

Existing tests vs. novel non-invasive assays for detection of invasive aspergillosis in patients with respiratory diseases

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Abstract

Background: Although existing mycological tests (bronchoalveolar lavage [BAL] galactomannan [GM], serum GM, serum (1,3)- β -D-glucan [BDG], and fungal culture) are widely used for diagnosing invasive pulmonary aspergillosis (IPA) in non-hematological patients with respiratory diseases, their clinical utility in this large population is actually unclear. We aimed to resolve this clinical uncertainty by evaluating the diagnostic accuracy and utility of existing tests and explore the efficacy of novel sputum-based *Aspergillus* assays.

Methods: Existing tests were assessed in a prospective and consecutive cohort of patients with respiratory diseases in West China Hospital between 2016 and 2019 while novel sputum assays (especially sputum GM and *Aspergillus*-specific lateral-flow device [LFD]) in a case-controlled subcohort. IPA was defined according to the modified European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria. Sensitivity and specificity were computed for each test and receiver operating characteristic (ROC) curve analysis was performed.

Results: The entire cohort included 3530 admissions (proven/probable IPA = 66, no IPA = 3464) and the subcohort included 127 admissions (proven/probable IPA = 38, no IPA = 89). Sensitivity of BAL GM (≥ 1.0 optical density index [ODI]: 86% [24/28]) was substantially higher than that of serum GM (≥ 0.5 ODI: 38% [39/102]) ($\chi^2 = 19.83$, $P < 0.001$), serum BDG (≥ 70 pg/mL: 33% [31/95]) ($\chi^2 = 24.65$, $P < 0.001$), and fungal culture (33% [84/253]) ($\chi^2 = 29.38$, $P < 0.001$). Specificity varied between BAL GM (≥ 1.0 ODI: 94% [377/402]), serum GM (≥ 0.5 ODI: 95% [2130/2248]), BDG (89% [1878/2106]), and culture (98% [4936/5055]). Sputum GM (≥ 2.0 ODI) had similar sensitivity (84% [32/38]) (Fisher's exact $P = 1.000$) to and slightly lower specificity (87% [77/89]) ($\chi^2 = 5.52$, $P = 0.019$) than BAL GM (≥ 1.0 ODI). Area under the ROC curve values were comparable between sputum GM (0.883 [0.812–0.953]) and BAL GM (0.901 [0.824–0.977]) ($P = 0.734$). Sputum LFD had similar specificity (91% [81/89]) ($\chi^2 = 0.89$, $P = 0.345$) to and lower sensitivity (63% [24/38]) ($\chi^2 = 4.14$, $P = 0.042$) than BAL GM (≥ 1.0 ODI), but significantly higher sensitivity than serum GM (≥ 0.5 ODI) ($\chi^2 = 6.95$, $P = 0.008$), BDG ($\chi^2 = 10.43$, $P = 0.001$), and fungal culture ($\chi^2 = 12.70$, $P < 0.001$).

Conclusions: Serum GM, serum BDG, and fungal culture lack sufficient sensitivity for diagnosing IPA in respiratory patients. Sputum GM and LFD assays hold promise as rapid, sensitive, and non-invasive alternatives to the BAL GM test.

Keywords: Invasive pulmonary aspergillosis; Sputum; Bronchoalveolar lavage; Galactomannan; Lateral-flow device

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Introduction

Invasive pulmonary aspergillosis (IPA) is increasingly reported in non-hematological patients with respiratory diseases, ranging broadly from chronic lung diseases (eg, chronic obstructive pulmonary disease [COPD], asthma, lung cancer, pulmonary fibrosis, or bronchiectasis) to acute lung diseases (eg, community-acquired pneumonia, influenza, or coronavirus disease 2019).^[1-5] Given the widespread underlying conditions, the lethality, and the elusive clinical presentation of IPA in immunocompetent patients (frequent absence of host factors and typical radiological features),^[1,6] diagnostic assessment of suspected IPA has been commonly and indispensably implemented in routine clinical practice in respiratory care facilities. However, this process can be lengthy, costly, or invasive (when bronchoscopy or biopsy is arranged), and notably the accuracy and utility of existing mycological tests including bronchoalveolar lavage (BAL) galactomannan (GM), serum GM, serum (1,3)- β -D-glucan (BDG), and fungal culture for diagnosing IPA in respiratory patients are actually unclear, yet they are widely used. Such clinical uncertainty would result in diagnostic chaos in which inappropriate ordering or interpretation of diagnostic tests leads to delayed or missed diagnosis or even misdiagnosis, accounting for increased mortality, morbidity, and healthcare costs for a large group of patients with a clinical suspicion of IPA.^[7-9] Therefore, improving the diagnosis of IPA in patients with respiratory diseases by defining the clinical utility of currently available fungal tests and exploring new alternatives remains a crucial unmet clinical need.

