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The performance of galactomannan in combination with 1,3-β-D-glucan or aspergillus-lateral flow device for the diagnosis of invasive aspergillosis: Evidences from 13 studies

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ABSTRACT

Galactomannan (GM), 1,3-β-D-glucan (BDG) and aspergillus-lateral flow device (LFD) are recognized as diagnostic tools for invasive aspergillosis (IA). The combined performance of these assays, however, is inconsistent in various studies. We undertook a meta-analysis of 13 studies involving 1513 patients to evaluate the utility of GM in combination with BDG or LFD for diagnosing IA. The pooled SEN, SPE, PLR, NLR and diagnostic odds ratio (DOR) were calculated and constructed to summarize the overall combined performance. Combining both positive results of GM and BDG assays leaded to the pooled SEN 0.49 (95%CI 0.27–0.72), SPE 0.98 (95%CI 0.94–1.00), PLR 31.68 (95%CI 5.36–187.37), NLR 0.52 (95%CI 0.32–0.84) and DOR 61.23 (95%CI 6.96–538.90). Comparing with GM and BDG assays, both positive results of GM and LFD leaded to high SEN, similar SPE, low PLR and NLR. At least one positive result of GM or LFD conferred great SEN 0.93 and low NLR 0.08. Both positive results of GM and BDG or LFD assay were in favor of confirming the existence of IA. And both negative results of GM and LFD were beneficial to rule out IA. Further studies with sufficient sample size should focus on the diagnostic performance and cost-effectiveness of these combined tests in clinical setting.

Contents

The diagnosis of invasive aspergillosis (IA) remains a challenge among immunosuppressed and clinical critical patients. In spite of several available antifungal drugs, the mortality rate for IA does not go down, largely owing to the difficulty of the diagnosis based on nonspecific clinical presentation, delayed radiological findings and insensitive mycological methods (Nucci et al., 2013). The traditional microbiological culture has limited performance due to low sensitivity (usually

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<50%) and a long turnaround time (Cuenca-Estrella et al., 2011; Hope et al., 2005). Invasive procedures relying on histopathological evidence are usually recognized as the gold standard to make a proven diagnosis of IA (De Pauw et al., 2008). However, these procedures are frequently contraindicated, especially in clinical critical patients and thrombocytopenic immunocompromised populations because of hemorrhagic diathesis or other complications. In recent years, non-culture diagnosis tools based on fungal antigens have therefore been developed and demonstrated to have tremendous potential for the diagnosis of IA (Mikulska et al., 2015).

Fungal antigens detection related to IA mainly include galactomannan, 1,3-β-D-glucan and aspergillus-lateral flow device. Galactomannan (GM) is a circulating cell-wall polysaccharide component of *Aspergillus* species and is released into the bloodstream during hyphal growth (Hope et al., 2005). The widespread acceptable assay is a double-sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia™ *Aspergillus*; BioRad, Marnes-La-Coquette, France) with samples of serum, cerebrospinal fluid or bronchoalveolar lavage fluid (BALF). The 1,3-β-D-glucan (BDG) is a polysaccharide located in the cell wall of most fungi (e.g., *Aspergillus* spp., *Candida* spp.), with the main exception of Mucorales and Cryptococcus species (Marty and Koo, 2009). Currently, four commercial tests have been used to detect serum BDG, which differ in the detection method (turbidimetric/colorimetric assays) or the reaction substrate, leading to different cut-off values (Mikulska et al., 2015). GM and BDG have been included as microbiological criteria in the 2002 and 2008 guidelines of European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) for the probable IA and opportunistic invasive fungal diseases (IFD) in immunocompromised patients, respectively (Ascioglu et al., 2002; De Pauw et al., 2008). During the last decade, the performances of GM and BDG for diagnosing IA have been comprehensively evaluated. Although GM and BDG have been widely recognized as crucial markers for supervising patients at risk of IA, some studies showed variable sensitivity or specificity of GM and BDG assay for patients with different underlying diseases. Additionally, Some drawbacks may limit GM or BDG performance, which include false-positive results, variable turnaround times and decreased sensitivity in cases of antifungal prophylaxis/empirical therapy (Asano-Mori et al., 2008; Marr et al., 2005; Martin-Rabadan et al., 2012; Racil et al., 2010; Theel and Doern, 2013).

Meanwhile the common limitations of GM and BDG assay include the time and special laboratory equipment required to perform the assays, which may restrain clinical validity. The aspergillus lateral flow device (LFD), a point-of-care immunochromatographic assay for the diagnosis of IA, can overcome the defects and provide the result in approximately 15 min without specific equipped laboratory. *Aspergillus*-LFD uses the monoclonal antibody JF5 to detect an extracellular glycoprotein secreted during the active growth of *Aspergillus* spp. (Thornton, 2008). Some studies have demonstrated the significant diagnostic potential of LFD assay for IA by using serum or BALF samples (Prattes et al., 2014; Thornton et al., 2012; Willinger et al., 2014). It is noteworthy that the simplicity of the LFD format makes it to be used without requiring special trained staffs and performed in clinical departments to promote the management of IA in high-risk populations.

Two recent meta-analyses, which demonstrated the performance of GM or LFD testing for the diagnosis of IA, concluded that GM and LFD were useful biomarkers to detect IA (Leeflang et al., 2015; Pan et al., 2015). It is worth noting that the three biomarkers may have highly variable diagnostic accuracy in different clinical settings. Additionally, more and more studies have focused on the combination of different assays to expand the efficient of diagnosis for IA. However, to our knowledge, no meta-analysis focusing on the combined performance of GM, BDG and LFD testing has been conducted. Therefore, the objective of the systematic review and meta-analysis is to evaluate the utility of GM assay in combination with BDG or LFD for the diagnosis of IA.

