Journal of Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Bronchoalveolar lavage *Aspergillus* Galactomannan lateral flow assay versus *Aspergillus*-specific lateral flow device test for diagnosis of invasive pulmonary Aspergillosis in patients with hematological malignancies

Dear Editor,

We read with interest the paper by Heldt and colleagues[1] who report on the benefit of combining multiple biomarkers and tests including galactomannan (GM) ELISA assay from bronchoalveolar lavage fluid (BALF) as well as the BALF *Aspergillus* specific Lateral Flow Device Test (LFD) for the diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies.

Importantly, early diagnosis and treatment of IPA remains the most important factor to reduce mortality and improve outcome.[2–4] As an important limitation, GM testing is limited in particular by varying turnaround times (up to 3 days and more in some centers), dependent on the number of specimens to be tested and the distance/duration of transport between the clinical setting and the laboratory where the test is performed.[5] This limitation has in part been overcome by the newly formatted and European conformity (CE)-marked *Aspergillus* specific LFD, which detects an extracellular mannoprotein antigen secreted exclusively during active growth of *Aspergillus* species via the JF 5 monoclonal antibody.[6–9] This test has been shown to increase performance for the diagnosis of IPA when used in combination with the galactomannan assay.[6–9] Very recently a second point-of-care test for IPA, which detects galactomannan, was developed and CE-marked (*Aspergillus* Galactomannan LFA). This test may overcome the limitation of long turnaround time by conventional galactomannan ELISA testing and may further facilitate point-of-care diagnosis of IPA. The objective of this study was to evaluate the new *Aspergillus* Galactomannan Lateral Flow Assay (LFA) and compare its performance against the *Aspergillus* specific LFD and other biomarkers for the diagnosis of IPA in patients with hematological malignancies.

A total of 24 BALF samples obtained from 24 patients with underlying hematological malignancies (1 patient with proven IPA, 8 patients with probable IPA, 5 patients with possible IPA, two patients meeting mycological and host criteria for IPA but without typical radiological signs [as defined by the revised European Organization for Research and Treatment of Cancer / Mycoses Study Group (EORTC/MSG) definitions], and eight patients not fulfilling IPA criteria) were included in this analysis. IPA was classified according to the revised EORTC/MSG criteria with one modification: exclusion of beta-D-Glucan as mycological criterion.[10] BALF samples were obtained between September 2016 and September 2018 at the University of California San Diego, United States. GM (Platelia Aspergillus Ag ELISA; Bio-Rad Laboratories, Munich, Germany) and culture were performed prospectively in all BALF samples. Randomly selected GM positive and negative samples were thereafter stored at −20 °C and tested between August and September 2018 for the *Aspergillus*-specific LFD (OLM Diagnostics, Newcastle upon Tyne, UK), and the *Aspergillus* Galactomannan LFA (IMMY, Norman, Oklahoma, USA). Stored BALF samples where thawed, vortexed, and tested according to the manufacturer's instructions. For the *Aspergillus*-specific LFD, clear BALF was centrifuged only, while not clear BALF was pretreated according to the manufacturer's instructions, and 70 μL of supernatant was added to the test. Results were read 15 and 25 min later and scored as either -, +, ++, or +++. For the *Aspergillus* Galactomannan LFA, BALF samples were pretreated, heated, and centrifuged. Test strips were then inserted into 80 μL of sample following the manufacturer's instructions. Results were read after 30 min and scores given ranging from 0 (i.e. negative), to 4 (highly positive). Results of both the LFD and LFA were each read by two interpreters who were blinded to IPA status, GM ELISA, and culture results.

Statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA). A two-sided P-value of less than 0.05 was considered statistically significant. The Human Research Protections Program at the University of California, San Diego approved the study protocol and all study-related procedures.

A total of 24 samples from 24 patients were included in the analysis. Demographic characteristics and underlying diseases of the study population are displayed in Table 1. A total of 10/24 (42%) of patients were receiving mold-active antifungal prophylaxis/therapy at the time of the BALF procedure. Performance of the *Aspergillus*-specific LFD, *Aspergillus* Galactomannan LFA, BALF culture, Galactomannan ELISA, as well as combinations of the assays are depicted in Table 2.

