Diagnostic accuracy of a novel lateral-flow device in invasive aspergillosis: a meta-analysis

Zhijie Pan,1† Mengjiao Fu,1† Jiaojiao Zhang,2 Hua Zhou,1 Yiqi Fu1 and Jianying Zhou1

1Respiratory Department, The First Affiliated Hospital, College of Medicine, Zhejiang University, No. 79 Qingchun Road, Hangzhou, Zhejiang, PR China
2Cancer Institute, The Second Affiliated Hospital, College of Medicine, Zhejiang University, No. 88 Jiefang Road, Hangzhou, Zhejiang, PR China

A novel lateral-flow device (LFD) has been invented for use as a diagnostic tool for invasive aspergillosis (IA). We conducted a meta-analysis to assess the diagnostic accuracy of the device. Published studies that used the European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria and provided sufficient data were included. Two reviewers independently collected the data from each study and assessed the risk bias using the Quality Assessment of Diagnostic Accuracy Studies-2. The pooled sensitivity, specificity and diagnostic odds ratio (DOR) were computed and reported with a 95 % confidence interval (CI). Seven studies published between 2008 and March 2015 were included. The pooled sensitivity, specificity and DOR for the proven/probable versus no IA cases were 0.86 (95 % CI, 0.76–0.93), 0.93 (95 % CI, 0.89–0.96) and 65.94 (95 % CI, 27.21–159.81) in the LFD test using bronchoalveolar lavage (BAL) fluid, and 0.68 (95 % CI, 0.52–0.81), 0.87 (95 % CI, 0.80–0.92) and 11.90 (95 % CI, 3.54–39.96) in the LFD test using serum. We concluded that the Aspergillus LFD had a good diagnostic value in immunocompromised patients at risk of IA. The BAL LFD might have a better performance than the serum LFD test.

Introduction

Invasive aspergillosis (IA) is the most frequently occurring invasive fungal infection in immunocompromised patients (Kontoyiannis et al., 2010; Neofytos et al., 2009), with a high mortality rate ranging from 27 to 89% (Cornillet et al., 2006; Pagano et al., 2010). Early and accurate diagnosis followed by appropriate antifungal therapy is vital for a favourable outcome (Walsh et al., 2008). However, in the absence of an accurate ‘gold standard’, the diagnosis of IA remains challenging (Guinea & Bouza, 2014; Johnson et al., 2014; Thornton, 2010). Microbiological tests have become the most important factor of the diagnostic criteria, as tissue biopsy has contra-indications and fungal cultures are neither highly sensitive nor highly specific. Development of tests not based on cultures, such as the galactomannan assay (GM assay), (1→3)-β-D-glucan assay (G assay) and PCR has received significant attention from researchers. Meta-analyses have shown a sensitivity of 0.78, 0.77 and 0.75 and a specificity of 0.81, 0.83 and 0.87 for the GM assay, G assay and PCR, respectively, using serum (Leeflang et al., 2008; Mengoli et al., 2009; Onishi et al., 2012). Despite their wide usage, these tests have limitations, such as time and equipment requirements as well as the occurrence of significant performance variation. In addition, (1→3)-β-D-glucan is not specific for Aspergillus spp. (Guinea & Bouza, 2014).

A novel lateral-flow device (LFD) was developed for the detection of the Aspergillus antigen in human serum (Thornton, 2008) and later in bronchoalveolar lavage (BAL) fluid (Hoenigl et al., 2012). Aspergillus LFD is an immunochromatographic assay that uses JF5, a mAb that binds to an extracellular glycoprotein secreted during active growth of Aspergillus. The LFD test is hypothesized to be highly specific because mAb JF5 displays great specificity. It is a rapid test (15 min) with easy manipulation that ensures its usefulness as a potential point-of-care assay. We conducted this meta-analysis to assess the diagnostic accuracy of the index test and to evaluate which variables affect its performance.

†These authors contributed equally to this work.

Abbreviations: AUC, area under curve; BAL, bronchoalveolar lavage; CI, confidence interval; DOR, diagnostic odds ratio; EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM, galactomannan; LFD, lateral-flow device; IA, invasive aspergillosis; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies-2; SROC, summary receiver operating characteristic.
Methods

Literature search. Two investigators independently searched the published English language literature using PubMed, Web of Science and Clinical Trials from January 2008 to March 2015. The search phrases included ‘Aspergillus’, ‘aspergillosis’, ‘lateral flow’, ‘LFD’ and their combinations. Additionally, the references of the included studies and reviews were examined.

