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Original article

Performance of lateral flow device and galactomannan for the detection of *Aspergillus* species in bronchoalveolar fluid of patients at risk for invasive pulmonary aspergillosis

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Summary

Early diagnosis of invasive pulmonary aspergillosis (IPA) remains difficult due to the variable performance of the tests used. We compared the performance characteristics of *Aspergillus* lateral flow device (LFD) in bronchoalveolar lavage (BAL) vs. BAL-galactomannan (GM), for the diagnosis of IPA. 311 BAL specimens were prospectively collected from patients who underwent bronchoscopy from January to May 2013. Patients at risk for IPA were divided into haematological malignancy (HEM) and non-HEM groups: solid organ transplants (SOT) (lung transplant (LT) and non-LT SOT); chronic steroid use (CSU); solid tumour (STU) and others. We identified 96 patients at risk for IPA; 89 patients (93%) were in the non-HEM groups: SOT 57 (LT, 46, non-LT SOT, 11); CSU 21; STU 6, other 5. Only three patients met criteria for IA (two probable; one possible). Overall sensitivity (SS) was 66% for both and specificity (SP) was 94% vs. 52% for LFD and GM respectively. LFD and GM performance was similar in the HEM group (SS 100% for both and SP 83% vs. 100% respectively). LFD performance was better than GM among non-HEM SOT patients ($P = 0.02$). Most false-positive GM results occurred in the SOT group (50.8%), especially among LT patients (56.5%). LFD performance was superior with an overall SP of 95.6% in SOT ($P < 0.002$) and 97% in LT patients ($P = 0.0008$). LFD is a rapid and simple test that can be performed on BAL to rule out IPA.

Key words: *Aspergillus*, lateral flow device, diagnosis, brochoalveolar lavage.

Introduction

Invasive pulmonary aspergillosis (IPA) is an important cause of morbidity and mortality among immunocompromised patients.[1] Overall, IPA predominates among patients with acute leukaemia and haematopoietic stem cell transplant recipients.[2] Among solid organ transplant (SOT) recipients, the highest rates occur in lung transplant (LT) recipients.[3] Early diagnosis and prompt initiation of antifungal therapy improves survival of patients with invasive aspergillosis (IA).[4,5]

The diagnosis of IPA remains challenging given the lack of a single gold standard test. Current diagnosis of IPA is based on host factors and clinical, radiological and microbiological criteria which are neither sensitive nor specific.[6] For instance, a definitive diagnosis of IPA requires isolating the organism from a normally sterile site and/or evidence of tissue invasion on histopathology. Obtaining these clinical specimens require

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invasive procedures that are often contraindicated in patients at risk for IPA due to bleeding diathesis or other comorbidities.

New strategies for diagnosis and management of IPA include the use of non-invasive biomarkers (i.e. galactomannan (GM) and 1,3 beta-d-glucan). The role of serum and bronchoalveolar lavage (BAL) GM for the diagnosis of IPA has been best established in patients with haematological malignancies.[7] Conversely, the role of these biomarkers in other patients at risk for IPA such as SOT recipients has not been established mainly because of the significant variation in performance observed in these patients.[8] In addition, factors such as cost and outsourcing to reference laboratories limits testing frequency and timely result availability.

Aspergillus immuno-chromatographic lateral flow device (LFD) is a novel technique that uses an *Aspergillus* – specific monoclonal antibody (mAb JF5).[9] *Aspergillus* LFD enables the point-of-care testing using the same technology used for the rapid diagnosis of pregnancy.[9,10] Indeed, recent studies report sensitivity, specificity, positive and negative predictive values up to 100%, 95%, 80% and 100%, respectively, for the diagnosis of IPA.[11–13]

We sought to determine the performance characteristics of the LFD using BAL for the diagnosis of IPA in patients at risk of invasive fungal infection (IFI) who underwent bronchoscopy at the Henry Ford Health System. Second, we compared the performance of BAL-LFD to that of BAL-GM.

