Prospective evaluation of the turbidimetric β -D-glucan assay and two lateral flow assays on serum in invasive aspergillosis

Toine Mercier^{1,2}, Ellen Guldentops², Katrien Lagrou^{1,3}, Johan Maertens^{1,2*}

¹ KU Leuven, Department of Microbiology, Immunology and Transplantation, Leuven, Belgium

² University Hospitals Leuven, Department of Hematology, Leuven, Belgium

³ University Hospitals Leuven, Department of Laboratory Medicine and National Reference Centre for Mycosis, Leuven, Belgium

* Corresponding author:

Johan Maertens, Herestraat 49, 3000 Leuven, Belgium

johan.maertens@uzleuven.be

* Alternate corresponding author:

Toine Mercier, Herestraat 49, 3000 Leuven, Belgium

toine.mercier@uzleuven.be

RUNNING TITLE: Combination serum tests in aspergillosis

SUMMARY: A fast and accurate diagnosis improves the outcome in invasive aspergillosis. Two recently approved lateral flow tests and the Wako beta-D-glucan assay were evaluated on sera. The IMMY assay performed as good as galactomannan detection. Combined beta-D-glucan detection is advantageous.

KEYWORDS: Invasive aspergillosis; diagnosis; lateral flow assay; beta-D-glucan; serum

ABSTRACT

Background: Invasive aspergillosis (IA) remains a potentially lethal disease and requires timely diagnosis and initiation of antifungal therapy. Recently, the IMMY lateral flow assay (LFA), the OLM Diagnostics lateral flow device (LFD) and the Wako turbidimetric beta-D-glucan assay have been approved for use as a diagnostic aid. However, their performance in diagnosing IA on serum samples from at-risk patients and the added value to the existing detection of serum galactomannan remain to be investigated.

Methods: We prospectively collected serum samples from 239 hematology patients and evaluated the diagnostic performance of these three assays while using the 2019 EORTC/MSG definitions.

Results: We identified five cases of proven IA, 36 cases of probable IA and 188 controls. The LFA had the highest negative predictive value (NPV) and sensitivity (0.90 and 0.49, respectively) while galactomannan detection had the highest positive predictive value (PPV) and specificity (0.93 and 0.99, respectively). Sensitivity was not significantly different between both tests. When used in combination, the highest NPV was seen in patients with a negative LFA and a negative beta-D-glucan test. The sensitivity of the LFD was significantly lower than the LFA. After omitting serum galactomannan from the definitions to control for incorporation bias, the sensitivity of the LFA outperformed galactomannan detection (0.41 versus 0.31, $p=0.046$).

Conclusions: The LFA is a fast and effective alternative to serum galactomannan detection for the diagnosis of IA and is especially useful for centers with low sample throughputs. The addition of the Wako beta-D-glucan assay further improves the diagnostic performance.

Background

Invasive aspergillosis (IA) affects over 300,000 immunocompromised patients yearly worldwide[1]. Unfortunately, diagnosis is still often delayed or not made at all in a significant portion of these cases[2,3], highlighting the importance of sensitive diagnostic tools. Furthermore, such tests should ideally have a short turnaround time as early therapy is associated with improved survival[4]. However, classical microbiological tests such as culture or microscopy suffer from a low sensitivity [5]. In recent years, more sensitive tests based on detection of galactomannan (GM) and *Aspergillus*-specific DNA have been developed. Although these latter assays have higher sensitivities, especially in bronchoalveolar lavage fluid (BALf) [6,7], it is not always feasible to perform bronchoscopy in at-risk patients due to hypoxia or thrombocytopenia. In addition, these tests are not always available locally and may need to be sent off-site, or have to be batched to remain cost-effective, thereby further increasing the diagnostic delay, potentially leading to increased mortality[8].

Recently, an *Aspergillus*-specific lateral flow device (LFD, OLM Diagnostics, Newcastle Upon Tyne, United Kingdom) and lateral flow assay (LFA, IMMY, Norman, OK, USA) that could potentially alleviate these issues became commercially available. Recent studies with these assays have reported good sensitivities on BALf[9,10]. However, the performance of the LFD on sera was only reported by two smaller studies using a prototype version of the LFD[11,12]. To date, no studies have evaluated the diagnostic properties of the reformatted version of the LFD or of the LFA on serum samples. In addition, a new assay for the detection of the fungal cell wall component (1→3)- β -D-glucan (BDG, FUJIFILM Wako Chemicals, Osaka, Japan) in serum was recently commercialized in Europe. This turbidimetric assay overcomes some of the limitations of the more widely used colorimetric Fungitell assay (Associates of Cape Cod): the Wako assay is not influenced by lipemia, icterus or hemolysis and can be run as a random-access test. This decreases the turnaround time and diagnostic delay and may reduce costs in settings with low sample numbers. All three assays have recently obtained a Conformité Européenne (CE) marking, allowing their use in patient care in Europe.