Inclusion criteria. The studies that provided sensitivity and specificity data and used the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria in the 2002 edition or the 2008 revised edition were included (Ascioglu et al., 2002; De Pauw et al., 2008). Review articles, case reports and meeting abstracts were excluded. If a study had a patient population that appeared to overlap with that of another study, we selected the larger study.

Risk of bias assessment. The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool was used to assess the risks of bias, although studies were not excluded on the basis of quality (Whiting et al., 2011). The item regarding the threshold in domain 2 (the index test) was deleted because the result of the LFD test was bivariate, either positive or negative, according to whether there was a test line. Three months was considered an ‘appropriate interval’ between the LFD test and the EORTC/MSG criteria.

Data collection. Two reviewers independently evaluated the included studies for the following characteristics: the study type (case-control, prospective cohort or retrospective cohort); description of the study population, including the size of the population and its age/sex composition and clinical characteristics (haematological malignancy, haematopoietic stem-cell transplantation, solid organ transplantation or respiratory diseases); use of the EORTC/MSG diagnosis criteria as the reference standard; the type of sample (serum or BAL fluid); and the use of antifungal therapy.

Statistical analysis. The data from the BAL LFD and serum LFD were processed separately. We calculated the measures including the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV) and diagnostic odds ratio (DOR), with a 95% confidence interval (CI) for proven/probable/possible IA versus no IA, proven/probable IA versus possible/no IA, and proven/probable IA versus no IA. PPV and NPV were computed on the basis of the pooled sensitivity and specificity at a range of prevalence from 0.10 to 0.20.

We assessed the heterogeneity by the Cochrane Q method and the test of inconsistency (the $I^2$ test) (Cochran, 1954; Higgins et al., 2003). To account for heterogeneity, a subgroup analysis of underlying diseases was performed. We computed the area under the curve of the summary receiver operating characteristic (AUC-SROC) to compare the diagnostic accuracy of the BAL LFD and serum LFD. All statistical analyses were conducted using RevMan v.5.3.

Results

Overall analysis

We identified 44 studies from the literature search, 18 of which had the full text reviewed. One study was added from the reference search and 12 studies were excluded for the reasons shown in Fig. 1. A total of seven studies were included in our analysis (Held et al., 2013; Hoenigl et al., 2012, 2014; Prattes et al., 2014; Thornton, 2008; White et al., 2013; Willinger et al., 2014). The characteristics of the studies are shown in Table 1. Three studies used serum as the test sample, whilst the other four used BAL fluid. All the studies used the EORTC/MSG criteria as the reference standard, although with slight variances. Antifungal therapy was discussed in five studies; however, none of them examined the effect of prior or current antifungal therapy on the performance of the LFD.

A total of 650 cases from 603 patients at risk for IA were included, of whom 278 had a haematological malignancy, 233 had respiratory diseases, 68 had solid organ transplantation, eight had other diseases and 16 were not reported with details. 115 (19.1%) patients were diagnosed with proven/probable IA; and 220 cases were tested in serum and 430 were in BAL fluid.

The risk of bias and applicability concerns summary assessed using QUADAS-2 showed that the major risk came from the flow-and-timing domain for six studies that did not report the interval of the LFD test and the EORTC/MSG criteria. Only one case–control study was at high risk of bias and high concern of applicability regarding the patient selection.

Performance of the LFD

The sensitivity and specificity of each study for proven/probable versus no IA cases are shown in Fig. 2. The sensitivity showed considerable heterogeneity ($I^2 = 63.3\%$) in the serum LFD test compared with the BAL LFD test ($I^2 = 46.3\%$). The specificity was relatively uniform in both tests. The pooled sensitivity and specificity of the serum LFD were 0.68 (95% CI, 0.52–0.81) and 0.87 (95% CI, 0.80–0.92), respectively. The pooled sensitivity and specificity of the BAL LFD were 0.86 (95% CI, 0.76–0.93) and 0.93 (95% CI, 0.89–0.96), respectively.
Table 1. Characteristics of the studies included in the meta-analysis of the diagnosis of IA using an LFD