Material and methods

We prospectively collected aliquots of all BAL specimens obtained from bronchoscopies performed at HFHS from January 2013 to May 2013. These BAL aliquots were stored at −20 °C for GM and LFD testing. All BAL specimens were stored at room temperature for up to 2 h or at 4 °C for up to 24 h before being stored at −20 °C. Specimens remained frozen up to 6 months before GM and LFD testing was performed. Clinical information regarding risk factors and diagnostic criteria for IPA was collected by retrospective chart review. Patients were classified as risk for IPA according to the current European Organization for Research and Treatment of Cancer /Mycoses Study Group (EORTC/MSG) guidelines for IFI.[6] For this study, we extended the revised EORCT/MSG host criteria to include a heterogeneous group of otherwise immunosuppressed patients at risk of contracting IPA (i.e. critically ill patients, severe burns and HIV patients with CD4 ≤ 200 cells/mm^3).[14] Isolation of *Aspergillus* in culture was utilised as mycological criteria for the diagnosis of IPA. Serum and BAL *Aspergillus* GM were not used as mycological criterion for the study. Only patients at risk for IPA were included in the study and were subsequently classified based on their underlying risk factor for IFI into haematological malignancy (HEM) group and non-HEM group. The non-HEM group included SOT recipients (who were further subdivided into LT group and non-LT SOT group), chronic steroid use, solid tumour and others.

Retrospective *Aspergillus* GM (Platelia™ *Aspergillus* EIA) testing of BAL fluid (BAL-GM) from patients at risk for IPA was performed at Mira Vista Diagnostics (Indianapolis, IN, USA) according to manufacturers' procedure. An optical density (OD) index ≥0.5 was considered positive.

The *Aspergillus*-LFD was performed on stored BAL fluid from all patients included in the study. The BAL-LFD testing was performed in the Infectious Disease Research Laboratory at HFHS, following the manufacturer's method process (Isca Diagnostics, Truro, Cornwall, UK). Results of BAL-LFD testing were read at 10 min after applying the fluid to the LFD sample well.[15] Qualitative results were interpreted as a positive [both the test (T) and the control (C) line were present] or negative [only red line appeared in the control (C) zone] (Fig. 1).

We compared the diagnostic performance of LFD and GM in BAL fluid from patients at risk for IPA. The BAL-LFD results were blindly analysed by one of the investigators in this study.

Fisher's exact test was used to test the null hypothesis that GM and LFD have the same specificity.

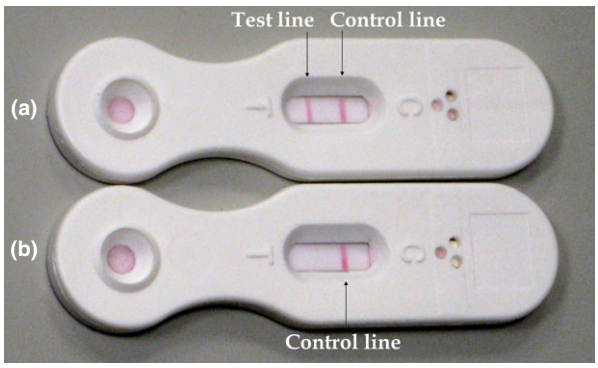

Figure 1 *Aspergillus* lateral flow device (LFD) qualitative results were interpreted as a 'positive' if both the test (T) and the control (C) line were present or 'negative' if only red line appeared in the control (C) zone. (a) Positive LFD test result. (b) Negative LFD test.

