring (at 405 and 490 nm), and a computer equipped with kinetic assay software (http://www.acciusa.com/pdfs/accProduct/Fungitell_multilang_pisheets/Fungitell%20Insert%20 EN.pdf. Accessed August 7, 2017).

The overall sensitivity, positive predictive value, specificity, and negative predictive values of the Fungitell assay for diagnosis of invasive fungal infection are reported as 64.3, 91.1, 86.6, and 74.1%, respectively (http://www.acciusa.com/pdfs/accProduct/Fungitell_multilang_pisheets/Fungitell%20Insert%20 EN.pdf. Accessed September 11, 2016), based on a multicenter study of the test in patients with proven or probable invasive fungal infection. Intra- and inter-assay variations range from 0.9% to 28.9% and 3.9% to 23.8%, respectively. The cutoff values in current use, which were found to provide the greatest diagnostic accuracy, are the following: $<$60 pg/mL are "negative," 60 pg/mL to 79 pg/mL are "indeterminate" suggesting possible invasive fungal infection, and those $\geq$80 pg/mL are "positive." Depending on the positive and negative cutoff values chosen for BDG level, various other studies reported wider ranges of these values: 64.4% to 100% sensitivity, 10.4% to 90.0% positive predictive value, 10.4% to 89.0% specificity, and 75.1% to 100% negative predictive value (http://www.medscape.com/viewarticle/752221. Accessed September 11, 2016). A meta-analysis of 2,979 patients combined from 16 studies found an overall sensitivity of 76.8% (95% confidence interval, 67.1%–84.

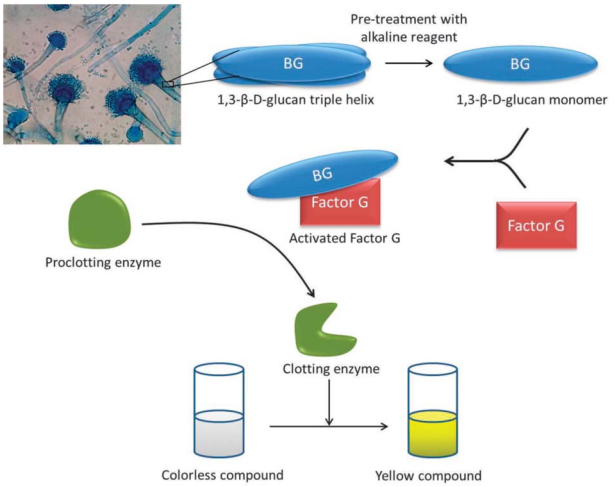

Fig. 3. Mechanism of beta-D-glucan biochemical calorimetric reaction (permission granted by College of American Pathologists).

3%) and specificity of 85.3% (95% confidence interval, 79.6%–89.7%) for diagnosing invasive fungal infection.[8]

Beta-D-Glucan Testing in Ophthalmology

In the ophthalmic literature, elevated levels of BDG have been detected in vitreous fluid of patients undergoing vitrectomy for fungal endophthalmitis,[23] tear fluid of patients with mycotic keratitis,[24] and serum of a patient with bilateral endogenous subretinal abscesses (Table 1).[25] Larger scale retrospective studies have found elevated serum BDG levels to be one of the most frequent clinical characteristics associated with endogenous fungal endophthalmitis.[26–28]

Fungal Endophthalmitis and Chorioretinitis

Shimbo et al[23] quantified BDG levels in the vitreous fluid of 26 patients in an attempt to establish the utility of this test in the diagnosis of fungal endophthalmitis. They showed high vitreous BDG concentration in two patients with fungal endophthalmitis, and below threshold (10.0 pg/mL) levels in all but one patient without fungal endophthalmitis. Based on these results, the authors concluded that vitreous BDG levels may be a useful adjunctive to vitreous culture in the diagnosis of fungal endophthalmitis.

Tanaka et al[26] reported 46 patients (79 eyes; 74% males; mean age, 57.2 years; mean follow-up, 3.5 years) with endogenous fungal endophthalmitis encountered over a 12-year period. Intraocular fungal infiltration was as follows: budding in the vitreous cavity (40%), vitreous opacity only (29%), vitreous opacity with retinal detachment (18%), and chorioretinal involvement (13%). Twenty-six eyes (33%) underwent PPV with six (23%) classified as "postoperatively blind" (i.e., light perception vision) at final visit. The authors identified the following as characteristics of patients with fungal endophthalmitis: serum BDG level >20 pg/mL (90%), receipt of intravenous hyperalimentation (87%), fever ≥38°C (76%), major operation (76%), male gender (74%), history of cancer (72%), neutropenia of <500/mm^2 (67%), and *Candida* detection system (an antigen-based test) >4 units (57%). The study reported the combination of elevated serum BDG level and fever as associated with the highest diagnostic rate of endogenous fungal endophthalmitis.