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Sex (M/F)</th>
<th>Age (range)</th>
<th>Underlying disease</th>
<th>Reference standard</th>
<th>Sample</th>
<th>Antifungal therapy</th>
<th>Study type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thornton (2008)</td>
<td>16</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>EORTC, 2002</td>
<td>Serum</td>
<td>NR</td>
<td>Case–control</td>
</tr>
<tr>
<td>Held et al. (2013)</td>
<td>101</td>
<td>NR</td>
<td>51 (21–70)</td>
<td>HM with HSCT</td>
<td>EORTC, 2008</td>
<td>Serum</td>
<td>Yes</td>
<td>Prospective</td>
</tr>
<tr>
<td>White et al. (2013)</td>
<td>103</td>
<td>73/30</td>
<td>53 (NR)</td>
<td>HM</td>
<td>EORTC, 2008</td>
<td>Serum</td>
<td>NR</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Hoenigl et al. (2012)</td>
<td>37</td>
<td>26/11</td>
<td>53 (14–75)</td>
<td>HM, SOT</td>
<td>EORTC, 2008</td>
<td>BALF</td>
<td>Yes</td>
<td>Prospective</td>
</tr>
<tr>
<td>Hoenigl et al. (2014)</td>
<td>78</td>
<td>54/24</td>
<td>58 (24–77)</td>
<td>HM, SOT, others</td>
<td>EORTC, 2008</td>
<td>BALF</td>
<td>Yes</td>
<td>Prospective</td>
</tr>
<tr>
<td>Prattes et al. (2014)</td>
<td>221</td>
<td>128/93</td>
<td>64 (18–92)</td>
<td>Respiratory diseases</td>
<td>Modified EORTC, 2008</td>
<td>BALF</td>
<td>Yes</td>
<td>Prospective</td>
</tr>
<tr>
<td>Willinger et al. (2014)</td>
<td>47</td>
<td>29/18</td>
<td>51 (18–71)</td>
<td>SOT</td>
<td>EORTC, 2008</td>
<td>BALF</td>
<td>Yes</td>
<td>Semi-prospective</td>
</tr>
</tbody>
</table>

M, male; F, female; NR, not reported; HM, haematological malignancy; HSCT, haematopoietic stem-cell transplantation; SOT, solid organ transplantation; BALF, BAL fluid.

Test samples
Three and four studies reported on the diagnostic accuracy of the serum LFD and BAL LFD, respectively. The pooled sensitivity, specificity, DOR, NLR, PLR, NPV and PPV of both tests are shown in Table 2. The SROC plot of the tests is shown in Fig. 3. The DORs and AUCs were 11.90 (95% CI, 3.54–39.96) and 0.85 (95% CI, 0.78–0.93) for serum LFD, and 65.94 (95% CI, 27.21–159.81) and 0.95 (95% CI, 0.92–0.98) for BAL LFD, respectively. All the measures suggested that the BAL LFD test might have a better diagnostic accuracy than the serum LFD test.

Underlying diseases
Patients with haematological malignancy, solid-organ transplantation and respiratory diseases were included for a subgroup analysis of underlying diseases. The sensitivity and specificity of the BAL LFD were 1.00 (95% CI, 0.59–1.00) and 1.00 (95% CI, 0.79–1.00) in patients with haematological malignancy, 0.94 (95% CI, 0.70–1.00) and 0.90 (95% CI, 0.73–0.98) in patients with solid-organ transplantation, and 0.77 (95% CI, 0.59–0.90) and 0.88 (95% CI, 0.69–0.97) in patients with respiratory diseases, as shown in Table 3.

Discussion
We examined the accuracy of the LFD test for the diagnosis of IA in high-risk patients and found that the LFD test had a high diagnostic value in IA patients, especially the BAL LFD. The pooled sensitivity, specificity and DOR of the BAL LFD were 0.86 (95% CI, 0.76–0.93), 0.93 (95% CI, 0.89–0.96) and 65.94 (95% CI, 27.21–159.81), respectively, for proven/probable versus no IA cases.

Proven and probable cases were both regarded as positive cases because the EORTC/MSG criteria for proven IA are so strict that they exclude many actual IA patients. The criteria require evidence from a pathogen culture or histopathology; however, pathogen cultures have a poor

![Fig. 2. Forest plots of the BAL LFD and serum LFD tests. TP, true positives; FP, false positives; FN, false negatives; TN, true negatives.](image-url)
sensitivity, and a tissue biopsy, as an invasive diagnostic method, is not always possible, particularly in patients with thrombocytopaenia. Possible cases were incorporated in three ways, as cases, as controls and excluded from the analysis, and we preferred the last criterion. The false-negative cases increased when possible cases were incorporated as IA cases and the sensitivity was consequently underestimated; likewise, the false-positive cases increased when possible cases were incorporated as controls and the specificity was underestimated, as shown in Table 2.

