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Original Article

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Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: A comparative multicenter study

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Abstract

Fast diagnosis of invasive pulmonary aspergillosis (IPA) is essential as early adequate therapy improves survival. However, current microbiological methods suffer from a low sensitivity or a long turnaround time, often as a result of batching. Recently, two lateral flow assays for diagnosing IPA have been CE (Conformité Européenne)-marked and commercialized. These assays can be used for fast single sample testing. However, clinical validation and comparative studies are lacking. We therefore sought to evaluate and compare these assays in adult hematology patients. We retrospectively tested 235 bronchoalveolar lavage fluid (BALf) samples of adult hematology patients from four centers using the AspLFD (OLM Diagnostics) and the sōna *Aspergillus* galactomannan LFA (IMMY). Both tests were read out independently by two researchers and by a digital reader. We included 11 patients with proven IPA, 64 with probable IPA, 43 with possible fungal disease, and 117 controls with no signs of IPA. In cases of proven IPA, the performance of both assays was similar. In cases of proven and probable IPA, we found an identical specificity for both assays, but a higher sensitivity (0.83 vs 0.69, $P = .008$) and a better negative predictive value (0.89 vs 0.82, $P = .009$) for the LFA. Digital readout improved the diagnostic performance of both tests. In conclusion, both assays showed a good performance for the diagnosis of IPA in BALf from adult hematology patients. Results were further improved by using a digital reader, especially for weakly positive results.

Key words: invasive aspergillosis, lateral flow device, lateral flow assay, hematology, diagnostics.

Introduction

Invasive pulmonary aspergillosis (IPA) carries a significant morbidity and mortality.[1] Hematology patients, especially those receiving allogeneic hematopoietic cell transplantation or intensive chemotherapy for acute leukemia and myelodysplastic syndromes, are at increased risk.[1] Fortunately, crude mortality rates

Figure 1. Examples of the OLM diagnostics AspLFD (left two cassettes) and IMMY sōna *Aspergillus* galactomannan LFA (right two strips) showing a strongly positive (left) and negative result (right). This Figure is reproduced in color in the online version of *Medical Mycology*.

of IPA have decreased from 86% in 1995[2] to 29% more recently.[3] This is partly due to the introduction of more effective and less toxic mould-active triazole antifungals in the 1990s, compared to previously used conventional amphotericin B.[4] Furthermore, the widespread use of diagnostic tools (such as the galactomannan [GM] enzyme immunoassay or *Aspergillus* spp. specific DNA detection by polymerase chain reaction [PCR]) that are more sensitive than fungal culture or direct microscopy, and the early implementation of thoracic computed tomography (CT) scanning, have led to an improved diagnosis. This in turn leads to earlier initiation of adequate therapy with subsequently improved survival.[5] Importantly, to be cost-efficient, these indirect mycological techniques usually require multiple samples to be batched in the same run. In addition, significant hands-on time results in an increased turnaround time.

Recently, two lateral flow assays for the diagnosis of IPA were CE-marked and commercialized. Both assays allow for single sample testing and have results available in less than 1 hour (Fig. 1). The diagnostic performance of the first assay, the AspLFD (OLM Diagnostics, Newcastle Upon Tyne, United Kingdom [LFD]) has been evaluated in different studies in hematology patients over the past decade.[6–9] However, apart from one brief report,[9] all studies used a prototype device, which differs in several aspects from the currently available commercial assay. One study compared the performance of the prototype device to the commercial version in 14 cases and 14 controls and found a higher specificity for the commercial version.[10] However, the small sample size makes it impossible to draw further conclusions regarding diagnostic performance from this study. The second assay, the sōna *Aspergillus* galactomannan LFA (IMMY, Norman, OK, USA; [LFA]) has only been evaluated in one recent brief report.[9]

In a retrospective multicenter study, the Dutch-Belgian Mycosis Study Group recently reported on the diagnostic performance of the LFD compared to the Platelia™ *Aspergillus* antigen assay using stored bronchoalveolar lavage fluid (BALf) samples of hematology patients at risk of IPA.[11] In the current multicenter study, we sought to compare the diagnostic performance of both lateral flow assays using the same BALf sources as used in the previous study, allowing for within-patient comparison.