Both the *Aspergillus*-specific LFD and the *Aspergillus* Galactomannan LFA showed high sensitivities and specificities for probable/proven IPA versus no IPA, with sensitivities of close to 90% for the *Aspergillus*-specific LFD read after 25 min (LFD 25 min) and the *Aspergillus* Galactomannan LFA, and a specificity of 100% of the *Aspergillus*-specific LFD read after 15 min (LFD 15 min) (Table 1). Sensitivity reached 100% when either the *Aspergillus*-specific LFD (25 min) or the *Aspergillus* Galactomannan LFA resulted positive, with a specificity of 75% when the assays were used in combination. The *Aspergillus*-specific LFD and the *Aspergillus* Galactomannan LFA also gave a positive result in a proportion of the possible cases (e.g. in one of the possible cases both tests gave strong positive results, ++ and 2, respectively) and the cases who fulfilled host criteria, had a positive BALF GM (4.77 and 1.51 ODI respectively), but did not have typical radiological criteria on chest CT (tests results were +++ and 2 in the case with the higher BALF GM level). This indicates that these POC tests may be useful in differentiating between those possible IPA cases.

The strength of the result also provided some information for both the LFD and LFA. The single false positive test results for the LFD and the LFA were only low level positive (i.e., + and 1,

https://doi.org/10.1016/j.jinf.2018.10.014

Table 1
Demographic data and underlying diseases of the study population.

	Probable or proven IPA ($n=9$)	No evidence for IPA ($n=8$)	Possible IPA ($n=5$)	Mycological and host factors for IPA but no typical radiological signs ($n=2$)
Female (n, %)	3 (33%)	5 (63%)	2 (40%)	0 (0%)
Age, years (median, range)	70 (24–78)	56 (32–75)	49 (20–62)	34 (21–46)
Underlying diseases (n, %)				
Acute myeloid leukemia	2 (22%)	3 (38%)	–	–
Multiple myeloma	2 (22%)	1 (13%)	1 (20%)	–
Acute lymphocytic leukemia	1 (11%)	2 (25%)	2 (40%)	1 (50%)
Myelofibrosis	1 (11%)	–	–	–
Non-hodgkin lymphoma	1 (11%)	2 (25%)	1 (20%)	1 (50%)
Melodysplastic syndrome	2 (22%)	–	1 (20%)	–
Allogeneic Stem Cell Transplantation	4 (44%)	2 (25%)	1 (20%)	1 (50%)
Autologous Stem Cell Transplantation	2 (22%)	1 (13%)	1 (20%)	1 (50%)

Table 2
Performance of the *Aspergillus*-specific Lateral Flow Device Test (LFD), the *Aspergillus* Galactomannan Lateral Flow Assay (LFA), Galactomannan (GM), and fungal culture in bronchoalveolar lavage (BALF) for diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies. Sensitivity and specificity for probable/proven IPA versus no IPA, as well as test positivity in cases of possible IPA and those who fulfilled mycological and host criteria of IPA but not clinical criteria are displayed.

Biomarkers/tests and combinations	Probable/proven IPA ($n=9$) versus no IPA ($n=8$)		Test positivity in cases with possible IPA ($n=5$)	Test positivity in cases with mycological and host factors for IPA but no typical radiological signs ($n=2$)
	Sensitivity	Specificity		
Aspergillus-specific LFD 15 Min	78% (7/9)	100% (8/8)	40%	100%
Aspergillus-specific LFD 25 Min	89% (8/9)	88% (7/8)	40%	100%
Aspergillus Galactomannan LFA 30 Min	89% (8/9)	88% (7/8)	20%	50%
BAL GM 0.5 ODI cut-off	89% (8/9)	100% (8/8)	0%	100%
BAL GM 1.0 ODI cut-off	78% (7/9)	100% (8/8)	0%	100%
BAL culture	11% (1/9)	100% (8/8)	0%	0%
Aspergillus-specific LFD 15 MinAND/OR *Aspergillus* Galactomannan LFA 30 Min	89% (8/9)	88% (7/8)	40%	100%
Aspergillus-specific LFD 25 Min AND/OR *Aspergillus* Galactomannan LFA 30 Min	100% (9/9)	75% (6/8)	40%	100%

respectively). BALF GM levels were significantly higher in those with at least a ++ positive test result ($n=7$) versus + positive test results ($n=4$) with the *Aspergillus*-specific LFD 15 min (median 4.77 ODI versus median 1.05 ODI; $p=0.042$). The same was true for the *Aspergillus*-specific LFD 25 min (median 3.62 ODI in the 8 cases with a ++ or stronger positive test result versus median < 0.5 ODI in the 5 cases with a + test result; $p=0.019$). In contrast there was no significant difference BALF GM levels when comparing scores of 1 versus 2 or higher for the *Aspergillus* Galactomannan LFA (median 2.46 ODI versus median 1.37 ODI; $p=0.8$).

