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Malgorzata Mikulska,[1,2] Elisa Furfaro,[1] and Matteo Bassetti[1,2]

[1]Division of Infectious Diseases, Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy; and [2]Division of Infectious Diseases, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

References

1. Jenks JD, Prattes J, Frank J, et al. Performance of the bronchoalveolar lavage fluid *Aspergillus* galactomannan lateral flow assay with cube reader for diagnosis of invasive pulmonary aspergillosis: a multicenter cohort study. Clin Infect Dis **2021**; 73:e1737–44.
2. Prattes J, Lackner M, Eigl S, et al. Diagnostic accuracy of the *Aspergillus*-specific bronchoalveolar lavage lateral-flow assay in haematological malignancy patients. Mycoses **2015**; 58:461–9.
3. Hoenigl M, Prattes J, Spiess B, 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**; 52:2039–45.
4. Pan Z, Fu M, Zhang J, Zhou H, Fu Y, Zhou J. Diagnostic accuracy of a novel lateral-flow device in invasive aspergillosis: a meta-analysis. J Med Microbiol **2015**; 64:702–7.
5. Linder KA, Kauffman CA, Zhou S, Miceli MH. Clinical application of *Aspergillus* lateral flow device in bronchoalveolar lavage fluid of patients with classic risk factors for invasive pulmonary aspergillosis. Mycoses **2019**; 62:1189–93.
6. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis **2020**; 71:1367–76.
7. Ullmann AJ, Aguado JM, Arikan-Akdagli S, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clinical Microbiol Infect **2018**; 24(Suppl 1):e1–38.
8. Blot SI, Taccone FS, Van den Abeele AM, et al.; AspICU Study Investigators. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med **2012**; 186:56–64.

Correspondence: Malgorzata Mikulska, Department of Health Sciences (DISSAL)- University of Genoa, Infectious Diseases Division, Ospedale Policlinico San Martino, L.go R. Benzi, 10 – 16132 Genoa, Italy (m.mikulska@unige.it).

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Reply to Mikulska et al

To the Editor—We thank Mikulska, Furfaro, and Bassetti for their letter and interest in our manuscript evaluating the *Aspergillus* Galactomannan (GM) Lateral Flow Assay (LFA) with automated digital reader from bronchoalveolar lavage fluid (BALF) for the diagnosis of invasive aspergillosis (IA) [1]. We and others have previously evaluated the *Aspergillus* GM LFA with manual readout from BALF fluid in patients both with [2] and without [3, 4] hematologic malignancy. According to the manufacturer's (IMMY, Norman, OK, USA) instructions for running and interpreting the *Aspergillus* LFA with manual readout, results are interpreted as either positive (based on the presence of both the test and control line) or negative (based on the presence of only the control line), regardless of the intensity of the test line, although further instruction available from the testing kits provides guidance on grading the intensity of the test line from 1+ to 4+.

As Mikulska et al note, a visual readout positive result from the *Aspergillus* GM LFA correlates with an optical density index (ODI) of more than 0.5. Using digital readout, they provide new data in a patient cohort showing a false-positive rate of 33% in BALF samples when an *Aspergillus* LFA ODI cutoff of greater than 0.5 is used, while false positivity decreased to 10.5% with a cutoff of more than 1.0 ODI, with sensitivities for diagnosing IA remaining unchanged [1]. Our study found an even larger effect on specificity when testing BALF samples, which increased from 44% to 73% once the LFA ODI cutoff was elevated from more than 0.5 ODI to more than 1.0 ODI [5]. While our study found a small decrease in sensitivity for diagnosing IA (89% with 0.5 ODI vs 82% with 1.0 ODI), the false-positivity rate of LFA results with ODIs between 0.5 and 1.0 was 88.5% (46/52), clearly indicating that, in fact, BALF LFA results between an ODI of 0.5 and 1.0 are most likely false positives. However, interpretation of these values in the "gray zone" will likely depend on patients' characteristics, presence or absence of antifungal agents, and status of IA.

Importantly, we have previously found that there was no significant difference between GM enzyme-linked immunosorbent assay (ELISA) ODIs and the intensity of the *Aspergillus* LFA test line of 2+ or higher versus 1+ with manual readout (median ODI of 2.46 vs median ODI of 1.37, $P = .80$) when evaluating BALF from hematology patients and a median ODI of 5.62 versus 1.14 ODI in other patient groups ($P = .068$) [3, 6]. Simply increasing the visual readout cutoff from 1+ to 2+ may therefore not solve the issue and, as Mikulska et al state, obtaining precise quantitative LFA ODIs using digital readout may be mandatory for testing BALF specimens.

While BALF LFA testing is preferable for the diagnosis of IA, BALF cannot always be obtained safely, as shown recently for patients with coronavirus disease 2019–associated pulmonary aspergillosis, where fears of aerosol transmission may limit the use of bronchoscopies in some centers [7, 8]. In such settings, GM testing of serum samples may be preferred to BALF, and the *Aspergillus* GM LFA has shown very promising results from serum samples, with an overall sensitivity of 70% and specificity of 96% in patients with underlying hematological malignancies [4, 9, 10]. Evaluation of the *Aspergillus* GM LFA with visual and digital readout from serum samples of other patient groups without hematological malignancy is currently lacking.

In conclusion, we agree with Mikulska et al that, in *Aspergillus* GM LFA testing from BALF, increasing the ODI cutoff to 1.0 or even 1.5 ODI is preferable and, for implementation of these higher

cutoffs, digital quantitative ODI readout is likely inevitable. These higher cutoffs offer improved specificity compared with an ODI cutoff of 0.5 for diagnosing IA, without significantly sacrificing sensitivity. Future studies are needed to evaluate whether the same is also true when testing serum specimens with the *Aspergillus* GM LFA.