Takebayashi et al[27] followed 58 patients (103 eyes; 69% male; mean age, 58.2 years; mean follow-up, 3.8 years) with endogenous fungal endophthalmitis in an attempt to identify a relationship between stage of fungal disease at initial examination and prognosis. They showed that stage of endogenous fungal endophthalmitis at presentation was associated with final visual acuity. Similar predisposing factors were identified as in the study by Tanaka et al,[26] with serum BDG levels >20 pg/mL measured in 95% of patients. Vitreous levels of BDG were measured in two patients (both with serum BDG values >20 pg/mL) and were <20 pg/mL in both cases, with the authors commenting on the difficulty in interpreting BDG vitreous levels given normal values are not known.

Nagao et al[28] diagnosed ocular candidiasis in 27% of patients (54/204) with *Candida* fungemia. Using multiple regression analysis, they showed that these 54 patients with ocular candidiasis were significantly more likely to be fungemic with *Candida albicans* species (odds ratio = 3.68) and have elevated serum BDG levels (odds ratio = 9.99).[28] However, the authors noted that BDG levels did not correlate with the etiologic fungal organism (i.e., *Candida* species) or prognosis of *Candida* fungemia in terms of mortality.

De Pascale et al[29] described three patients admitted to the intensive care unit with candidemia and BDG levels >500 pg/mL. They were started on appropriate antifungal therapy with systemic echinocandins, all central and peripheral lines were removed, and cardiac and ophthalmologic evaluations were performed. Despite these measures, BDG levels did not decrease, which prompted the authors to repeat systemic surveillance for an infectious focus as well as a dilated ophthalmologic examination. This lead to the diagnosis of fungal chorioretinitis in two patients and fungal endophthalmitis in one patient. Although the authors opined that persistently elevated BDG levels could represent a "surrogate marker of residual fungal burden," there are no current recommendations regarding interpretation of BDG levels after initiation of antifungal therapy.

Table 1. Summary of Primary Literature on Beta-D-Glucan Testing in Ophthalmology

Study	Reference Number	No. of Patients	Type of Fungal Infection	Type of Sample	Main Findings
Endophthalmitis and chorioretinitis					
Tanaka et al, 2001	26	46	Endophthalmitis	Serum	33% underwent PPV of which 23% had light perception vision at final visit Predisposing factors for fungal endophthalmitis: serum BDG >20 pg/mL (90%), intravenous hyperalimentation (87%), fever ≥38° C (76%), and others Combination of BDG level and fever had the highest "index of diagnosis"
Shimbo et al, 2002	23	26	Endophthalmitis	Vitreous fluid	High vitreous BDG concentration in two patients with fungal endophthalmitis BDG levels below threshold (10 pg/mL) levels in all but one patient without fungal endophthalmitis
Takebayashi et al, 2006	27	58	Endophthalmitis	Serum (n = 58), vitreous (n = 2)	Endogenous fungal endophthalmitis stage at presentation was associated with final vision Serum BDG >20 pg/mL in 95% Vitreous BDG levels were <20 pg/mL in 2 patients with serum level >20 pg/mL
Nagao et al, 2012	28	54	Endophthalmitis	Serum	27% of patients with *Candida* septicemia developed ocular candidiasis Ocular candidiasis associated with *Candida albicans* and elevated BDG level BDG levels did not correlate with type of *Candida* species or prognosis

Table 1. (*Continued*)

Study	Reference Number	No. of Patients	Type of Fungal Infection	Type of Sample	Main Findings
De Pascale et al, 2013	29	3	Chorioretinitis (n = 2), endophthalmitis (n = 1)	Serum	Patients with BDG >500 pg/mL and increasing BDG levels despite antifungals and line removal Repeat surveillance performed and intraocular involvement diagnosed Increasing BDG levels despite therapy may represent a "surrogate marker of residual fungal burden"
Keratitis and subretinal abscess					
Kaji et al, 2009	24	Control, fungal, bacterial keratitis (n = 4 each)	Keratitis	Tear film	Mean ± SD BDG levels in patients with fungal keratitis 184 ± 128 pg/mL before corneal scraping and >1,000 pg/mL afterward BDG levels <10 pg/mL in controls and patients with bacterial keratitis irrespective of corneal scraping
Inoda et al, 2015	25	1 (case report)	*Scedosporium prolificans* subretinal abscesses	Serum	74-year-old man on chemotherapy for acute myeloid leukemia with negative blood cultures and positive BDG levels Right eye was enucleated as vision was light perception despite antifungal and antibacterial medications Left eye received intravitreal antifungal injections and the vision stabilized at 20/200 at the 6-month follow-up

