β-D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis

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We aimed to assess the accuracy of measuring serum or plasma (1→3)-β-D-glucan (BDG) for the diagnosis of invasive fungal infections (IFIs) by means of a meta-analysis of relevant studies. We searched in bibliographic databases for relevant cohort or case-control studies. We primarily compared BDG between patients with proven or probable IFIs (excluding *Pneumocystis jirovecii* infections), according to the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group or similar criteria, and patients without IFIs (excluding healthy individuals as controls). A total of 2979 patients (594 with proven or probable IFIs), included in 16 studies, were analyzed. The pooled sensitivity of BDG was 76.8% (95% confidence interval [CI], 67.1%–84.3%), and the specificity was 85.3% (95% CI, 79.6%–89.7%). The area under the summary receiver operating characteristic curve was 0.89. Marked statistical heterogeneity was noted. BDG has good diagnostic accuracy for distinguishing proven or probable IFIs from no IFIs. It can be useful in clinical practice, if implemented in the proper setting and interpreted after consideration of its limitations.

Invasive fungal infections (IFIs) comprise a rather heterogeneous group of yeast and mold infections [1]. They have acquired increasing clinical importance because of an increase in the population at risk, which generally includes patients with disease-related or iatrogenic immunosuppression [2, 3] and patients dependent on various types of supportive care [4, 5]. Despite the advent of novel antifungal agents [6], the mortality rate associated with IFIs is considerably high [7]. Early administration of antifungal therapy is important in this regard, yet a definitive diagnosis of IFI often cannot be made promptly, as this may require the use of invasive procedures [8]. Many patients with clinical suspicion for the presence of an IFI are treated empirically with antifungal therapy that may involve the unnecessary use of potentially toxic and costly drugs.

There is increasing interest in preemptive antifungal therapy, which is defined as treatment that is deferred until there is substantial evidence for the presence of an IFI [9]. Early detection of diagnostic markers of a fungal infection, such as fungal nucleic acids, antigens, antibodies, or cell wall components, is essential in this regard [10, 11]. The main relevant example is the use of galactomannan in the diagnosis of invasive aspergillosis [12, 13]. Another serum marker for the presence of IFIs is (1→3)-β-D-glucan (BDG), which has been included in the relevant European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) diagnostic criteria [12].

BDG is a component of the cell wall of most fungi. The main exceptions are Zygomycetes and cryptococci, which release no or little BDG to be detected in human serum [14]. The measurement of BDG is based on the *Limulus* test [15]. BDG activates factor G, a serine protease zymogen of the *Limulus* amebocyte lysate, which is extracted from amebocytes of horseshoe crab species. This in turn activates a coagulation cascade. The activity of this reaction can be measured with use of

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colorimetric or turbidimetric methods. We aimed to evaluate the diagnostic accuracy of this test by performing a meta-analysis of data extracted from relevant studies.

METHODS

Study Selection

We searched in PubMed and Scopus (Health Sciences subject area) for records through February 2010 and in the Cochrane Library for records through April 2010 of studies that evaluated the diagnostic performance of serum or plasma BDG for IFIs. The search terms used were "glucan AND infection" for PubMed and Scopus and "glucan OR beta-glucan" for the Cochrane Library. We also hand-searched the bibliographies of relevant articles. We selected for inclusion studies that, using diagnostic criteria that were in accordance with the 2002 or 2008 relevant EORTC/MSG criteria [12, 16], compared patients with IFIs with patients without IFIs. We excluded studies that were presented only as abstracts at scientific conferences, included fewer than 10 cases of IFI, or were published in languages other than English, Spanish, German, French, Italian, or Greek.

Data Extraction

From each of the included studies, we extracted data regarding the study design, characteristics of the patients studied, criteria used for the diagnosis of IFI, types of IFI included, assay used for BDG detection, strategy followed for BDG sampling, and administration of antifungal therapy. We also extracted patient data regarding the number of true- and false-positive and true- and false-negative results obtained with the BDG assay. Where such data were not specifically reported, we calculated them from the related data on sensitivity and specificity and positive and negative predictive value with use of known mathematical formulas. We also extracted specific data regarding the sensitivity of BDG specifically for diagnosis of invasive infections caused by *Candida* or *Aspergillus*.

Quality Assessment of the Included Studies

The methodological quality of the included studies was assessed with the QUADAS tool [17]. Eight of the 14 QUADAS items were considered relevant for our meta-analysis. The spectrum of patients included in a study was considered to be representative of the target population if they had an IFI or were at risk for developing an IFI. The acceptable reference standard consisted of diagnostic criteria matching the 2002 or 2008 EORTC/MSG relevant criteria [12, 16]. Partial and differential verification bias was considered to have been avoided if all the included patients were assessed with the same reference standard. Incorporation bias was considered to have been avoided if the diagnosis of IFI was established regardless of the BDG test result. The RevMan software, version 5, was used to plot the output data of the methodological quality assessment [18].

Data Analysis

To evaluate the diagnostic performance of BDG testing for IFI, we performed 2 comparisons. First, we performed a proof-of-concept comparison evaluating the performance of BDG assays for case patients with proven IFIs compared with control patients without IFIs. In our main comparison, case patients with proven or probable IFIs were compared with control patients without IFIs. We did not include data on patients with *Pneumocystis jirovecii* infection. We also did not include data on healthy individuals. We excluded data on patients with possible IFIs from our analysis. For studies that did not report specific data to allow for the exclusion of these patients, we considered such patients to be control patients if they made up <15% of the total control group.

We did not exclude any study from the analysis on the basis of the BDG assay used. We included in the analysis diagnostic data referring to the BDG cutoff level used in each study. If a study reported BDG data for different cutoff values, we selected for inclusion those referring to a cutoff of 80 pg/mL for the Fungitell or Glucatell assay, 30 pg/mL for the Fungitec G-Test MK, 20 pg/mL for the (Fungitec) G-Test [19], and 11 pg/mL for the Wako β-Glucan assay, which were considered to be equivalent [19, 20]. If a study reported BDG data obtained with different sampling strategies, we included those for which a positive test result was defined by 1 positive BDG value. Subgroup analyses were performed on studies using the same or similar BDG assays.

We performed a diagnostic test meta-analysis regarding the performance of BDG testing for the diagnosis of IFIs. We calculated the pooled sensitivity and specificity, positive and negative likelihood ratios, and the diagnostic odds ratio of BDG, along with the respective 95% confidence intervals (CIs), using a bivariate meta-analysis model [21]. We constructed a hierarchical summary receiver operating characteristic (HSROC) curve plotting sensitivity versus specificity and calculated the area under the curve [22]. We evaluated the degree of interstudy statistical heterogeneity using the I^2 test [23]. We evaluated the presence of a threshold effect on the accuracy of the BDG with the Spearman correlation coefficient between the logits of sensitivity and specificity. For all the above analyses we used the Midas module in the Stata software, version r.10 (StataCorp) [24, 25].

RESULTS

Characteristics of the Included Studies

As a result of our searches in bibliographic databases and the reference lists of relevant articles, we included in our review 23 individual studies that comparatively assessed the value of BDG

testing for the diagnosis of IFIs [19, 20, 26–46]. The process of selection of studies that were eligible for inclusion is shown graphically in Figure 1.

