

# $\beta$ -Glucan Antigenemia Assay for the Diagnosis of Invasive Fungal Infections in Patients With Hematological Malignancies: A Systematic Review and Meta-Analysis of Cohort Studies From the Third European Conference on Infections in Leukemia (ECIL-3)

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**Background.** Invasive fungal infections (IFIs) are life-threatening complications in patients with hematological malignancies, and early diagnosis is crucial for outcome. The compound 1,3- $\beta$ -D-glucan (BG), a cell wall component of most fungal species, can be detected in blood during IFI. Four commercial BG antigenemia assays are available (Fungitell, Fungitec-G, Wako, and Maruha). This meta-analysis from the Third European Conference on Infections in Leukemia (ECIL-3) assessed the performance of BG assays for the diagnosis of IFI in hematological patients.

**Methods.** Studies reporting the performance of BG antigenemia assays for the diagnosis of IFI (European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria) in hemato-oncological patients were identified. The analysis was focused on high-quality cohort studies with exclusion of case-control studies. Meta-analysis was performed by conventional meta-analytical pooling and bivariate analysis.

**Results.** Six cohort studies were included (1771 adult patients with 414 IFIs of which 215 were proven or probable). Similar performance was observed among the different BG assays. For the cutoff recommended by the manufacturer, the diagnostic performance of the BG assay in proven or probable IFI was better with 2 consecutive positive test results (diagnostic odds ratio for 2 consecutive vs one single positive results, 111.8 [95% confidence interval {CI}, 38.6–324.1] vs 16.3 [95% CI, 6.5–40.8], respectively; heterogeneity index for 2 consecutive vs one single positive results, 0% vs 72.6%, respectively). For 2 consecutive tests, sensitivity and specificity were 49.6% (95% CI, 34.0%–65.3%) and 98.9% (95% CI, 97.4%–99.5%), respectively. Estimated positive and negative predictive values for an IFI prevalence of 10% were 83.5% and 94.6%, respectively.

**Conclusions.** Different BG assays have similar accuracy for the diagnosis of IFI in hemato-oncological patients. Two consecutive positive antigenemia assays have very high specificity, positive predictive value, and negative predictive value. Because sensitivity is low, the test needs to be combined with clinical, radiological, and microbiological findings.

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Early recognition and treatment of life-threatening invasive fungal infections (IFIs) in patients with hemato-oncological malignancies are crucial for positive outcomes [1]. Uncertain detection of IFI, due to limited performance of cultures and imaging, results in prolonged empirical antifungal therapy [2, 3]. New tools are thus needed for early diagnosis and prompt, targeted therapy.

The glucose polymer 1,3- $\beta$ -D-glucan (BG), a major cell wall component of most fungal species (with exception of zygomycetes and *Cryptococcus* spp.), is released in blood and tissues in the course of IFI. Measurement of BG levels is based on activation by BG of factor G of the coagulation cascade in the amoebocyte lysate from the horseshoe crab, which leads to quantifiable transformation of a chromogenic substrate [4]. BG assays with different thresholds of detection that use reagents from different horseshoe crab species are commercially available: *Limulus polyphemus* from North America and *Tachypleus tridentatus* from Japan. BG has been evaluated in case-control studies (patients with proven or probable IFI vs healthy controls or distinct patient populations at low risk of IFI) with 50%–90% sensitivity and 70%–100% specificity [5–8]. Monitoring BG antigenemia has also been assessed in prospective cohorts of homogeneous populations at high risk of IFI [9–11], which better reflect the real performance of the test.

Recently, the BG test has been included in the revised European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG) consensus definitions of probable IFI [2]. However, no recommendations have been formulated regarding the use of different assays, timing and number of measurements, and criteria of positivity.

This study was conducted on behalf of the Third European Conference on Infections in Leukemia (ECIL-3; Juan-les-Pins, France, September 2009). The objective was to assess the performance of BG assays for diagnosis of IFI in hemato-oncological patients by a systematic literature review and meta-analysis of cohort studies.

## METHODS

### Search Strategy and Selection Criteria

A systematic search of the literature was performed using the following sources from inception through the end of December 2009: Medline database through PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), Embase through Ovid, and Scopus (<http://www.scopus.com/home.url>). The following keywords were used: glucan, beta-glucan, fungal infection, mycoses, aspergillosis, *Aspergillus*, candidiasis, and *Candida*. The following syntax was used: “glucan” OR “beta-glucan” AND “fungal infections” OR “mycoses,” AND/OR “Aspergillus” OR “aspergillosis,” AND/OR “candidiasis” OR “Candida.” This search was completed by the consultation of abstracts from international infectious diseases or hemato-oncology

meetings and reviews (2005–2009). No language restriction was applied. The abstracts identified through the above search strategy were screened by 3 authors (F. L., M. C., and O. M.). Full text of potentially relevant publications of clinical studies reporting measurement of BG antigenemia for the diagnosis of IFI was analyzed on the basis of the following inclusion criteria: (1) The test used was a commercially available BG assay with evaluation of the cutoff value for positivity recommended by the manufacturer: Fungitell (formerly GlucateLL; Associates of Cape Cod, East Falmouth, MA), Fungitec-G (Seikagaku, Kogyo, Tokyo, Japan), Wako turbidimetric assay (Wako Pure Chemical Industries, Tokyo, Japan), and Maruha colorimetric assay (Maruha-Nichiro Foods, Tokyo, Japan). (2) The study population was mainly (>50%) composed of adult or pediatric hemato-oncological patients at risk of IFI (prolonged neutropenia after myelosuppressive chemotherapy for acute leukemia or hematopoietic stem cell transplantation). (3) The EORTC-MSG criteria [2, 12] were used as a reference standard for classification of IFI, invasive candidiasis (IC), and invasive aspergillosis (IA) as proven, probable, or possible, independently of BG test results (2002 criteria were used in all studies with the exception of 1 study that applied 2008 criteria after exclusion of BG test results). Studies conducted before 2002 could be included if the reported information allowed post hoc classification of IFI according to EORTC-MSG criteria. Studies reporting the use of BG in patients with *Cryptococcus* spp. or *Pneumocystis jirovecii* infections only were excluded, because these fungal pathogens are beyond the scope of this analysis. (4) Data on true-positive, true-negative, false-positive, and false-negative results of the BG test were reported separately or could be calculated from the manuscript. (5) BG measurements were performed in a homogenous cohort of patients at risk of IFI according to a monitoring strategy or based on clinical suspicion of IFI (case-control studies comparing IFI with healthy controls or controls from distinct patient populations at low risk of IFI were excluded). If needed, corresponding authors were contacted for retrieval of complementary information. Agreement of 3 authors (F. L., M. C., and O. M.) on the above criteria was required for inclusion in the analysis.