Results

We prospectively collected BAL samples from 311 patients. Of these, 96 patients were identified at risk for IPA. The enrolment and distribution of patients is summarised in Fig. 2. Mean patient age was 61 years (range: 35–81), 59 were female. Most patients, 89 of 96 (93%) were in the non-HEM groups: 57 SOT patients (LT: 46 patients, non-LT SOT: 11 patients), chronic steroid use: 21 patients, solid tumour: six patients and other: five patients. Only three patients in the study met criteria for IPA (2 probable; 1 possible IPA). The two patients with probable IPA had compatible clinical and radiographical findings and a positive BAL culture with *Aspergillus* species. One of these two cases was a haematological cancer patient with positive BAL-LFD and positive serum and BAL-GM (OD index 0.86 and 7.7 respectively). The patient was started on treatment with voriconazole but died 6 days later. The other patient had HIV infection with a CD4 cell count of 110/mm^3. The patient was treated with voriconazole. Serum and BAL-GM were negative. Subsequent workup revealed *Pseudomonas aeruginosa* pneumonia and oesophageal squamous cell carcinoma with metastasis to the lungs. Given the alternative diagnosis and the development of QTc segment prolongation, voriconazole was stopped after 2 weeks. The patient was alive at 30 day follow-up. Retrospective BAL-LFD testing was negative. Lastly, one critically ill patient in the surgical intensive care unit met criteria for possible IPA. Fungal culture of BAL fluid was not performed. Clinically, the suspicion for IPA was low and the patient was not treated with antifungals. This patient died within 2 weeks of admission. Reported cause of the death was multifactorial (stage IV congestive heart failure secondary to ischaemic cardiomyopathy complicated by refractory cardiogenic and septic shock, multiorgan failure). Serum GM result received postmortem was positive (OD index 2.67). Retrospective BAL-LFD and BAL-GM testing for this research study were positive (GM OD index was 4.02).

The overall sensitivity and specificity of BAL-GM for IPA was 66% and 52% respectively. Sensitivity and specificity of BAL-LFD were 66% and 94% respectively. Test performance was similar for BAL-LFD and BAL-GM in the HEM group (sensitivity 100% for both and specificity 83% vs. 100% respectively). However, BAL-LFD showed a better performance than BAL-GM in the non-HEM groups, particularly in the SOT group ($P = 0.002$) (Table 1). Forty-four of 96 (45%) BAL-GM showed false-positive results, using a BAL-GM cut-off ≥0.5 OD index. False-positive BAL-GM were more frequent in the SOT group (29/57 patients; 50.8%), especially among LT recipients (26/46 patients; 56.5%). False-positive BAL-GM results were associated with lower OD index values (average: 1.49; range: 0.5–7.18) compared to true positive results (average: 5.86; range 4.02–7.7). Increasing the BAL-GM cut-off to ≥1.0 OD index improved the specificity compared to BAL-GM cut-off at

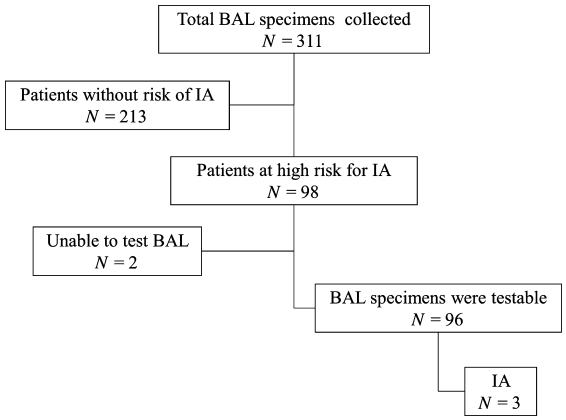

Figure 2 Patients Breakdown. BAL: bronchoalveolar; IA: invasive aspergillosis; N: number of patients.

Table 1 Specificity of *Aspergillus* lateral flow device test and galactomannan in bronchoalveolar lavage fluid by patient groups.