In Table 1, we present the characteristics of the 23 studies included in our review, and in Table 2, the data on the diagnostic performance of BDG assays for IFIs that we extracted from each of the included studies, according to our prespecified criteria. Among the 23 reviewed studies, 16 were eligible for inclusion in the meta-analysis [19, 20, 26–29, 31–33, 35, 37–39, 41, 43, 46]. The remaining 7 studies were not included in the meta-analysis, because they used healthy individuals as controls [34, 36, 40, 44, 45], included patients with possible IFIs as case patients [30], or provided data only for sample-based BDG analysis [42]. We present data from these 7 studies, with regard to their characterstics and the diagnostic performance of BDG, in Tables 1 and 2.

Among the 16 studies included in the meta-analysis, 10 had a cohort study design [19, 20, 26, 28, 29, 32, 33, 39, 41, 46], and the remaining 6 had a case-control design (Table 1) [27, 31, 35, 37, 38, 43]. Eleven studies evaluated mainly (>50%) patients with hematological malignancy or other serious hematological disease [19, 20, 26–28, 31, 32, 37, 39, 41, 46], whereas the remaining 5 studies evaluated hospitalized patients who primarily had systemic *Candida* infections [29, 35, 38], had received liver transplants [33], or had miscellaneous underlying diseases [43].

Four of the 16 studies used the Fungitell assay [27, 28, 31, 38], and 3 studies used the Glucatell assay [20, 29, 37]; these are the

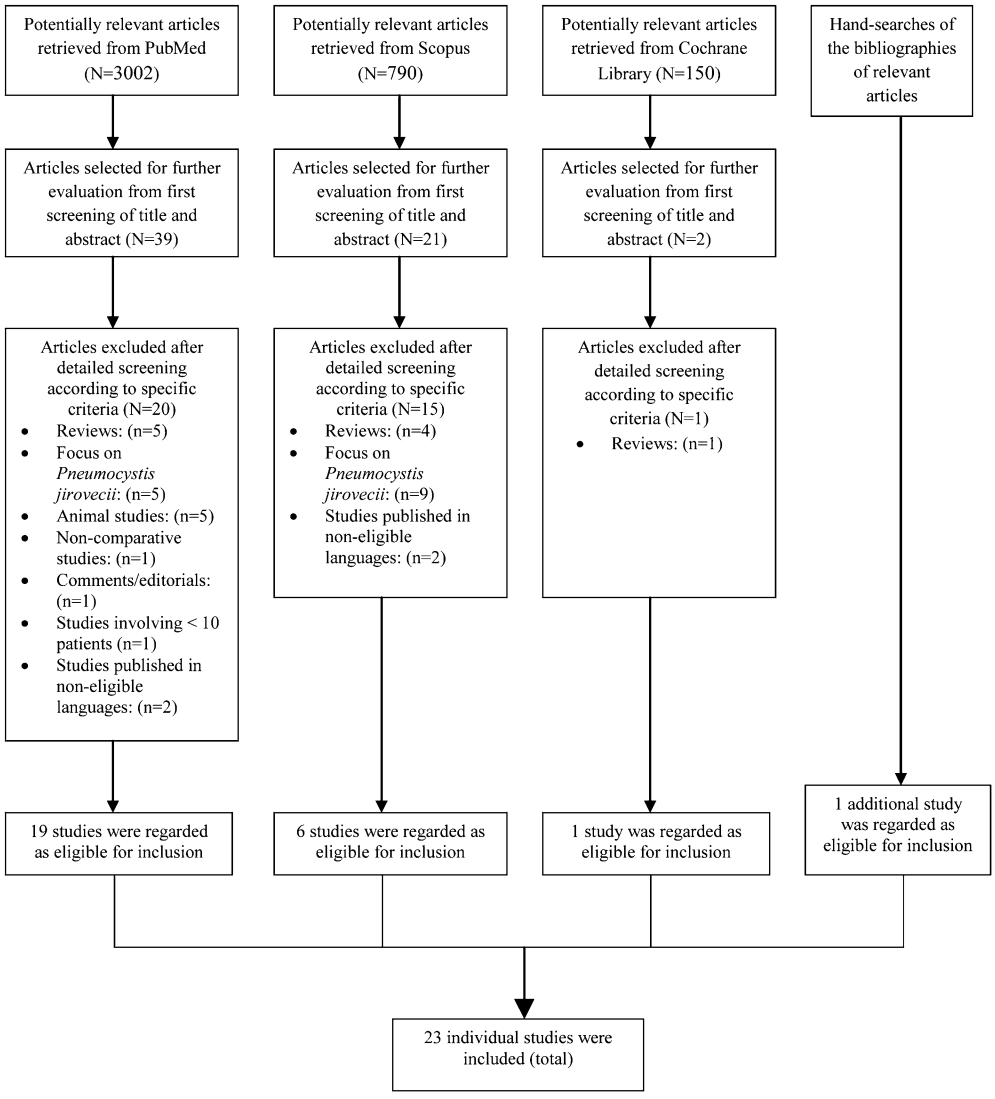

Figure 1. Flow diagram of the process of selection of studies for inclusion in the review.

Table 1. Main Characteristics of Studies Included in Review Evaluating (1→3)-β-D-Glucan (BDG) Testing for the Diagnosis of Invasive Fungal Infection (IFI)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Zhao et al 2010 [26]	Cohort study	Pediatric patients with hematologic (n = 89) or other malignant disorders (n = 11) and critically ill pediatric patients with risk factors for IFI (n = 30)	Proven IFI, 2 (candidemia); probable IFI, 20 (pulmonary fungal infection, 11; chronic disseminated candidiasis, 4; IA, 4; fungal UTI, 1); possible IFI, 7	Twice weekly sampling	EORTC-MSG (2002), IFI diagnosis standards in patients with hematologic disease/cancer of China, 2007 guidelines for the diagnosis and treatment of IFI in critically ill patients of China	GKT-5M Set Kinetic Fungus Detection Kit/plasma	Empirical therapy prior to BDG testing: half of the samples
Hachem et al 2009 [27]	Prospective case-control study	Case patients: (1) 22 patients with hematological malignancy and IA, (2) 17 patients with hematological malignancy and other mold infections, (3) 23 patients with candidemia (solid tumors, 12; hematological malignancy, 11); control patients: 20 nonneutropenic patients with solid tumors and without any radiological or clinical evidence of IA or risk factors for IFI	IA, 22 (proven or probable); other mold infections, 17 (*Fusarium,* zygomycosis, *Scedosporium*); candidemia, 23 (proven)	Twice in week 1 and once every other week for a total of 12 weeks	EORTC-MSG criteria (2002)	Fungitell/serum	Antifungal therapy before BDG testing: all patients with IA or other mold infections
Koo et al 2009 [28]	Retrospective cohort study	All patients who had ⩾1 BDG assay result at a cancer institute: 112 patients with proven or probable IFI (within 1 week after initial BDG assay)** and 759 patients with possible IFI or at risk for IFI	Proven IFI, 69; probable IFI, 43 (*Candida*, 41; *Aspergillus*, 32; *Pneumocystis*, 14; zygomycetes, 4; other molds, 8; other yeasts, 8; endemic fungal species, 2; coinfections, 3); possible IFI, 97	⩾1 BDG assay result during the study period	EORTC-MSG criteria (2008), independently of BDG results	Fungitell/serum	Antifungal therapy at the time of initial BDG testing: 248 (28.5%) of 871 patients