1. Materials and methods

1.1. Literature search strategy and study selection criteria

Two investigators independently performed a comprehensive literature search from PubMed and Embase databases (up to October 2017). The following search terms were used, ("aspergillosis" or "Aspergillosis") AND ("galactomannan" or "GM") AND/OR ("1,3-β-D-glucan" or "BDG") AND/OR ("*Aspergillus* lateral flow device" or "LFD"). Additionally, the references of the included studies were screened for any potentially relevant articles. Studies were included if they met the following predefined criteria: (1) the manuscript was a full-text publication on human subjects; (2) the study used the definition criteria of IA proposed by EORTC/MSG as the diagnostic standard, with slight variance permissible; (3) the study evaluated the combined performance of GM and BDG or LFD assay for IA diagnosis; (4) the manuscript had sufficient published data for two-by-two table. It's noteworthy that if the same population was included in different publications, only the study with the largest dataset was selected. Exclusion criteria were: (1) reviews, case reports and meeting abstracts; (2) insufficient data for two-by-two table; (3) sample size smaller than 20 patients; (4) duplicate publications; (5) the study did not have patients without IA.

1.2. Data extraction and quality assessment

For every eligible study, the following data were extracted: the first author, publication year, original country of study participants, population characteristics, age group, sample size, sample type, study design, data collection, sampling method, reference standard, data for two-by-two tables, threshold for a positive result and the use of antifungal therapy. Two investigators independently performed data extraction and resolved any discrepancies by consensus. The quality of the included studies was evaluated by using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting et al., 2011), which assessed the risk of bias and applicability concerns. Each item scored a "yes", "no" or "unclear" if information was insufficient for accurate judgment.

1.3. Methodological approach

The combined performance of GM and BDG, GM and LFD testing were separately analyzed. The true positive result was defined by two ways: one for having at least one positive result of these tests, the other one with both positive results of GM and BDG, or GM and LFD. According to the revised EORTC/MSG consensus criteria, the population in this study was divided into four groups for their diagnostic status of IA, namely proven, probable, possible and non-IA. We analyzed the pooled sensitivity (SEN), specificity (SPE), positive and negative likelihood ratio (PLR and NLR, respectively) and diagnostic odds ratio (DOR), with a 95% confidence interval (95% CI) for proven or probable IA vs. possible or no IA.

1.4. Statistical analysis

The data for two-by-two table, namely true positive, false positive, false negative and true negative, were extracted from every eligible study. Based on a bivariate random effects model, the potential between-study heterogeneity was investigated and the possible association of the SEN and SPE was incorporated to calculate the pooled estimates of SEN, SPE, PLR and NLR with 95% CI. Generally, PLR >10 and NLR <0.1 show convincing evidence to rule in or rule out a disease, while PLR >5 and NLR <0.2 show moderate diagnostic evidence. The summary receiver operating characteristic (SROC) curve was constructed to evaluate the presence of a threshold effect, and the respective area under the curve (AUC) was measured to evaluate diagnostic accuracy of the test. A perfect diagnostic assay has the value of AUC close to 1.0, whereas AUC close to 0.5 suggests a poor assay. Diagnostic odds ratio (DOR) indicates the pooled accuracy of a diagnostic test, which is

expressed as the odds of positive test results in participants with the disease compared with those without (Glas et al., 2003).

Potential between-study heterogeneity was explored by univariable meta-regression and subgroup analyses. Covariates reported by more than 80% included studies were analyzed: neutropenia (yes vs. no), study design (cohort vs. case–control), data collection (prospective vs. retrospective), sampling method (consecutive vs. non-consecutive/unclear), antifungal therapy (yes vs. no/unclear). The potential publication bias was inspected using Deek's funnel plot, with $P < 0.1$ for the slope coefficient indicating significant asymmetry (Deeks et al., 2005). The post-test probability was calculated by using the prevalence of 12% with the Fagan nomograms (Fagan, 1975). All analyses were performed using STATA, version 12 (Stata Corporation, College Station, Tex) with the module "midas". All statistical tests in the meta-analysis were two-tailed, and *P*-value less than 0.05 denoted statistical significance unless otherwise noted.

2. Results

2.1. Study characteristics

According to the literature search strategy, 378 citations were identified. After screening titles and abstracts, 26 potentially relevant citations were retrieved for full-text review. Among these citations, 13 articles were excluded due to small sample size, reviews or insufficient data. Ultimately, 13 studies enrolling a total of 1513 patients (373 with proven/probable IA) met the inclusion criteria and were included in the systematic review (Acosta et al., 2012; Cai et al., 2014; Farina et al., 2014; Held et al., 2013; Hoenigl et al., 2012, 2014; Miceli et al., 2015; Pazos et al., 2005; Pini et al., 2016; Prattes et al., 2014, 2015; Sulahian et al., 2014; White et al., 2013). The flow diagram of study selection procedure was shown in Fig. 1. Of these studies, seven studies evaluated the combined performance of GM and BDG tests with serum or BALF samples for IA diagnosis (Acosta et al., 2012; Cai et al., 2014; Farina et al., 2014; Hoenigl et al., 2014; Pazos et al., 2005; Pini et al., 2016; Sulahian et al., 2014). Meanwhile, the combined diagnostic accuracy of GM and LFD tests was demonstrated in seven studies (Held et al., 2013; Hoenigl et al., 2012, 2014; Miceli et al., 2015; Prattes et al., 2014, 2015; White et al., 2013). Of note is that one study (Hoenigl et al., 2014) simultaneously evaluated the diagnostic properties of GM, BDG and LFD test for IA. The characteristics of these studies were summarized in Table 1. The objects of two studies (Hoenigl et al., 2012, 2014) were not overlapping due to different time periods. Two articles of Prattes et al. (Prattes et al., 2014, 2015) studied patients with different underlying diseases. Most patients of the included studies were neutropenic adults with hematological malignancy. Additionally, the participants of three studies (Acosta et al., 2012; Cai et al., 2014; Prattes et al., 2014) were nonneutropenic patients underlying respiratory diseases or at a high risk of IFD. The prevalence of proven and probable IA across included cohort studies ranged from 2.1% to 32.4%, with an overall average of 21.2%.