Group at risk (n = 96)	GM (≥0.5 ODI)	LFD	*P* value	GM (≥1.0 ODI)	LFD	*P* value
HEM group (n = 7)	0.66	1.00	0.45	0.83	1.00	1.0
Non-HEM group (n = 89)						
SOT group (n = 57)	0.49	0.96	<0.000001	0.75	0.96	<0.0021
Lung TX (n = 46)	0.43	0.97	<0.0001	0.71	0.97	0.0008
Non-lung (n = 11)	0.72	0.90	0.59	0.90	0.90	1.0
Solid tumour (n = 6)	0.16	1.00	0.02	0.66	1.00	0.45
Chronic steroid use (n = 21)	0.71	0.90	0.24	0.76	0.90	0.40
Other (n = 5)	0.30	1.00	0.4	0.33	1.00	0.4

GM, galactomannan; LFD, lateral flow device; ODI, optic density index; HEM group, haematological malignancy and peripheral stem cell transplant group; SOT group, solid organ transplant recipients, further divided into lung transplant (lung Tx) group and non-lung groups).

Table 2 Summary of seven patients with positive bronchoalveolar lateral flow device test results.

Risk factor	IPA	BAL-LFD result	BAL-GM result	BAL culture result
SOT (non-lung)	No-IPA	False positive	False positive (7.18 ODI)	No growth
Chronic steroid use	No-IPA	False positive	False positive (1.67 ODI)	No growth
Chronic steroid use	No IPA	False positive	False positive (5.0 ODI)	No growth
HEM group	Probable IPA	True positive	True positive (7.7 ODI)	*Aspergillus fumigatus*
Solid tumour	No IPA	False positive	False positive (0.94 ODI)	No growth
SOT (lung transplant)	No IPA	False Positive	True negative (0.27 ODI)	No growth
Other (critically ill patient)	Possible IPA	True Positive	True positive (4.02 ODI)	Not available

BAL, bronchoalveolar lavage; GM, galactomannan; HEM group, haematological malignancy and peripheral stem cell transplant group; IPA, invasive pulmonary aspergillosis; LFD, lateral flow device; ODI, optic density index; SOT group, solid organ transplant recipients, further divided into lung transplant non-lung groups).

≥0.5 OD index (74% vs. 52%). Conversely, BAL-LFD performance was superior with an overall specificity of 95.6%, particularly among SOT and LT recipients ($P < 0.002$ and 0.0008 respectively).

Seven patients had positive BAL-LFD test results. Two of them met criteria of IPA (true positive results) and were described above. False-positive BAL-LFD results were observed in five patients from the non-Hem group (SOT group, two; chronic steroid use, two; solid tumour, one). Four of these five patients also tested falsely positive for BAL-GM. The BAL fluid fungal cultures from all five patients were negative. Results are summarised in Table 2.

Eight patients grew mould from fungal cultures of BAL fluid (*Aspergillus* species four, other moulds: four). Among the four patients with *Aspergillus* species in BAL fungal culture, only two patients met criteria of probable IPA by EORTC/MSG definition. These two patients were described above. One of them tested positive for BAL-LFD (true positive) and BAL-GM (7.7 OD index) (true positive), whereas the other patient tested negative for BAL-LFD and GM (false negative). The remaining two patients with *Aspergillus* species in BAL fungal culture did not meet criteria for IPA. Both patients had a positive BAL-GM at 0.83 and 1.35 OD index, respectively (false positive), and both had negative BAL-LFD (true negative).

We also evaluated cross-reactivity of BAL-GM and BAL-LFD with other moulds growing from BAL cultures of 4 LT recipients. Fungal BAL cultures in these patients yielded *Fusarium* sp, *Exophiala jenselmei*, *Emmonsia* sp, and *Penicillium* sp respectively. None of these patients met EORCT/MSG definition criteria for IPA and isolated moulds were regarded as contaminant. The results of BAL-LFD were negative in all four patients (true negative), whereas BAL-GM results were positive in two of these four patients (false positive). Cross-reaction of BAL-GM was observed with *Fusarium* sp and *Emmonsia* sp.