We performed a prospective study to evaluate the performance and added value of these novel assays for diagnosing IA using serum samples from at-risk hematology patients.

Methods

Study design: This study was performed between January 2017 and June 2019 at the Belgian National Reference Center for Mycosis (University Hospitals Leuven, Leuven, Belgium). We prospectively screened consecutive adult patients (≥ 18 years of age) with underlying hematological disorders at risk for developing IA. This study was approved by the Ethics Committee Research UZ/KU Leuven (S59863/S61797, NCT03004092). All subjects provided written informed consent before any study-specific procedure.

The prospectively screened study population included patients receiving (1) intensive chemotherapy for acute leukemia or high-risk myelodysplastic syndromes, (2) allogeneic cell transplant recipients (up to 1 year after transplantation) and (3) patients with severe aplastic anemia receiving ATG-based immunotherapy. We also included (4) patients who were undergoing autologous stem cell transplantation, as these individuals typically also develop severe mucositis and receive antibacterial therapy for neutropenic fever, though with a significantly lower risk of IA compared to the previous patient groups. We also allowed (5) hematology patients who developed proven or probable IA outside of the screening program (e.g. acute myeloid leukemia patients receiving hypomethylating agents) to be enrolled from the time of fungal diagnosis onward. Patients were excluded from the study if they were receiving treatment for a previous episode of fungal disease or if they had completed antifungal therapy less than 6 weeks before the time of enrollment.

All participants in the screening group (groups 1-4) were treated according to a standardized departmental protocol. All patients received fluconazole (400 mg per day) with daily screening for serum galactomannan. After 98-120 h of fever unresponsive to broad-spectrum antibiotics or clinical signs or symptoms suggestive of pulmonary disease (e.g. dry cough, pleuritic chest pain or hemoptysis) or serum GM detection (two consecutive GM indices of ≥ 0.5 or one GM index ≥ 0.8), a

CT scan of the chest was performed. In case of any abnormality, bronchoscopy and collection of BALf was performed for extensive microbiological (including GM detection by the Platelia™ assay [Bio-Rad, Marnes-la-Coquette, France] using a GM index threshold of ≥ 1.0) and microscopic analysis.

Patients were classified independently by two physicians as having proven IA, probable IA, or possible invasive fungal disease as per the 2019 European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group (EORTC)/Mycoses Study Group of the National Institute of Allergy and Infectious Diseases (MSG) consensus definitions[13]. To overcome incorporation bias, we also classified patients while excluding serum GM as a mycological criterion (i.e. only based on a positive respiratory culture or a positive BALf GM) in a secondary analysis.

After inclusion, we prospectively collected serum samples twice weekly whenever the patient was hospitalized or during outpatient visits. After antifungal therapy was initiated for IA, serum sampling continued twice weekly up until 6 weeks after diagnosis. Patients that received mould-active antifungal therapy for clinically suspected IA without fulfilling the 2019 EORTC/MSG definitions (“unclassifiable”) were excluded from further analysis.

Only patients with proven or probable IA were considered as ‘cases’. The serum sample taken at diagnosis before the start of anti-*Aspergillus* therapy was used for analysis of the diagnostic performance of the three tests. Patients who were suspected of having invasive fungal disease because of fever or because they underwent a diagnostic work-up, but never received anti-*Aspergillus* therapy due to lack of supportive evidence were considered as ‘controls’. The diagnostic performance of the different tests was assessed using the serum sample that was drawn on the day that the patient had one of the following clinical events (in descending order of likelihood for IFD): on the day of bronchoscopy, day of high-resolution CT scanning of the chest, or on the day that the patient developed a fever of $>38.2^{\circ}\text{C}$. Patients not having one of these events were excluded from further analysis.

Lateral flow tests: Both assays were performed in accordance with the manufacturer's instructions. Briefly, for the LFD (AspLFD, OLM Diagnostics, Newcastle Upon Tyne, United Kingdom), 150 µL of serum was pretreated by addition of 300 µL of EDTA-containing buffer, heating at 100°C for 3 minutes, and centrifugation. Seventy µL of the resulting supernatant was then pipetted on the sample port of the LFD. Results were recorded after 15 minutes and 30 minutes.

For the LFA (sōna *Aspergillus* galactomannan LFA, IMMY, Norman, Oklahoma, United States of America), 300 µL of serum was pretreated by addition of 100 µL of EDTA-containing buffer, heating at 120°C for 6 – 8 minutes, and centrifugation. Eighty µL of the resulting supernatant was transferred to a separate test tube, to which 40 µL of running buffer was added. A test strip was then inserted into this tube. Results were recorded after 30 minutes.