Methods

We used remaining fractions from BALf samples that were previously collected for a study on the LFD. Briefly, we used BALf samples of patients with IPA ("cases") and without IPA ("controls") that were collected as part of routine clinical care and subsequently stored at ≤ −20°C in two centers in Belgium (University Hospitals Leuven, Leuven, and AZ St Jan Bruges, Bruges) and two centers in the Netherlands (Erasmus University Medical Center, Rotterdam, and Radboud University Medical Center, Nijmegen). Eligible samples were shipped frozen and analyzed centrally at the research laboratory of the National Reference Center for Mycosis, Leuven, Belgium. Upon receipt, BALf samples were thawed and split into two aliquots (provided at least 750 μl were available). Storage of separate aliquots for both tests ensured an identical number of freeze-thaw cycles for both tests, removing possible bias due to repeated thawing. One aliquot of at least 450 μl was used for testing both the Platelia™ GM assay and the LFD in parallel; the second aliquot of at least 300 μl for testing the LFA afterward.

Patient selection and data collection

We included patients that (i) were at least 18 years of age; (ii) had an underlying hematological disease or underwent hematopoietic stem cell transplantation (HSCT); (iii) had a chest CT scan performed within 7 days of BALf sampling; and (iv) had at least 750 μl of remaining BALf stored at ≤−20°C. All four participating centers followed a similar integrated care pathway for managing invasive fungal diseases in hematology patients: persistent fever unresponsive to 3–5 days of broad-spectrum antibiotics triggered a high resolution chest CT scan. Patients with pathological CT findings underwent bronchoscopy with lavage for extensive microbiologic (including GM detection) and microscopic analysis. Mould-active antifungal prophylaxis was given per institutional policy. To reflect the estimated incidence of IPA in hematology patients referred for bronchoscopy in these four centers (±30%), we aimed for a case:control ratio of 1:2. We collected demographic data, the underlying disease, host factors, serum, and BALf GM as determined by the local laboratory, fungal culture results, other microbiological findings, microscopy

(with the use of optical brighteners), histopathology (including autopsy) results, use of mould-active antifungals more than 24 hours before bronchoscopy, use of mould-active prophylaxis, absolute neutrophil count, and findings from chest CT scan and bronchoscopy. Survival and date of last follow-up were recorded through 12 weeks after initiation of *Aspergillus*-specific therapy.

Case definitions

Patients were classified as having proven IPA, probable IPA, or possible invasive fungal disease according to the revised European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group (EORTC)/Mycoses Study Group of the National Institute of Allery and Infectious Diseases (MSG) consensus definitions.[12] Patients not fulfilling any of the EORTC/MSG clinical and mycological criteria, patients with aberrant findings on pulmonary imaging but with BALf GM optical density index (ODI) <1.0 and a documented alternative diagnosis (e.g., bacterial) not receiving mould-active therapy, and patients not receiving any specific anti-mould therapy at all who survived for more than 6 months after bronchoscopy, were defined as control patients.

Study procedures

Both assays were performed at the Belgian National Reference Centre for Mycosis in accordance with the manufacturer's instructions. Samples were thawed at room temperature and vortexed briefly prior to testing. Both assays were read out after the manufacturer-recommended time by two researchers who were otherwise blinded to the final disease classification of the sample (T.M. and E.G.). After independent readout, discordant results were immediately resolved by consensus. We defined visual results as positive when both the test and control line were visible, as negative when only the control line was clearly visible, or as invalid when only a very weak or no control line was visible. Both assays were simultaneously read out using a digital lateral flow assay reader (aLF Reader, QIAGEN Lake Constance, Stockach, Germany) to provide an objective qualitative and quantitative result. The digital reader protocol containing the positions of the test and control lines was determined using positive and negative controls provided with both kits. The diagnostic cutoffs that performed best for each of the assays were determined using Youden's index within each subanalysis. The LFD and LFA kits were provided by OLM Diagnostics and IMMY respectively. The manufacturers had no role in the design of this study, its execution, analysis, interpretation of the data, or decision to publish.

OLM Diagnostics AspLFD

Hemorrhagic or highly viscous samples were pretreated by addition of 300 μl of EDTA-based buffer to 150 μl of BALf sample, followed by heating at 100°C for 3 minutes. The denatured samples were centrifuged for 5 minutes at 14,000 *g*. Seventy microliters of the resulting supernatant were pipetted onto the sample port of the LFD. Nonhemorrhagic, nonviscous samples were pipetted onto the LFD without pretreatment. The LFD was removed from its protective packaging just prior to pipetting of the sample. Results were read out after exactly 15 minutes of incubation at room temperature and recorded qualitatively as visually positive, negative, or invalid. Digital readout was performed in parallel with visual readout.