Although recommended by the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) for diagnosing invasive fungal diseases in immunocompromised patients, the existing mycological tests have not yet been officially recommended for immunocompetent patients.^[10,11] Development of clinical recommendations has been hampered by a lack of robust and informative evidence. To our knowledge, previous studies of diagnostic accuracy of the existing tests for IPA in respiratory patients either had small sample sizes or were retrospective reviews of hospital records, leading to considerable between-study variation in diagnostic yields of a test and failure to accurately compare different tests. Therefore, a large-scale prospective comparison of diagnostic performance of existing tests in patients with respiratory diseases is required to determine their clinical utility in this specific but large population, allowing change in clinical practice and decision-making.

Despite the paucity of reports on diagnostic effectiveness of the existing tests in patients with respiratory diseases, serum GM generally showed lower sensitivity than BAL GM,^[12,13] supporting the notion that immunocompetent patients tend to develop airway-invasive aspergillosis rather than angio-invasive forms.^[14] Compared with BAL, sputum is a noninvasive and readily available lower respiratory tract specimen that most patients with lung diseases can produce for microbiologic evaluation.

Therefore, the discovery of novel tests by detecting *Aspergillus* biomarkers in sputum presents an opportunity to improve the early diagnosis of IPA. To date, *Aspergillus*-specific biomarkers including GM,^[15] the JF5 antigen (assayed by a lateral-flow device [LFD]),^[16] *Aspergillus* DNA (detected by polymerase chain reaction [PCR]),^[17] triacetylfusarinine C (T AFC),^[18] and bis(methylthio)gliotoxin (bmGT)^[19] have been investigated in serum or BAL. However, no study has detected these biomarkers in sputum and compared them to existing tests for IPA in patients with respiratory diseases.

The primary objectives of this study were to evaluate and compare the clinical utility of BAL GM, serum GM, serum BDG, and fungal culture for detection of IPA in patients with respiratory diseases, and to discover the diagnostic potential of sputum GM, LFD, PCR, T AFC, and bmGT tests in this population. We hypothesized that the clinical utility of sputum-based tests could be superior to that of existing tests in diagnosis of IPA.

Methods

Study design

This prospective diagnostic study was conducted at the West China Hospital of Sichuan University. We evaluated the diagnostic accuracy of the existing tests in a consecutive cohort of patients admitted to our general respiratory wards (entire cohort), and that of novel sputum-based tests in a subcohort (sputum biomarker subcohort). Tests performed before antifungal treatment were analyzed. The study was approved by the Clinical Trial and Biomedical Ethics Committee of West China Hospital of Sichuan University (No. 2016-234) and registered at <http://www.chictr.org.cn> (ChiCTR-DPD-16009070). All patients provided written informed consent.

Patient enrolment, diagnosis, and follow-up

For the entire cohort, adult (≥ 18 years) patients with underlying respiratory diseases were screened and enrolled by clinical researchers at admission. Exclusion criteria were hematological malignancy, receipt of solid organ transplant, neutropenia, or unwillingness to provide informed consent. Following consent, demographics, chest imaging results, and medical history of the patient were recorded. During hospitalization, diagnostic tests and treatment regimens were prescribed by the attending physicians and were not affected by study participation.

IPA was diagnosed according to the 2008 EORTC/MSG criteria^[10] modified for patients with respiratory diseases, with underlying respiratory disease added as a host factor and pulmonary infiltrate added as a radiological criterion.^[3-6,9] Particularly, the presence of clinical criteria was determined by a panel of three clinicians specializing in respiratory medicine and infectious diseases who were masked to mycological findings. No IPA was defined as

patients who did not fulfill the clinical criteria and was further confirmed by significant clinical improvement at discharge without receiving antifungals.

For sputum biomarker subcohort, induced sputum samples were obtained from patients with proven/probable IPA without receiving antifungals and randomly selected patients with no IPA (1:2).