All tests were performed by the same researcher (TM) and were read using a digital reader (Cube reader, Chembio Diagnostics GmbH, Berlin, Germany) using lot-specific reader settings as provided by the manufacturer. For the LFD, a slightly modified readout protocol was used which also provided a numerical result; in the commercial version, only a textual result (negative, very low positive, or positive) is provided by the reader. No other parameters were modified. The optical intensity of each lateral flow test is provided as a unitless number based on the manufacturer's protocol. The scale of this number is different for the LFA and the LFD. A positive test results was defined as the presence of a visual line at the test site, or as a qualitative positive result according to the Cube reader using the manufacturer's protocol.

Beta-D-glucan: BDG was measured in serum using the Wako β-Glucan Test (FUJIFILM Wako Chemicals, Osaka, Japan) in accordance with the manufacturer's instructions. Briefly, 100 µL of serum was pretreated by addition of 900 µL of pretreatment buffer, heating to 70°C for 10 minutes, and cooling on ice. Next, 200 µL of pretreated sample was added to a vial containing freeze-dried Limulus Amebocyte lysate. The turbidity of the resulting mix was then continuously measured over 90 minutes using a dedicated MT-6500 Toxinometer (FUJIFILM Wako Chemicals, Osaka, Japan). A lot-

specific preset calibration curve provided by the manufacturer is then used to calculate the concentration of BDG in the sample based on the speed of increase in turbidity of the sample.

Statistical analysis: Diagnostic performance parameters (sensitivity, specificity, positive and negative predictive values) were calculated for all evaluable patients, including their 95% confidence intervals (CI's). Sensitivity and specificity of both assays were compared using McNemar's chi-square test; positive predictive value (PPV) and negative predictive value (NPV) using the weighted generalized score statistic; and area under the curve (AUC) using DeLong's test for two correlated receiver operating characteristic (ROC) curves, as all these comparisons are pairwise observations. A 2-sided p-value of ≤ 0.05 was considered statistically significant. In case a specific assay was positive at diagnosis, we evaluated if decline in intensity after 1 week and 2 weeks was predictive of survival at week 6 using Cox regression. All statistical tests were calculated using R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

The flow of patients throughout the study is shown in Figure 1 and a summary of patient characteristics at enrollment is shown in Table 1. A detailed overview of all cases of proven and probable IA is shown in Table 2. In cases of IA (n=41), the median serum GM was 0.3 (interquartile range [IQR] 0.1 - 1.05) and the median BALf GM was 4.4 (IQR 2.15 - 5.4) at the time of diagnosis (Figure 2). The median serum GM value at diagnosis in the high-risk screening group (groups 1 – 4) was 0.3 versus 0.4 ($p=0.679$) in patients that were included upon diagnosis (group 5).

Test results

Serum GM and the LFA had the best overall performance as estimated by the AUC (0.83 and 0.82 respectively, $p=0.738$). The AUC of the LFD (0.63, $p=0.003$) and of BDG (0.69, $p=0.003$) was significantly lower than that of serum GM. Based on ROC curve analysis, we found any positive BDG

above the limit of quantification (2.359 pg/mL) to have the best overall performance as indicated by Youden's index.

Used alone, GM had the highest specificity and the LFA the highest sensitivity (Table 3). However, the difference in sensitivity between the LFA and serum GM was not statistically significant (0.49 versus 0.44, $p=0.180$), whereas the difference in specificity was significant (0.95 versus 0.99, $p=0.014$). The LFD performed significantly worse than the LFA on all parameters ($p=0.012$, 0.034, 0.004 and 0.001 for sensitivity, specificity, NPV and PPV respectively). The rate of conversion from a negative LFD readout at 15 minutes to a positive readout at 30 minutes (as recently recommended by the manufacturer) was similar in cases and in controls (15% conversion versus 9%, $p=0.244$). The diagnostic performance of the LFD, LFA and serum GM when excluding serum GM as a mycological criterion ($n=34$) is shown in Table 4. In this sub-analysis, the LFA showed a significantly higher sensitivity than serum GM or the LFD ($p=0.046$ and 0.021, respectively). Conversely, the specificity of serum GM was significantly higher than the LFA or the LFD ($p=0.014$ and <0.001 , respectively).

By combining different tests, the diagnostic performance could be improved depending on the specific combination used. The highest sensitivity and NPV was seen in patients with a positive LFA or $\text{BDG} \geq 2.359 \text{ pg/mL}$. However, this combination leads to a lower specificity of 0.86, second to last only to the combination of a positive LFD or $\text{BDG} \geq 2.359 \text{ pg/mL}$ (0.83, $p=0.18$). The highest PPV was obtained in patients that had a serum GM ≥ 0.5 , or both a positive LFA and BDG, or both a positive GM and BDG. The highest NPV was obtained in patients that had a negative BDG combined with either a negative LFA or GM.

We found a significant though not perfect correlation between serum GM values and LFA intensity (adjusted R^2 0.446, $p<0.001$), and serum GM values and LFD intensity (adjusted R^2 0.457, $p<0.001$), although the LFD showed a large variability in GM negative samples (Figure 3).