IMMY sōna *Aspergillus* galactomannan LFA

All samples were pretreated by addition of 100 μl of EDTA-containing buffer to 300 μl of BALf sample, followed by heating at 120°C for 7 minutes, and centrifuged at 14,000 *g* for 5 minutes. Eighty microliters of the supernatant were transferred to a separate tube, to which 40 μl of running buffer was added. An LFA strip was then removed from its protective tube and placed in the tube containing the sample – running buffer mix. Results were recorded after 30 minutes of incubation at room temperature, and recorded qualitatively as visually positive, negative, or invalid, as well as semi-quantitatively using the calibrated reading card provided by the manufacturer on a scale of 1–4 for positive samples. Discordant semi-quantitative results were averaged if both researchers independently considered the assay to be positive. Digital readout was performed in parallel with visual readout.

Statistical analysis

Exact binomial 95% confidence intervals (CIs) of the negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity were calculated for each assay. The 95% CIs of the positive and negative likelihood ratios (PLR and NLR) were calculated.[13] Sensitivity and specificity of both assays were compared using McNemar's χ^2 test; PPV and NPV using the weighted generalized score statistic[14]; PLR and NLR using a regression model approach[15]; and area under the curve using DeLong's test for two correlated receiver operating characteristic (ROC) curves, as all these comparisons are pairwise observations. A two-sided *P*-value of ≤.05 was considered statistically significant. Cox regression was used to determine the relation between the LFA result (both quantitative and qualitative) and outcome, controlling for age, sex, HSCT, neutropenia, and prednisone-equivalent dose of ≥0.3 mg/kg/day for >21 days. Cohen's kappa coefficient (with 95% CI) was calculated to measure the agreement between the LFA and LFD (digital or consensus of visual readout), between the visual readings of the LFA of the two evaluators, and between visual and digital readout of the LFA. Kappa values of >0.8 represent an almost perfect agreement, while values between 0.61 and 0.80 represent substantial

agreement, in accordance with the classification by Landis and Koch.[16]

For primary analysis, we defined true positives as patients with proven IPA according to the EORTC/MSG definitions, and true negatives as patients without any evidence of IPA (as defined above). We restricted our primary analysis to this narrow subgroup as possible and probable IPA are an assessment of the likelihood of disease (by consensus), rather than reflecting a definite diagnosis.

To allow comparison with previous reports, we also assessed the performance of the LFA with cases of EORTC/MSG-defined proven or probable as true positives in a secondary analysis. The EORTC/MSG definitions were used both with and without BALf and serum GM test results included. Indeed, as the GM assay itself is an accepted microbiologic criterion in these definitions, a comparison of the diagnostic performance of the LFA (which contains an antibody similar to the one used in the Platelia™ GM assay) to EORTC/MSG probable cases without the removal of GM from the definitions leads to incorporation bias.

Statistical analysis was performed using R v3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

We analyzed a total of 235 BALf samples from 11 patients with proven IPA, 64 with probable IPA, 43 with possible invasive fungal disease, and 117 with no signs of IPA ("controls"). *Aspergillus* spp. was cultured from 24 (10.2%) samples, and 71 (30.2%) had a GM ODI ≥ 1.0. Patient characteristics are summarized in Table 1. Contingency tables for all subanalyses are provided in Supplement 1.

Table 1. Patient characteristics.

N	235
Center (%)	
Belgium 1	130 (55.3)
Belgium 2	38 (16.2)
Netherlands 1	34 (14.5)
Netherlands 2	33 (14.0)
Age, years (median [IQR])	64 [52, 71]
Male (%)	143 (60.9)
Underlying disease (%)	
Acute myeloid leukemia	74 (31.5)
Allogeneic HSCT	59 (25.1)
Lymphoma	57 (24.3)
Acute lymphoblastic leukemia	10 (4.3)
Multiple myeloma	14 (6.0)
Myelodysplastic syndrome	8 (3.4)
Autologous HSCT	6 (2.6)
Other	7 (3.0)
Neutropenia (%)	115 (48.9)
High dose corticosteroids (%)	82 (34.9)
T-cell suppression (%)	119 (50.6)
Severe inborn immune deficiency (%)	1 (0.4)
Serum GM ODI (median [IQR])	0.10 [0.07, 0.20]
BALf GM ODI (median [IQR])	0.20 [0.10, 1.65]
Fungal culture (%)	
A. fumigatus	23 (9.8)
A. flavus	3 (1.3)
A. versicolor	1 (0.4)
Negative	208 (88.5)
Absolute neutrophil count, cells/μl (median [IQR])	200.00 [0.00, 3150.00]
Mould-active prophylaxis (%)	15 (6.4)

BALf, bronchoalveolar lavage fluid; GM ODI, galactomannan optical density index; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range.

