Diagnosis of Invasive Fungal Disease Using Serum \((1\rightarrow 3)-\beta-D\)-Glucan: A Bivariate Meta-Analysis

Yuan Lu¹, Yi-Qiang Chen², Ya-Ling Guo³, Shou-Ming Qin², Cong Wu² and Ke Wang²

Abstract

**Background** The \((1\rightarrow 3)-\beta-D\)-Glucan (BG) assay has been approved for diagnosing invasive fungal disease (IFD). However, the test performance has been variable. We conducted a meta-analysis to determine the overall accuracy of BG assay for diagnosing IFD.

**Methods** The sensitivity, specificity, and positive and negative likelihood ratios (PLR and NLR, respectively) of BG for diagnosing IFD were pooled using a bivariate meta-analysis. We also performed subgroup analyses.

**Results** Twelve reports, including 15 studies, were included for the analysis (proven and probable IFD vs possible or no IFD). The sensitivity, specificity, PLR and NLR were 0.76 (95% CI, 0.67-0.83), 0.85 (95% CI, 0.73-0.92), 5.05 (95% CI, 2.71-9.43), and 0.28 (95% CI, 0.20-0.39), respectively. Subgroup analyses showed that the BG assay had higher specificities for patients with hematological disorders and a positive BG result with two consecutive samples. The combination of galactomannan and BG increased the specificity value to 0.98 (95% CI, 0.95-0.99) for diagnosing invasive aspergillosis.

**Conclusion** Serum BG determination is clinically useful for diagnosing IFD in at-risk patients, especially for hematology patients. The combination of galactomannan and BG was sufficient for diagnosing invasive aspergillosis. Since the BG assay is not absolutely sensitive and specific for IFD, the BG results should be interpreted in parallel with clinical findings.

**Key words:** invasive fungal disease, \(\beta-D\)-Glucan, diagnosis

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Introduction

During the past two decades, there has been an increase in the frequency of invasive fungal diseases (IFDs) among immunosuppressed patients (1-6). Changes in hosts, prevention strategies, and treatment modalities, and other expanding potential pathogens have likely impacted the epidemiology of IFD. According to recent reports, the most commonly encountered IFDs are invasive aspergillosis (IA) and invasive candidiasis (IC), followed by cryptococcosis, endemic mycoses, and zygomycosis (3-6).

IFDs have been associated with considerable morbidity and mortality (1-6). The high morbidity and mortality rates result in part from difficulties in establishing an early diagnosis. Nonspecific clinical manifestations, delayed radiologic findings and poor yield of cultures have necessitated the need for developing sensitive and specific methods for the early diagnosis of IFD. Nowadays, the non-invasive diagnostic tools using \((1\rightarrow 3)-\beta-D\)-Glucan (BG) have become the focus of clinical study.

BG is a common cell wall constituent of most pathogenic fungi, including *Candida* spp. and *Aspergillus* spp. The BG assays are commercially available [Wako test (Wako Pure Chemical Industries Ltd., Tokyo, Japan), Fungitec G test MK (Seikagaku, Tokyo, Japan), Fungitell (Associates of Cape Cod, Inc., Falmouth, MA)]. The potential role of BG has been acknowledged by its inclusion in the revised Euro-

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European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria definitions (7). A recent meta-analysis has shown that the value of BG measurements for the diagnosis of IFD was good (8); however, there were some serious limitations in the meta-analysis, such as the inappropriate definition of sensitivity (SEN) and specificity (SPE), the indefinite exclusion/inclusion criteria for all studies, per-episode data instead of per-person data. Since their conclusions were not supported by the methodology and the data they used, we performed the meta-analysis to characterize the clinical usefulness of the BG assay in at-risk patients and to evaluate which variables affect its performance.

Materials and Methods

Study identification and selection

Two investigators (Y. L. and Y. L. G.) searched the published English language literature using the MEDLINE and EMBASE databases from January 2002 to January 2011. Search terms included “invasive fungal disease,” “IFD,” “invasive fungal infection,” “IFI,” “deep mycotic infection,” “invasive mycoses,” “invasive mycosis,” “(1-3)-β-D-Glucan,” “β-D-Glucan,” “β-Glucan,” “D-Glucan,” “BG,” and “BDG.” The syntax for the MEDLINE searches was as follows: (“invasive fungal disease” OR “invasive mycoses” OR “invasive fungal infection” OR “deep mycotic infection” OR “invasive mycosis” OR “IFD” OR “IFI”) AND (“[1-3]-β-D-Glucan” OR “β-D-Glucan” OR “β-Glucan” OR “D-Glucan” OR “BG” OR “BDG”). We screened the reference lists of included studies and related publications. The results were hand searched for eligible trials. Conference abstracts and letters were excluded because of the limited data. Results were arbitrated by a second investigator (K. W.).