Of the IA patients that were LFA positive at diagnosis with a prior sample available (the screening group), 12 / 14 (86%) were already previously positive (median time between diagnostic

and previous sample 3 days, IQR 3 – 4 days) with a median increase in intensity of 0.09 ($p=0.490$). For BDG, 7/12 had a prior positive result (58%). For the LFD, only 1 / 10 (10%) had a prior positive result. After initiation of therapy, 17 / 18 patients (94%) still had a positive LFA (median decline of 0.02) after one week and 11 / 8 (61%) after two weeks (median decline 0.17). For BDG, 11 / 17 patients (65%) still had a positive BDG test after 1 week (median decline 5.777 pg/mL), and 9 / 17 (53%) after 2 weeks (median decline 5.893 pg/mL). For the LFD, 3 / 15 (20%) were still positive after one week (median decline 6.4) and remained positive after 2 weeks (median decline 7.4).

The intensity at diagnosis of the LFA or the LFD were significant predictors ($p<0.001$) for survival at week 6 in Cox regression, after controlling for age and neutropenic status. However, the level of BDG was not a significant predictor. In cases of IA that were positive at baseline, the decline in intensity after 1 week was not predictive for survival at week 6 for the LFD ($p=0.277$), BDG ($p=0.640$) or the LFA ($p=0.621$), after controlling for age and neutropenic status. This effect remained non-significant when looking at the decline after 2 weeks of antifungal therapy.

In cases of possible IA, the LFA was positive in 4 / 10 (40%), the LFD in 1 / 10 (10%) and BDG in 0 / 10 (0%). All positive LFA tests had a low optical intensity (median 0.65, IQR 0.6125 - 0.665). The optical intensity of the single positive LFD was 31.4.

Discussion

In this prospective study, we evaluated the performance of two lateral flow tests and a BDG assay for diagnosing IA in serum of hematology patients. When used as a standalone test, the LFA was as sensitive as GM testing by the Platelia™ assay. Performance of the LFD seemed to be suboptimal and overall had the lowest diagnostic characteristics of all assays that were evaluated. The low specificity of the LFD seems to be largely due to a large test variability, which is evident from Figure 3. For the Wako BDG assay, the manufacturer's recommended cutoff of 11 pg/mL appeared to be too high, leading to a low sensitivity. By decreasing the cutoff to 2.359 pg/mL, sensitivity was increased significantly, with only a minor decrease in specificity. A similar effect was seen in two

recent studies on the Wako BDG assay in invasive candidiasis and *Pneumocystis pneumonia*, where a lower threshold resulted in an improved diagnostic performance[14,15].

Previous studies have shown an increase in diagnostic performance when combining tests such as GM, PCR and/or BDG[16–18]. Indeed, in our study, the optimal combination appeared to be BDG combined with either GM or the LFA: patients had the highest probability of having proven or probable IA if both tests were positive; conversely, if both tests were negative, patients had the highest probability of not having IA.

Furthermore, we found the lateral flow optical intensity at diagnosis to be predictive of the mortality, similar to what is seen with GM[19]. However, contrary to GM, we did not find a correlation between decline in lateral flow intensity and survival.

Nowadays, many hematology centers screen their patients at high-risk for IA two or three times a week for circulating GM [20]. As the GM assay uses a 96-well microtiter plate, it is best suited for screening large numbers of patients. The LFA is better suited for situations with low sample throughput as it can be used for single sample testing. Our data suggest that the LFA might replace GM testing as the diagnostic sensitivity was similar and the majority of patients with a positive LFA at diagnosis already had a positive LFA test a median of 3 days prior to the initiation of antifungal therapy. However, this needs to be validated further in a prospective screening study.

The manufacturer of the LFD recently modified the recommendation for reading the results of serum samples, now instructing to perform readout after 30 minutes instead of after 15 minutes. Although this does result in a higher sensitivity, it comes at a cost of a lower specificity. Indeed, we found a similar rate of conversion from a negative test at 15 minutes to a positive result at 30 minutes in cases and controls, which is in line with 2 previous studies in BALf[21,22].

Our results should be interpreted with caution as we use an intensive GM-based screening approach for the high-risk patients or an aggressive diagnostic approach (including high-resolution chest CT, bronchoscopy and BALf GM testing) for the lower risk patients. The inclusion of serum GM

in the screening population could lead to an incorporation bias as GM is used as a microbiological criterion in the consensus definitions. Indeed, when excluding serum GM from the classification, we found the LFA to statistically outperform both the LFD and serum GM. This is an interesting finding given that 40% of the cases of possible IA (based on a suggestive CT scan with a negative GM result) had a (low) positive LFA result. It is unclear if these results represent false-positive results rather than an improved sensitivity over the serum GM assay. Furthermore, we could not investigate the impact of mold-active antifungals on the diagnostic assays in this study as patients received fluconazole prophylaxis. However, it is already known that antifungal treatment significantly decreases the sensitivity of the LFD and LFA in BALf, similar to what is seen with other fungal diagnostic tests[10,22–24]. We have therefore no reason to suspect otherwise for the LFD, LFA or Wako BDG in serum.