Visual readout

The diagnostic performance of both assays when read out visually is summarized in Table 2. In the small subgroup of cases of proven IPA versus controls, we found a similar sensitivity (0.91 vs 0.82, $P = .317$), while specificity was identical. In the larger group of cases of proven and probable IPA versus controls, the LFA performed significantly better regarding sensitivity (0.83 vs 0.69, $P = .008$), NPV (0.89 vs 0.82, $P = .009$), and NLR (0.20 vs 0.35, $P = .010$). Even when excluding GM from the mycological criteria, these differences remained significant ($P = .046$; 0.048, and 0.052 for sensitivity, NPV, and NLR, respectively).

The inter-rater agreement of the two investigators for qualitative visual readout (positive vs negative) of the LFA was substantial but not perfect (11% mismatch, Cohen's kappa 0.75 [95% CI 0.67–0.84]). The inter-rater agreement for semi-quantitative visual readout was slightly lower (17% mismatch, Cohen's kappa 0.70 [95% CI 0.62–0.78]). Cases of proven and probable IPA with a positive fungal culture were not significantly more likely to have a positive LFA result (92% vs 78%, $P = .204$) but did have a higher intensity of the test line (median semi-quantitative result 2.5 vs 1, $P < .001$). The agreement between the LFD and the LFA was only moderate, with disagreement on 22% of all samples (Cohen's kappa 0.52 [95% CI 0.4–0.63]). Cox regression in cases of proven and probable IPA using the LFA results as a binary outcome did not show a significant relation with mortality after controlling for age, sex, allogeneic HSCT, use of high-dose corticosteroids and neutropenia (hazard ratio 0.622 [95% CI 0.252–1.535], $P = .303$). Nine out of the 43 cases of possible invasive fungal disease had a positive LFA result, all with low intensity (semiquantitative result of 1). The number of patients on mold-active prophylaxis (6.4%) was too low for any relevant subgroup analysis. The sensitivity of the LFA was significantly lower in patients receiving empiric antifungal therapy compared to those who had not received therapy (0.63 vs 0.88, $P = .026$), while no impact on specificity could be demonstrated (0.88 vs 0.87, $P = 1.00$). This lowered sensitivity was not significantly different from that of the LFD in these patients (0.62 vs 0.44, $P = .180$).

Table 2. Diagnostic performance of visual readout of both lateral flow assays in bronchoalveolar lavage fluid from hematology patients.

		Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Proven IPA versus control ($n = 128$)	LFA	0.91 (0.59–1.00)	0.87 (0.80–0.93)	0.40 (0.21–0.61)	0.99 (0.95–1.00)	7.09 (4.27–11.79)	0.10 (0.02–0.68)
	LFD	0.82 (0.48–0.98)	0.87 (0.80–0.93)	0.38 (0.19–0.59)	0.98 (0.93–1.00)	6.38 (3.69–11.04)	0.21 (0.06–0.73)
Proven and probable IPA versus control ($n = 192$)	LFA	0.83 (0.72–0.90)	0.87 (0.80–0.93)	0.81 (0.70–0.89)	0.89 (0.81–0.94)	6.45 (3.98–10.46)	0.20 (0.12–0.33)
	LFD	0.69 (0.58–0.79)	0.87 (0.80–0.93)	0.78 (0.66–0.87)	0.82 (0.74–0.88)	5.41 (3.29–8.88)	0.35 (0.25–0.50)
Proven and probable IPA (GM excluded) versus control ($n = 147$)	LFA	0.87 (0.69–0.96)	0.87 (0.80–0.93)	0.63 (0.47–0.78)	0.96 (0.91–0.99)	6.76 (4.13–11.07)	0.15 (0.06–0.38)
	LFD	0.73 (0.54–0.88)	0.87 (0.80–0.93)	0.59 (0.42–0.75)	0.93 (0.86–0.97)	5.72 (3.40–9.62)	0.31 (0.17–0.56)
Probable IPA vs controls ($n = 181$)	LFA	0.81 (0.70–0.90)	0.87 (0.80–0.93)	0.78 (0.66–0.87)	0.89 (0.82–0.94)	6.34 (3.89–10.31)	0.22 (0.13–0.36)
	LFD	0.67 (0.54–0.78)	0.87 (0.80–0.93)	0.74 (0.61–0.85)	0.83 (0.75–0.89)	5.24 (3.17–8.66)	0.38 (0.26–0.54)

CI, confidence interval; GM, galactomannan; IPA, invasive pulmonary aspergillosis; LFA, IMMY sōna *Aspergillus* galactomannan LFA; LFD, OLM diagnostics AspLFD.