Quality of included studies was assessed by 3 authors (F. L., M. C., and C. M.) according to recommendations of Standards for Reporting of Diagnostic Accuracy [13] by use of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool based on 14 items (Figure 2) specifically developed for assessing quality of studies on diagnostic tests [14]. Each item was scored as “yes,” “no,” or “unclear,” and agreement of 3 authors was required.

### 1,3- $\beta$ -D-Glucan Data and Statistical Analyses

Raw data of positive and negative BG test results were extracted for all cutoffs and evaluated for the following diagnostic criteria:

(1) proven or probable IFI according to EORTC-MSG classification [2, 12] versus no IFI, possible IFI being excluded; (2) proven, probable, or possible IFI versus no IFI; (3) proven or probable IC versus no IFI, other IFI being excluded; and (4) proven or probable IA versus no IFI, other IFI being excluded. Criteria defining a positive test were as follows: (1) a single value above a given cutoff (criterion A), and (2) 2 consecutive values above a given cutoff (criterion B). Analyses of receiver operating characteristic (ROC) curves were performed for studies reporting results for different BG cutoffs.

For meta-analysis, 2 distinct approaches were used: (1) a conventional meta-analytical method after logit transformation (based on the inverse variance method for weight evaluation) [15], and (2) a bivariate random effects approach, which preserves the bidimensional nature of sensitivity and specificity data [16, 17]. Cumulative estimates of sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratios (DORs) were calculated. The DOR ( $[\text{true positives} \times \text{true negatives}] / [\text{false positives} \times \text{false negatives}]$ ) was adopted as a diagnostic accuracy index that simultaneously integrates sensitivity and specificity. The hierarchical summary ROC (hsROC) curve method was used [18] in order to model the trade-off between sensitivity and specificity for various cutoffs. The hsROC curve method overcomes some limitations of the traditional summary ROC curve procedure indicated by Moses and Littenberg [19] and is closely related to bivariate random-effects meta-analysis. Heterogeneity was assessed by visual inspection of forest plots and hsROC plots of raw data. Moreover, heterogeneity was formally assessed by a test of inconsistency ( $I^2$ ). Because some studies explored several BG cutoffs, only data on cutoffs recommended by the manufacturers for each single assay were included in meta-analysis (Fungitell cutoff, 60–80 pg/mL; Fungitec-G cutoff, 20 pg/mL; Wako and Maruha cutoff, 11 pg/mL).

Positive and negative predictive values of BG were determined over a wide range of prevalence of IFI by use of the Bayes' theorem with summary of sensitivity and specificity data. The following statistic software were used: RevMan 5 (<http://ims.cochrane.org/revman/about-revman-5>), MetaDisc ([http://www.hrc.es/investigacion/metadisc\\_en.htm](http://www.hrc.es/investigacion/metadisc_en.htm)), and Stata 11 (<http://www.stata.com/>).

## RESULTS

### Study Selection

A total of 1076 studies were identified by systematic review of the literature: 1036 were excluded because they did not address the study question or did not fulfill the inclusions criteria, and 40 were reviewed as full papers (Figure 1). Ten cohort studies were identified, of which 4 did not meet the inclusion criteria: 2 were performed in populations other than hemato-oncological patients [20, 21], and 2 evaluated a single cutoff value different

from that recommended by the manufacturer in a limited number of patients [22, 23].

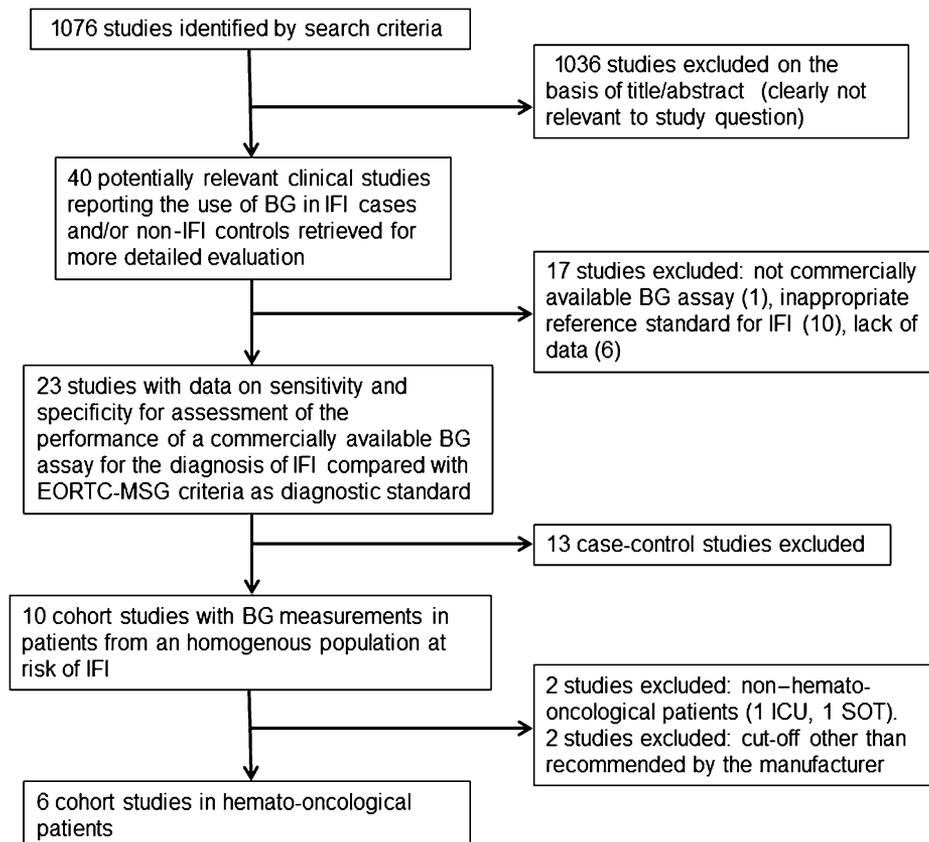
The characteristics of the 6 cohort studies [9–11, 24–26] fulfilling the inclusion criteria are listed in Table 1. The BG levels were measured in blood samples on the basis of a screening strategy (once or twice weekly) in 5 studies. In 1 study [26], all patients who had at least 1 BG measurement for suspected IFI were enrolled. Five studies included exclusively patients with hematological malignancies at high risk of IFI (prolonged neutropenia after myelosuppressive chemotherapy or allogeneic hematopoietic stem cell transplantation), whereas the population was mixed (60% hemato-oncological patients) in the remaining study [26]. The individual quality assessments of each study, as well as the summary for each item across all the included studies, are presented in Figure 2.