Laboratory procedures

Blood, BAL, and spontaneous sputum samples collected for the existing mycological tests were sent to the Department of Laboratory Medicine. GM testing on BAL or serum was performed with the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad, Hercules, CA, USA). Serum BDG testing was performed with the Dynamiker[®] Fungus BDG Assay kit (Dynamiker Biotechnology, Tianjin, China), with BDG <70 pg/mL considered negative.^[20] Fungal culture on BAL or qualified spontaneous sputum (squamous cells <10 and leukocytes >25 per low power field) was done by observing fungal growth on Sabouraud Dextrose Agar medium (Oxoid, Basingstoke, Hampshire, UK) for 7 days.^[21]

Induced sputum samples were collected and processed as previously described^[22] for GM, LFD, PCR, TAFC, and bmGT assays. Specimens with a squamous cell percentage >50% or cell viability <40% were deemed unqualified and excluded. GM test on sputum was performed as per that on BAL or serum. The reproducibility of sputum GM assay was previously validated by Baxter *et al.*,^[23] showing an intra-assay coefficient variation of 5% and inter-assay coefficient variation of 9%. Sputum LFD was performed with the commercialized *AspLFD* kit (OLM Diagnostics, Newcastle upon Tyne, UK). *Aspergillus* DNA was detected with a quantitative PCR protocol using the pan-*Aspergillus* primers designed by Walsh *et al.*^[24] PCR result, that is, *Aspergillus* load, was expressed as numbers of copies per μ L of the specimen. Sputum TAFC and bmGT were simultaneously detected by using the method of high-performance liquid chromatography-tandem mass spectrometry.^[22] The method has a limit of detection of ≤ 1 ng/mL and a lower limit of quantitation of 1.56 ng/mL for both TAFC and bmGT.^[22]

The laboratory personnel were blinded to the study design and performed each type of test separately.

Sample size calculation

We calculated the sample size based on the diagnostic accuracy of the sputum GM assay to ensure sufficient statistical power for the assessment of novel tests in the sputum biomarker subcohort. Since the receiver operating characteristic (ROC) curves show the trade-off between sensitivity and specificity, and the area under the ROC curve (AUC) is considered as an overall index of accuracy for quantitative biomarkers, the sample size was computed according to the AUC value of sputum GM. We set the ratio of proven/probable IPA to no IPA as 1:2 according to our pilot study, which included 20 patients

with proven/probable IPA and 40 patients with no IPA and generated an AUC value of 0.854 for sputum GM. The sample size was then computed according to the method described in our study protocol,^[22] revealing the required total number of cases for sputum biomarker cohort was 114, including 38 cases of proven/probable IPA and 76 cases of no IPA. Anticipating a sampling failure rate of approximately 40% (insufficient or unqualified sputum samples), cases of no IPA were expanded to 108.

Statistical analysis

Continuous variables were expressed as the median (interquartile range) or mean \pm standard deviation and compared with the *t*-test or Mann-Whitney *U* test where appropriate. Categorical variables were compared by χ^2 test or Fisher's exact test. Patients with proven/probable IPA were considered reference standard positive and patients with no IPA were reference standard negative. The sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio including 95% confidence intervals (CIs) were calculated for all diagnostic tests. Sensitivities and specificities between diagnostic tests were compared by χ^2 test or Fisher's exact test. ROC curve analysis was performed and AUC values (95% CIs) were computed for BAL, serum, and sputum GM as well as sputum PCR. AUC values between the diagnostic tests from different datasets were compared by using Cleves's method.^[25] Cut-offs for GM, BDG, and PCR tests were determined according to respective ROC curves, kit instructions, and previous literature.^[3,11,20] All determinate test results were included for analysis. *P* < 0.05 was considered statistically significant. All analyses were performed in Stata 15 (StataCorp, College Station, TX, USA).

Results

Study population

Between August 17, 2016 and May 31, 2019, 3940 admissions were consecutively screened and 3685 admissions were enrolled [Figure 1]. Of 3530 admissions included in the analysis as the entire cohort, 66 had proven/probable IPA (four proven, 62 probable) and 3464 had no IPA. Overall, proven/probable IPA had an incidence of 1.79% (66/3685). Of 127 admissions for the sputum biomarker subcohort, 38 had proven/probable IPA (one proven, 37 probable) and 89 had no IPA.

Patients characteristics

Characteristics of the entire cohort are shown in Table 1. COPD was the most common (61% [40/66]) underlying respiratory disease among patients with proven/probable IPA. Approximately, half of the patients with proven/probable IPA (47% [31/66]) presented less-circumscribed infiltrate. The majority of patients with proven/probable (89% [59/66]) IPA received voriconazole treatment during hospitalization. Characteristics of the sputum biomarker subcohort were similar to that of the entire cohort [Table 1].