Digital readout

The diagnostic performance of both assays when read out using a digital reader is summarized in Table 3. In cases of proven IPA versus controls, we found no statistically significant differences for any of the diagnostic parameters. Overall, both tests performed similar in this subgroup (area under the curve [AUC] 0.90 vs 0.92, $P = .570$), although the small number of cases does not allow any firm conclusions. The ROC curves for both assays are shown in Fig. 2. When comparing cases of proven and probable IPA versus controls, we found the LFA to outperform the LFD on all diagnostic measures, although this difference was only significant for the sensitivity ($p = 0.005$), NPV ($p = 0.003$), NLR ($P = .003$) and overall performance as assessed by the AUC (0.92 vs 0.82, $P = .002$). These differences remained statistically significant even when excluding GM from the mycological criteria.

Digital readout of the LFA in cases of proven IPA versus controls showed a trend towards improved diagnostic performance as the digital readout increased the specificity (0.87 vs 0.92, $P = .058$). In cases of proven and probable IPA versus controls, digital readout improved all diagnostic parameters, although this was only statistically significant for the PPV ($P = .040$) and PLR (.042). Overall, the agreement between digital readout (using a cutoff of 49.05 mV) and consensus of visual readout was excellent (7.7% mismatch, Cohen's kappa 0.83 [95% CI 0.76–0.91]).

There was a high degree of correlation between the OI's of the LFD and the LFA ($R^2 = 0.80$, Fig. 3a). The correlation of the LFA OI and the GM ODI was lower, with an exponential relation (Fig. 3b). Upon visual inspection of the correlation between the GM ODI and the LFA OI, we noticed a breakpoint around a GM ODI of 3. Indeed, the sensitivity was significantly lower in cases of proven and probable IPA with a BALf GM ODI < 3 (0.64 vs 0.98, $P < .001$) while specificity was similar (0.88 vs 0.87, $P = 1.000$).

Cox regression analysis on the predictive value of the LFA regarding outcome of cases of proven and probable IPA using the LFA OI as a continuous predictor was not significant after controlling for age, sex, allogeneic HSCT, use of high-dose corticosteroids, and neutropenia (hazard ratio 1.089 [95% CI 0.933–1.271], $P = .278$).

Similar to visual readout, we found a significantly higher OI (310.36 mV vs 92.90 mV, $P = .001$) and GM (6.4 vs 2.75, $P = .048$) in culture positive cases of proven and probable IPA. In cases of possible IPA, the median OI was 52.07 mV (interquartile range 41.78–59.47 mV). All LFA's visually classified as negative still had a detectable test line with a mean OI of 31.62 mV (95% CI 29.93–33.30 mV).

Discussion

In this multicenter retrospective study, we compared the diagnostic characteristics of two recently CE-marked lateral flow devices in 235 hematology patients who had undergone BALf sampling in order to diagnose or rule out IPA. As the diagnosis of IPA can only be made with certainty in cases of proven IPA as defined by the EORTC/MSG definitions, we first evaluated the assays in the (albeit smaller) subgroup of cases of proven IPA

Table 3. Diagnostic performance of digital readout of both lateral flow assays in bronchoalveolar lavage fluid from hematology patients.