Funding

This research was funded by Research Foundation-Flanders [T004517N], and the Michaël Van Waeyenberge Fund & King Baudouin Foundation [J5810650].

Conflict of Interest

T.M. received an unrestricted research grant from Gilead Sciences, served as a consultant for Gilead Sciences, Pfizer and Celgene, and received non-financial support from MSD/Merck, Gilead Sciences, Pfizer, OLM Diagnostics, IMMY and FUJIFILM Wako. K.L. received consultancy fees from MSD, SMB Laboratoires Brussels and Gilead, travel support from Pfizer and MSD and speaker fees from Gilead, MSD, FUJIFILM WAKO and Pfizer. J.M. 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 served on the speaker's bureau of Astellas, Gilead Sciences, Bio-Rad, Merck/MSD, Pfizer, F2G, Schering-Plough, Basilea, Mundipharma and Shire/Takeda. E.G. has no conflicts to report.

Acknowledgments

The authors wish to thank OLM Diagnostics, FUJIFILM Wako Chemicals Europe, and IMMY for providing the test kits used in this study. The manufacturers had no role in the design of this study, its execution, analysis, interpretation of the data, or decision to publish.

References

1. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. *J Fungi (Basel)* **2017**; 3.
2. Chamilos G, Luna M, Lewis RE, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003). *Haematologica* **2006**; 91:986–989.
3. Sharma S, Nadrous HF, Peters SG, et al. Pulmonary Complications in Adult Blood and Marrow Transplant Recipients: Autopsy Findings. *Chest* **2005**; 128:1385–1392.
4. von Eiff M, Roos N, Schulten R, Hesse M, Zühlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration* **1995**; 62:341–347.
5. Lamoth F, Calandra T. Early diagnosis of invasive mould infections and disease. *J Antimicrob Chemother* **2017**; 72:i19–i28.
6. Leeflang MMG, Debets-Ossenkopp YJ, Wang J, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* **2015**; :CD007394.
7. Zou M, Tang L, Zhao S, et al. Systematic Review and Meta-Analysis of Detecting Galactomannan in Bronchoalveolar Lavage Fluid for Diagnosing Invasive Aspergillosis. *PLoS One* **2012**; 7.
8. Schauwvlieghe AFAD, Jonge N de, Dijk K van, et al. The diagnosis and treatment of invasive aspergillosis in Dutch haematology units facing a rapidly increasing prevalence of azole-resistance. A nationwide survey and rationale for the DB-MSG 002 study protocol. *Mycoses* **2018**; 61:656–664.
9. 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. *Journal of Infection* **2019**; 78:249–259.
10. 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* **2019**;
11. White PL, Parr C, Thornton C, Barnes RA. Evaluation of Real-Time PCR, Galactomannan Enzyme-Linked Immunosorbent Assay (ELISA), and a Novel Lateral-Flow Device for Diagnosis of Invasive Aspergillosis. *J Clin Microbiol* **2013**; 51:1510–1516.