A total of 1771 patients were analyzed, including 414 episodes of IFI, of whom 215 (52%) were classified as proven or probable according to EORTC-MSG criteria: 90 (42%) had IA, 80 (37%) had IC, and 45 (21%) had IFI attributed to other fungi (including mixed episodes); (Table 1).

The performance of the BG assay for the diagnosis proven or probable IFI could be assessed in all but 1 study [9], which reported only sensitivity and specificity for proven, probable, or possible IFI. Performance for different cutoffs was assessed in 4 studies [9, 11, 25, 26]. Different numbers of test results above a given cutoff to define positivity (single test, criterion A;  $\geq 2$  consecutive tests, criterion B) were assessed in 4 studies [9–11, 25]. The 6 primary publications generated several comparisons to the reference standard: 15 for the Fungitell assay (cutoff, 31–500 pg/mL), 18 for the Wako and Maruha assays (cutoff, 2–11 pg/mL), and 1 for the Fungitec-G assay (cutoff, 20 pg/mL).

### ROC Curve Analyses

The ROC curve analyses of studies reporting different cutoffs of positivity in proven or probable IFI are shown in Figure 3. For the Wako and Maruha assays, cutoffs of 2–11 pg/mL were tested in 2 studies [11, 25]. The areas under the curves (AUCs) obtained by pooling the results of both studies for criteria A and B did not differ considerably (0.84 and 0.88, respectively). For the Fungitell assay, different cutoffs were evaluated in 1 study (31–500 pg/mL) [26]. Analysis was possible only for criterion A: the AUC for this criterion was similar to that obtained with the Wako and Maruha assays (0.80 vs 0.84, respectively). ROC curve analysis was not possible for the Fungitec-G assay because data were lacking on the diagnostic performance of different BG cutoffs. On the basis of the ROCs, the optimal cutoffs (ie, the shortest distance between the point of a given cutoff on the ROC curve and the left upper corner of the graph representing 100% sensitivity and 100% specificity) were as follows: 5 pg/mL (sensitivity, 76%; specificity, 77%) for criterion A and 3 pg/mL



**Figure 1.** Flowchart of study selection. Abbreviations: BG,  $\beta$ -glucan assay; EORTC-MSG, European Organization for Research and Treatment of Cancer and Mycoses Study Group; ICU, intensive care unit; IFI, invasive fungal infection; SOT, solid-organ transplant recipient.

(sensitivity, 85%; specificity, 74%) for criterion B with the Wako and Maruha assays, and 60 pg/mL (sensitivity, 70%; specificity, 81%) for criterion A with the Fungitell assay.

### Meta-Analysis

For the meta-analysis, data obtained with the cutoff recommended by the manufacturer (see “Methods”) were selected for each study. Results of the bivariate analysis for criterion A are shown in Figure 4 (not performed for criterion B because of lacking data). Figure 5 shows the forest plot of the DOR obtained by meta-analytical pooling. For the diagnosis of proven or probable IFI, a higher DOR was obtained for criterion B when compared with criterion A (111.8 vs 16.3, respectively). Heterogeneity was absent for criterion B, but it was substantial for criterion A ( $I^2$ , 0% and 71.6 %, respectively). The overall performance of the BG test was lower for proven, probable, or possible IFI. Results of cumulative estimates of DOR, sensitivity, specificity, and positive and negative likelihood ratios obtained by both methods (bivariate analysis and meta-analytical pooling) are shown in Table 2.

Data on the performance of BG for the diagnosis of proven or probable IA and IC were reported separately in 4 studies

[10, 11, 24, 25] and 2 studies [10, 11], respectively. The pooled DOR, sensitivity, and specificity in IA and IC were as follows: (1) criterion A, 79.1 (21.0–298.6), 0.57 (0.33–0.83), and 0.97 (0.96–0.98) in IA and 177.6 (16.1–1954.2), 0.73 (0.57–0.85), and 0.97 (0.95–0.98) in IC; (2) criterion B, 24.1 (6.8–84.5), 0.46 (0.21–0.73), and 0.97 (0.95–0.98) in IA, and 124.7 (33.6–462.1), 0.75 (0.42–0.94), and 0.97 (0.96–0.98) in IC.

The estimated positive and negative predictive values of the BG test for the diagnosis of proven or probable IFI according to the IFI prevalence are shown in Figure 6. The median IFI prevalence in the analyzed studies was 10% (range, 7%–27%), which resulted in an estimated positive predictive value of 46.1% and negative predictive value of 97.1% for criterion A, and a positive predictive value of 83.5% and negative predictive value of 94.6 % for criterion B.