Discussion

We evaluated the diagnostic performance of the novel *Aspergillus* LFD and GM done on BAL obtained from a large and heterogeneous group of patients at high risk for IPA. In our study, the overall performance of BAL-LFD for the diagnosis of IPA was superior to BAL-GM, particularly among patients in the non-Hem group. Indeed, specificity of BAL-LFD was higher in the SOT group, especially among LT patients. These findings confirm those of Willinger *et al.*[13], that performance of BAL-LFD from 47 SOT patients demonstrated very high specificity and a NPV greater than 97% for diagnosis of IPA. Similarly, a smaller study reported on the performance of 10 SOT patients, with emphasis on the accuracy of the BAL-LFD to diagnose IPA and the ability of the test to exclude the diagnosis of IPA in this patient population.[11]

Classic diagnostic criteria for the diagnosis of IPA are difficult to apply in the SOT patients.[3] For instance, the halo sign on computed tomography (CT) scan of the lungs is rarely observed in SOT recipients. Most SOT patients with IPA will present with focal consolidation or infiltrates and nodular lesions in CT of the chest.[16] The clinical utility of culture methods is also limited. Airway colonisation with *Aspergillus* sp within 6 months after lung transplantation could be as high as 46% despite prolonged anti-mould prophylaxis.[16,17] Similarly, diagnostic performance of non-culture diagnostic assays such as BAL-GM is variable in this patient population. A recent meta-analysis study conducted to determine the role of BAL-GM for the diagnosis IPA showed that test sensitivity and specificity varied (up to 84% and 95% respectively) depending on the population tested and the cut-off point value used.[8] The GM assay has been approved by the Food and Drug Administration (FDA) for serum and BAL.[18,19] In the United States, serum or BAL- GM value ≥ 0.5 OD index is considered positive. Using this

suggested cut-off point, the reported sensitivity and specificity of the serum GM assay was 80.7% and 89.2% respectively.[20] However, the threshold for positive BAL-GM remains under debate due to its great impact on test performance. In our study, increasing the BAL-GM cut-off to ≥1.0 OD index improved test specificity from 52% to 74%. This is in accordance with prior reports showing that increasing the cut-off value to ≥1.0 OD index improved sensitivity and specificity of the test (up to 60% and 98% respectively).[21]

False-positive BAL-GM test results in SOT particularly among LT recipients are not uncommon.[3,8] Our study showed a high rate of false-positive results using BAL-GM in SOT patients (50.8%), particularly among LT recipients (56%). Only two of 44 false-positive BAL-GM results were due to cross-reactivity with other moulds. Cross-reactivity of GM with other fungal antigens has been reported.[22,23] In our study, BAL-GM cross-reacted with *Fusarium* sp and *Emmonsia* sp. The cause of false-positive BAL-GM results in the remaining 42 patients could not be determined. These findings are consistent with prior reports on the high incidence of false-positive BAL-GM testing in SOT patients (50%), particularly among LT recipients (up to 56%) likely due to frequent *Aspergillus* colonisation in these patients.[24] On the basis of these data, we recommend BAL-GM should be used as an adjunctive tool for the diagnosis of IPA and test results should be interpreted with caution in this patient population. It is critical that non-invasive biomarkers for the diagnosis of IPA are used in the right clinical context.

In our study, no cross-reactivity was observed with BAL-LFD. Five BAL-LFD results were falsely positive, but the cause for false-positive results could not be determined. False-positive test results and potential cross-reactivity of BAL-LFD with other fungal antigens have been described. Initial *in vitro* studies showed that the MAb JF5 used in the LFD cross-reacted with antigens from *Paecilomyces variotti* and non-pathogenic *Penicillium* species.[9,11,13] In a study of 47 BAL samples from SOT recipients, Willinger *et al.*[13] reported six false-positive BAL-LFD results. Four of these patients had positive BAL culture. One patient had possible invasive infection caused by *Penicillium* sp, the remaining three patients had *Aspergillus* species growing in BAL culture, but none of them met criteria for IPA. Similarly, Hoenigl *et al.*[11] reported cross-reactivity with *Penicillium* species in 1 of 37 clinical BAL samples.