Full-text publications were included if 1) they used the revised EORTC/MSG criteria in 2008 (7), the EORTC/MSG criteria in 2002 (9), or autopsy as diagnostic criteria; 2) they provided data for two-by-two tables; and 3) they included immunocompromised or at-risk patients. Studies with fewer than 10 patients were excluded to avoid selection bias. Two reviewers (Y. Q. C. and S. M. Q.) judged study eligibility while screening the citations. Disagreements were arbitrated by a second investigator (C. W.).

Data extraction and quality assessment

The final set of articles was assessed by two reviewers (Y. Q. C. and S. M. Q.). Disagreements were resolved by a third author (K. W.). The following information was obtained: population, study, and assay characteristics; reference standard; methodological quality; threshold for a positive result; and data for two-by-two tables. The number of healthy volunteers and individuals without risk factors was excluded, because they may lead to overestimation of diagnostic accuracy (10). When the same population was analyzed in several publications, the study’s results were accounted for only once. If the publication reported two different definitions for a BG-positive result: a single positive BG result and two consecutive positive BG results, we divided this article into two studies and extracted data separately. If several cut-offs were reported in one study, we used the manufacturer-recommended cut-off value.

We assessed the methodological quality using the standards for reporting diagnostic accuracy (STARD) tool and the quality assessment for studies of diagnostic accuracy (QUADAS) tool (11, 12). We requested the relevant information from the authors if data were unreported. The “unclear” items were treated as “no” if we received no answers.

Data synthesis

In most clinical practice, the at-risk patients are classified into four groups: proven, probable, possible and no cases (7, 9). The rates of correct identification of patients with and without the target disorder are known as test SEN and test SPE, respectively (13). Thus, when we defined “the target disorder” as “proven and probable IFD”, we transformed the 4×2 table into the 2×2 table (proven and probable cases vs possible or no cases) (13). Because the revised EORTC/MSG probable and possible classifications were inherent uncertain, we also analyzed the two-by-two table (proven, probable, or possible vs no IFD). We did not perform an episode-based meta-analysis, because various definitions of episode were reported.

With a bivariate regression model, we estimated the overall SEN and SPE as the main outcome measures, constructed hierarchical summary receiver operating characteristic (SROC) curves, and calculated the area under the SROC curve (AUC) (14). Based on random-effects models, this bivariate regression model accounts for potential between-study heterogeneity and incorporates the possible correlation between the SEN and the SPE (14). Briefly, rather than using the diagnostic odds ratio, the bivariate model preserves the two-dimensional nature of diagnostic data in a single model. We also calculated positive and negative likelihood ratios (PLR and NLR, respectively) (13, 14). PLRs>10 and NLRs<0.1 have been noted as providing convincing diagnostic evidence, whereas those >5 and <0.2 give strong diagnostic evidence (13, 15).

The impact of unobserved heterogeneity was assessed statistically by the quantity ² (16). If there were enough reports (>10), we performed subgroup analyses to explore the potential between-study heterogeneity (17). Covariates requiring that at least 80% of studies reported on a particular item were analyzed: hematological disorders (only vs mixed/other), neutropenia (only vs mixed/other), solid-organ transplant recipient (only vs mixed/other), intensive care unit (ICU) patients (only vs mixed/other), data collection (prospective vs retrospective), sampling method (consecutive vs non-consecutive), design (cohort vs case-control), blinded status (yes vs no/unclear), Fungitell (yes vs Fungithec), fungitell (yes vs WAKO), EORTC/MSG criteria (2002 vs 2008), IFD (only IA/IC vs mixed/other), and positive sam-
Eligible study characteristics and quality assessment

The literature search resulted in 320 publications, 65 of which were evaluated for more details. Of these publications, we eventually pooled 13 articles. The process of selection of flow diagram is not shown (Table 1) (20-32). Four articles had data from two and more definitions for a simultaneous BG-positive result (Table 2); therefore, there were 17 BG-related studies.