### DISCUSSION

This ECIL-3 meta-analysis of the diagnostic performance of BG antigenemia in IFI was focused on adult hemato-oncological patients at high risk of IFI in whom preemptive antifungal strategies guided by biological markers are being intensively

**Table 1. Characteristics of Cohort Studies Assessing the Performance of  $\beta$ -Glucan Assays for the Diagnosis of Invasive Fungal Infection in Patients With Hematological Malignancies**

Study Reference	BG Assay	Biological Sample	Study Design	Frequency of BG Screening	Study Population	Antifungal Therapy	No. of Patients					
							Total	Any IFI	Proven or Probable IFI	Invasive Candidiasis	Invasive Aspergillosis	Other IFI
Odabasi et al (2004) [10]	Fungitell (ACC, United States)	Serum	Prospective, consecutive	Twice per week	Adults (100% with H, no aHSCT)	Prophylaxis (all)	283	53	20	11	3	6 <sup>a</sup>
Ellis et al (2008) [9]	Fungitell (ACC, United States)	Serum	Prospective, consecutive	Every 2 days	Adults (100% with H, no aHSCT)	Empirical <sup>b</sup> (all)	80	38	26	12	14	0
Koo et al (2009) [26]	Fungitell (ACC, United States)	Serum	Retrospective, consecutive	No screening <sup>c</sup>	Adults (60% with H, 23% with aHSCT)	NA	871	209 <sup>d</sup>	112	41	32	39 <sup>e</sup>
Kami et al (2000) [24]	Fungitec-G (Seikagaku, Japan)	Plasma	Prospective, consecutive	Once per week	Adults (100% with H, 30% with aHSCT)	Prophylaxis (all)	215	30	16	0	16	0
Kawazu et al (2004) [25]	Wako (Japan)	Plasma	Prospective, consecutive	Once per week	Adults (100% with H, 38% with aHSCT)	NA	149	24	11	0	11	0
Senn et al (2008) [11]	Maruha and Wako (Japan)	Serum	Prospective, consecutive	Twice per week	Adults (100% with H, no aHSCT)	Prophylaxis (22% of patients)	173	60	30	16	14	0

Abbreviations: ACC, Associates of Cape Cod (see "Methods"); aHSCT, allogeneic hematopoietic stem cell transplantation; BG, 1,3- $\beta$ -D-glucan; H, hematological malignancies; IFI, invasive fungal infection; NA, data not available.

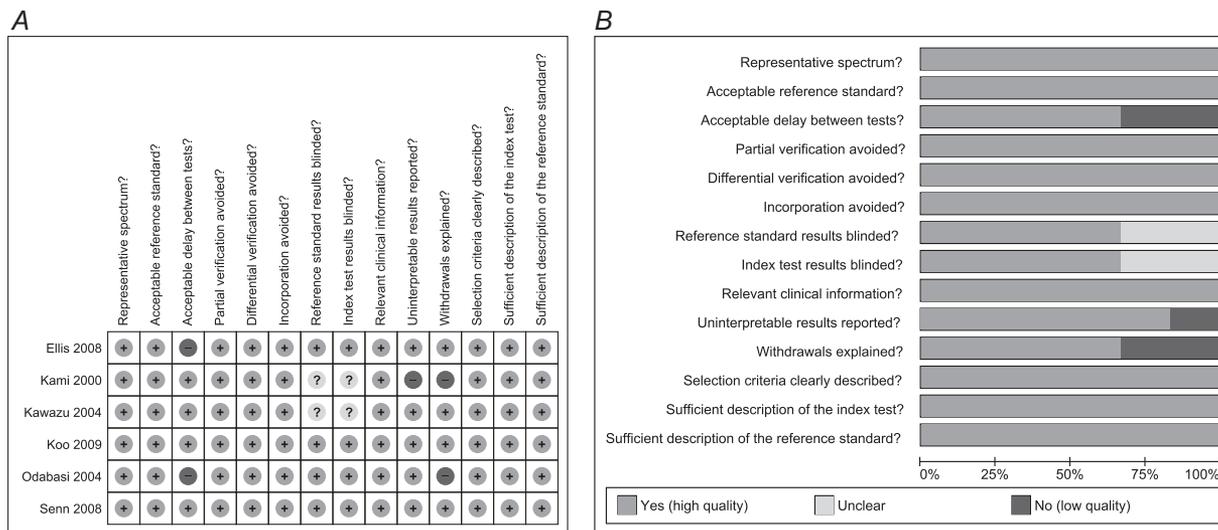
<sup>a</sup> Other molds, 5 patients; mixed species, 1 patient.

<sup>b</sup> All patients had persistent neutropenic fever of unknown origin for >3 days.

<sup>c</sup> The first BG measurement performed according to clinical suspicion of IFI was used for analysis.

<sup>d</sup> IFI occurring within 1 week after initial BG testing.

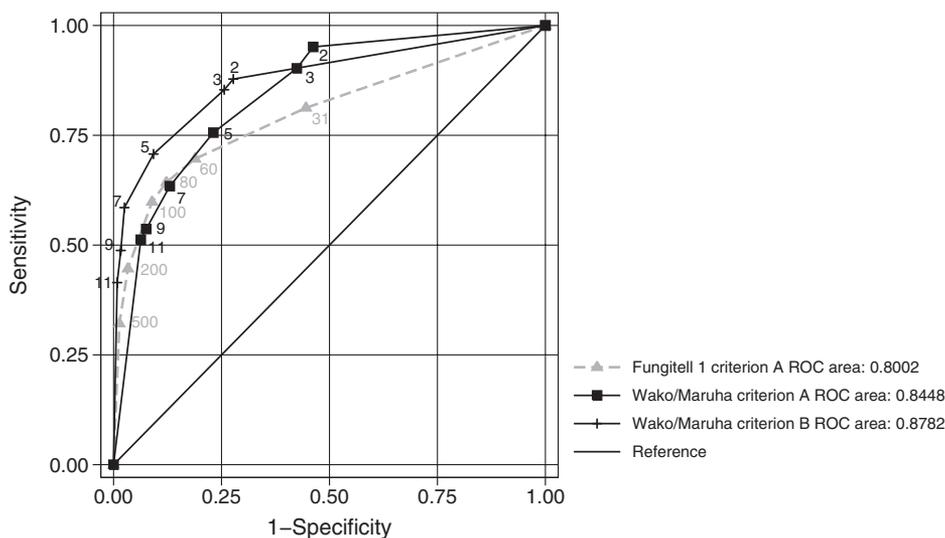
<sup>e</sup> *Pneumocystis jirovecii*, 14 patients; zygomycetes, 4 patients; other molds or yeasts, 16 patients; mixed species, 3 patients; dimorphic fungi, 2 patients.