- 298 12. Held J, Schmidt T, Thornton CR, Kotter E, Bertz H. Comparison of a novel *Aspergillus* lateral-flow
299 device and the Platelia galactomannan assay for the diagnosis of invasive aspergillosis following
300 haematopoietic stem cell transplantation. *Infection* **2013**; 41:1163–1169.
- 301 13. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of
302 Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer
303 and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* **2019**;
- 304 14. Friedrich R, Rappold E, Bogdan C, Held J. Comparative Analysis of the Wako β -Glucan Test and
305 the Fungitell Assay for Diagnosis of Candidemia and *Pneumocystis jirovecii* Pneumonia. *J Clin*
306 *Microbiol* **2018**; 56.
- 307 15. Mercier T, Guldentops E, Patteet S, Beuselinck K, Lagrou K, Maertens J. Beta-d-Glucan for
308 Diagnosing *Pneumocystis* Pneumonia: a Direct Comparison between the Wako β -Glucan Assay
309 and the Fungitell Assay. *J Clin Microbiol* **2019**; 57.
- 310 16. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β -
311 D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis. *Clin Infect Dis*
312 **2011**; 52:750–770.
- 313 17. Aguado JM, Vázquez L, Fernández-Ruiz M, et al. Serum galactomannan versus a combination of
314 galactomannan and polymerase chain reaction-based *Aspergillus* DNA detection for early
315 therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled
316 trial. *Clin Infect Dis* **2015**; 60:405–414.
- 317 18. Rogers TR, Morton CO, Springer J, et al. Combined real-time PCR and galactomannan surveillance
318 improves diagnosis of invasive aspergillosis in high risk patients with haematological
319 malignancies. *Br J Haematol* **2013**; 161:517–524.
- 320 19. Mercier T, Guldentops E, Lagrou K, Maertens J. Galactomannan, a Surrogate Marker for Outcome
321 in Invasive Aspergillosis: Finally Coming of Age. *Front Microbiol* **2018**; 9:661.
- 322 20. Maertens J, Verhaegen J, Lagrou K, Eldere JV, Boogaerts M. Screening for circulating
323 galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged
324 neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood*
325 **2001**; 97:1604–1610.
- 326 21. Jenks JD, Mehta SR, Taplitz R, Aslam S, Reed SL, Hoenigl M. Point-of-care diagnosis of invasive
327 aspergillosis in non-neutropenic patients: *Aspergillus* Galactomannan Lateral Flow Assay versus
328 *Aspergillus*-specific Lateral Flow Device test in bronchoalveolar lavage. *Mycoses* **2019**; 62:230–
329 236.
- 330 22. Mercier T, Schauwvlieghe A, de Kort E, et al. Diagnosing invasive pulmonary aspergillosis in
331 hematology patients: a retrospective multicenter evaluation of a novel lateral flow device.
332 *Journal of Clinical Microbiology* **2019**; :JCM.01913-18.
- 333 23. Castillo CG, Kauffman CA, Zhai J, Jiang H, Agozino SM, Miceli MH. Testing the performance of a
334 prototype lateral flow device using bronchoalveolar lavage fluid for the diagnosis of invasive
335 pulmonary aspergillosis in high-risk patients. *Mycoses* **2018**; 61:4–10.
- 336 24. Duarte RF, Sánchez-Ortega I, Cuesta I, et al. Serum Galactomannan–Based Early Detection of
337 Invasive Aspergillosis in Hematology Patients Receiving Effective Antimold Prophylaxis. *Clin*
338 *Infect Dis* **2014**; 59:1696–1702.

339

340

Tables

Table 1. Characteristics of the study population at baseline (N=239)

Male gender (%)	150 (62.8)
Age (median [IQR]), years	60 [51.5, 66]
Neutropenia at baseline (%)	42 (17.6)
Active GvHD (%)	7 (2.9)
Reason for inclusion	
ALL/LBL (group 1)	8 (3.5)
AML (group 1)	49 (21.4)
High-risk MDS (group 1)	1 (0.4)
Allogeneic SCT (group 2)	75 (32.8)
ATG for AA (group 3)	1 (0.4)
Autologous SCT (group 4)	80 (34.9)
Proven/probable IA (group 5)	15 (6.6)

IQR = Interquartile range, SCT = Stem cell transplantation, GvHD = Graft versus host disease, AA =

aplastic anemia, ATG = Anti-thymocyte globuline, AML = acute myeloid leukemia, ALL = Acute

lymphoblastic leukemia, LBL = Lymphoblastic lymphoma, MDS = Myelodysplastic syndrome, PCD =

Plasma cell disorder, COPD = Chronic obstructive pulmonary disease, ILD = Interstitial lung disease

Table 2. Characteristics of cases of proven and probable invasive aspergillosis at time of diagnosis