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Presterl et al 2009 [29]	Multicenter cohort study	Consecutive long-term ICU patients with severe underlying diseases,[††] extensive surgery, and length of stay ⩾7 days: 24 patients with proven IFI and 58 patients without evidence of fungal infection	Proven IFI, 24 (candidemia, 11; hepatic candidiasis, 9; *Candida* peritonitis, 2; IPA, 2)	Twice weekly sampling	Growth of fungi in blood cultures and abscesses or aspirates from otherwise sterile compartments (pleura, CSF)	Glucatell/serum	NR
Ellis et al 2008 [30]	Prospective cohort study	Consecutive patients with hematological malignancy@ and neutropenic fever for which they received antifungal therapy: case patients with IFI, 38; control patients without evidence of IFI, 42	Candidiasis, 14 (proven, 5; probable, 7; possible, 2); aspergillosis, 24 (proven, 0; probable, 14; possible, 10)	Sampling on first day of neutropenic fever unresponsive to antibiotics and on subsequent alternate days for 14 days[‡‡]	EORTC-MSG criteria (2002), without use of galactomannan or PCR	Fungitell/serum	Empirical treatment: 80 (100%) of 80
Obayashi et al 2008 [19]	Retrospective study	All consecutive cases at single institution with autopsy records available ($N = 456$) and serum tested for BDG within 2 weeks of death ($n = 104$): 41 cases with IFI[#] and 63 cases without IFI	IA, 28; *Pneumocystis jirovecii* pneumonia, 6; systemic candidiasis, 2; systemic candidiasis and invasive aspergillosis, 1; cryptococcosis, 2; systemic trichosporonosis, 1; systemic zygomycosis, 1	BDG test within 2 weeks before death	Autopsy	Fungitec G-Test MK/ serum	Prophylactic or empirical treatment: 40 of 41 cases, 41 of 63 controls
Persat et al 2008 [31]	Multicenter retrospective case-control study	Case patients: 117 patients with IFI; control patients: (1) 122 patients from hematology wards or intensive care units, at risk for IFI, but no identified IFI; (2) 40 healthy blood donors	IP,: 70 (7 proven, 63 probable); bloodstream FI, 27 (*Candida*, 26; *Geotrichum capitatum*, 1); *Pneumocystis jirovecii* pneumonia, 20	Several samples were available for some patients	EORTC-MSG criteria (2002)	Fungitell/serum	Empirical therapy: 10 (14%) of 70 patients with IPA

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Senn et al 2008 [32]	Prospective cohort study	95 adult patients with acute myeloid or lymphoblastic leukemia, hospitalized for myeloablative chemotherapy, who had 190 neutropenic episodes (173 analyzed)	Proven IFI, 9 episodes (aspergillosis, 5; candidiasis, 4); probable IFI, 21 episodes (pulmonary aspergillosis, 10; hepatosplenic candidiasis, 13); possible IFI, 30 episodes	Twice weekly sampling while patients remained afebrile; sampling at fever onset and on days 1–4, 7, 10, and 14, for each febrile episode; twice weekly sampling until subsequent febrile episode or hospital discharge	EORTC-MSG criteria (2002)	Wako colorimetric assay/s	Prophylaxis: 10 (17%) of 60 proven or probable cases, 6 (20%) of 30 possible cases, 29 (26%) of 113 cases with no IFI
Akamatsu et al 2007 [33]	Prospective study	180 consecutive adult living-donor liver transplant recipients monitored for 1 year after surgery	Proven IFI, 24 (candidemia, 2; aspergillosis, 5; cryptococcosis, 3); probable IFI, 14 (candidemia, 12; *Pneumocystis jirovecii*, 2)	BDG was measured once a week for 3 months and then once a month for 1 year	EORTC-MSG criteria (2002)	Fungitec G-Test/ plasma	Preemptive therapy: when 2 consecutive BDG tests had positive results
Alam et al 2007 [34]	Retrospective case-control study	Cases: (1) 27 patients with culture-proven candidemia, (2) 39 patients with clinically suspected/ possible systemic candidiasis; control patients: (1) 10 *Candida* vaginitis patients, (2) 16 healthy subjects without complains of oral or vaginal *Candida* infection	Proven IFI, 27 (candidemia)	Case patients: (1) 32 serum samples, (2) 51 serum samples	(1) Positive blood culture result, signs and symptoms suggestive of septicemia, plus ⩾1 risk factors; (2) fever that did not respond to 4 days of broad-spectrum antibiotic treatment plus ⩾3 of the following risk factors: extended period of hospitalization (>2 weeks), isolation of *Candida* species from ⩾1 anatomic site, presence of intravenous catheter/line, recent history of a surgical procedure, and administration of immunosuppressive therapy	Fungitell/ serum	Most samples were obtained before antifungal therapy

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Fujita et al 2006 [35]	Prospective case-control study	Case patients: 105 candidemic patients who had available ⩾2 serum samples during index fever episode;[‡] control patients: 175 febrile patients who had no clinical evidence of fungal findings and were treated successfully without systemic antifungal drugs (105 bacteremic and 70 nonbacteremic)	Proven IFI, 76 (candidemia)	BDG was measured in 76 of the case patients (90 serum samples)	Blood culture positive for *Candida* species and fever refractory to antibiotics for ⩾2 days	Wako BG test/serum	Systemic antifungal drugs before candidemia: 2 (2%) of 105
Ostrosky-Zeichner et al 2005 [36]	Prospective multicenter case-control study	163 patients with IFI:[§§] 170 control patients: healthy volunteers or outpatients with minor medical problems ($n = 98$) and hospitalized patients with medical problems other than IFI	Proven IFI, 142 (*Candida*, 107; *Aspergillus*, 10; *Fusarium*, 3; *Mucor*, 2; *Rhizopus*, 1; *Cryprococcus*, 12; other fungi, 7); probable IFI, 21 (*Candida*, 4; *Aspergillus*, 12; other fungi, 5)	Single sample per patient (within 72 h after diagnosis of IFI)	EORTC-MSG criteria (2002)	Fungitell/serum	Prior antifungal treatment: 118 (83%) of 142
Pazos et al 2005 [37]	Retrospective case-control study	40 neutropenic patients selected from a cohort of 154 adult hematological cancer patients as high-risk individuals for developing IFI: case patients: 11 with proven, probable, or possible IA; control patients: 29 patients	11 patients with IA (5 proven, 3 probable, 3 possible)	Twice weekly until no risk for IFI	EORTC-MSG criteria (2002)	Glucatell/serum	Prophylaxis: 11 (28%) of 40

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Pickering et al 2005 [38]	Case-control study	15 patients with blood cultures positive for yeast; 25 bacteremic patients (gram positive, 15; gram negative, 10); 6 histoplasma antigen–positive patients; 32 *Aspergillus* galactomannan-positive patients; 36 healthy blood donors	Proven or probable IFI, 16	⩾1 sample (depending on patient group) 5 days before or after positive blood culture	Blood culture positive (1 bacteremic patient had concurrent candidiasis proven by means of tissue biopsy)	Fungitell/serum	NR
Kawazu et al 2004 [39]	Prospective cohort study	Consecutive adult patients with hematological disorders at high risk for IA: case patients: 24 IA episodes; control patients: 125 no IA episodes	Proven IA, 9; probable IA, 2; possible IFI, 13	Once weekly sampling whenever patients were at risk for IFI	EORTC-MSG criteria (2002), without use of plasma galactomannan	β-Glucan Wako/plasma	Empirical therapy when IFI was suspected
Kondori et al 2004 [40]	Prospective case-control study	14 patients with systemic candidiasis:††† control patients: (1) 9 lactating mothers with superficial *Candida albicans* infection of nipples; (2) 10 healthy blood donors	Proven systemic candidiasis, 14	Sampling on 2 or 3 consecutive occasions, starting on day when candidiasis was culture proven	(1) Positive culture result from normally sterile sites, (2) presence of risk factors (cancer and chemotherapy, abdominal surgery, or the use of broad-spectrum antibiotics, (3) presence of an infectious syndrome (fever) that did not respond to antibacterial therapy	Gluspecy kit/serum	All of the systemic candidiasis cases were treated with flucytosine during sample collection