		Cutoff (mV)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Proven IPA versus controls ($n = 128$)	LFA	49.05	0.90 (0.72–1.00)	0.91 (0.59–1.00)	0.92 (0.86–0.96)	0.53 (0.29–0.76)	0.99 (0.95–1.00)	11.82 (6.14–22.75)	0.10 (0.02–0.64)
	LFD	33.15	0.92 (0.81–1.00)	0.82 (0.48–0.98)	0.96 (0.90–0.99)	0.64 (0.35–0.87)	0.98 (0.94–1.00)	19.15 (7.77–47.17)	0.19 (0.05–0.67)
Proven and probable IPA versus controls ($n = 192$)	LFA	49.05	0.92 (0.87–0.97)	0.87 (0.77–0.93)	0.92 (0.86–0.96)	0.88 (0.78–0.94)	0.92 (0.85–0.96)	11.27 (5.98–21.24)	0.14 (0.08–0.26)
	LFD	16.62	0.82 (0.76–0.88)	0.72 (0.60–0.82)	0.87 (0.80–0.93)	0.78 (0.67–0.87)	0.83 (0.75–0.89)	5.62 (3.43–9.20)	0.32 (0.22–0.46)
Proven and probable IPA (GM excluded) versus controls ($n = 147$)	LFA	43.26	0.97 (0.94–1.00)	0.97 (0.83–1.00)	0.86 (0.79–0.92)	0.64 (0.49–0.78)	0.99 (0.95–1.00)	7.07 (4.46–11.20)	0.04 (0.01–0.27)
	LFD	26.70	0.86 (0.77–0.95)	0.73 (0.54–0.88)	0.93 (0.87–0.97)	0.73 (0.54–0.88)	0.93 (0.87–0.97)	10.73 (5.31–21.66)	0.29 (0.16–0.52)
Probable IPA versus controls ($n = 181$)	LFA	52.49	0.92 (0.88–0.97)	0.84 (0.73–0.92)	0.95 (0.89–0.98)	0.90 (0.79–0.96)	0.92 (0.85–0.96)	16.45 (7.49–36.12)	0.16 (0.09–0.29)
	LFD	16.62	0.81 (0.74–0.87)	0.70 (0.58–0.81)	0.87 (0.80–0.93)	0.75 (0.62–0.85)	0.84 (0.77–0.90)	5.48 (3.33–9.03)	0.34 (0.23–0.50)

AUC, area under the curve; CI, confidence interval; GM, galactomannan; IPA = invasive pulmonary aspergillosis; LFD, OLM Diagnostics AspLFD; LFA, IMMY sōna *Aspergillus* galactomannan LFA.

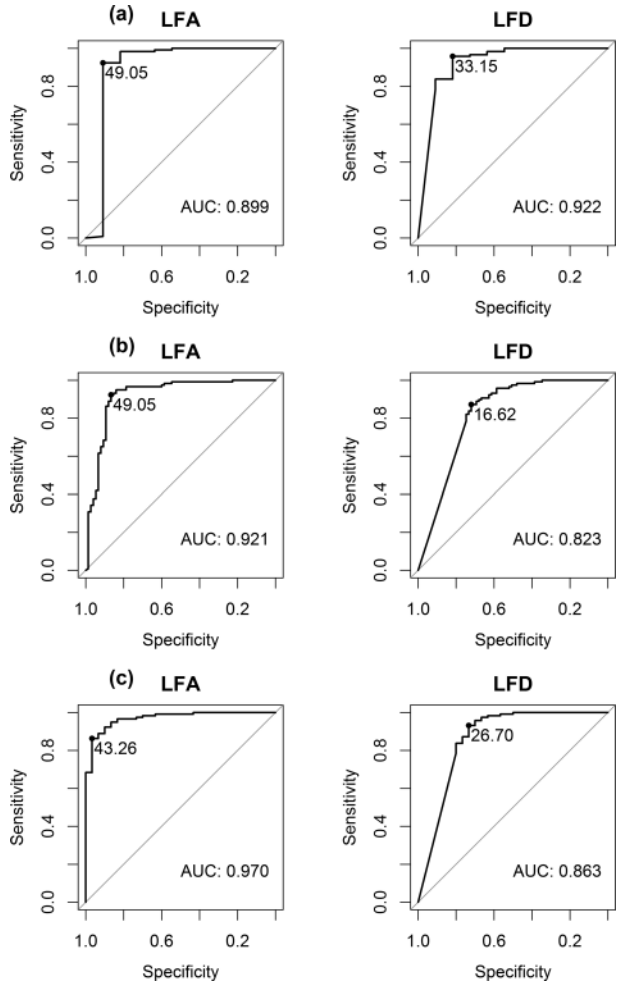

Figure 2. Receiver operating characteristic curves for digital readout of both assays in different subgroups. (a) Proven invasive pulmonary aspergillosis (IPA) versus controls. (b) Proven and probable IPA versus controls. (c) Proven and probable IPA, with exclusion of galactomannan as mycological criterion, versus controls.

versus controls with no signs of IPA and found a good diagnostic performance for both assays. The specificity of both assays was improved further by using a digital reader, as compared to visual readout. This could be explained by variations in visual readout as we found disagreement between two independent researchers in 11% of the samples. However, by reading out the LFA in consensus, we could decrease the disagreement with the digital (objective) readout to 7.7%.