The bar graph of quality assessment was shown in Fig. 2, which indicated that the major risk came from the flow-and-timing domain and patient selection. Eight studies did not include all patients into the analysis of the diagnostic performances of GM, BDG or LFD tests for IA. However, the applicability concerns were high. The symmetry of all funnel plots (not shown) indicated no publication bias in the eligible studies, with all $P > 0.1$ for the slope coefficient.

2.2. Studies evaluating GM and BDG for proven/probable IA cases

Five studies evaluated the combined diagnostic accuracy of GM and BDG test in serum samples with an index cutoff value of 0.5OD and 80 pg/ml, respectively. One study used BAL samples to detect GM with 1.0 cutoff value (Hoenigl et al., 2014). Another study demonstrated the performance of GM and BDG, with cutoff value of 1.5OD and 120 pg/ml, respectively (Pazos et al., 2005).

Combining both positive results of GM and BDG assays (GM + BDG) in four studies (Cai et al., 2014; Farina et al., 2014; Pazos et al., 2005; Sulahian et al., 2014) leaded to SPE 0.98, PLR 31.68 and DOR 61.23, but the pooled SEN and NLR were only 0.49 and 0.52, respectively (Fig. 3). Seven studies evaluated the diagnostic accuracy of the two assays with at least one positive result (GM/BDG), in which the pooled SEN, SPE, PLR, NLR and DOR were 0.88, 0.84, 5.60, 0.14 and 41.03, respectively. The area under the SROC curve (AUC) for these studies was 0.92 (Fig. 4). The Fagan nomogram (Fagan, 1975) was used to assess the clinical utility of the combined performance of GM and BDG assays, which demonstrated that the possibility of having IA was heightened 3.6-fold when the two tests had at least one positive result, and declined to 2% with both negative results (Fig. 5).

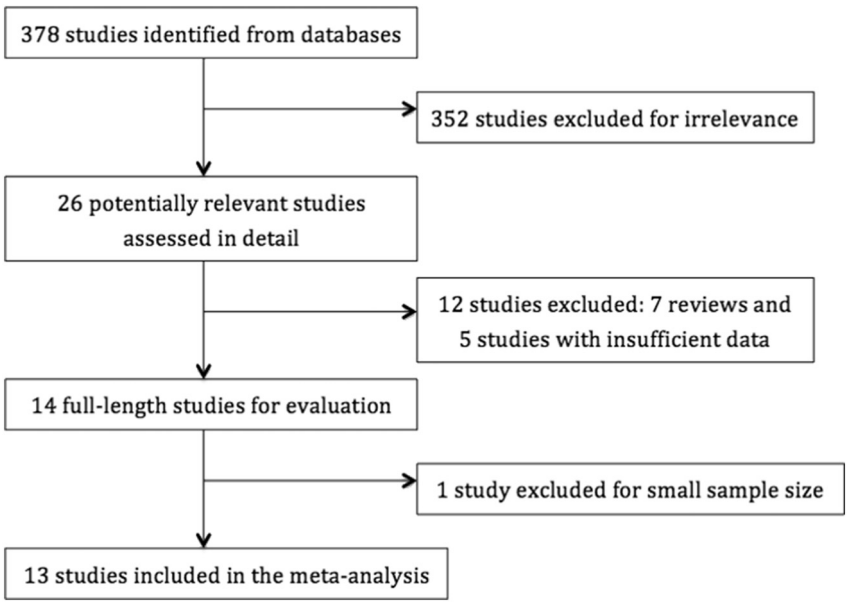

Fig. 1. Flow diagram for the study selection process.

Table 1
Main characteristics of 13 studies included in the meta-analysis for the diagnosis of IA.

Study (year)	Country	Patients	Age group	Sample size	Proven and Probable IA	Sample type	Study design	Sample processing	Sample method	Diagnostic standard	Antifungal prophylaxis/ therapy
Pazos et al. (2005)	Spain	HM	Adults	40	8	Serum	Cohort	Retrospective	Consecutive	2002 EORTC/MSG	Yes
Acosta et al. (2012)	Spain	Critically ill, nonneutropenic patients	Adults	98	12	Serum	Cohort	Prospective	Unclear	2008 modified EORTC/MSG	Yes
Hoenigl et al. (2012)	Austria	HM, SOT	Adults, children	37	12	BALF	Cohort	Retrospective	Unclear	2008 EORTC/MSG	NA
Held et al. (2013)	Germany	HSCT	Adults	101	10	Serum	Cohort	Prospective	Consecutive	2008 modified EORTC/MSG	Yes
White et al. (2013)	UK	Hematology patients	Adults	103	22	Serum	Case–control	Retrospective	Unclear	2008 modified EORTC/MSG	NA
Prattes et al. (2014)	Austria	Non-neutropenic patients with respiratory diseases	Adults	221	31	BALF	Cohort	Retrospective	Consecutive	2008 modified EORTC/MSG	Yes
Cai et al. (2014)	China	Non-neutropenic patients with respiratory diseases	Adults	97	27	Serum	Cohort	Prospective	Unclear	2008 modified EORTC/MSG	No
Hoenigl et al. (2014)	Austria Germany	Immunocompromised patients with HM, SOT	Adults	78	17	BALF	Cohort	Prospective	Unclear	2008 EORTC/MSG	NA
Sulahian et al. (2014)	France	HM	NA	252	105	Serum	Case–control	Retrospective	Unclear	2008 modified EORTC/MSG	NA
Pini et al. (2016)	Italy	Neutropenic patients with HM, SOT	Adults	232	58	Serum	Cohort	Prospective	Consecutive	2008 modified EORTC/MSG	NA
Farina et al. (2014)	Italy	HM, SOT, AIDS, ICU patients	Adults	63	42	Serum	Cohort	Retrospective	Unclear	2008 EORTC/MSG	NA
Miceli et al. (2015)	USA	HM, HOST, SOT and Solid Tumor	Adults	96	2	BALF	Cohort	Prospective	Unclear	2008 modified EORTC/MSG	NA
Prattes et al. (2015)	Austria	HM	Adults	95	27	BALF	Cohort	Prospective	Unclear	2008 EORTC/MSG	Yes