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Odabasi et al 2004 [20]	Prospective cohort study	Consecutive neutropenic patients with AML or MDS undergoing initial induction chemotherapy and receiving antifungal prophylaxis: case patients: 53 patients with proven, probable, or possible IFI; control patients: 230 patients	Proven fungemia, 16 (*Candida*, 12; *Fusarium*, 1; *Trichosporon*, 3; *Aspergillus*, 1); probable fungal pneumonia, 4 (*Aspergillus*, 2; *Aspergillus* and *Candida*, 1; *Fusarium*, 1); possible fungal pneumonia, 33	Twice weekly sampling during period of neutropenia	EORTC-MSG criteria (2002)	Glucatell/serum	Prophylaxis: all patients
Kami et al [41] 2000	Prospective cohort study	215 consecutive patients with hematological malignancy who underwent cytotoxic chemotherapy: case patients: 30 patients with IPA; control patients: 185 patients with without IPA	Proven, 16; suspected, 14	Once weekly sampling	Proven IPA: histologic evidence of tissue invasion and culture positive for *Aspergillus* species from the sputum or lung; suspected: suggestive CT signs and persistent fever unresponsive to broad-spectrum antibiotics, or histopathologic evidence of IPA	Fungi-Tec/plasma	Empirical therapy was added when fever persisted ⩾5–7 days
Hiyoshi et al 1999 [42]	Cohort study	62 immune-compromised patients* suspected of having deep *Candida* mycosis, who provided 212 plasma samples	Proven *Candida* deep mycosis, 46 samples; suspected deep mycosis, 31 samples	3.4 mean samples per patient/sample-based analysis	All cases had unexplained fever for ⩾7 days, despite antibacterial therapy. Proven *Candida* deep mycosis: cultures from blood or site of infection positive for *Candida;* suspected deep mycosis: radiographic findings suggesting deep mycosis and *Candida* antigenemia	Wako β-Glucan test (Wako Pure Chemicals)/plasma	NR

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Mori et al 1997 [43]	Multicenter case-control study	Case patients: patients with deep mycosis, well diagnosed or suspected, or patients with febrile episodes of unknown origin that did not respond to antibacterial chemotherapy;¶ control patients: (1) 26 patients with nonmycotic diseases, (2) 92 healthy controls	Candidemia, 12; IPA, 4; pulmonary cryptococcosis, 3	⩾1 value per patient	Culture, histopathological examination of biopsy findings, diagnostic serologic tests, and/or clinical examination (particularly chest radiograph)	Wako WB003/ plasma	NR
Mitsutake et al 1996 [44]	Case-control study	Case patients†: (1) 39 patients with candidemia, (2) 10 patients with deep mycoses, (3) 10 patients with superficial *Candida* colonization; control patients: 20 healthy volunteers	Candidemia, 32; invasive pulmonary aspergillosis, 5; cryptococcal meningitis, 5	NR	(1) Blood cultures positive; (2) autopsy/ CSF cultures positive	β-D-Glucan represented the difference in titers between 2 chromogenic *Limulus* assays, the conventional and the endotoxin-specific *Limulus* tests/serum	NR
Miyazaki et al 1995 [45]	Prospective case-control study	24 HIV-negative patients with clinical evidence of invasive mycosis; 36 healthy volunteers	11 patients with candidemia, 3 patients with IA, 10 patients with pulmonary cryptococcosis	NR	Candidemia: ⩾2 positive blood culture results, temperature, ⩾38.0°C refractory to antibiotics for ⩾3 days; cryptococcosis: positive culture of sputum, bronchoalveolar lavage fluid, transbronchial lung biopsy specimens, or CSF/positive cryptococcal antigen latex agglutination test; invasive aspergillosis: autopsy	G-test/plasma	NR

Table 1. (*Continued*)

Study	Design	Population	Types of IFIs	BDG sampling strategy	Reference diagnostic standard	Assay used/ specimen	Antifungal prophylaxis/therapy
Obayashi et al 1995 [46]	Multicenter cohort study	202 febrile episodes in 179 consecutive patients:[§] case patients: 41 febrile episodes of deep mycoses; control patients (1) 59 febrile episodes of other than FI known causes: autopsy-verified systemic bacterial infections, microbiologically documented bacterial infections, nonfungal nonbacterial infections, or fevers of noninfectious origin; (2) 102 febrile episodes of unknown etiology; (3) 21 noninfectious controls; (4) 60 healthy controls	Autopsy-verified deep mycoses, 7; fungemia, 22; fungemia and bacteremia, 2; fungal catheter fever, 2; fungal meningitis, 2; *Aspergillus* mediastinitis, 1; *Aspergillus* pyothorax, 2; candidal UTI, 3	Once per episode sampling in 121 episodes, ⩾2 times in the remainder	Autopsy or microbiological documentation	G-test/serum	NR

NOTE. AML, acute myelogenous leukemia; CSF, cerebrospinal fluid; EORTC/MSG criteria, European Organization of the Research and Treatment of Cancer/Mycoses Study Group; FI, (invasive) fungal infection; HIV, human immunodeficiency virus; IC, invasive *Candida* infection; ICU, intensive care unit; I(P)A, invasive (pulmonary) aspergillosis; MDS, myelodysplastic syndrome; NR, not reported; UTI, urinary tract infection .

[*] Underlying diseases: hematopoietic malignancy, 24 patients; solid malignant tumors, 8; brain tumors or cerebrovascular disease, 6; serious bacterial infections, 5; collagen vascular disease, 6; diabetes mellitus, 4; post-cardiovascular surgery, 3; multiple organ failure of unknown cause, 2; pancreatitis, 2; Crohn's disease, 1.

[**] Underlying conditions: malignancy, 69 patients; hematologic malignancy, 51; hematopoietic stem cell transplantation, 16; recent prolonged neutropenia, 20; recent receipt of T cell immunosuppressants or prolonged corticosteroid use, 77; hemodialysis, 9.

[#] Underlying diseases in all 54 patients with autopsy-proven IFI: leukemia, 28 patients; malignant lymphoma, 7; AIDS, 7; solid malignancy, 6; autoimmune disease, 6.

[§] Underlying diseases: hematological malignancy or other hematological disease with a poor prognosis, 67%; cerebrovascular disease, 9%; tuberculosis, 5%; malignancy, 5%; collagen disease, 4%; other, 10%.

@ Acute myelogenous leukemia, 49 patients; lymphocytic leukemia, 33; other, 13.

[‡] 69 of the 105 candidemic patients had a solid tumor, 6 cardiovascular disease, 5 leukemia, 3 malignant lymphoma, and 22 had other disorders.

[‡‡] In a subgroup of patients, sampling was performed daily from the first day of hospital admission.

[§§] Common comorbidities: hematological malignancy, 33 patients; cardiovascular disease, 13; gastrointestinal surgery, 14; solid tumor, 14; receipt of organ transplant, 20; diabetes mellitus, 9; other infections, 9; none, 9.