One study reports on a patient (1 of 47) with false-negative BAL-LFD in a patient with diagnosis of probable IPA in a kidney transplant recipient, with a BAL-GM of 24.9 OD index and negative BAL culture.[13] In another studies, no false-negative results were observed, even among patients receiving antifungal prophylaxis.[11] We were unable to fully evaluate the occurrence of false-negative results of either BAL-GM or BAL-LFD because of our study design. However, we observed one BAL-LFD false-negative result in an HIV patient with probable IPA who also tested negative for BAL-GM. Interestingly, an alternative diagnosis (i.e. *Pseudomonas aeruginosa* pneumonia and oesophageal squamous cell carcinoma with metastasis to the lungs) was found to explain this patient's symptoms and antifungals were stopped within 2 weeks. Divergent results in this patient may be explained by the fact that he did not have probable IPA in the first place and it could be inferred that BAL-LFD and GM test results were indeed accurate.

Our findings suggest that *Aspergillus* LFD in BAL fluid may be considered as a valuable aid to rule out IPA in high-risk population, especially in LT recipients that have a high prevalence of airway colonisation with *Aspergillus* species. Indeed, having a rapid and accurate test to help rule out the presence of IPA in these patients would be important to prevent overtreatment with broad-spectrum antifungal agents and the potential drug–drug interactions and toxicities associated with these agents. The four studies evaluating the performance of BAL-LFD for the diagnosis of IPA vs. no-IPA are summarised in Table 3.

Our study has limitations that should be acknowledged, including the small sample size, with only three cases of probable/possible IPA. Another important limitation is that classic EORTC/MSG definition criteria for the diagnosis of IA are difficult to apply in non-neutropenic patients, particularly SOT recipients which represent the largest group in our study. Furthermore, we used very inclusive eligibility criteria, we have included all patients at risk for IPA who underwent bronchoscopy and had BAL fluid obtained regardless the indication for this procedure (e.g.: surveillance bronchoscopy following LT, mucus plug removal, suspected pneumonia, aspiration, etc.). This means that most of our patients did not undergo bronchoscopy for suspected IPA. Thus, it is not surprising that only three patients in our cohort met criteria of possible/probable IPA. Consequently, our statistical analysis was limited to specificity. More patients with probable/proven IPA (true positive) should be included in future studies to evaluate sensitivity of the test. Only after evaluating both sensitivity and specificity would we will be able to make a more definitive comparison of

Table 3 Summary of studies on the performance of lateral flow device testing in bronchoalveolar fluid of patients at risk of invasive pulmonary aspergillosis.