Table 1, 2 list the characteristics and quality assessment of the eligible articles. Eight articles used the EORTC/MSG criteria in 2002, whereas four studies used the revised EORTC/MSG criteria in 2008. We found no incorporation bias of using a positive BG result as part of the revised EORTC/MSG criteria. Seven articles reported using one single BG sample, two reported the results of two consecutive BG samples, and four reported both. Two articles were limited to critically ill patients in ICU, two were limited to solid-organ transplant populations, and five were limited to patients with hematological disorders. Two articles mentioned that the mixed cases were immunocompromised patients. In these immunocompromised patients, the most underlying diseases were solid and hematologic malignancies.

In all, the quality of study design and reporting diagnostic accuracy of most studies were high, as all studies had higher STARD scores (≥13) and 11 studies had higher QUADAS scores (≥10).

**SEN, SPE, PLR, NLR, and SROC curve**

Twelve reports, including 15 studies, met the analysis method A (proven and probable IFD vs possible or no IFD) (20-22, 24-32) (Table 3). When methods B, C and D were used to define true-positive cases and true-negative cases, 8 reports (20, 21, 24, 26, 28-31), 9 reports (20-22, 24-26, 30-32), and 6 reports (21, 23, 24, 26, 30, 31) were included, respectively. The test performances of BG assay were markedly higher in method B (proven IFD vs no IFD). The SEN and SPE for method A

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient population</th>
<th>Neutropenic status</th>
<th>Cohort design</th>
<th>Prospective collection</th>
<th>Consecutive sampling</th>
<th>Blinded status</th>
<th>Assay method</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acosta /2010 [20]</td>
<td>Adult, ICU</td>
<td>Yes, part</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unclear</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Alexander /2010 [21]</td>
<td>Adult, LTR</td>
<td>Unclear</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Akamatsu /2007 [22]</td>
<td>Adult, LDLTR</td>
<td>Unclear</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Ellis /2008 [23]</td>
<td>Adult, HD</td>
<td>Yes, all</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Hirata /2010 [24]</td>
<td>Adult, HD</td>
<td>Yes, all</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>WAKO</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Hachem /2009 [25]</td>
<td>Mixed age, HD and ST</td>
<td>Yes, part</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Kawazu /2004 [26]</td>
<td>Adult, HD</td>
<td>Yes, part</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>WAKO</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Koo /2009 [27]</td>
<td>Adult, at-risk patients</td>
<td>Yes, part</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Leon /2009 [28]</td>
<td>Adult, ICU</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unclear</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Obayashi /2008 [29]</td>
<td>At-risk patients</td>
<td>Unclear</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Fungitell</td>
<td>Autopsy</td>
</tr>
<tr>
<td>Odabasi /2004 [30]</td>
<td>Adult, HD</td>
<td>Yes, all</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Pazos /2005 [31]</td>
<td>Adult, HD</td>
<td>Yes, all</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
</tr>
<tr>
<td>Persat /2008 [32]</td>
<td>Adult, HD and ICU</td>
<td>Yes, part</td>
<td>No</td>
<td>No</td>
<td>Unclear</td>
<td>Fungitell</td>
<td>EORTC/MS</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. EORTC/MSG, European Organization of the Research and Treatment of Cancer/Mycoses Study Group; HD, hematological disorders; ICU, intensive care unit; LDLTR, living donor liver transplant recipients; LTR, lung transplant recipients; ST, solid tumor

* BG detection was not used as a microbiologic criterion for invasive fungal disease

**Results**
defining a suitable denominator of SPE, we did not calculate the SEN of the BG assay for IA or IC. Because most reports (20, 21, 23, 25, 27, 28, 30-32) provided data for patients with IA or IC. The SEN of the BG assay in patients with IA (149/194, 76.8%) was similar with that in patients with IC (127/164, 77.9%). Because of the difficulty of defining a suitable denominator of SPE, we did not calculate the SPE of the BG assay for IA or IC.

On the other hand, 6 reports (20, 25-27, 31, 32) provided the combination data of *Aspergillus galactomannan* (GM) antigen and BG. When we combined the GM and BG assays for diagnosing IA, the SEN decreased to 0.55 (0.44-0.61), whereas the SPE increased to 0.98 (0.95-0.99) (Table 3). Because there was only one report with the combination of Candida mannan and BG, we did not perform further analysis.

**Investigations of heterogeneity, publication bias**

Because most reports (20, 21, 23, 25, 27, 28, 30-32) provided the data for the Fungitell assay, we showed the results of this kit according to different analysis methods (Table 3).