[¶] Underlying diseases: acute myeloblastic leukemia, 7 patients; malignant lymphoma, 5; tuberculosis, 9; solid cancer, 11; collagen diseases, 2; pneumonia, 4; ileus, 3; aortic aneurysm, 3; others, 35.

[†] Underlying diseases: hematological malignancy, 5 patients; solid tumors, 5; cerebral infarction, 13; infections, 13; cerebral bleeding, 2; chronic renal failure, 2; sick sinus syndrome, 1; Parkinson syndrome, 3; ileus, 2; burn, 2; acute pancreatitis, 1; alcoholism, 1; chronic heart failure, 1; NR, 5.

[††] Underlying diseases: cardiovascular disease, 10 patients; trauma, 22; acute respiratory distress syndrome, 3; solid neoplasm, 10; AIDS, 1; past organ transplantation, 2; recent organ transplantation, 15; cerebral hemorrhage, 14; liver disease, 11; lung disease, 8; leukemia/lymphoma, 3; renal disease, 1.

[†††] Underlying diseases: neoplasms, 4 patients; pancreaticoduodenectomy, 1; aneurysm, 2; diabetes mellitus hemorrhagic pancreatitis, 1; diabetes mellitus, 1; diabetic angiopathy and nephropathy, 1; atrial fibrillation, 1; diabetic coma, 1; hepatitis B and hepatitis C, 1.

Table 2. Diagnostic Performance of (1→3)-β-D-Glucan (BDG) Testing for Proven or Probable Invasive Fungal Infections (IFIs), According to Data Extracted from Different Studies

Study	Subgroups of patients compared for BDG testing	BDG values required for positive result	BDG cutoff value (pg/mL)	Adjusted[a] BDG cutoff	Sensitivity, %	Specificity, %	True +	False +	False –	True –	True + (proven IFIs)	False – (proven IFIs)
Zhao et al 2010 [26]	Patients with proven or probable IFI vs patients with possible IFI or at risk for IFI	Any	10	1	82	82	18	19	4	89	NR	NR
Hachem et al 2009 [27]	Patients with proven or probable IFI vs control patients	2 consecutive	80	1	64	90	37	2	21	18	13	8
Koo et al 2009 [28]	Patients with proven or probable IFI (excluding those with *Pneumocystis jirovecii* infection) vs patients with possible IFI or patients at risk for IFI	First BDG value	80	1	60	84	59	124	39	635	34	19
Presterl et al 2009 [29]	Patients with proven IFI vs ICU patients without IFI at day 7	BDG value at day 7 after ICU admission	40	0.5	52	76	12	14	11	44	Same	Same
Ellis et al 2008 [30]	Patients with proven, probable, or possible IFI vs patients at risk for IFI	Any	80	1	95	45	36	23	2	19	NA	NA
Obayashi et al 2008 [19]	Patients with postmortem evidence of IFI (excluding patients with *P. jirovecii* infection) vs control patients without postmortem evidence of IFI	Single	30	1	94	86	33	9	2	54	Same	Same

Table 2. (*Continued*)

Study	Subgroups of patients compared for BDG testing	BDG values required for positive result	BDG cutoff value (pg/mL)	Adjusted[a] BDG cutoff	Sensitivity, %	Specificity, %	True +	False +	False –	True –	True + (proven IFIs)	False – (proven IFIs)
Persat et al 2008 [31]	Patients with proven or probable IFI excluding *P. jirovecii* pneumonia vs patients at risk for IFI	The one closest to time of IFI diagnosis	80	1	73	70	71	36	26	86	29	5
Senn et al 2008 [32]	Neutropenic episodes in patients with proven or probable IFI vs neutropenic episodes in patients without IFI	Any	11	1	50	89	15	12	15	101	5[b]	4[b]
Akamatsu et al 2007 [33]	Patients with proven or probable IFI excluding *P. jirovecii* pneumonia vs patients at risk	Any (before the IFI diagnosis)	40	2	55	83	12	26	10	130	5	8
Alam et al 2007 [34]	Patients with proven candidemia vs patients with *Candida albicans* vaginitis plus healthy control patient)	Any	80	1	52	100	14	0	13	26	NA	NA
Fujita et al 2006 [35]	Candidemic patients vs control patients	Any	11	1	95	84	72	28	4	147	Same	Same
Ostrosky-Zeichner et al 2005 [36]	All patients with proven or probable IFI vs healthy and nonhealthy control patients	Single, defined	80	1	64	92	105	13	58	157	NA	NA
Pazos et al 2005 [37]	Patients with proven or probable IA vs patients at risk for IA	Any	120	1.5	88	90	7	3	1	26	5	0

Table 2. (*Continued*)

Study	Subgroups of patients compared for BDG testing	BDG values required for positive result	BDG cutoff value (pg/mL)	Adjusted[a] BDG cutoff	Sensitivity, %	Specificity, %	True +	False +	False –	True –	True + (proven IFIs)	False – (proven IFIs)
Pickering et al 2005 [38]	Patients with blood cultures positive for yeast (proven or probable IFI) vs bacteremic patients	Any	80	1	88	46	14	13	2	11	NR	NR
Kawazu et al 2004 [39]	Episodes in patients with proven or probable IA vs episodes in patients without IA	Any	11	1	55	98	6	3	5	122	6	3
Kondori et al 2004 [40]	Patients with proven systemic candidiasis vs both control groups	Any	20	1	100	100	14	0	0	19	NA	NA
Odabasi et al 2004 [20]	Patients with proven or probable IFI vs patients with possible IFI or without IFI	Any	80	1	90	94	18	15	2	248	14	2
Kami et al. 2000 [41]	Patients with definite IPA or suspected IPA and culture positive for IPA vs control patients	Any	20	1	67	76	12	44	6	139	10	6
Hiyoshi et al 1999 [42]	Samples from cases of proven or suspected deep mycosis vs no deep mycosis	Value for each sample	10.6	1	84	86	65	19	12	116	NA	NA
Mori et al 1997 [43]	Patients with deep mycosis vs patients with nonmycotic diseases	Any	1000	1	79	96	15	1	4	25	Same	Same

Table 2. (*Continued*)

Study	Subgroups of patients compared for BDG testing	BDG values required for positive result	BDG cutoff value (pg/mL)	Adjusted[a] BDG cutoff	Sensitivity, %	Specificity, %	True +	False +	False –	True –	True + (proven IFIs)	False – (proven IFIs)
Mitsutake et al 1996 [44]	Patients with IFI vs patients with superficial *Candida* colonization and healthy volunteers	NR	60	1	86	100	32	0	5	30	NA	NA
Miyazaki et al 1995 [45]	Patients with candidemia, aspergillosis and cryptococcosis vs healthy volunteers	The first BDG value	10	2	71	100	17	0	7	36	NA	NA
Obayashi et al 1995 [46]	Febrile episodes in patients with deep mycoses vs febrile episodes in patients with other known causes or in patients with fever of unknown etiology	Any	20	1	90	84	37	26	4	135	Same	Same

NOTE. ICU, intensive care unit; I(P)A, invasive (pulmonary) aspergillosis; NA, nonanalyzed (see text); NR, not reported; Same, same data as for proven/probable IFIs; +, positive; –, negative.