Classification	Culture	Disease	Reason for inclusion	W6M	Serum GM	BALf GM	Neutropenia	LFD @ 15'	LFD @ 30'	LFA	BDG (pg/mL)
Probable IA		AML	Allogeneic SCT	Alive	0	3.7	yes	7.8 (NEG)	13 (NEG)	0.33	2.358
Probable IA	<i>A. fumigatus</i>	AML	AML	Alive	0.1	5.4	yes	7.7 (NEG)	9 (NEG)	0.84	2.358
Probable IA	<i>A. fumigatus</i>	AML	Allogeneic SCT	Dead	0.2	4.7	no	6 (NEG)	3 (NEG)	0.44	39.84
Proven IA	<i>A. fumigatus</i>	AML	Proven/probable IA	Alive	0	5.4	no	9.1 (NEG)	22.2 (VLP)	0.01	2.358
Proven IA		MF	Allogeneic SCT	Alive	0.2	4.7	yes	6.6 (NEG)	15.4 (NEG)	0.19	2.358
Probable IA		AML	AML	Alive	0.4	4.4	yes	6.2 (NEG)	6.3 (NEG)	0.35	3.376
Probable IA		AML	Autologous SCT	Alive	0.1	4.4	yes	6.9 (NEG)	7.3 (NEG)	0.34	2.358
Probable IA		MDS	Allogeneic SCT	Dead	4.7	5	yes	33.5 (POS)	53.1 (POS)	2.66	77
Probable IA	<i>A. fumigatus</i>	MDS	Allogeneic SCT	Dead	0.1	5.5	no	5.6 (NEG)	14.5 (NEG)	0.17	2.358
Probable IA		AML	AML	Alive	0.2	0.8	yes	2.9 (NEG)	5.5 (NEG)	0.52	2.358
Probable IA		Lymphoma	Allogeneic SCT	Alive	0.1	0.7	no	4 (NEG)	7.3 (NEG)	0.32	11.76
Probable IA	<i>A. fumigatus</i>	ALL	Proven/probable IA	Dead	3.9	4.2	yes	63 (POS)	82.5 (POS)	11.39	69.18
Probable IA		AML	AML	Alive	6.2	ND	yes	13.3 (NEG)	22 (VLP)	0.47	2.358
Proven IA	<i>A. fumigatus</i>	AML	Allogeneic SCT	Alive	0.2	4.2	yes	7 (NEG)	7.6 (NEG)	0.55	2.358
Probable IA		MM	Proven/probable IA	Dead	1.2	4	no	5 (NEG)	11.3 (NEG)	0.67	74.93
Probable IA		AML	AML	Dead	1.1	ND	yes	7.3 (NEG)	11.8 (NEG)	1.07	4.742
Probable IA		Lymphoma	Proven/probable IA	Alive	0	0.7	no	2.5 (NEG)	10.9 (NEG)	0.24	2.358
Probable IA		ALL	ALL/LBL	Alive	0	1.5	yes	4.6 (NEG)	8.6 (NEG)	0.21	2.358
Probable IA		Lymphoma	Allogeneic SCT	Alive	0.5	6.4	no	7.1 (NEG)	14.8 (NEG)	0.53	32.21
Probable IA		AML	AML	Dead	0.3	4.8	yes	6.4 (NEG)	8.8 (NEG)	0.28	3.247
Proven IA		Lymphoma	Allogeneic SCT	Alive	0.1	0.5	no	6 (NEG)	15.3 (NEG)	0.03	776.5
Probable IA		AML	Proven/probable IA	Dead	0.9	5.4	yes	7.8 (NEG)	13.8 (NEG)	1.1	3.265
Probable IA		AML	AML	Alive	0.5	4.4	yes	8.3 (NEG)	9.5 (NEG)	0.91	2.358
Probable IA		ALL	ALL/LBL	Alive	0.1	3.3	no	13.2 (NEG)	23.1 (POS)	0.42	2.358
Probable IA	<i>A. fumigatus</i>	ALL	Allogeneic SCT	Dead	3.7	4.7	yes	25.6 (POS)	42.6 (POS)	2.89	9.247

Probable IA		Lymphoma	Proven/probable IA	Alive	0	5.1	yes	4 (NEG)	3.6 (NEG)	0.28	3.473
Probable IA		Lymphoma	Allogeneic SCT	Dead	7.1	ND	no	36.1 (POS)	65.2 (POS)	5.54	39.04
Probable IA		AML	AML	Alive	0.2	6.1	yes	5.7 (NEG)	5.7 (NEG)	0.61	4.585
Probable IA		AML	AML	Alive	2.7	7.3	yes	9.7 (NEG)	15 (NEG)	1.07	2.425
Probable IA		AML	AML	Alive	3.8	0.2	no	13.3 (NEG)	16.1 (VLP)	1.99	22.62
Probable IA	<i>A. fumigatus</i>	AML	Proven/probable IA	Alive	0.5	ND	no	5.3 (NEG)	12.3 (NEG)	0.76	64.05
Probable IA	<i>A. fumigatus</i>	AML	AML	Dead	1	1.1	no	14.4 (NEG)	28.1 (POS)	0.75	203.9
Proven IA		MDS	Proven/probable IA	Alive	0.3	6.1	yes	4.1 (NEG)	11.6 (NEG)	0.37	2.358
Probable IA		AML	AML	Dead	0.8	ND	yes	13.1 (NEG)	19.3 (VLP)	0.64	2.358
Probable IA		AML	AML	Alive	0	6.1	yes	3.2 (NEG)	9 (NEG)	0.3	2.358
Probable IA		AML	AML	Alive	0.1	2.6	yes	6.4 (NEG)	10.5 (NEG)	0.06	2.358
Probable IA		CML	Allogeneic SCT	Alive	0.1	2	no	2.7 (NEG)	5.7 (NEG)	0.24	2.358
Probable IA		LBL	ALL/LBL	Dead	0.9	0.3	yes	6.4 (NEG)	12.7 (NEG)	1.13	2.358
Probable IA	<i>A. fumigatus</i>	Lymphoma	Autologous SCT	Alive	0.4	ND	yes	5.1 (NEG)	6.3 (NEG)	0.35	2.358
Probable IA	<i>A. terreus</i>	Lymphoma	Proven/probable IA	Dead	0.1	5.5	no	5.3 (NEG)	6.8 (NEG)	0.24	2.358
Probable IA		MM	Proven/probable IA	Alive	1.1	ND	yes	7.6 (NEG)	7.6 (NEG)	0.64	2.358

IA = invasive aspergillosis, AML = acute myeloid leukemia, ALL = acute lymphoblastic leukemia, LBL = lymphoblastic lymphoma, MDS = myelodysplastic

syndrome, MF = myelofibrosis, MM = multiple myeloma, CML = chronic myeloid leukemia, SCT = stem cell transplantation, LFD = OLM Diagnostics AspLFD,

LFA = IMMY *Aspergillus* galactomannan LFA, BDG = β -D-glucan, POS = positive, NEG = negative, VLP = very low positive, ND = not done, GM =

galactomannan, BALf = bronchoalveolar lavage fluid, W6M = Week 6 mortality

Table 3. Diagnostic performance of the different tests in serum in proven and probable invasive aspergillosis versus controls.