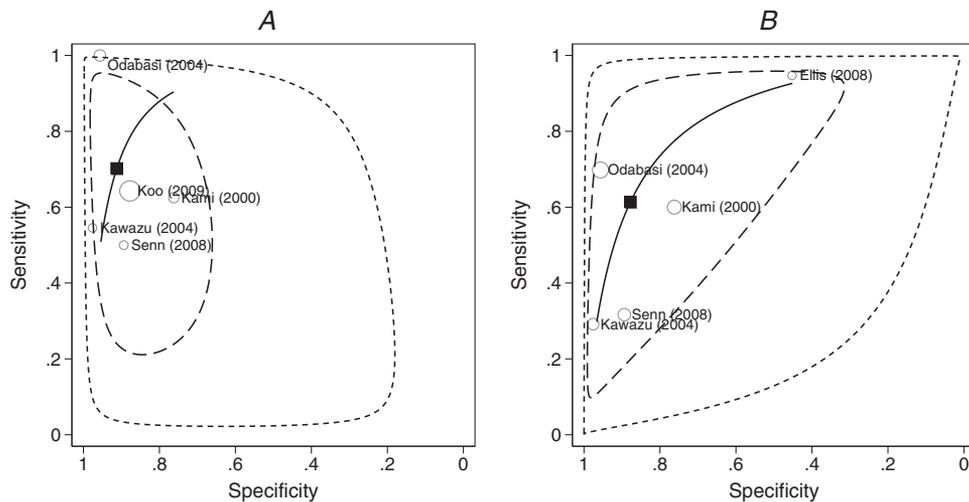
**Figure 2.** Methodological quality graph summary of all included studies with the authors' appreciation about each methodological quality item (QUADAS tool) according to the recommendations of the Standards for Reporting of Diagnostic Accuracy. Studies are identified by first author and year. Quality assessment of each individual study by the 14 items (A), and summary of quality assessment for each of the 14 items across all the included studies (B).

investigated. Data on 1771 patients, including 414 IFI cases (215 proven or probable), were extracted from 6 high-quality cohort studies fulfilling strict criteria for patient enrollment, data collection, and data analysis. In particular, the selection of

patients with homogenous underlying risk profiles makes them highly representative of the real clinical conditions in the hemato-oncological setting. Because of their implications for BG use in these patients, some findings of this meta-analysis



**Figure 3.** Receiver operating characteristic (ROC) curve analyses of  $\beta$ -glucan (BG) assays for the diagnosis of invasive fungal infections (IFIs; European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria). Analyses have been performed for the diagnostic standard proven or probable IFI for 2 different types of BG assay (Fungitell and Wako/Maruha). Analysis for each test is shown according to the number of BG test results above a given cutoff: 1 single positive value (criterion A) and 2 positive consecutive values (criterion B). Different cutoffs were assessed in 3 studies: Koo et al (2009) [26] for Fungitell (analysis for criterion B was not possible for this study because of lacking data), Senn et al (2008) [11] for Wako/Maruha, and Kawazu et al (2004) [25] for Wako/Maruha. Each point of the ROC is labeled by the corresponding cutoff (pg/mL).