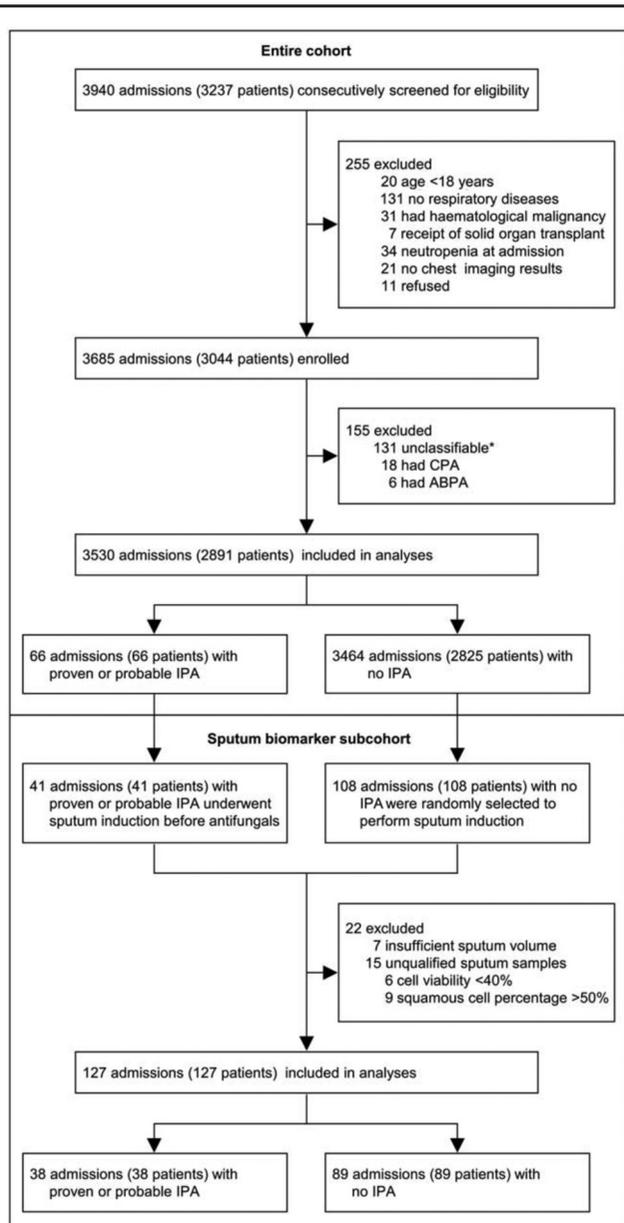


Figure 1: Study flow diagram for assessing existing tests vs. novel non-invasive assays for detection of invasive aspergillosis in patients with respiratory diseases. Unclassifiable patients were those who fulfilled the clinical criteria but had negative test results or did not undergo mycological tests. ABPA: Allergic bronchopulmonary aspergillosis; CPA: Chronic pulmonary aspergillosis; IPA: Invasive pulmonary aspergillosis.

Diagnostic performance of mycological tests

Test results are shown in Table 2. BAL and serum GM were significantly elevated in proven/probable IPA groups, in which higher proportion of patients presenting positive serum BDG and fungal culture were also found (all $P < 0.05$). Sputum GM index, LFD positive rate, *Aspergillus* gene copy numbers from the quantitative PCR assays and TAFC detectable rate were significantly higher in proven/probable patients than no IPA patients (all $P < 0.05$). Diagnostic performance of mycological tests is shown in Table 3. Sensitivity of BAL GM (≥ 1.0 optical density index [ODI]: 86% [24/28]) was substan-

tially higher than that of serum GM (≥ 0.5 ODI: 38% [39/102]) ($\chi^2 = 19.83, P < 0.001$), serum BDG (≥ 70 pg/mL: 33% [31/95]) ($\chi^2 = 24.65, P < 0.001$), and fungal culture (33% [84/253]) ($\chi^2 = 29.38, P < 0.001$). BAL GM (≥ 1.0 ODI) had a specificity of 94% (377/402), which was comparable to serum GM (≥ 0.5 ODI: 95% [2130/2248]) ($\chi^2 = 0.63, P = 0.428$), significantly higher than serum BDG (89% [1878/2106]) ($\chi^2 = 7.90, P = 0.005$), and slightly lower than fungal culture (98% [4936/5055]) ($\chi^2 = 21.65, P < 0.001$).