HM: hematological malignancy. HSCT: hematopoietic stem cell transplant. SOT: solid organ transplantation. NA: not available. BALF: Bronchoalveolar lavage fluid. AIDS: acquired immune deficiency syndrome. ICU: Intensive Care Unit.

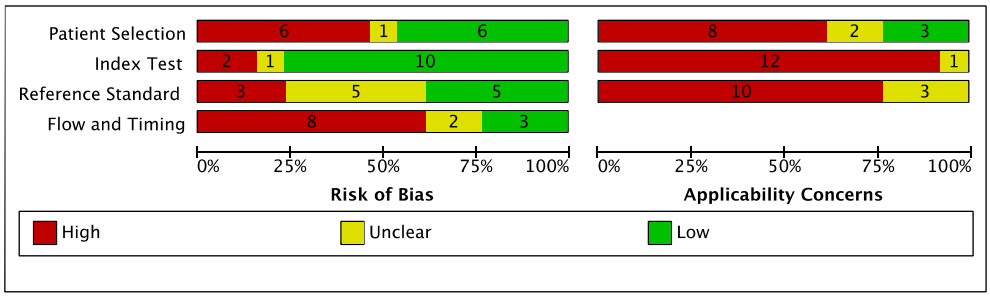

Fig. 2. Overall quality assessment of included studies (QUADAS-2 tool).

2.3. Studies evaluating GM and LFD for proven/probable IA cases

Seven studies provided combined diagnostic data of GM and LFD test for proven/probable IA versus possible or no IA, of which five studies (Hoenigl et al., 2012, 2014; Miceli et al., 2015; Prattes et al., 2014, 2015) evaluated the two assays with BAL samples and two studies (Held et al., 2013; White et al., 2013) with serum samples. When at least one positivity of GM or LFD (GM/LFD) was defined for the true positive result, the pooled SEN, SPE, PLR, NLR, DOR and AUC were 0.93, 0.82, 5.11, 0.08, 62.77 and 0.91, respectively, with substantial heterogeneity (Q-test = 7.69, $P = 0.011$, $I^2 = 73.98\%$) (Figs. 6 and 7). To explore potential heterogeneities, meta-regression and subgroup meta-analysis were conducted (Fig. 8). Overall, the test performances were affected by study design, antifungal agents, sample type, sample size and neutropenia. Subgroup analyses based on BAL samples demonstrated that the combination use of GM and LFD assay improved the SEN to 0.97, with slightly declined SPE 0.77. When both positive results of GM and LFD (GM + LFD) were defined for the true positive result, the overall SEN obviously reduced to 0.59 while the SPE was significantly improved to 0.94. Meanwhile, the PLR and NLR increased to 10.12 and 0.43, respectively. The Fagan nomogram showed that at least one positive result of GM or LFD increased the probability of proven/probable IA from 12% to 47% while both positivity made the probability improved nearly 6-fold (from 12% to 70%), whereas the probability reduced to only 1% when results of the two assays were negative. The combined diagnostic performances of GM, BDG and LFD tests for proven or probable IA were showed in Table 2.

3. Discussion

Some studies demonstrated the diagnostic accuracy of GM and BDG test for IA, with wide dispersion for the SEN and SPE of the two assays. The range of reported SEN for the GM assay was from 23% to 100%, with SPE ranging from 25% to 98% (Leeflang et al., 2015). Similarly, discrepant value of SEN for the BDG test ranged from 80% to 90% and SPE ranging from 36% to 92% (Sulahian et al., 2014). Additionally, *Aspergillus*-LFD is a novel diagnostic test for IA, with certain advantage and characteristics. Obviously, the three assays detect different fungal targets, and their combination may have the potential to increase the chance of IA diagnosis. Overall, our study demonstrated that both positive results of GM and BDG assays (GM + BDG) conferred the pooled high SPE, PLR and AUC (0.98, 31.68 and 0.94, respectively), indicating the