For the 15 eligible studies in the primary analysis, the negative correlation between SEN and SPE was relatively low (Spearman rank correlation=-0.15), and the proportion of heterogeneity likely due to threshold effect was very low (Spearman rank correlation=-0.15), indicating the absence of a diagnostic threshold effect on the performance of BG assay. We performed subgroup analyses to explore the potential between-study heterogeneity (Fig. 4). Overall, the test performances of the BG

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**Table 2. Quality and Results of 13 Reports Included in the Meta-Analysis**

<table>
<thead>
<tr>
<th>Study/Year</th>
<th>IA/IC only</th>
<th>No. of patients</th>
<th>Proven cases</th>
<th>Probable cases</th>
<th>Possible cases</th>
<th>STAD scores</th>
<th>QUADA scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acosta /2010 [20]</td>
<td>No</td>
<td>80 pg/ml</td>
<td>1</td>
<td>51</td>
<td>7</td>
<td>6</td>
<td>Unclear</td>
</tr>
<tr>
<td>Alexander /2010 [21]</td>
<td>No</td>
<td>80 pg/ml</td>
<td>1</td>
<td>73</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Akamatsu /2007 [22]</td>
<td>No</td>
<td>20 pg/ml</td>
<td>2</td>
<td>180</td>
<td>10</td>
<td>14</td>
<td>Unclear</td>
</tr>
<tr>
<td>Ellis /2008 [23]</td>
<td>Yes</td>
<td>80 pg/ml</td>
<td>1 or 2</td>
<td>80</td>
<td>5</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Hirata /2010 [24]</td>
<td>No</td>
<td>7 pg/ml</td>
<td>1</td>
<td>70</td>
<td>3</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Hachem /2009 [25]</td>
<td>No</td>
<td>80 pg/ml</td>
<td>2</td>
<td>82</td>
<td>62</td>
<td>62 for proven and probable</td>
<td>Unclear</td>
</tr>
<tr>
<td>Kawazu /2004 [26]</td>
<td>Yes</td>
<td>11 pg/ml</td>
<td>1 or 2</td>
<td>96</td>
<td>9</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Koo /2009 [27]</td>
<td>Yes</td>
<td>80 pg/ml</td>
<td>1</td>
<td>116</td>
<td>80</td>
<td>36</td>
<td>93</td>
</tr>
<tr>
<td>Leon /2009 [28]</td>
<td>Yes</td>
<td>75 pg/ml</td>
<td>1</td>
<td>240</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Obayashi /2008 [29]</td>
<td>No</td>
<td>20 pg/ml</td>
<td>1</td>
<td>104</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Odabasi /2004 [30]</td>
<td>No</td>
<td>60 pg/ml</td>
<td>1 or 2</td>
<td>283</td>
<td>16</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>Pazos /2005 [31]</td>
<td>Yes</td>
<td>120 pg/ml</td>
<td>1 or 2</td>
<td>40</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Persat /2008 [32]</td>
<td>Yes</td>
<td>80 pg/ml</td>
<td>1</td>
<td>239</td>
<td>117</td>
<td>117 for proven and probable</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

**NOTE.** IA, invasive aspergillosis; IC, invasive candidiasis; IFD, invasive fungal disease; QUADA, the quality assessment for studies of diagnostic accuracy; STARD, the standards for reporting diagnostic accuracy.