All the measures including sensitivity, specificity, DOR and AUC suggested that the BAL LFD test might have a better diagnostic accuracy than the serum LFD test. Compared with the BAL LFD test, the serum LFD test had a much lower sensitivity (0.68 vs 0.86) and a comparable specificity (0.87 vs 0.93). There are three explanations for the low sensitivity of the LFD test in serum. First, IA originates mostly from the lung, and the targeted antigen, JF5, has a higher concentration and is easier to detect in BAL fluid. Secondly, the presence of serum proteins inhibits the detection of the Aspergillus antigen. The analytical sensitivity was shown to be inferior (1.25 ng ml\(^{-1}\) in PBS vs 37 ng ml\(^{-1}\) in serum) in the development of the LFD (Thornton, 2008). Therefore, it was necessary to dilute, heat and centrifuge serum samples to optimize performance, preventing serum LFD from being a point-of-care test. Although C. Thornton, the inventor of the device, and his colleagues provided a procedure protocol (Thornton et al., 2012), the three studies included in our analysis performed serum LFD in their own way. This difference led to an inevitable influence on the performance of the test in serum. BAL fluid does not have this problem and could be tested without pre-treatment. Thirdly, as with the GM test whose sensitivity was markedly reduced in patients receiving antifungal

### Table 2. Measures of accuracy of the BAL LFD and serum LFD tests in diagnosis of IA

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>DOR</th>
<th>NLR</th>
<th>PLR</th>
<th>NPV/PPV for prevalence of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10% 15% 20%</td>
</tr>
<tr>
<td>BAL LFD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPP vs N</td>
<td>0.72 (0.64–0.80)</td>
<td>0.93 (0.89–0.96)</td>
<td>32.13 (15.03–68.67)</td>
<td>0.32 (0.22–0.45)</td>
<td>9.59 (6.28–14.66)</td>
<td>0.97/0.32 0.95/0.31 0.93/0.39</td>
</tr>
<tr>
<td>PP vs PN</td>
<td>0.86 (0.76–0.93)</td>
<td>0.84 (0.80–0.88)</td>
<td>27.35 (13.20–56.64)</td>
<td>0.21 (0.12–0.35)</td>
<td>5.20 (3.99–6.79)</td>
<td>0.98/0.40 0.97/0.52 0.96/0.60</td>
</tr>
<tr>
<td>PP vs N</td>
<td>0.86 (0.76–0.93)</td>
<td>0.93 (0.89–0.96)</td>
<td>65.94 (27.21–159.81)</td>
<td>0.19 (0.11–0.32)</td>
<td>10.70 (6.96–16.44)</td>
<td>0.98/0.40 0.97/0.52 0.96/0.60</td>
</tr>
<tr>
<td>Serum LFD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPP vs N</td>
<td>0.52 (0.41–0.63)</td>
<td>0.87 (0.80–0.92)</td>
<td>6.13 (1.50–25.09)</td>
<td>0.52 (0.24–1.12)</td>
<td>3.27 (1.66–6.45)</td>
<td>0.94/0.11 0.91/0.16 0.88/0.21</td>
</tr>
<tr>
<td>PP vs PN</td>
<td>0.68 (0.52–0.81)</td>
<td>0.81 (0.75–0.87)</td>
<td>8.67 (3.62–20.75)</td>
<td>0.43 (0.22–0.85)</td>
<td>3.18 (2.18–4.64)</td>
<td>0.96/0.19 0.93/0.27 0.91/0.35</td>
</tr>
<tr>
<td>PP vs N</td>
<td>0.68 (0.52–0.81)</td>
<td>0.87 (0.80–0.92)</td>
<td>11.90 (3.54–39.96)</td>
<td>0.41 (0.19–0.87)</td>
<td>4.65 (2.76–7.82)</td>
<td>0.96/0.19 0.94/0.27 0.92/0.35</td>
</tr>
</tbody>
</table>

Table 2. Measures of accuracy of the BAL LFD and serum LFD tests in diagnosis of IA

PPP vs N, proven/probable/possible versus no IA; PP vs PN, proven/probable versus possible/no IA; PP vs N, proven/probable versus no IA.