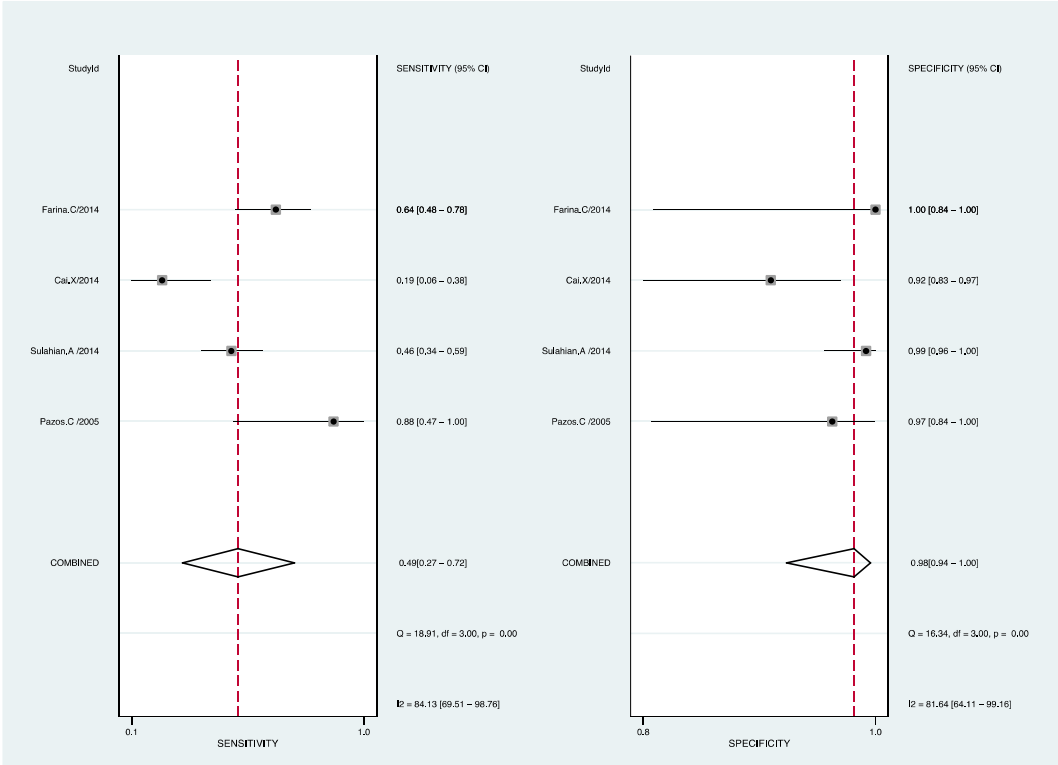

Fig. 3. Forest plot of sensitivities and specificities from test accuracy studies with both positive results of GM and BDG assays for the diagnosis of IA.

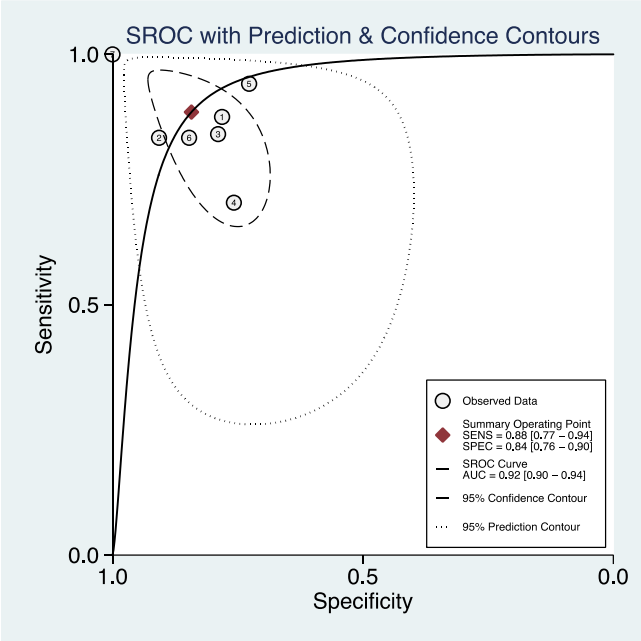

Fig. 4. SROC curve for individual studies on the accuracy of diagnosing IA using GM or BDG assay.

existence of IA. Compared with GM and BDG, both positive results of GM and LFD (GM + LFD) leaded to lightly high SEN but lightly low SPE, and were also beneficial to rule in the possibility of IA. Meanwhile, Our study showed that positivity of either GM or LFD (especially BAL-GM or BAL-LFD), due to the pooled high SEN, could be useful for screening patients with the risk of IA, while both negative results might rule out the possibility of IA.

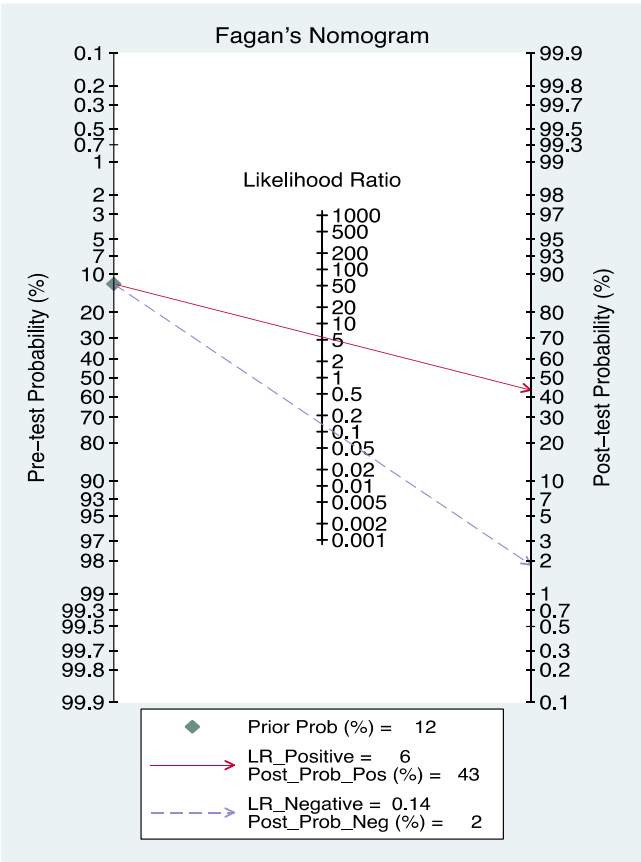

Fig. 5. Fagan plot analysis to evaluate the clinical utility of combination of GM and BDG assay.