Notes

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Jeffrey D. Jenks,[1,2,3] Juergen Prattes,[4] Dieter Buchheidt,[5] and Martin Hoenigl[2,3,4]

[1]Division of General Internal Medicine, University of California, San Diego, San Diego, California, USA, [2]Division of Infectious Diseases and Global Public Health, University of California, San Diego, San Diego, California, USA, [3]Clinical and Translational Fungal-Working Group, University of California, San Diego, La Jolla, California, USA, [4]Section of Infectious Diseases and Tropical Medicine and Division of Pulmonology, Medical University of Graz, Graz, Austria, and [5]Department of Hematology and Oncology, Mannheim University Hospital, Heidelberg University, Mannheim, Germany

References

1. Mikulska MFE, Furfaro E, Bassetti M. The importance of quantitative results of lateral flow Aspergillus assay–the significant impact on the specificity. Clin Infect Dis **2021;** 73:e1783–4.
2. Mercier T, Dunbar A, de Kort E, et al. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: a comparative multicenter study. Med Mycol **2020**; 58:444-52.
3. Jenks JD, Mehta SR, Taplitz R, Aslam S, Reed SL, Hoenigl M. Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: Aspergillus Galactomannan Lateral Flow Assay versus Aspergillus-specific Lateral Flow Device test in bronchoalveolar lavage. Mycoses **2019**; 62:230–6.
4. Jenks JD, Hoenigl M. Point-of-care diagnostics for invasive aspergillosis: nearing the finish line. Expert Rev Mol Diagn **2020**; 20:1009–17.
5. Jenks JD, Prattes J, Frank J, et al. Performance of the bronchoalveolar lavage fluid aspergillus galactomannan lateral flow assay with cube reader for diagnosis of invasive pulmonary aspergillosis: a multicenter cohort study. Clin Infect Dis **2021**; 73:e1737–44.
6. Jenks JD, Mehta SR, Taplitz R, Law N, Reed SL, Hoenigl M. 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. J Infect **2019**; 78:249–59.
7. Hoenigl M. Invasive fungal disease complicating COVID-19: when it rains it pours. Clin Infect Dis **2020**; 73:e1645–8.
8. Arastehfar A, Carvalho A, van de Veerdonk FL, et al. COVID-19 associated pulmonary aspergillosis (CAPA)—from immunology to treatment. J Fungi (Basel) **2020**; 6:91. doi:10.3390/jof6020091
9. Mercier T, Guldentops E, Lagrou K, Maertens J. Prospective evaluation of the turbidimetric β-D-glucan assay and two lateral flow assays on serum in invasive aspergillosis. Clin Infect Dis **2020**; 72:1577–84.
10. White PL, Price JS, Posso R, Cutlan-Vaughan M, Vale L, Backx M. Evaluation of the performance of the IMMY Sona Aspergillus Galactomannan Lateral Flow Assay when testing serum to aid in diagnosis of invasive aspergillosis. J Clin Microbiol **2020**; 58:e00053-20.

Correspondence: M. Hoenigl, Division of Infectious Diseases, Department of Medicine, 200 West Arbor Drive #8208, San Diego, CA 92103 (mhoenigl@ucsd.edu).

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Trends in the Incidence of Reported Adverse Events After Changing the Bacillus Calmette-Guérin Vaccination Age in Japan

To the Editor—I read with great interest the article by Wei et al regarding inoculation age and BCG vaccine–related adverse events in Taiwan [1]. With the revision of Japan's Immunization Act in April 2013, the upper age limit for bacillus Calmette-Guérin (BCG) vaccination was changed from 6 months to 1 year and, as postponed BCG inoculation age from 24 hours after birth to 5–8 months of age in Taiwan from 2016, the government recommended administration at age 5–8 months.

Data from the National Council on Vaccine Safety [2], which periodically reviews reported adverse events, show that from April 2013, when the amended act required full reporting of adverse events following routine vaccination, an estimated 6 723 862 doses of BCG vaccine had been administered by the end of February 2020. In that period, 29 cases of osteitis/osteomyelitis were reported as adverse events by healthcare providers and 36 cases were reported by vaccine manufacturers. In the absence of any overlap between the 2 sources, the maximum number of reported cases stands at 65, giving an incidence of BCG-related osteitis/osteomyelitis of 9.7 cases per million doses. Koyama et al [3] reported a lower incidence of BCG-related osteitis at 2 cases per million between 1998 and 2007 in Japan. However, they might have underestimated the incidence rate because the reporting of all adverse events was not mandated before 2013, and the rate was calculated based on a literature search of case reports. Thus, it is not straightforward to determine the impact of Japan's change in the BCG vaccination age.

To try to determine this, I used the following approach. Given that Chiu et al [4] reported an average time between BCG vaccination and osteomyelitis onset of 12.4 ± 6.1 months, I defined the end of February 2014 (the closest assessment point to 12 months after the system changed in April 2013) as the period during which BCG-induced osteomyelitis was most likely to be reported as an adverse event in infants vaccinated with BCG vaccine at an earlier age. I then compared the frequency of adverse events reported before and after this time point (Table 1). In the 11-month period from April 2013 to February 2014 [5], 13 cases of osteitis/osteomyelitis were reported, giving an incidence of 16.3 cases per million. In the subsequent period, 52 cases were reported, indicating a significantly lower frequency of 8.8 cases per million. Considering that there were no significant reductions in other reported adverse reactions subject to comprehensive reporting and even accounting for some probable early omissions in reporting after the system changed when the total reporting requirement was not yet fully implemented, the data suggest