To allow comparison with other assays and previous reports on the LFD,[6–9] we further evaluated the performance in cases of proven and probable IPA versus controls. Overall, the LFA was found to be superior to the LFD, with significantly higher AUC, sensitivity, NPV and NLR, both when read out visually or digitally. As GM was significantly higher in culture positive cases, this could be a contributing factor for the very high AUC of the LFA in the subanalysis on proven/probable IPA with GM excluded as mycological criterion (Fig. 2c). However, this is not the sole explanation, as the AUC of the LFA remains significantly higher than that of the LFD even in patients from this subgroup with negative GM (AUC 0.898 vs 0.482, $P = .001$, $n = 5$). In part, these differences may be explained by the difference in antigens targeted by both assays. The LFD uses the JF5 mouse monoclonal antibody (mAb), which binds to an extracellular glycoprotein released by Aspergillus during active growth.[17] The LFA, on the other hand, uses a proprietary mix of two different mAb's: the ME-A5 human immunoglobulin G (IgG) monoclonal, and an undisclosed proprietary mAb with an unknown target. The ME-A5 mAb targets a similar or identical epitope as the EB-A2 rat mAb used in the Platelia™ GM assay, as it is inhibitory to EB-A2 binding. As the LFA includes a highly similar mAb as the GM assay, this could positively bias the results when including cases of probable IPA, as GM is used as one of the mycological criteria in the EORTC/MSG definitions.

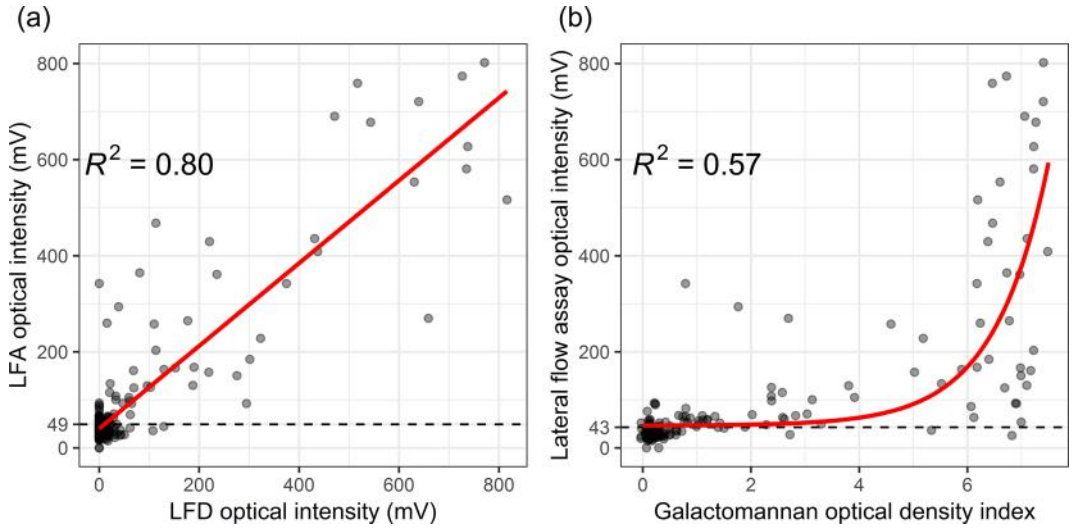

Figure 3. Correlation of the optical intensity of the sōna Aspergillus galactomannan LFA and the AspLFD (a) and galactomannan (b). This Figure is reproduced in color in the online version of *Medical Mycology*.

However, after excluding GM from these definitions, differences in diagnostic performance remained significant. Another possible source of differences in performance may be found in the different test procedures (e.g., sample volume, pretreatment buffer, temperature, and time of heating steps, etc).

The sensitivity of both assays was decreased significantly by empiric antifungal therapy. We could not show a significant difference between the two assays, although this is likely due to the small number of patients receiving empiric antifungal therapy in our study ($n = 24$), as our centers use a diagnostics-driven approach.

During testing, we noticed the presence of a very faint line at the site of the test lines of the LFA, including on LFA strips that were run using buffer only (i.e., strictly negative tests). This line is not detected by regular cameras (as is evident from Fig. 1) but is visible to the naked eye. Furthermore, its presence was confirmed using the digital reader, with an intensity of around 32 mV. This means that a comparator is required in cases with a weak test line to determine if this is due to the background effect seen in negative tests, or due to the presence of an actual yet weak line. During testing, we therefore used the included reading card and considered any line fainter than a line of semiquantitative intensity 1 to be negative. Alternatively, plain running buffer can be used to create a negative control LFA to which any clinical samples can be compared. Any line that is more intense than the negative control can be considered positive. This of course introduces additional uncertainty, variability and cost (when creating a negative control for every sample run). The use of a digital reader with predefined cutoffs can help solve this problem.