References	Objective	Study patients	Test performance for the diagnosis of IPA vs. no-IPA	Comments
Hoenigl *et al.* [11]	Evaluation of BAL-LFD vs. BAL-GM (cut-off ≥1.0 ODI) and BAL fungal culture for the diagnosis of probable IPA	37 BAL from patients at risk of IPA [HM, 27; SOT, 10 (LT, 5)] IPA 21 patients: probable (12), possible (9) Indication for BAL testing: clinical suspicion of IPA	BAL-LFD: SS 100%; SP 81%; PPV 71%; NPV 100% Cross-reaction: *Penicillium* sp (1) False-negative BAL-LFD results: none False-negative BAL-GM results: five (all five patients met criteria of possible IPA, had positive BAL-LFD and were on anti-mould prophylaxis)	BAL *Aspergillus* GM and fungal culture were used as mycological criterion for the diagnosis of IPA
Hoenigl *et al.* [12]	Evaluation of BAL-GM, BAL fungal culture, BAL-BDG, BAL-LFD and BAL *Aspergillus* PCR assay for the diagnosis of IPA	67 BAL from patients at risk for IPA (HM, 43; SOT, 4; CLD, 12; ICU, 4; AIDS, 2; other, 2) IPA 26 patients: proven (3), probable (7), possible (16) Indication for BAL testing: clinical suspicion of IPA	51 of 67 had all 5 test results available BAL-GM ODI ≥0.5: SS 80%; SP 98%; PPV 89%; NPV 95% BAL-GM ODI ≥1.0: SS 70%; SP 98%; PPV 88%; NPV 93%; BAL fungal culture: SS 50%; SP 95%; PPV 71%; NPV 89% BAL-BDG: SS 80%; SP 76%; PPV 44%; NPV 94% BAL *Aspergillus* PCR: SS 70%; SP 100%; PPV 100%; NPV 93% BAL-LFD: SS 80%; SP 95%; PPV 80%; NPV 95%	Cases of possible IPA were excluded from the analysis. Cross-reactivity, false positive and false-negative test results were not discussed
Willinger *et al.* [13]	Evaluation of BAL-LFD vs. BAL-GM (cut-off >1.0 ODI) and BAL culture for the diagnosis of proven or probable IPA.	47 BAL from SOT patients (LT, 26). IPA 22 patients: probable or proven (11) and possible (11) Indication for BAL testing: clinical suspicion of IPA	BAL-LFD: SS 91%; SP 83%; PPV 63%; NPV 97% False-positive results (6/25): Cross-reactivity with *Penicillium* sp (1), LT patient with *Aspergillus* sp cultured from BAL (3), unknown (2) False-negative results (1/22)	BAL-GM and fungal culture were used as mycological criterion for the diagnosis of IPA. Cases of possible IPA were excluded
Miceli *et al.* (this study)	Evaluation of BAL-GM (cut-off ≥0.5 and ≥1.0) vs. BAL-LFD the diagnosis of proven, probable, or possible IPA in patients at risk.	96 BAL from patients at risk of IPA {HM, 7; non-HM, 89 [SOT 57, (LT: 46; non-LT SOT: 11), CSU 21, STU 6, other 5]} IPA 3 patients (probable 2, possible 1) Indication for BAL testing: medical necessity including clinical suspicion of IPA	BAL-LFD: SS 66%; SP 94% BAL-GM ODI >0.5: SS 66%; SP 52% BAL-GM ODI ≥1.0: SS 66%; SP 74% False-positive results BAL-LFD: 5; cross-reaction: none False-negative results BAL-LFD: 1 False-positive BAL-GM ≥0.5: 44 (LT, 26 patients)	Serum and BAL *Aspergillus* GM were not used as mycological criterion for the diagnosis of IPA. Analysis was limited due to small number of patients with IPA

AIDS, acquired immune deficiency syndrome; BAL, bronchoalveolar lavage; BDG, beta-D-glucan; CLD, chronic lung disease; CSU, chronic steroid use; GM, galactomannan; HM, haematological malignancy and peripheral stem cell transplant recipients; ICU, intensive care unit patients; IPA, invasive pulmonary aspergillosis; LFD, lateral flow device; LT, lung transplant recipients; NPV, negative predictive value; ODI, optic density index; PPV, positive predictive value; SOT, solid organ transplant recipients; SP, specificity; SS, sensitivity; STU, solid tumour.

the diagnostic performance of both methods (BAL-LFD vs. BAL-GM).

Finally, retrospective BAL-GM testing should be mentioned as a potential limitation of this study. However, a recent study comparing prospective real-time vs. retrospective BAL-GM testing showed minimal change in the BAL-GM OD index when BAL specimens were stored at −20 °C for up to 11 months.[25]

In summary, our data demonstrate that BAL-LFD is a promising tool for the diagnosis of IPA. *Aspergillus* LFD is a rapid, simple, potential point-of-care and inexpensive test performed on BAL that could help to

rule out IPA in high-risk population, particularly LT recipients. More studies are needed to further determine the role of BAL-LFD in patients with suspected diagnosis of IPA.

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Conflict of interest

No funding was obtained for this study. The *Aspergillus* LFD tests used in this study were provided by Dr. Christopher Thornton, University of Exeter. Dr. Thornton is the inventor of the *Aspergillus* LFD test. All other authors declared no conflict of interest.

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