The utility of GM test or LFD for diagnosing IA was evaluated in several meta-analyses (Guo et al., 2010; Leeflang et al., 2015; Pan et al., 2015; Zou et al., 2012). BAL-GM or serum-GM assay had variable performances due to different cutoff values among patients with underling different conditions. Leeflang et al. (2015) investigated the diagnostic accuracy of GM assay in serum samples for IA among immunocompromised patients, and demonstrated that increasing threshold (from 0.5 to 1.5) decreased the SEN by 21% (from 82% to 61%), but increased the SPE by12% (from 81% to 93%). Compared with serum-GM, BAL-GM assay had high accuracy for increasing SEN and SPE, especially with cutoff value 1.0 (e.g. 0.86 SEN and 0.95 SPE) (Guo et al., 2010; Zou et al., 2012). Furthermore, a recent meta-analysis drew the conclusion that the BAL-LFD might have a better diagnostic performance than the serum-LFD with SEN 0.86, SPE 0.93, PLR 10.70 and NLR 0.19 for proven/probable IA versus no IA (Pan et al., 2015). However, the both assays have certain disadvantages, and could lead to false positive or false negative results. Therefore, our study evaluated the combined performance of GM and LFD assays for IA diagnosis. Our study found that the SPE was 0.94 when at least one negative result of the two assays was defined as true negative, which was in line with the study of White et al. (White et al., 2013). Furthermore, the pooled PLR was 10.12, which indicated that the both positive results of GM and LFD would rule in IA. Additionally, Negative results of these assays should be reconfirmed by another assay to prevent delayed diagnosis in cases of false-negative results.

Our study showed that both positive results of GM and BDG assays had almost 100% SPE but only 49% SEN, which was consistent with other studies (Cai et al., 2014; Pazos et al., 2005). A possible reason for the low SEN might be that the serum samples collected prematurely made BDG assay tend to become positive earlier than GM test (Pazos et al., 2005). Similarly, A recent study provided the evidence that the combined determination of GM and BDG made sensitivity significant increase with compared to the use of GM alone (Pini et al., 2016). Furthermore, our investigation demonstrated that at least one positive result of the two tests for IA diagnosis leaded to a relatively low SPE 0.84, but an obviously high SEN 0.88, especially for serum samples with SPE 0.86 and SEN 0.89. In terms of the high PLR with both consecutive positive results, the combined assay performance was beneficial for the diagnosis of IA.

One strength of our meta-analysis flowed from the direct comparison of combined performances for GM and BDG or LFD test. However, significant heterogeneity was observed in the combination of GM and LFD assays, and the potential causes were explored in the univariable meta-regression and subgroup analysis. Some study characteristics including study design, sample size and sample type were the covariates that had effects on the pooled SEN. The subgroup analysis showed that the SEN of the combined tests with BALF was superior to that with serum samples, which may be partly due to high fungal burden in the bronchial tree of patients with pulmonary IA (Desai et al., 2009). Additionally, the time taken for galactomannan releasing from infection site and binding to substances present in the blood accounted for delayed serum galactomannan and lower SEN with serum samples in early infection (Mennink-Kersten et al., 2004).

Because of small sample sizes in our study, antibiotic and antifungal agents were not significant covariates influencing the SEN or SPE of combination use of GM, BDG and LFD. The problem whether there is association of false galactomannan positivity with β-lactam antibiotics such as amoxicillin-clavulanate and piperacillin-tazobactam (PTZ) has been discussing. Some previous studies demonstrated that the administration of PTZ was responsible for false galactomannan positivity (Sulahian et al., 2003; Viscoli et al., 2004), which was inconsistent with recent evidences that the association was no longer systematic (Gerlinger et al., 2012; Vergidis et al., 2014). The influence of antifungal