**Table 3. Pooled Test Performance of the Included Studies in the Meta-Analysis**

<table>
<thead>
<tr>
<th>Analysis method</th>
<th>No. of reports</th>
<th>Pooled SEN (95% CI)</th>
<th>Pooled SPE (95% CI)</th>
<th>Pooled PLR (95% CI)</th>
<th>Pooled NLR (95% CI)</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The BG assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Proven or probable IFD vs possible or no IFD)</td>
<td>12</td>
<td>0.76 (0.67-0.83)</td>
<td>0.85 (0.73-0.92)</td>
<td>5.05 (2.71-9.43)</td>
<td>0.28 (0.20-0.39)</td>
<td>0.85 (0.81-0.88)</td>
</tr>
<tr>
<td>B (Proven IFD vs no IFD)</td>
<td>8</td>
<td>0.88 (0.75-0.95)</td>
<td>0.89 (0.73-0.96)</td>
<td>8.36 (2.97-23.51)</td>
<td>0.13 (0.06-0.30)</td>
<td>0.94 (0.91-0.96)</td>
</tr>
<tr>
<td>C (Proven, probable IFD vs no IFD)</td>
<td>9</td>
<td>0.73 (0.64-0.81)</td>
<td>0.91 (0.78-0.96)</td>
<td>7.80 (3.09-19.70)</td>
<td>0.29 (0.21-0.41)</td>
<td>0.83 (0.80-0.86)</td>
</tr>
<tr>
<td>D (Proven, probable, or possible IFD vs no IFD)</td>
<td>6</td>
<td>0.65 (0.47-0.80)</td>
<td>0.90 (0.72-0.97)</td>
<td>6.73 (2.33-19.41)</td>
<td>0.39 (0.25-0.59)</td>
<td>0.83 (0.79-0.86)</td>
</tr>
<tr>
<td><strong>The GM + BG assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven, probable or possible IFD vs no IFD</td>
<td>7</td>
<td>0.55 (0.44-0.61)</td>
<td>0.98 (0.95-0.99)</td>
<td>27.98 (10.00-78.34)</td>
<td>0.48 (0.40-0.58)</td>
<td>0.66 (0.62-0.70)</td>
</tr>
<tr>
<td><strong>The Fungitell assay only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Proven or probable IFD vs possible or no IFD)</td>
<td>5</td>
<td>0.75 (0.67-0.82)</td>
<td>0.79 (0.61-0.90)</td>
<td>3.59 (1.72-7.51)</td>
<td>0.32 (0.22-0.46)</td>
<td>0.80 (0.76-0.83)</td>
</tr>
<tr>
<td>(Proven IFD vs no IFD)</td>
<td>5</td>
<td>0.90 (0.69-0.98)</td>
<td>0.84 (0.54-0.96)</td>
<td>5.71 (1.53-21.33)</td>
<td>0.11 (0.03-0.45)</td>
<td>0.94 (0.91-0.96)</td>
</tr>
<tr>
<td>(Proven, probable IFD vs no IFD)</td>
<td>6</td>
<td>0.77 (0.65-0.86)</td>
<td>0.86 (0.63-0.96)</td>
<td>5.57 (1.72-17.99)</td>
<td>0.27 (0.16-0.45)</td>
<td>0.84 (0.81-0.87)</td>
</tr>
<tr>
<td>(Proven, probable, or possible IFD vs no IFD)</td>
<td>4</td>
<td>0.73 (0.56-0.85)</td>
<td>0.83 (0.51-0.96)</td>
<td>4.33 (1.27-14.74)</td>
<td>0.33 (0.20-0.54)</td>
<td>0.82 (0.78-0.85)</td>
</tr>
</tbody>
</table>

**NOTE.** AUC, the area under the summary receiver operating characteristic curve; CI, confidence interval; galactomannan, GM; IA, invasive aspergillosis; IFD, invasive fungal disease; NLR, negative likelihood ratio; PLR, positive likelihood ratio; SEN, sensitivity; SPE, specificity.

were 0.76 (0.67-0.83) and 0.85 (0.73-0.92), respectively (Fig. 1). Compared with method A, method D incorporated “possible IFD” into the truly positive groups, leading to a decreased SEN value (from 0.76 to 0.65) and an increased SPE value (from 0.85 to 0.90). High heterogeneity was noted in all indices, irrespective of analysis methods used. For methods A and C, hierarchical SROC curves are displayed in Fig. 2, 3. The AUC values were 0.85 (0.81-0.88), 0.83 (0.80-0.86) and 0.83 (0.79-0.86) for method A, C and D, respectively. An AUC close to 1.0 signifies that the test has almost perfect discrimination while an AUC close to 0.5 suggests poor discrimination. Thus, as compared with method C and D, method A had the better discrimination ability.

**BG assay for patients with IA or IC**

In the eligible reports, 11 reports (20-22, 24-26, 27, 29, 30-32) and 8 reports (21, 24, 25, 27-30, 32) provided data for patients with IA or IC. The SEN of the BG assay in patients with IA (149/194, 76.8%) was similar with that in patients with IC (127/164, 77.9%). Because of the difficulty of defining a suitable denominator of SPE, we did not calculate the SPE of the BG assay for IA or IC.