Although sputum GM (≥ 2.0 ODI) had a slightly lower specificity of 87% (77/89) compared with BAL GM (≥ 1.0 ODI; 94% [377/402]) ($\chi^2 = 5.52, P = 0.019$), their sensitivities (84% [32/38] vs. 86% [24/28]) were comparable (Fisher’s exact $P = 1.000$). The trends remained when higher thresholds were designated for sputum GM (≥ 3.0 ODI) and BAL GM (≥ 2.0 ODI) (specificity: 92% [82/89] vs. 97% [390/402], Fisher’s exact $P = 0.060$; sensitivity: 63% [24/38] vs. 57% [16/28], $\chi^2 = 0.24, P = 0.621$). Sputum LFD had a comparable specificity of 91% (81/89) ($\chi^2 = 0.89, P = 0.345$) but a lower sensitivity of 63% (24/38) ($\chi^2 = 4.14, P = 0.042$) than BAL GM (≥ 1.0 ODI). Nevertheless, it had significantly higher sensitivity than serum GM (≥ 0.5 ODI) ($\chi^2 = 6.95, P = 0.008$), BDG ($\chi^2 = 10.43, P = 0.001$), and fungal culture ($\chi^2 = 12.70, P < 0.001$). Sputum PCR (≥ 300 copies/ μ L: 40% [15/38]) showed low sensitivity as that for serum GM (≥ 0.5 ODI) ($\chi^2 = 0.02, P = 0.894$). TAFC and bmGT were detectable only in three and two patients with proven/probable IPA, respectively.

Thirty sputum samples were tested twice (over 1 week) with LFD to evaluate inter-assay reproducibility. Twelve samples were positive and 17 were negative in both aliquots, while one sample was positive in one aliquot but negative in another. The inter-assay agreement of sputum LFD was 97% (29/30). Of 127 sputum LFD tests performed across the study, five (3.9%) failed on the first attempt but succeeded on the second attempt, resulting in a total overall failure rate of 3.8% (5/132). Reproducibility of other sputum tests has been reported previously.^[22-24]

ROC analysis

ROC analysis revealed an AUC value of 0.883 (95% CI: 0.812–0.953) for sputum GM for differentiating proven/probable IPA from no IPA, which was similar to that for BAL GM (0.901 [0.824–0.977]) ($P = 0.734$), and was significantly higher than that for serum GM (0.766 [0.712–0.819]) ($P = 0.009$) [Figure 2A].

Clinical utility of mycological tests

Clinical utility of existing and sputum-based tests including five dimensions (sensitivity, specificity, invasiveness, turnaround time, and cost) was summarized by radar charts [Figure 2B], roughly reflecting the overall superiority of sputum GM and LFD tests relative to existing tests.

Table 1: Characteristics of the entire cohort and sputum biomarker subcohort.