Fungal Keratitis

Kaji et al[24] quantified BDG levels in tear fluid of patients with fungal (n = 4) and bacterial (n = 4) keratitis before and after corneal scraping using the Fungitec G test (Seikagaku CO, Ltd, Tokyo, Japan). The authors showed that mean ± SD BDG levels in patients with fungal keratitis were 184 ± 128 pg/mL before corneal scraping and >1,000 pg/mL after corneal scraping. However, tear fluid BDG levels were <10 pg/mL in controls (n = 4) and in patients with bacterial keratitis irrespective of corneal scraping.

Fungal Subretinal Abscess

Inoda et al[25] reported a case of bilateral endogenous fungal (*Scedosporium prolificans*) subretinal abscesses in a 74-year-old man on chemotherapy for acute myeloid leukemia. Despite negative blood cultures, serum BDG levels were highly elevated. Vision worsened to light perception in the right eye despite initiation of antifungal and antibiotic medications, the eye was enucleated, and the fungus was isolated from culture of the subretinal lesion. The left eye received intravitreal antifungal injections, the subretinal abscess abated, and the vision stabilized at 20/200 6 months later.

Beta-D-Glucan Testing: Potential Recommendations for Clinical Use

Serum BDG testing may be useful in the diagnosis of invasive fungal infections either as an ancillary test in conjunction with additional clinical data (culture or histopathology of tissue or body fluid and radiographic data), in cases with high clinical suspicion, or where additional culture data are negative or tissue is unable to be obtained (http://www.acciusa.com/pdfs/accProduct/Fungitell_multilang_pisheets/Fungitell%20Insert%20 EN.pdf. Accessed September 11, 2016). Diagnostic testing should ideally precede initiation of empiric antifungal therapy. In patients considered particularly "high risk" for invasive fungal infection, serial serum BDG testing may be warranted.[5] A positive test should be confirmed with a second specimen due to false positive results.[30] Once antifungal therapy is initiated, subsequent serial BDG testing may be helpful in determining response to treatment. Although there are no definitive guidelines with regards to rapidity or percent change in BDG levels in relation to time after treatment initiation, the general literature consensus suggests that a trend toward decline rather than an absolute value change in BDG levels may imply appropriate treatment response.[12] For example, Sims et al[5] defined successful systemic treatment as a negative slope of the best-fit curve for serologic quantification of BDG levels. However, if the levels are stable or are increasing (i.e., positive slope), this may indicate suboptimal treatment response or prompt need for continued work-up to identify an additional source of infection.[5,29]

Beta-D-Glucan Testing: General Limitations

When interpreting results of the Fungitell BDG assay, a clinician should keep a number of important caveats in mind (http://www.acciusa.com/pdfs/accProduct/Fungitell_multilang_pisheets/Fungitell%20Insert%20 EN.pdf. Accessed September 11, 2016). A positive result is not organism or species specific and does not necessarily indicate presence of disease; therefore, it is recommended that this test be used in conjunction with blood and tissue cultures and pathology, as well as other pertinent clinical tests and findings. There are no formal guidelines with regards to BDG testing before or after initiation of fungal therapy, frequency of testing when used for surveillance of high-risk patients, or while tracking response to treatment. The effects of different antifungal agents on change in measured serum BDG levels are not known. As noted prior, BDG testing is not useful for certain fungal organisms such as *Cryptococcus*, *Zygomycetes* (*Absidia*, *Mucor*, and *Rhizopus*), and *Blastomyces dermatitidis* that either produce very low levels or do not produce BDG, and location of infection and presence of encapsulation may affect the amount of BDG produced. False negative reactions may be due to lipemic and hemolyzed blood samples. False positive reactions have been found in patients with hemodialysis, those treated with fractionated blood products, in patients exposed to glucan-containing gauze and sponges, and in those with high triglycerides or bilirubin. In addition, false positive reactions are associated with the use of certain antibiotics[31] and bacteremia with select organisms (e.g., *Streptococcus*, *Escherichia coli*, and *Pseudomonas*).[32] Finally, it is recommended that blood samples not be obtained from heel or finger stick, and data range for those <6 months of age has not been established.