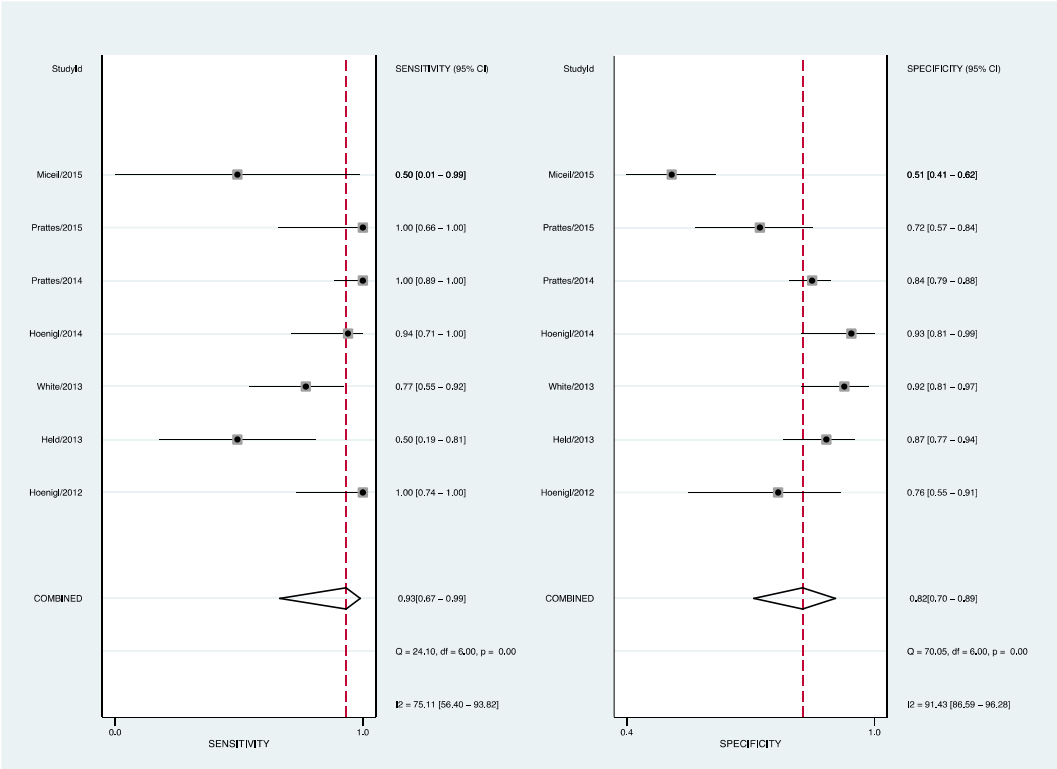

Fig. 6. Forest plot of sensitivities and specificities from test accuracy studies with at least one positive result of GM and LFD test for the diagnosis of IA.

agents on the SEN of these assays is always the focus of clinical studies, but the results are discrepant. Some studies noted that mold-active agents decreased the SEN of these assays (Acosta et al., 2012; Marr et al., 2005; McCulloch et al., 2012; Wiederhold et al., 2013), whilst others demonstrated indifference (Hoenigl et al., 2013; Koo et al., 2009; Wingard et al., 2010). Additionally, the SEN may associate with duration of antifungal therapy. One report elaborated that SEN of GM increased in the preliminary stage of antifungal therapy but decreased beyond 2–3 days of treatment (Racil et al., 2011), yet another study did not find the association (Frealle et al., 2009).

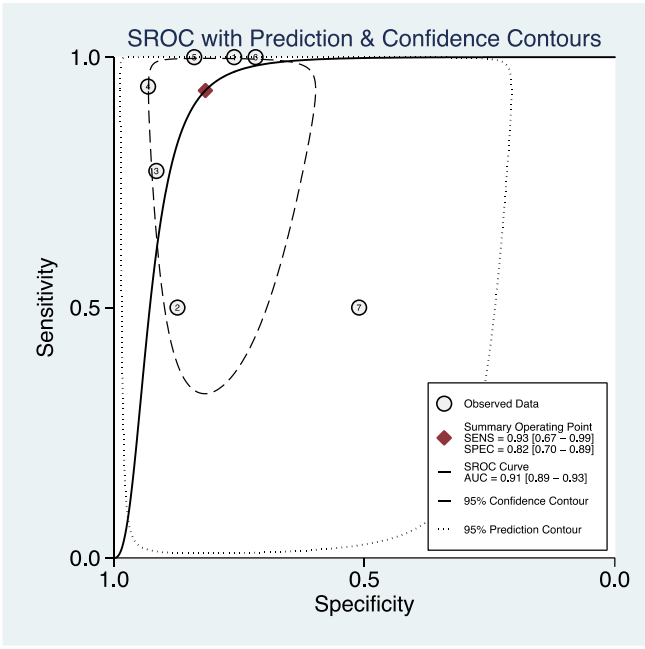

Fig. 7. SROC curve for individual studies on the accuracy of diagnosing IA using GM or LFD test.

Similarly, our study showed that neutropenia did not significantly influence the diagnostic ability of the combined assays, especially for GM assay. However, a previous study elaborated the association of neutropenia with GM assay performance (Racil et al., 2011). It has been maintained that neutrophils eliminate galactomannan from the blood through mannose-binding receptors (Mennink-Kersten et al., 2004). Moreover, there were inconsistent results for the effect of neutropenia on the SEN of BAL-GM assay, ranging from indifference to 2-fold greater in patients with neutropenia (Bergeron et al., 2010; Racil et al., 2011). Neutropenia was even indicated to have an effect on the SEN of serum-GM but no BAL-GM (D'Haese et al., 2012). Meanwhile, BAL-GM was proposed as a more sensible assay than serum-GM for non-neutropenic patients with airway-invasive signs (Bergeron et al., 2012). Different *Aspergillus* species may release different quantity of galactomannan and change SEN of GM assay (Swanink et al., 1997). And non-*Aspergillus* molds, such as *Penicillium marneffei*, *Histoplasma capsulatum* and *Fusarium oxysporum*, may have cross-reaction with *Aspergillus* GM epitopes or MAb JF5, which may lead to false-positive results of GM assay or LFD (Hoenigl et al., 2012; Rimek et al., 1999; Tortorano et al., 2012; Wheat et al., 2007). Our study did not analyze these covariates due to lack of corresponding data. It is worth mentioning that the LFD test has now been formatted for large-scale manufacture and marked as an in vitro diagnostic (IVD) device by CE. A latest study showed that the previous LFD should be described as prototype LFD, as it was technically not the same LFD that is now commercially available, and demonstrated that the newly formatted LFD was equally sensitive but more specific than the prototype LFD (Hoenigl et al., 2018). However, it needs further studies for utility of the new LFD and its combined performance with BDG or GM in the clinical setting.