A subgroup of special interest is that of patients with possible IPA, where all conventional microbiologic tests (β-D-glucan, GM, and culture) are negative despite a clinical and radiological suspicion of IPA. In these cases, an additional test that could differentiate between actual IPA and other diseases is needed. However, as we still lack a perfect standard for these patients, we cannot assess the diagnostic performance of the LFA in this subgroup. Since only a minority of possible cases (9 out of 43) had a low-intensity positive LFA test result, we assume that many of these patients have radiological findings not related to *Aspergillus* disease.

Our study has its limitations. All samples had been frozen and thawed at least once. To avoid any impact of this freeze-thaw step on the results of our study, we repeated the GM measurement on the thawed BALf samples. It was reassuring to see that the results of this GM measurement were very consistent with the values measured at the time of BALf sampling (data not shown). The pretest probability of IPA as well as the number of controls was chosen by us. We therefore assumed that the 1:2 case:control ratio reflects the ratio of IPA in patients undergoing BAL sampling for pulmonary infiltrates in hematology centers that treat patients with acute leukemia and allogeneic HSCT and use mould-active azole prophylaxis infrequently. This case:control ratio may be lower in other centers; this would result in a higher overall NPV.

While the LFA and LFD are both relatively easy to use, the LFD is somewhat more straightforward in its use. Indeed, no pretreatment steps are needed in nonbloody, nonviscous BALf samples: the sample can be pipetted directly onto the LFD and the result is available within 15 minutes. In case of a bloody or viscous sample, a pretreatment by heating (3 minutes) and centrifugation (5 minutes) is required, bringing the total test time to around 25 minutes. This is still faster than the LFA, which always requires pretreatment consisting of heating (6–8 minutes) and centrifugation (5 minutes), bringing the total test time of the LFA to around 45 minutes.

In conclusion, both lateral flow tests show a good performance for the diagnosis of IPA in BALf from hematology patients. The performance can be further improved by using a digital reader. Both tests can be used as a fast screening test with a short turnaround time, although confirmation by other diagnostic tests such as GM, PCR, or culture is still warranted.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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Author contributions

A.S., J.M., K.L., and T.M. designed the experiment. A.D., A.S., E.d.K., and T.M. collected the clinical data. A.D., A.S., A.V., B.R., E.G., M.R., P.V., and T.M. collected the BALf samples. E.G. and T.M. performed the experiments. T.M. analyzed the data and wrote the initial draft. All authors critically revised the initial draft and final manuscript.

Declaration of interest

A.S. has received nonfinancial travel support from Abbvie, Amgen, Roche, Gilead Sciences, and Pfizer. B.R. has received research grants from Gilead Sciences and MSD outside the context of this work and served as a speaker for Gilead, MSD, BMS, ViiV, Pfizer. J.M. has received research grants from Merck/MSD, Gilead Sciences, and Pfizer; is a consultant to Astellas, Basilea, Bio-Rad, Merck/MSD, Pfizer, Schering-Plough, F2G, Gilead Sciences, Cidara, Scynexis, Amplyx, and Luminex; and has served on the speaker's bureau of Astellas, Gilead Sciences, Bio-Rad, Merck/MSD, Pfizer, Schering-Plough, Basilea and Viropharma/Shire. K.L. has received research grants from Gilead Sciences, MSD, and Pfizer; has received consultancy fees from Gilead Sciences, Pfizer, Abbott, MSD, and SMB Laboratoires Brussels; has received travel support from Pfizer, Gilead Sciences, and MSD; and has received speaker fees from Gilead Sciences, Roche, Abbott. M.R. has received nonfinancial support from Gilead Sciences and Pfizer. N.B. has received research grants from Merck/MSD, Gilead Sciences, and Pfizer. P.V. has received research grants from Merck/MSD, Gilead Sciences, F2G, and Pfizer; is a consultant to Merck/MSD, Pfizer, F2G, Cidara, Scynexis, and Siemens; and has served on the speaker's bureau of Gilead Sciences, Merck/MSD, and F2G. T.M. has received nonfinancial support

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