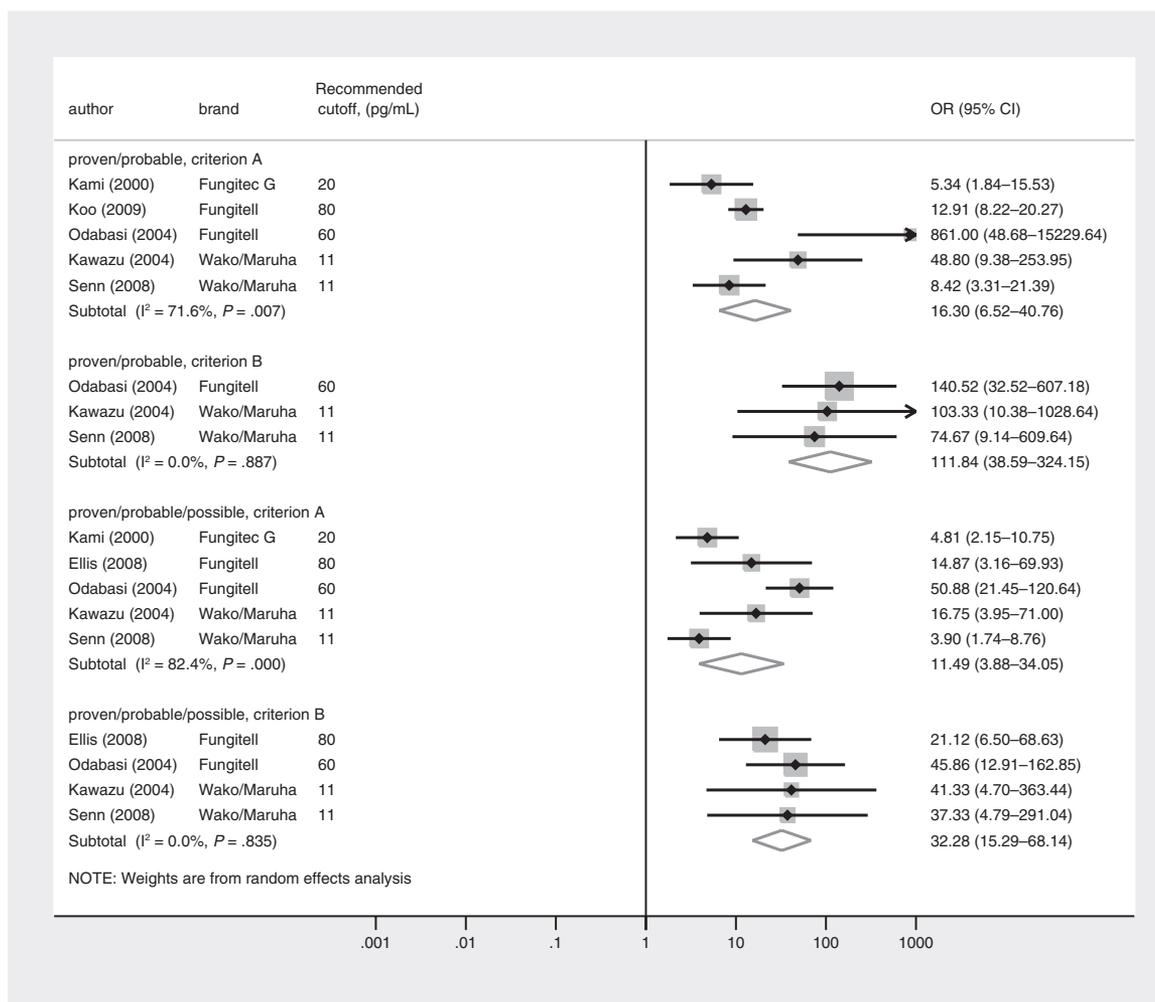


**Figure 4.** Bivariate analysis of the sensitivity and specificity of  $\beta$ -glucan (BG) assays for the diagnosis of invasive fungal infection (IFI; European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria) using the cutoffs recommended by the manufacturer (Fungitell cutoff, 60–80 pg/mL; Fungitec-G cutoff, 20 pg/mL; Wako and Maruha cutoff, 11 pg/mL). *A*, Diagnostic standard proven or probable IFI with a single value above the recommended cutoff (criterion A). *B*, Diagnostic standard proven, probable, or possible IFI with a single value above the recommended cutoff (criterion A). The bivariate analysis presents studies as sensitivity and specificity data, which are identified by the coordinates of corresponding points in sensitivity-specificity space. Ellipses indicate the bidimensional limits of confidence of sensitivity and specificity for each diagnostic criterion: dashed line, 95% confidence area; dotted line, 95% prediction area. The hierarchical summary ROC (hsROC) curve is a quasi-equivalent summary ROC that is coherent with the bivariate estimate. The analysis was not possible for criterion B (2 consecutive values above the recommended cutoff) because of lacking data. Studies are identified by first author and year. Solid line, hsROC curve; square, central estimate.

should be highlighted. First, similar diagnostic accuracy was found among commercial BG assays based on different reagents (*L. polyphemus* and *T. tridentatus* horseshoe crabs). Second, higher diagnostic performance and better homogeneity of test results were found for 2 consecutive positive BG antigenemia assays than for a single one; although derived from a limited number of studies that used different screening strategies, this finding is clinically relevant because no recommendation regarding the number of measurements required for defining a BG test result as positive (1 single vs 2 consecutive positive antigenemia assays) has been formulated so far. Third, regardless of the criteria for positivity and despite very high negative predictive values, the sensitivity of BG remains low; this implies that a negative result is not reliable to rule out this life-threatening disease and that therapeutic decisions should only be based on combining BG with classical clinical, radiological, and microbiological findings. Fourth, very high specificity (99%) and positive predictive values of 2 consecutive positive BG antigenemia assays suggest that such a finding is sufficient per se to rule in diagnosis of probable IFI according to EORTC-MSG criteria and to preemptively start antifungal therapy. Fifth, the present meta-analysis shows that important issues remain unanswered. Due to differences in screening strategies (BG monitoring during the period at risk for IFI vs punctual testing based on clinically driven suspicion of IFI) and

in frequencies of BG testing (once to thrice weekly), optimal timing of blood sampling and time interval between 2 BG measurements to confirm a positive result could not be assessed. Lack of data also preclude drawing conclusions on the optimal cutoff for BG positivity. The ROC curve analyses suggested a lower threshold than that recommended for the Fungitell assay (60 vs 80 pg/mL) and the Wako and Maruha assays (3–5 vs 11 pg/mL). However, these findings were derived from limited data in few studies. The meta-analysis was thus based on cutoffs recommended by manufacturers to avoid bias due to unreliable accuracy of extreme values. Data on performance of BG in IFI due to specific fungal pathogens are also unclear or lacking. Despite apparent superiority of BG performance in IC compared with that in IA, this result should be interpreted cautiously because separate analysis in IC was available from only 2 studies. The low number of IFI due to less common fungal pathogens did not allow any specific subgroup analysis.

A recent meta-analysis on BG performance for the diagnosis of IFI pooled hemato-oncological patients, nonhematological patients in the intensive care unit, and transplant recipients from case-control and cohort studies. With important data heterogeneity, the sensitivity and specificity of a single positive BG value was 76.8% and 85.3%, respectively [27]. The broad mix of both cases controls and study designs could explain the better



**Figure 5.** Forest plot of diagnostic odds ratios (DORs) of  $\beta$ -glucan (BG) assays for the diagnosis of invasive fungal infections (IFIs; European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria) using the cutoffs recommended by the manufacturer (Fungitell cutoff, 60–80 pg/mL; Fungitec-G cutoff, 20 pg/mL; Wako and Maruha cutoff, 11 pg/mL). Analyses were performed for the diagnostic standards proven or probable IFI, and proven, probable, or possible IFI. Analysis for each test is shown according to the number of BG test results above the recommended cutoff: 1 single positive value (criterion A) and 2 positive consecutive values (criterion B). The sizes of the squares are proportional to the weights of the studies. Studies are identified by first author and year. Abbreviations: CI, confidence interval.

sensitivity and lower specificity compared with the present meta-analysis strictly derived from cohorts of hemato-oncological patients. Moreover, diagnostic performance was not assessed for 2 consecutive positive BG values.