[a] The adjusted cutoff was calculated by dividing the BDG cutoff selected from each of the included studies by 80 for the Glucatell/Fungitell assay, 30 for the Fungitec G-test MK, 20 for the (Fungitec) G-test, 11 for the Wako β-Glucan test, and by the actual cutoff used for the other assays (see text for details).

[b] Data for proven IFIs are for a cutoff of 7 pg/mL and 2 consecutive positive values required for positivity.

commercial and the research version, respectively, of the same assay marketed by Associates of Cape Cod. Four other studies used BDG assays developed by Seikagaku Corporation (the parent company of the one above), namely, the Fungitec G-Test MK [19], Fungitec G-Test [33], Fungi-Tec [41], and G-Test [46]. All 11 assays listed above are colorimetric. Four studies used assays developed by Wako Pure Chemicals Industries—turbidimetric assays in 3 studies [35, 39, 43] and a colorimetric assay in 1 study [32]. One study used the GKT-5M Set Kinetic Fungus Detection Kit, another turbidimetric BDG assay [26].

Various cutoff levels for the definition of a positive BDG test result were used in the 16 included studies (Table 2). Eleven studies required at least 1 positive BDG value in cases in which multiple samples per patient were measured [20, 26, 32, 33, 35, 37–39, 41, 43, 46]; 4 studies required a single positive BDG value for a specific measurement [19, 28, 29, 31]; and 1 study required 2 consecutive positive BDG values [27].

In 7 of the analyzed studies, more than 50% of the patients received antifungal therapy before BDG sampling or IFI diagnosis (Table 1) [19, 20, 26, 27, 33, 39, 41]. In 5 studies, fewer than 50% of the patients received such therapy [28, 31, 32, 35, 37], whereas no specific relevant information was clearly reported in the remaining 4 studies [29, 38, 43, 46].

Methodological Quality of the Included Studies

Figure 2 presents our assessment of whether each of the 23 studies included in our review satisfied the 8 items of the QUADAS tool that were considered relevant to our review. Figure 3 summarizes the findings of our methodological quality assessment for all 23 studies cumulatively.

Diagnostic Accuracy of BDG Measurement (Patients with Proven IFIs)

Fourteen studies provided specific data regarding the measurement of BDG for case patients with proven IFIs ($n = 365$) and control patients without IFIs ($n = 2253$) [19, 20, 27–29, 31–33, 35, 37, 39, 41, 43, 46]. The pooled sensitivity of BDG was 79.1% (95% CI, 68.9%–86.7%), and the specificity was 87.7% (95% CI, 82.4%–91.6%). The pooled diagnostic odds ratio was 27.0 (95% CI, 13.8–52.8), the positive likelihood ratio was 6.4 (95% CI, 4.4–9.4), and the negative likelihood ratio was 0.24 (95% CI, 0.16–0.37). The area under the HSROC curve for BDG was 0.91 (95% CI, 0.88–0.93). High interstudy heterogeneity was noted; the I^2 index was 92% (95% CI, 84%–99%).

Diagnostic Accuracy of BDG Measurement (Patients with Proven or Probable IFIs)

The 16 studies included in our main analysis evaluated a total of 2979 patients (594 patients with proven or probable IFIs and 2385 patients without IFIs). The pooled sensitivity of BDG testing for the diagnosis of IFIs was 76.8% (95% CI, 67.1%–84.3%), and the specificity was 85.3% (95% CI, 79.6%–89.7%) (Figure 4). The pooled diagnostic odds ratio was 19.2 (95% CI, 10.5–35.4), the positive likelihood ratio was 5.2 (95% CI, 3.7–7.5), and the negative likelihood ratio was 0.27 (95% CI, 0.19–0.40). The area under the HSROC curve for BDG was 0.89 (95% CI, 0.86–0.91) (Figure 5). The I^2 index of

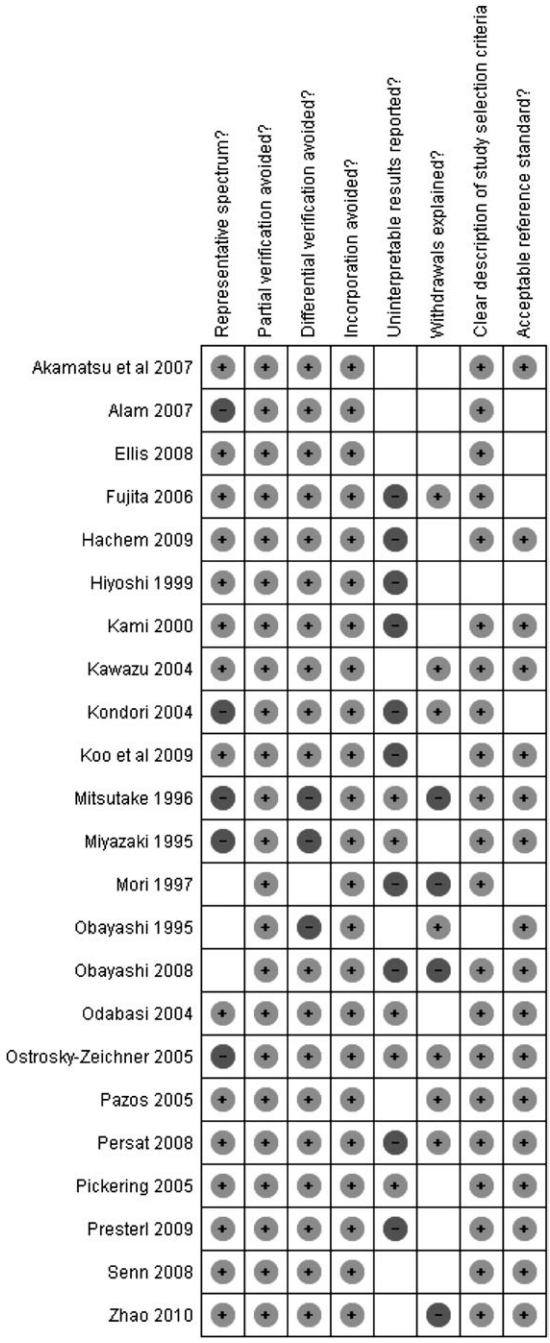

Figure 2. Methodological quality assessment of each of the 23 reviewed studies according to 8 QUADAS criteria. A positive sign denotes that a study meets the criteria, and a negative sign denotes that a study does not meet the criteria. A blank cell denotes that no adequate relevant data were provided.

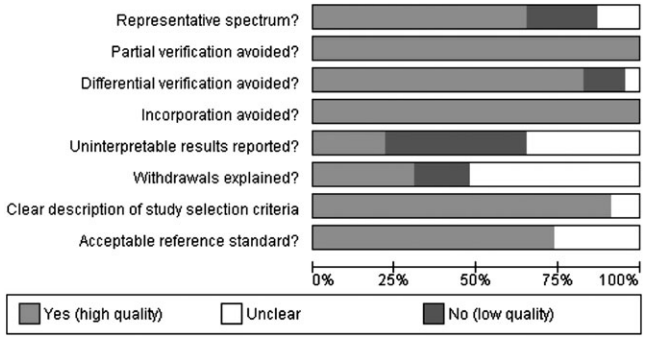

Figure 3. Summary of the methodological quality assessment of the reviewed studies according to 8 QUADAS criteria. The percentages of the 23 studies that meet the criteria (Yes), do not meet the criteria (No), and do not provide adequate relevant data (Unclear) are shown.

heterogeneity was 95% (95% CI, 91%–99%). There was a weak negative correlation between the logits of sensitivity and specificity (Spearman correlation coefficient, −0.09), indicating the absence of an important effect of the diagnostic threshold (cutoff level) on the performance of BDG measurement.