On the other hand, 6 reports (20, 25-27, 31, 32) provided the combination data of *Aspergillus galactomannan* (GM) antigen and BG. When we combined the GM and BG assays for diagnosing IA, the SEN decreased to 0.55 (0.44-0.61), whereas the SPE increased to 0.98 (0.95-0.99) (Table 3). Because there was only one report with the combination of Candida mannan and BG, we did not perform further analysis.
The SEN of the test was 0.66 (95% CI, 0.42-0.90), and the SPE was 0.45 (95% CI, 0.03-0.86) for studies limited to solid-organ transplant recipients. For ICU patients, the SEN was 0.82 (95% CI, 0.63-1.00), and the SPE was 0.67 (95% CI, 0.26-1.00). The SEN and SPE of the BG test for hematological patients was 0.76 (95% CI, 0.63-0.89) and 0.95 (95% CI, 0.90-0.99), respectively. SEN and SPE of the BG assay for two consecutive positive samples were 0.65 (95% CI 0.52-0.78) and 0.93 (95% CI 0.86-1.00), respectively, and if only a single positive sample was required, these values were 0.81 (95% CI 0.73-0.88) and 0.78 (95% CI 0.65-0.92), respectively. The SEN was significantly lower with some covariates, such as prospective design and only IA/IC reported. On the other hand, for studies using the WAKO assay, the SPE was significantly higher. According to the Deeks’ funnel plot asymmetry test, the statistically non-significant value (p=0.40) for the slope coefficient suggests symmetry in the data and a low likelihood of publication bias (Fig. 5).

Discussion

In a recent similar meta-analysis reported by Karageorgopoulou et al (8), the analysis was based on the 2×2 table

![Figure 1](image1.png)

**Figure 1.** Forest plot of the pooled sensitivity and specificity of (1→3)-β-D-Glucan for the diagnosis of invasive fungal diseases (proven and probable cases vs possible or no cases). a the data with two consecutive positive BG to define a BG-positive result.

![Figure 2](image2.png)

**Figure 2.** Summary receiver operating characteristic curve plots of sensitivity and specificity for the diagnosis of invasive fungal diseases (proven and probable cases vs possible or no cases).

![Figure 3](image3.png)

**Figure 3.** Summary receiver operating characteristic curve plots of sensitivity and specificity for the diagnosis of invasive fungal diseases (proven and probable cases vs possible or no cases).
Figure 4. Forest plots of subgroup analyses for sensitivity and specificity. Fungitell (Yes), the Fungitell kit; Fungitell (No), the Fungitec kit; fungitell (Yes), the Fungitell kit; fungitell (No), the WAKO kit; HD (Yes), only hematological disorders reported; ICU (Yes), only intensive care units patients reported; Neutropenia (Yes), only neutropenia reported; OnlyIAIC (Yes), only invasive aspergillosis and candidiasis reported; Transplant (Yes), only solid-organ transplant recipient reported.

Figure 5. Linear regression test of funnel plot asymmetry. The statistically non-significant value (p=0.40) for the slope coefficient suggests symmetry in the data and a low likelihood of publication bias.

(proven and probable cases vs no cases). According to our results (Table 3), we found that the possible status had a great influence on the diagnostic accuracy. Thus, for a meta-analysis of diagnostic accuracy, the excluded data of some special population (i.e. possible IFD cases) were inappropriate, and led to overestimation (10). Furthermore, the conclusions were not supported by the data they used. According to their exclusion/inclusion criteria in that meta-analysis, one study should not be omitted (28). Two studies should be excluded, for only 8 cases of invasive fungal infection were calculated (31), and other IFDs were included in the control groups (33). In addition, episode-related data were inappropriately used to calculate the test performances of patient-based analysis in two studies (26, 34). Thus, their results were severely biased (10), and their conclusions could not be applied to clinical practice.

Based on meta-analysis, we drew the conclusion that the BG assay was clinically useful for diagnosing IFD in at-risk patients, especially for patients with hematological disorders. However, the diagnostic performance of the BG assay was markedly decreased for solid-organ transplant recipients, for
whom it had poor SEN and SPE. The findings of this review are therefore applicable to some special clinical settings in which patients with hematological disorders are managed. However, this conclusion should be interpreted cautiously because significant heterogeneity was present.

An exploration of the reasons for heterogeneity, rather than the computation of summary measures, is an important goal of meta-analysis (16, 17). The subgroup analyses identified the study quality that accounted for some heterogeneity in our results. However, we noted that the difference did not reach statistical significance for the SPE, indicating that quality problems had little influence on the true-negativity of BG test. Further studies should aim to improve study quality and thus decrease the risk of bias.