Some intrinsic limitations due to the design of the included studies need to be discussed. Based on quality assessment (using the QUADAS tool), lack of precision on timing of BG measurements referring to time of diagnosis of IFI according to EORTC-MSG criteria was observed. In the majority of studies, information on blinded interpretation of BG test results, reporting of noninterpretable BG test results, and explanation of data withdrawals from analysis were unclear or lacking. In addition, most studies did not report data on potential causes

of false-negative BG test results (eg, antifungal prophylaxis or therapy at time of BG measurement, and type, location, and severity of IFI) or false-positive results (eg, concomitant  $\beta$ -lactam therapy; blood transfusions, blood-derived products, or hemodialysis or hemofiltration; and bacterial coinfections). Lack of data on specific types of patients (eg, allogeneic hematopoietic stem cell transplant recipients vs other hemato-oncological patients, patients receiving vs those not receiving antifungal agents, neutropenic vs nonneutropenic patients, and patients with vs those without gastrointestinal tract mucositis or graft-vs-host disease) did not allow differentiated subgroup analyses. None of the included studies reported data from pediatric populations, in whom higher BG levels and lower specificity

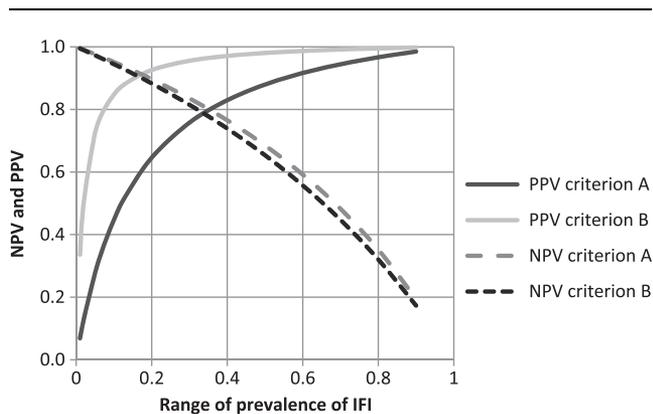
**Table 2. Cumulative Estimates of Diagnostic Odds Ratio, Sensitivity, Specificity, and Positive and Negative Likelihood Ratios of the  $\beta$ -Glucan Assay for the Diagnosis of Invasive Fungal Infections**

Criterion for Positivity, Analysis <sup>a</sup>	DOR (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PLR (95% CI)	NLR (95% CI)
Proven or probable IFI					
Criterion A					
Bivariate	24.4 (5.8–103.2)	70.2 (47.0–86.2)	91.2 (83.1–95.6)	7.99 (3.45–18.51)	0.33 (0.16–0.67)
MA pooling	16.3 (6.5–40.8)	61.5 (48.3–73.2)	90.8 (83.4–95.1)	7.30 (3.59–14.84)	0.46 (0.34–0.60)
Criterion B					
Bivariate	NA	NA	NA	NA	NA
MA pooling	111.8 (38.6–324.1)	49.6 (34.0–65.3)	98.9 (97.4–99.5)	50.10 (20.25–123.97)	0.53 (0.40–0.71)
Proven, probable, or possible IFI					
Criterion A					
Bivariate	11.4 (4.6–28.3)	61.3 (32.3–84.1)	87.8 (67.7–96.1)	5.02 (2.23–11.33)	0.44 (0.23–0.83)
MA pooling	11.5 (3.9–34.1)	59.4 (34.9–80.0)	87.3 (68.6–95.6)	4.38 (2.00–9.59)	0.52 (0.35–0.76)
Criterion B					
Bivariate	NA	NA	NA	NA	NA
MA pooling	32.3 (15.3–68.1)	44.1 (20.1–71.2)	97.5 (81.5–99.7)	14.98 (3.60–62.36)	0.64 (0.49–0.83)

Abbreviations: BG, 1,3- $\beta$ -D-glucan; CI, confidence interval; DOR, diagnostic odds ratio; IFI, invasive fungal infection; MA, meta-analytical; NA, not available; NLR, negative likelihood ratio; PLR, positive likelihood ratio.

<sup>a</sup> According to the European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria: criterion A of IFI, positivity defined by a single BG value above the recommended cutoff; criterion B, positivity defined by 2 consecutive BG values above the recommended cutoff. The cumulative estimates were calculated using data for the cutoff recommended by the manufacturer (Fungitell cutoff, 60–80 pg/mL; Fungitec-G cutoff, 20 pg/mL; Wako and Maruha cutoff, 11 pg/mL) of each study. Diagnostic accuracy indices were evaluated by bivariate analysis and meta-analytical pooling (random effects according to DerSimonian and Laird [36]; weights by the inverse variance method) for criterion A and criterion B. The bivariate analysis was not possible for criterion B due to lacking data.

have been suggested in small series [28, 29]. Moreover, only data on performance of the BG test in blood samples are



**Figure 6.** Estimated positive predictive values (PPVs) and negative predictive values (NPVs) of  $\beta$ -glucan (BG) assays for the diagnosis of proven or probable invasive fungal infections (IFIs; European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria) in patients with hemato-oncological malignancies, using the cutoffs recommended by the manufacturer (Fungitell cutoff, 60–80 pg/mL; Fungitec-G cutoff, 20 pg/mL; Wako and Maruha cutoff, 11 pg/mL; Bayesian approach). The x-axis is the range of prevalence of IFI (0%–100%); the y-axis is PPV and NPV (%). The analysis for each test is shown according to the number of BG test results above the recommended cutoff: 1 single positive value (criterion A) and 2 positive consecutive values (criterion B).

available. Its diagnostic utility in other biological samples (eg, bronchoalveolar lavage fluid, cerebrospinal fluid, and tissue biopsy specimens) remains unknown.

The BG test can be used as diagnostic tool for the detection of a broad spectrum of fungal pathogens. Compared with other fungal markers specific for *Aspergillus* spp. (galactomannan) or *Candida* spp. (mannan and antimannan), BG seems to have lower sensitivity and similar specificity [30, 31]. However, the few studies comparing or combining BG with other fungal markers do not allow any conclusion about the superiority of a single test or combination of tests [5, 23, 25]. Prospective randomized interventional studies assessing the role of a screening strategy that includes single or multiple fungal markers in combination with clinical, radiological, and microbiological findings for starting preemptive antifungal therapy are scarce [32–34]. Such studies may better define the clinical validity and cost effectiveness of a BG-driven strategy for management of IFI in hemato-oncological patients.