### Table 3. Sensitivity and specificity of the BAL LFD and serum LFD tests in subgroups with underlying diseases

<table>
<thead>
<tr>
<th>Subgroup (no. of cases)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL LFD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (368)</td>
<td>0.86 (0.76–0.93)</td>
<td>0.93 (0.89–0.96)</td>
</tr>
<tr>
<td>HM (20)</td>
<td>1.00 (0.59–1.00)</td>
<td>1.00 (0.79–1.00)</td>
</tr>
<tr>
<td>SOT (45)</td>
<td>0.94 (0.70–1.00)</td>
<td>0.90 (0.73–0.98)</td>
</tr>
<tr>
<td>RD (242)</td>
<td>0.77 (0.59–0.90)</td>
<td>0.88 (0.69–0.97)</td>
</tr>
<tr>
<td>Serum LFD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (178)</td>
<td>0.68 (0.52–0.81)</td>
<td>0.87 (0.80–0.92)</td>
</tr>
<tr>
<td>HM (162)</td>
<td>0.69 (0.50–0.84)</td>
<td>0.86 (0.79–0.92)</td>
</tr>
</tbody>
</table>

HM, haematomal disease; SOT, solid-organ transplantation; RD, respiratory diseases.
prophylaxis or therapy (Marr et al., 2005), the administration of antifungal drugs also resulted in reduced sensitivity of the test using serum samples but had little influence on the outcome using BAL fluid, which was presented in a guinea pig model of IA (Wiederhold et al., 2013).

The BAL LFD test showed a high sensitivity and specificity in patients with haematological malignancy or organ transplantation but moderate accuracy in patients with respiratory diseases, as shown in Table 3, although the population included in the subgroup analysis was small, especially the haematological malignancy group. The variance of performance in different disease groups needs to be confirmed in a larger population. A similar analysis was done for the serum LFD test. Patients with haematological malignancy composed the overwhelming majority (162/178 cases) of the included population and consequently the performance of the serum LFD test in this group showed little difference compared with that in all patients. Analysis of other underlying diseases was not conducted because there were not enough reported cases.

The PLR (10.70, 95% CI 6.96–16.44) and NLR (0.19, 95% CI 0.11–0.32) indicated that the BAL LFD test might perform better in confirmation than in exclusion, i.e. a positive result from the BAL LFD test could facilitate early diagnosis and timely therapy to get a better prognosis, whilst a negative result should be reconfirmed by a different test such as the GM test or PCR to prevent overtreatment with antifungal drugs, which results in unnecessary toxicity to patients and a heavy economic burden and leads to drug resistance (Azoulay et al., 2012; van der Linden et al., 2011).

We computed the NPVs and PPVs of the index test for prevalences of 10, 15 and 20%, which are shown in Table 2. The NPV was high, whereas the PPV was relatively low but increased with increasing prevalence. The low PPV was caused by the false-positive cases, which could partly be explained by the use of the GM test in the diagnostic criteria of probable IA. For example, the interpretation of LFD results is affected by the GM cut-off. A weak positive LFD result might correspond to a GM cut-off of 0.5; however, it would be taken as false positive when the cut-off was 1.0. In addition, antifungal prophylaxis or therapy would decrease the sensitivity of the GM test. Patients receiving antifungal therapy could have a negative GM outcome and be diagnosed with possible IA in spite of having a positive LFD result. Under these circumstances, the so-called false-positive cases might actually be true-positive cases, with the diagnosis accuracy of the LFD test being underestimated. This is another reason why possible cases should be excluded from the analysis. In addition, cross-reaction might be another cause of false-positive cases. Although the mAb JF5 displayed a great specificity for Aspergillus spp. when the LFD was invented, cross-reaction with certain fungi including Candida spp. and Penicillium sp. was reported later in two patients (Hoenigl et al., 2012).

Our analysis had its limitations. Although significant attention was paid to the newly invented Aspergillus LFD, it has not been commercialized and widely used. There were not as many studies reporting data on its accuracy, and the sample size in this analysis was not sufficient. Although the BAL LFD test showed variant accuracy among the groups of different underlying diseases, the outcome remained doubtful and needed to be checked in a larger population. The effects of GM cut-off and antifungal drugs on the diagnostic accuracy of the index test were not examined in our study because of incomplete data and are worth further studying.

We showed good specificity and sensitivity in the index test, suggesting that LFD has good value in the diagnosis of IA, especially in BAL fluid. The high PLR suggested that the LFD has a good ability to confirm the possibility of IA in those patients with positive results. In consideration of its great advantages as a rapid test with simple manipulation, the LFD is quite promising. Further studies should focus on the effect of underlying diseases on the test performance and assessment of the performance of the combined tests.

Acknowledgements

This work was supported by the Specialized Research Fund for the Doctoral Program of Higher Education (China) (20121011100008) and National Key Clinical Speciality.

References