In some situations, variations may occur among studies due to differences in cut-off values. The serum BG values measured with different kit showed some differences, possibly because various standard β-glucans, blood pretreatment methods, and different origins of lysates are used in each kit (29, 30). Yoshida et al (35) reported that the kit with the highest SEN was Fungitell, followed by Fungitec, whereas the WAKO kit had an extremely low SEN value. Conversely, compared with the Fungitell and Fungitec kits, the WAKO kit had a higher SPE value. According to our subgroup analyses, we also identified some heterogeneity due to the BG assay. For example, the WAKO assay was less sensitive (0.63 and 0.75, respectively) than the Fungitell assay but more specific (0.97 and 0.79, respectively). However, we found that the Fungitell kit had the highest SEN value (0.86), which was not in line with the results reported by Yoshida et al (35). The reasons for the discrepancies could be partly related to the retrospective design that they used, which may render the results to be biased. Because such differences among these kits could result in some clinical problems (35), further prospective cohort studies are necessary to clarify the clinical efficacy of these kits. Since these variations did not account fully for the heterogeneity, searching other differences was very important in our meta-analysis.

In other situations, variations in the interpretive criteria for a BG-positive result may occur among studies. For example, some have reported using one single BG sample, some reported the results of two consecutive BG samples, while others reported both. According to our subgroup analyses, we found that compared to a single positive result, two consecutive BG results reduced the overall SEN value, but enhanced the overall SPE value. Analysis of the kinetics of BG levels (provided that serial samples are obtained during the period of risk) helped in the identification of false-positive results since in these patients BG levels showed abrupt rises and falls (31, 36). Very recently, Hope et al investigated the kinetics of BG in persistently neutropenic rabbits with IA (37). The study suggested that the kinetics of BG provided important information regarding the extent and stage of infection. Thus, at least two sequential positive BG results are required for indirect mycological criteria to allow possible IFD to be upgraded to probable IFD (7). Considering that false-positive BG may result from various factors, further studies focused on the test performance of serial samples detection are needed.

In the revised EORTC/MSG guideline in 2008, the definition of probable IFD has been expanded, whereas the definition of possible IFD has been diminished (7). To clarify the relations between BG and IFD status, we used different methods to define the true-positive cases and true-negative cases, as described by Koo et al (27). Koo et al reported that there were no significant changes in the diagnostic indices, irrespective of the methods used. However, we found that these diagnostic indices varied according to different methods. Because there is an intrinsic uncertainty regarding the true disease status of IFD, the calculation of these indices could be significantly affected by the definition of the disease status. Except for the different number of included studies, the variable results of test performances might be explained on the basis that many false-negative results occurred in probable or possible IFD cases. However, due to lack of eligible data, we failed to perform subgroup analyses for false-negative or false-positive factors.

The present results for the combination of GM and BG assay showed that although it failed to improve the SEN of each assay, the combined use was very useful in confirming the diagnosis of IA. The results of our analyses might be explained on the basis that the kinetics of GM and BG in patients with IA was found to be similar (31, 36). Furthermore, the combined use of two assays could be useful to identify the false positive results. Thus, if clinical conditions are feasible, we recommend performing the combination assay to rule in diagnoses for at-risk patients.

The present study had several important limitations. First, infections due to Cryptococcus species or Zygomycetes account for approximately 10% of all IFDs (4-6). However, the BG assay is not useful in diagnosing these diseases (7), which led to biased results. Second, the BG assay appeared to be of particular relevance for the diagnosis of pneumocystis pneumonia (PCP), because nearly all of the reported PCP patients have presented highly positive BG levels (45/46, 97.8%) (20, 22, 27, 29, 32). However, the guidelines did not confirm “serum-BG” as microbiologic evidence for PCP (7). Further meta-analysis is needed to assess the overall accuracy of serum-BG for diagnosing PCP. Lastly, two important possible covariates could not be assessed due to lack of data: beta-lactam antibiotics and antifungal therapy, because most patients have been treated with antibiotics or antifungal drugs before the BG assay. Further studies focused on the impact of these factors are needed.

Conclusion

In conclusion, the current meta-analysis suggests that the BG assay can be used as a diagnostic adjunct tool for at-risk patients, especially for patients with hematological disorders. However, at least two sequential positive BG results are required for diagnosing probable IFD. Since the BG assay is
The authors state that they have no Conflict of Interest (COI).

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