In conclusion, commercially available BG assays display similar accuracies in hemato-oncological patients at high risk of IFI, with the highest diagnostic performance achieved with the use of 2 consecutive positive BG values. Despite the high negative predictive value, due to low sensitivity, a negative BG antigenemia assay should be interpreted with caution and only in combination with clinical, radiological, and microbiological findings. However, remarkably high specificity and positive

predictive values of 2 consecutive positive BG antigenemia assays make this test a reliable diagnostic marker of IFI. These findings are consistent with the B-II grading of recommendation and level of evidence proposed by the ECIL-3 expert panel for the use of BG in hemato-oncological patients [35]. Assessment of the optimal BG cutoff, best timing of BG measurements, and efficiency of combining BG with other fungal markers needs specifically designed clinical investigations.

## Notes

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## References

1. Neofytos D, Horn D, Anaissie E, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin Infect Dis* **2009**; 48:265–73.
2. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* **2008**; 46:1813–21.
3. Hughes WT, Armstrong D, Bodey GP, et al. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* **2002**; 34:730–51.
4. Obayashi T, Yoshida M, Tamura H, Aketagawa J, Tanaka S, Kawai T. Determination of plasma (1→3)-β-D-glucan: a new diagnostic aid to deep mycosis. *J Med Vet Mycol* **1992**; 30:275–80.
5. Hachem RY, Kontoyiannis DP, Chemaly RF, Jiang Y, Reitzel R, Raad I. Utility of galactomannan enzyme immunoassay and (1,3)-β-D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. *J Clin Microbiol* **2009**; 47:129–33.
6. Obayashi T, Negishi K, Suzuki T, Funata N. Reappraisal of the serum (1→3)-β-D-glucan assay for the diagnosis of invasive fungal infections—a study based on autopsy cases from 6 years. *Clin Infect Dis* **2008**; 46:1864–70.

7. Ostrosky-Zeichner L, Alexander BD, Kett DH, et al. Multicenter clinical evaluation of the (1→3)-β-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* **2005**; 41:654–9.
8. Persat F, Ranque S, Derouin F, Michel-Nguyen A, Picot S, Sulhian A. Contribution of the (1→3)-β-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* **2008**; 46:1009–13.
9. Ellis M, Al-Ramadi B, Finkelman M, et al. Assessment of the clinical utility of serial β-D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* **2008**; 57:287–95.
10. Odabasi Z, Mattiuzzi G, Estey E, et al. β-D-Glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* **2004**; 39:199–205.
11. Senn L, Robinson JO, Schmidt S, et al. 1,3-β-D-Glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. *Clin Infect Dis* **2008**; 46:878–85.
12. Ascoglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* **2002**; 34:7–14.
13. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *AJR Am J Roentgenol* **2003**; 181:51–5.
14. Whiting P, Rutjes AW, Dinnes J, Reitsma J, Bossuyt PM, Kleijnen J. Development and validation of methods for assessing the quality of diagnostic accuracy studies. *Health Technol Assess* **2004**; 8:iii, 1–234.
15. Deeks JJ. Systematic reviews in health care: systematic reviews of evaluations of diagnostic and screening tests. *BMJ* **2001**; 323:157–62.
16. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol* **2005**; 58:982–90.
17. van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med* **2002**; 21:589–624.
18. Rutter CM, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Stat Med* **2001**; 20:2865–84.
19. Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. *Stat Med* **1993**; 12:1293–316.
20. Akamatsu N, Sugawara Y, Kaneko J, Tamura S, Makuuchi M. Preemptive treatment of fungal infection based on plasma (1→3)-β-D-glucan levels after liver transplantation. *Infection* **2007**; 35:346–51.
21. Presterl E, Parschalk B, Bauer E, Lassnigg A, Hajdu S, Graninger W. Invasive fungal infections and (1,3)-β-D-glucan serum concentrations in long-term intensive care patients. *Int J Infect Dis* **2009**; 13:707–12.
22. Pazos C, Moragues MD, Quindos G, Ponton J, del Palacio A. Diagnostic potential of (1,3)-β-D-glucan and anti-*Candida albicans* germ tube antibodies for the diagnosis and therapeutic monitoring of invasive candidiasis in neutropenic adult patients. *Rev Iberoam Micol* **2006**; 23:209–15.
23. Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-β-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* **2005**; 43:299–305.
24. Kami M, Tanaka Y, Kanda Y, et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1→3)-β-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica* **2000**; 85:745–52.
25. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-β-D-glucan

- test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* **2004**; 42:2733–41.
26. Koo S, Bryar JM, Page JH, Baden LR, Marty FM. Diagnostic performance of the (1 → 3)-β-D-glucan assay for invasive fungal disease. *Clin Infect Dis* **2009**; 49:1650–9.
  27. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β-D-Glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* **2011**; 52:750–70.
  28. Mularoni A, Furfaro E, Faraci M, et al. High levels of β-D-glucan in immunocompromised children with proven invasive fungal disease. *Clin Vaccine Immunol* **2010**; 17:882–3.
  29. Smith PB, Benjamin DK Jr, Alexander BD, Johnson MD, Finkelman MA, Steinbach WJ. Quantification of 1,3-β-D-glucan levels in children: preliminary data for diagnostic use of the β-glucan assay in a pediatric setting. *Clin Vaccine Immunol* **2007**; 14:924–5.
  30. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. *Crit Care* **2010**; 14:R222.
  31. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* **2006**; 42:1417–27.
  32. Cordonnier C, Pautas C, Maury S, et al. Empirical versus preemptive antifungal therapy for high-risk, febrile, neutropenic patients: a randomized, controlled trial. *Clin Infect Dis* **2009**; 48:1042–51.
  33. Girmenia C, Micozzi A, Gentile G, et al. Clinically driven diagnostic antifungal approach in neutropenic patients: a prospective feasibility study. *J Clin Oncol* **2010**; 28:667–74.
  34. Maertens J, Theunissen K, Verhoef G, et al. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* **2005**; 41:1242–50.
  35. Marchetti O, Lamothe F, Mikulska M, Viscoli C, Verweij P, Bretagne S. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. *Bone Marrow Transplant* **2011** [Epub ahead of print].
  36. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* **1986**; 7:177–88.