In addition to the heterogeneity mentioned above, other limitations of our study should be thought over. First, the eligible studies and the

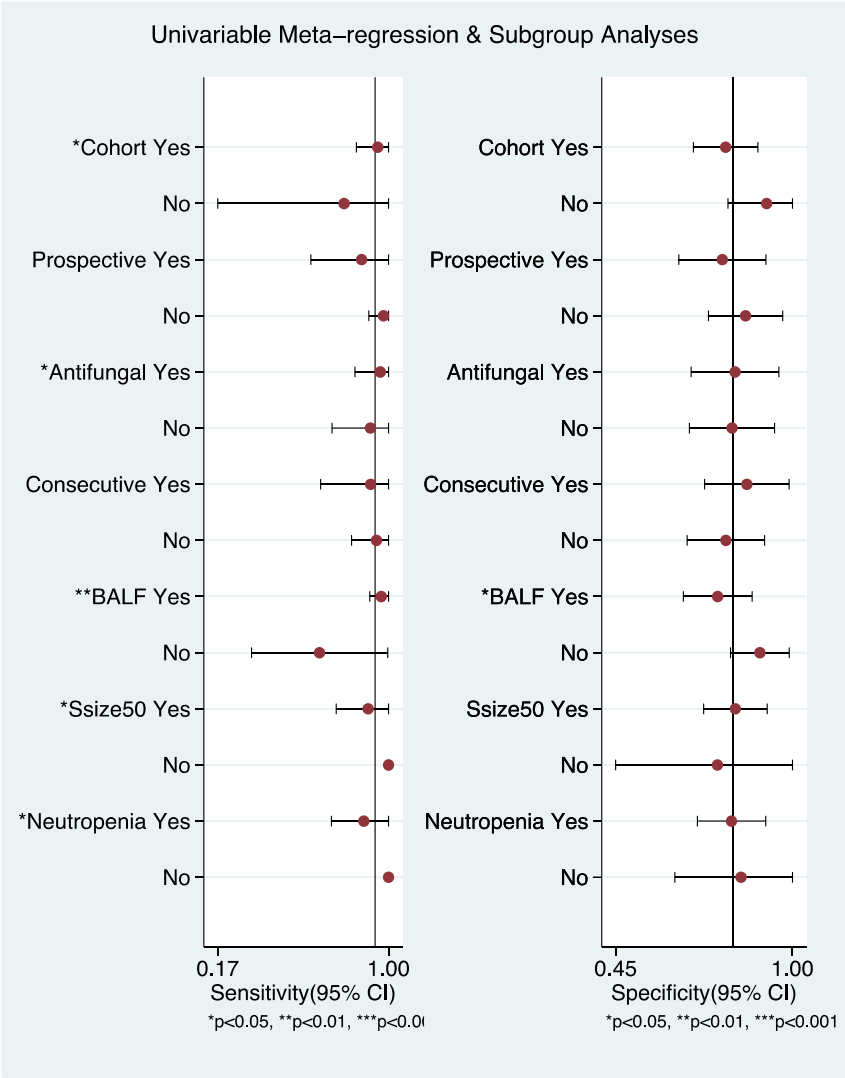

Fig. 8. Forest plot of multiple univariable meta-regression and subgroup analyses for sensitivities and specificities of the combination of GM and LFD test.

total number of patients included in the meta-analysis were small, partly because there were only a few literatures simultaneously investigating the diagnostic ability of these assays for IA. And studies with poor performance of these diagnostic tests may be more difficult to publish than studies with favorable performance. This might lead to overestimation of assay performances. Additionally, the exclusion of studies with small sample size ($n < 20$) and studies with inadequate data for two-by-two tables might give rise to selective bias. Misclassification bias for IA may also occur due to the subjective interpretations of the revised EORTC/MSG criteria. Due to low SEN and complications of the gold standard for IA diagnosis, the disease is not definitely excluded in spite of failure to meet the criteria. Furthermore, the effect of different cut-off value for GM and BDG assay was not investigated and the association of prevalence with diagnostic test accuracy was not discussed due to the paucity of data.

In conclusion, the present meta-analysis showed that both positive results of GM and BDG assays or LFD were in favor of confirming the existence of IA. And both negative results of GM and LFD were beneficial to rule out the possibility of IA. It is essential to make further investigation about the diagnostic performance of these combined tests for IA with sufficient sample size. Furthermore, further studies should focus on the cost-effectiveness of combining GM assay with BDG test or LFD for patients with different underlying diseases to confirm its position in clinical application.

Table 2
Pooled results of the included studies for the diagnosis of IA.

Studies	Number	Pooled SEN (95% CI)	Pooled SPE (95% CI)	Pooled PLR (95% CI)	Pooled NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
GM + BDG	4*	0.49(0.27–0.72)	0.98(0.94–1.00)	31.68(5.36–187.37)	0.52(0.32–0.84)	61.23(6.96–538.90)	0.94(0.92–0.96)
GM/BDG	7	0.88(0.77–0.94)	0.84(0.76–0.90)	5.60(3.40–9.26)	0.14(0.06–0.29)	41.03(12.33–136.52)	0.92(0.90–0.94)
	6*	0.89(0.75-0.95)	0.86(0.76–0.92)	6.28(3.30–11.95)	0.13(0.05–0.33)	47.31(10.62–210.62)	0.93(0.91–0.95)
GM + LFD	4	0.59(0.30–0.83)	0.94(0.90–0.97)	10.12(5.36–19.10)	0.43(0.21–0.88)	23.25(7.06–76.52)	0.94(0.92–0.96)
GM/LFD	7	0.93(0.67–0.99)	0.82(0.70–0.89)	5.11(2.99–8.73)	0.08(0.01–0.50)	62.77(8.22–479.22)	0.91(0.89–0.93)
	5#	0.97(0.81–0.99)	0.77(0.63–0.87)	4.21(2.41–7.33)	0.04(0.01–0.30)	94.92(9.34–964.55)	0.93(0.91–0.95)

* Number for studies with serum sample.
\# Number for studies with bronchoalveolar lavage fluid.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Author contributions

L.Z. and Z.G. conceived and designed the experiments; L.Z., S.X., J.Z., G.C., J.F., and Y.H. analyzed the data, L.Z. and Z.G. wrote the manuscript. All authors reviewed and approved the manuscript.

Ethical approval and informed consent

Ethical approval and formal consent was not required.

Competing financial interests

The authors declare no competing financial interests.

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