Beta-D-Glucan Testing: Additional Considerations for Ophthalmology

Several additional issues currently limit the use of BDG testing in ocular fluids. Although BDG levels have been measured in pleural fluid, cerebrospinal fluid, bronchoalveolar lavage samples, and joint fluid,[33–35] the vitreous concentration of BDG neither

in healthy individuals nor in patients with fungal endophthalmitis has been established. A quantitative study of BDG levels in the vitreous might be of high clinical value in furthering the diagnostic utility of this testing modality. In hospitals and resource-limited settings where an examination by an ophthalmologist is not readily available, particularly for high-risk individuals, an elevated serum BDG level may help to guide initiation of antifungal therapy based on the patient's clinical presentation.

The minimum recommended sample volume for BDG testing is 0.5 mL; however, testing on a smaller volume of sample may be possible (personal communication, Dr. Finkelman, Associates of Cape Cod). Approximately 5 μL of actual patient sample is added to reagents in a well of a 96-well plate (total volume of each well is ~100 μL), and this is typically performed in duplicate or triplicate to obtain a reliable quantitative measurement. Therefore, total minimum volume of sample could be as low as 20 μL. Although a PPV is likely the best way to acquire a large enough sample volume for BDG quantification, it is technically possible to perform measurements on samples from an anterior chamber or vitreous tap. Additional work is needed to establish and validate testing from ocular fluids before clinical use can be recommended.

Others Tests to Diagnose Fungal Endophthalmitis

Detailed discussion of diagnostics available for detection of invasive systemic fungal disease and endophthalmitis is beyond the scope of this review. In brief, tissue and blood culture, histopathologic examination, fungal-specific stains, and serologic studies (e.g., immunodiffusion, complement fixation, and enzyme immunoassay) have been used for decades, with tissue and blood culture and histopathology considered "gold standard" for diagnosis of invasive fungal infection.[36] Other diagnostic options include fungal antigen detection, polymerase chain reaction detection and sequencing of ribosomal RNA from body fluid samples, and proteomics assays such as matrix-assisted laser desorption/ionization time-of-flight, which are used to rapidly identify fungal organisms grown in culture.

Polymerase chain reaction testing of aqueous/vitreous fluid for detection of fungal endophthalmitis has been available for approximately two decades.[37] It has high sensitivity and specificity and can provide a result in as little as 90 minutes. As the availability of fungal polymerase chain reaction testing for clinical use is currently limited to select few large university-based academic centers, BDG testing may be of high clinical utility in those centers where fungal polymerase chain reaction testing is not readily available.

Conclusion

Beta-D-glucan testing is minimally invasive and has demonstrated test characteristics and reproducibility that can be useful in the diagnosis of certain invasive fungal infections. The results can be readily obtained, which aids in prompt diagnosis and initiation of appropriate pharmacologic treatment. As a serologic test, BDG has been extensively reported and validated in the infectious disease literature in early surveillance and diagnosis of fungal infections, particularly in patients with hematologic malignancy, critical illness, and the allogenic stem cell and solid organ transplant populations.[9,38,39] It may be useful in the monitoring of clinical response and may lead to a quicker diagnosis and management plan for fungal endophthalmitis. Results of this test must be interpreted in light of the fact that there are no established guidelines for initiation, frequency, or duration of BDG testing, and more limited data on testing of ocular fluids (compared with serum). In addition, there are a number of factors that may limit the utility of this test and/or result in false positive or negative results. Future clinical studies are necessary to fully characterize the utility of BDG testing in patients with fungal endophthalmitis.

As illustrated by our case presentation and based on the overall review of literature, we encourage the use of this test as an adjunct for rapid and early clinical assessment in patients with suspected fungal endophthalmitis. If interpreted within the context of the clinical scenario, this test has the potential to lead to a quicker diagnosis and management plan for fungal endophthalmitis. A positive BDG may support a prompt diagnostic vitrectomy, systemic workup, and initiation of antifungal therapy. Finally, in those patients with a high clinical suspicion for fungal infection in light of negative blood or vitreous cultures (i.e., false negatives), BDG testing may provide guidance for optimization of clinical management.

Key words: beta-D-glucan, fungal endophthalmitis, vitreous, ophthalmology.

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