Subgroup Analyses

Seven studies used either the Fungitell or Glucatell assay for the measurement of BDG [20, 27–29, 31, 37, 38]. The pooled sensitivity of BDG testing for the diagnosis of IFIs was 71.3% (95% CI, 59.9%–80.6%), and the pooled specificity was 82.0%

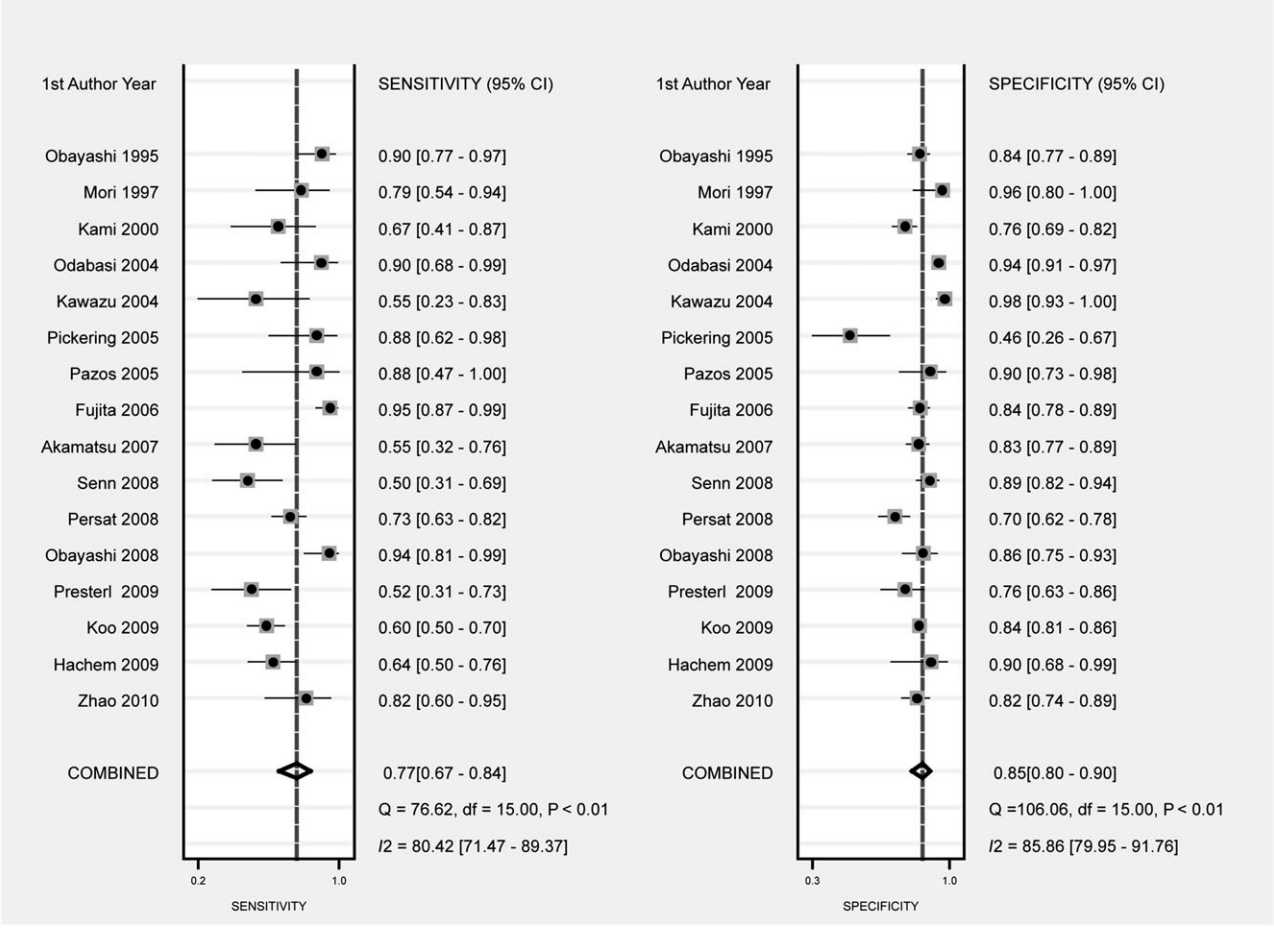

Figure 4. Forest plot of the pooled sensitivity and specificity of measuring serum or plasma (1→3)-β-D-glucan levels for the diagnosis of proven or probable invasive fungal infections. The circles in squares and the horizontal lines represent the point estimate and 95% confidence interval, respectively, for each included study; the dotted line represents the pooled estimate; and the diamond represents the 95% confidence interval of the pooled estimate.

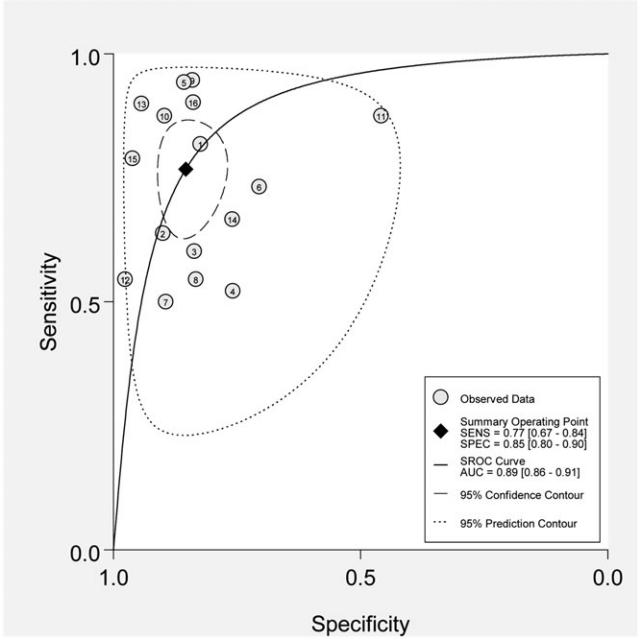

Figure 5. Summary hierarchical receiver operating characteristic curve of the sensitivity versus specificity of measuring serum (1→3)-β-D-glucan levels for the diagnosis of proven or probable invasive fungal infections. The straight line represents the curve; the diamond represents the summary point of the curve to which the pooled sensitivity and specificity correspond; the dashed line represents the 95% confidence area for the summary point; the circles represent the data from each of the included studies; and the dotted line represents the 95% confidence area in which a new relevant study will be located. SENS, sensitivity; SPEC, specificity.

(95% CI, 69.6%–90.1%). The pooled diagnostic odds ratio was 11.3 (95% CI, 4.7–27.5), the positive likelihood ratio was 4.0 (95% CI, 2.2–7.2), and the negative likelihood ratio was 0.35 (95% CI, 0.24–0.52). The area under the HSROC curve was 0.81 (95% CI, 0.78–0.85). The I^2 index was 85% (95% CI, 70%–100%).

BDG Measurement for Patients with *Candida* or *Aspergillus* Infection

Of the 23 overall included studies, 11 provided specific data regarding the sensitivity of BDG testing for both patients with invasive aspergillosis and patients with systemic *Candida* infections [19, 20, 27, 28, 31–33, 36, 43–45]. As can be inferred from Table 3, the performance of BDG testing for the detection of systemic *Candida* infection did not differ considerably from its performance for the detection of invasive aspergillosis.