Given the importance of early and reliable diagnosis of IPA for targeted and successful treatment, rapid tests allowing for point-of-care diagnosis of IPA are an extremely attractive tool. Here we evaluated for the first time a newly CE-marked *Aspergillus* Galactomannan LFA and compared performance to the recently CE-marked *Aspergillus*-specific LFD. We found that both tests were highly sensitive and specific for diagnosing IPA in patients with hematological malignancies. Future studies should investigate the diagnostic performance of both point-of-care tests in larger prospective patient cohorts.

Conflicts of interest

M. Hoenigl received research grants from Gilead and honoraria from the Research Practitioner Network and the Mycoses Study Group. R. Taplitz has served on a Merck Advisory Board. All other authors have nothing to declare.

Funding

Aspergillus-specific LFDs were provided by OLM Diagnostics, Newcastle upon Tyne, UK, while *Aspergillus* Galactomannan LFAs were provided by IMMY, Norman, Oklahoma, USA. Neither company had a role in study design, data collection, analysis, interpretation, decision to publish, in the writing of the manuscript, or in the decision to submit the manuscript for publication.

References

1. Heldt S, Prattes J, Eigl S, Spiess B, Flick H, Rabensteiner J, et al. Diagnosis of invasive Aspergillosis in hematological malignancy patients: performance of cytokines, asp LFD, and Aspergillus PCR in same day blood and Bronchoalveolar lavage samples. *J Infect* 2018;**77**(3):235–41.
2. Shannon VR, Andersson BS, Lei X, Champlin RE, Kontoyiannis DP. Utility of early versus late fiberoptic bronchoscopy in the evaluation of new pulmonary infiltrates following hematopoietic stem cell transplantation. *Bone Marrow Transpl* 2010;**45**(4):647–55.
3. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clin Microbiol Infect* 2018;**24**(1):e1–e38.
4. Hoenigl M, Gangneux JP, Segal E, Alanio A, Chakrabarti A, Chen SC, et al. Global guidelines and initiatives from the European confederation of medical mycology to improve patient care and research worldwide: new leadership is about working together. *Mycoses* 2018;**61**(11):885–94.
5. Chindamporn A, Chakrabarti A, Li R, Sun PL, Tan BH, Chua M, et al. Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: An Asia Fungal Working Group (AFWG) initiative. *Med Mycol* 2018;**56**(4):416–25.
6. Hoenigl M, Eigl S, Heldt S, Duettmann W, Thornton C, Prattes J. Clinical evaluation of the newly formatted lateral-flow device for invasive pulmonary Aspergillosis. *Mycoses* 2018;**61**(1):40–3.
7. Prattes J, Lackner M, Eigl S, Reischies F, Raggam RB, Koidl C, et al. Diagnostic accuracy of the Aspergillus-specific bronchoalveolar lavage lateral-flow assay in haematological malignancy patients. *Mycoses* 2015;**58**(8):461–9.
8. Hoenigl M, Prattes J, Spiess B, Wagner J, Prueller F, Raggam RB, et al. Performance of galactomannan, beta- D-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary Aspergillosis. *J Clin Microbiol* 2014;**52**(6):2039–45.
9. Orasch T, Prattes J, Faserl K, Eigl S, Duttmann W, Lindner H, et al. Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. *J Infect* 2017;**75**(4):370–3.

10. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**(12):1813–21.

Jeffrey D. Jenks*, Sanjay R. Mehta, Randy Taplitz, Nancy Law
Department of Medicine, University of California San Diego, San Diego, CA, United States

Sharon L. Reed
Department of Medicine, University of California San Diego, San Diego, CA, United States
Department of Pathology, University of California San Diego, San Diego, CA, United States

Martin Hoenigl**
Department of Medicine, University of California San Diego, San Diego, CA, United States
Department of Medicine, Medical University of Graz, Graz, Austria

*Corresponding author. Present address: Division of General Internal Medicine, Department of Medicine, University of California San Diego, San Diego, CA 92103, USA.
**Corresponding author. Present address: Division of Infectious Diseases, Department of Medicine, University of California San Diego, San Diego, CA 92103, USA.
E-mail addresses: jjenks@ucsd.edu (J.D. Jenks), mhoenigl@ucsd.edu (M. Hoenigl)

Accepted 15 October 2018