Test	Sensitivity (95% CI)	Specificity (95% CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)
GM	0.44 (0.28 - 0.6)	0.99 (0.96 - 1)	0.89 (0.86 - 0.91)	0.93 (0.66 - 0.99)
LFA	0.49 (0.33 - 0.65)	0.95 (0.91 - 0.98)	0.9 (0.86 - 0.92)	0.69 (0.52 - 0.82)
LFD	0.24 (0.12 - 0.4)	0.89 (0.84 - 0.93)	0.84 (0.82 - 0.87)	0.33 (0.2 - 0.5)
BDG $\geq$ 11	0.27 (0.14 - 0.43)	0.98 (0.95 - 1)	0.86 (0.84 - 0.88)	0.79 (0.52 - 0.93)
BDG $\geq$ 2.359	0.46 (0.31 - 0.63)	0.9 (0.85 - 0.94)	0.89 (0.85 - 0.91)	0.51 (0.38 - 0.65)
LFA AND GM	0.39 (0.24 - 0.55)	0.99 (0.97 - 1)	0.88 (0.85 - 0.91)	0.94 (0.68 - 0.99)
LFD AND GM	0.2 (0.09 - 0.35)	1 (0.98 - 1)	0.85 (0.83 - 0.87)	0.86 (0.51 - 0.96)
BDG $\geq$ 2.359 AND GM	0.3 (0.17 - 0.47)	0.99 (0.97 - 1)	0.87 (0.84 - 0.89)	0.92 (0.62 - 0.99)
LFA OR GM	0.54 (0.37 - 0.7)	0.94 (0.89 - 0.97)	0.9 (0.87 - 0.93)	0.66 (0.5 - 0.8)
LFD OR GM	0.49 (0.32 - 0.65)	0.86 (0.8 - 0.91)	0.89 (0.85 - 0.91)	0.44 (0.32 - 0.57)
BDG $\geq$ 2.359 OR GM	0.6 (0.43 - 0.75)	0.88 (0.82 - 0.93)	0.91 (0.87 - 0.94)	0.53 (0.4 - 0.65)
LFA AND BDG $\geq$ 2.359	0.32 (0.18 - 0.48)	0.99 (0.97 - 1)	0.87 (0.84 - 0.89)	0.93 (0.64 - 0.99)
LFD AND BDG $\geq$ 2.359	0.15 (0.06 - 0.29)	0.97 (0.93 - 0.99)	0.84 (0.82 - 0.86)	0.5 (0.25 - 0.75)
LFA OR BDG $\geq$ 2.359	0.63 (0.47 - 0.78)	0.86 (0.8 - 0.91)	0.92 (0.88 - 0.94)	0.5 (0.4 - 0.6)
LFD OR BDG $\geq$ 2.359	0.56 (0.4 - 0.72)	0.83 (0.77 - 0.88)	0.9 (0.86 - 0.92)	0.42 (0.32 - 0.52)

GM = galactomannan, LFA = IMMY *Aspergillus* galactomannan LFA, LFD = OLM Diagnostics AspLFD,

BDG = Wako β -D-glucan, CI = confidence interval

Table 4. Diagnostic performance of the different tests in serum in proven and probable invasive aspergillosis (excluding serum galactomannan as mycological criterion) versus controls. N = 34 cases.

Test	Sensitivity (95% CI)	Specificity (95% CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)
GM	0.31 (0.16 - 0.5)	0.99 (0.96 - 1)	0.89 (0.86 - 0.91)	0.89 (0.53 - 0.98)
LFA	0.41 (0.25 - 0.59)	0.95 (0.91 - 0.98)	0.9 (0.87 - 0.92)	0.61 (0.42 - 0.77)
LFD	0.18 (0.07 - 0.35)	0.89 (0.84 - 0.93)	0.86 (0.84 - 0.88)	0.23 (0.12 - 0.41)

GM = galactomannan, LFA = IMMY *Aspergillus* galactomannan LFA, LFD = OLM Diagnostics AspLFD, CI

= confidence interval

Figure legends

Figure 1. Flowchart of patients through the study

Figure 2. Histogram of galactomannan values in cases of proven and probable invasive aspergillosis

BALf = Bronchoalveolar lavage fluid

Figure 3. Correlation between serum galactomannan values and optical intensity of the two lateral flow tests