BDG Measurement for Patients with Possible IFIs and for Healthy Individuals

Five of the studies included in our review reported specific BDG data for patients with possible cases of IFI [20, 32, 34, 37, 39]. They included a total of 130 BDG results for such cases (range, 3–51 results), and the average rate of BDG positivity was 27.7% (range, 3%–51%). Eight of the studies reported BDG data for healthy individuals [31, 34, 38, 40, 43–46]. They included a total of 310 BDG results for such individuals (range, 10–92 results), and the average rate of BDG positivity was 1.0%. All 3 positive results were observed in a single study with 40 healthy controls [31].

DISCUSSION

The main finding of our meta-analysis is that serum BDG measurement has good diagnostic accuracy for IFIs diagnosed in accordance with the EORTC/MSG or similar criteria. In our main analysis comparing patients who had proven or probable IFIs with patients who did not have IFIs, the area under the HSROC curve was 0.89; a value between 0.80 and 0.90 has traditionally been considered to indicate good diagnostic test accuracy. The sensitivity of BDG measurement seems to be lower than the specificity, 77% compared with 85%. However, the above findings should be interpreted in light of the high statistical heterogeneity that we noted among the included studies for all analyses.

Potential sources of heterogeneity among the included studies are differences in study design, characteristics of the study population, type of pathogens evaluated, assay and cutoff level used, sampling method and definition of a positive test result, and prior administration of antifungal therapy [47]. The latter parameter might be important, because prior antifungal therapy can decrease the sensitivity of galactomannan [48] and—of particular relevance to the measurement of BDG—echinocandins act by inhibiting BDG synthesis.

The studies included in our meta-analysis evaluated various categories of patients. We excluded data on healthy individuals used as controls, because they are not representative of the population to which the test will be applied in clinical practice. Of note, BDG tended to yield negative results for almost all of the healthy controls included in the various studies. Their inclusion would therefore have caused an overestimate of the diagnostic accuracy of BDG testing [13].

We did not observe a considerable difference in the sensitivity of BDG testing for the detection of invasive *Candida* or *Aspergillus* infections. Some studies included IFIs caused by cryptococci or Zygomycetes, but they constituted a small minority of all IFIs evaluated [19, 27, 28, 33, 43], and BDG values were not always negative, particularly for infections due to cryptococci, which can release small amounts of BDG [19]. We did not include patients with *P. jirovecii* infections in this meta-analysis, which should be evaluated in the context of positive BDG results [19, 28, 31].

The available BDG assays differ in many aspects, such as the measurement method (colorimetric or turbidimetric), the horseshoe crab species used, the type of β-glucan used as standard, the

Table 3. Sensitivity of (1→3)-β-D-Glucan (BDG) Testing to Detect Proven or Probable Systemic *Candida* Infection in Comparison with Invasive Aspergillosis As Reported in Different Studies

Study	Cutoff(pg/mL)	Systemic *Candida* infections, proportion (%)	Invasive aspergillosis, proportion (%)
Hachem et al 2009 [27]	80 (2 consecutive values)	13/21 (62)	14/21 (67)
Koo et al 2009 [28]	80	26/41 (63)	24/32 (75)
Obayashi et al 2008 [19]	30	3/3 (100)	28/28 (100)
Persat et al 2008 [31]	80	22/26 (85)	48/70 (69)
Senn et al 2008 [32]	7 (2 consecutive values)	10/17 (59)	9/15 (60)
Akamatsu et al 2007 [33]	40	7/14 (50)	5/5 (100)
Ostrosky-Zeichner et al 2005 [36]	80	83/107 (78)	8/10 (80)
Odabashi et al 2004 [20]	80	9/11 (82)	4/4 (100)
Mori et al 1997 [43]	1000	11/12 (92)	4/4 (100)
Mitsutake et al 1996 [44]	60	27/32 (84)	5/5 (100)
Miyazaki et al 1995 [45]	10	11/11 (100)	3/3 (100)
Total from all studies[a]	…	222/295 (75)	152/197 (77)

[a] Total represents cumulative data

pretreatment method, and the cutoff level [19]. There are limited and inconclusive in vitro or in vivo data comparing the performance of different BDG assays [49, 50]. In the subgroup analyses that we performed on studies using the Fungitell or the Glucatell assay, findings seemed to be less favorable for the diagnostic accuracy of BDG testing than those of the rest of the studies.

An inherent limitation in the evaluation of diagnostic markers for IFIs lies in the absence of an accurate dichotomous reference standard. Establishing the presence of IFIs by using the EORTC/MSG criteria is not always feasible. Some cases of true IFIs are inevitably missed, according to data from autopsy investigations [51]. Moreover, proven IFI cases often represent cases of advanced infection. For such patients, the diagnosis and treatment of IFIs may not alter the clinical course [8]. In this context, we compared in our primary analysis patients with proven or probable IFI with patients without IFI.

We did not include patients with possible IFIs as case patients, although they represent a great number of patients in routine clinical practice and the main value of BDG testing would be to correctly reclassify a subset of them as having probable IFIs. Not all of these patients have true IFIs, however [20, 28]. We noted substantial variability in the rate of BDG positivity for patients with possible IFIs among the included studies providing relevant data. We believe that the inclusion of patients with possible IFIs as case patients would not serve the purpose of this meta-analysis to estimate the diagnostic accuracy of BDG testing in patients with true IFIs and those without IFIs.

The diagnostic performance of BDG testing for IFIs evaluated in this meta-analysis seems to be similar to that of galactomannan detection used for the diagnosis of invasive aspergillosis [13, 52]. Few studies have performed direct comparisons of the 2 tests for the diagnosis of invasive aspergillosis in the same patients. One study has favored BDG in this regard [27] and one study has favored galactomannan [39], but most studies have not showed a considerable difference between the 2 tests [31, 37, 41, 45]. An issue that warrants additional investigation is whether the 2 tests can be used as complements to each other, as their concordance might not be too high [27, 31, 37, 38]. It is likely that for some patients with invasive aspergillosis that is not identified with galactomannan testing, BDG testing could be used to make a diagnosis more quickly [32, 37, 41].

Certain issues regarding the clinical utility of BDG testing have not been well clarified, including the timing and the frequency of BDG testing for at-risk patients, as well as the criteria for the definition of a positive test result. Caution is also warranted in the presence of factors that could increase BDG levels for reasons other than IFI [14], such as hemodialysis with cellulose membranes [53], administration of human blood products (immunoglobulins or albumin) [54], use of such antibiotics as amoxicillin-clavulanate or piperacillin-tazobactam [27, 55], presence of serious bacterial infections [38], use of surgical gauzes containing glucan [56], or severe mucositis [30]. In addition, practical issues, such as the need for the allocation of trained personnel for adequate time to perform the BDG test (at least 1 h for all the steps), may be important for determining its clinical utility.

In conclusion, our meta-analysis suggests that measuring serum or plasma BDG levels has good accuracy to discriminate between patients with IFIs, mainly due to *Candida* or *Aspergillus,* and patients without IFIs, but there are important differences in the characteristics of the studies that we analyzed. In clinical practice, proper use of the test would require good knowledge of its characteristics, particularly the fungal pathogens that it does not detect and the factors associated with a false-positive test result. Some issues regarding the optimal utilization of BDG

testing necessitate further evaluation, such as the optimal sampling strategy for patients at risk, the criteria to define a positive test result, the optimal cutoff value, and the influence of concurrent bacteremia on diagnostic performance.

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