Items	Entire cohort				Sputum biomarker subcohort					
	Total	Proven or probable IPA	No IPA	Statistics	P value	Total	Proven or probable IPA	No IPA	Statistics	P value
No. of cases	3530	66	3464	-	-	127	38	89	-	-
Age (years)	66 (54-75)	67 (60-77)	66 (54-75)	1.244*	0.214	66 (54-75)	68 (62-77)	65 (54-75)	1.423*	0.156
Male	2252 (64)	53 (80)	2199 (63)	7.935†	0.005	93 (73)	29 (76)	64 (72)	0.264†	0.608
Underlying respiratory diseases [§]										
COPD	1677 (48)	40 (61)	1637 (47)	4.628†	0.031	72 (57)	21 (55)	51 (57)	0.045†	0.832
Bronchiectasis	569 (16)	15 (23)	554 (16)	2.172†	0.141	35 (28)	11 (29)	24 (27)	0.052†	0.819
Community-acquired pneumonia	592 (17)	13 (20)	579 (17)	0.413†	0.521	20 (16)	7 (18)	13 (15)	0.292†	0.589
Influenza	27 (1)	4 (6)	23 (1)	-‡	0.001	0 (0)	0 (0)	0 (0)	-	-
Pulmonary fibrosis	242 (7)	4 (6)	238 (7)	-‡	1.000	4 (3)	3 (8)	1 (1)	-‡	0.080
Lung cancer	367 (10)	3 (5)	364 (11)	2.472†	0.116	12 (9)	2 (5)	10 (11)	-‡	0.508
Asthma	198 (6)	1 (2)	197 (6)	-‡	0.181	5 (4)	0 (0)	5 (6)	-‡	0.321
Pulmonary tuberculosis	107 (3)	1 (2)	106 (3)	-‡	0.723	3 (2)	1 (3)	2 (2)	-‡	1.000
Charlson Comorbidity Index	3 (2-4)	4 (2-5)	3 (2-4)	2.041†	0.041	3 (2-5)	4 (2-5)	3 (2-5)	1.123†	0.263
Radiological signs										
Dense, well-circumscribed lesion	430 (12)	14 (21)	416 (12)	5.128†	0.024	20 (16)	12 (32)	8 (9)	10.242†	0.001
Cavity	135 (4)	14 (21)	121 (3)	55.285†	<0.001	9 (7)	5 (13)	4 (4)	-‡	0.126
Air-crescent sign	7 (0)	7 (11)	0 (0)	-‡	<0.001	4 (3)	4 (11)	0 (0)	-‡	0.007
Less-circumscribed infiltrate	1192 (34)	31 (47)	1161 (34)	5.241†	0.022	52 (41)	17 (45)	35 (39)	0.323†	0.570
Blood neutrophils ($\times 10^9$ cells/L)	4.8 (3.4-6.9)	6.7 (4.1-11.5)	4.7 (3.4-6.8)	3.793*	<0.001	4.7 (3.6-7.5)	6.1 (3.6-9.6)	4.7 (3.7-6.9)	1.691*	0.091
Serum procalcitonin (ng/mL)	0.05 (0.03-0.11)	0.12 (0.05-0.25)	0.05 (0.03-0.11)	4.669*	<0.001	0.06 (0.03-0.15)	0.12 (0.05-0.23)	0.05 (0.03-0.13)	1.924*	0.010
Diagnostic test performed										
BAL GM test	425 (12)	27 (41)	398 (11)	52.932†	<0.001	29 (23)	16 (42)	13 (15)	11.429†	0.001
Serum GM test	1890 (54)	61 (92)	1829 (53)	40.880†	<0.001	83 (65)	35 (92)	48 (54)	17.138†	<0.001
Serum BDG test	1923 (54)	61 (92)	1862 (54)	39.055†	<0.001	80 (63)	35 (92)	45 (51)	19.715†	<0.001
Fungal culture	2951 (84)	66 (100)	2885 (83)	13.196†	<0.001	116 (91)	38 (100)	78 (88)	-‡	0.033

Data are presented as n (%), or median (IQR). * Z values. † Fisher's exact test. ‡ Some patients were diagnosed with more than one respiratory disease. BAL: Bronchoalveolar lavage; BDG: (1,3)- β -D-glucan; COPD: Chronic obstructive pulmonary disease; GM: Galactomannan; IPA: Invasive pulmonary aspergillosis; IQR: Interquartile range.

Table 2: Results of all studied mycological tests.

Items	Proven/probable IPA	No IPA	Statistics	P values
Existing tests				
BAL GM index	2.46 (1.12–4.83)	0.15 (0.10–0.28)	7.095*	<0.001
Serum GM index	0.32 (0.17–0.72)	0.13 (0.09–0.22)	9.094*	<0.001
Serum BDG ≥70 pg/mL	33% (31/95)	11% (228/2106)	41.628†	<0.001
Fungal culture positive	33% (84/253)	2% (119/5055)	623.333‡	<0.001
Sputum-based tests				
Sputum GM index	4.38 (2.26–5.95)	0.78 (0.43–1.56)	6.630*	<0.001
Sputum LFD positive	63% (24/38)	9% (8/89)	41.458*	<0.001
Sputum PCR (copies/μL)	178 (32–489)	35 (8–125)	3.495*	<0.001
Sputum TAFC detectable§	8% (3/38)	0 (0/89)	–‡	0.025
Sputum bmGT detectable	5% (2/38)	0 (0/89)	–‡	0.088

Data are presented as median (IQR) or % (n/total). * Z values. † χ^2 values. ‡ Fisher’s exact test. § Of three samples from patients with proven/probable IPA detectable for TAFC (above the limit of detection), two were quantifiable and the concentrations were 5.04 ng/mL and 4.28 ng/mL, respectively. || Of two samples from patients with proven/probable IPA detectable for bmGT, both were quantifiable and the concentrations were 5.15 ng/mL and 3.67 ng/mL, respectively. BAL: Bronchoalveolar lavage; BDG: (1,3)- β -D-glucan; bmGT: Bis(methylthio)gliotoxin; GM: Galactomannan; IPA: Invasive pulmonary aspergillosis; IQR: Interquartile range; LFD: Lateral-flow device; PCR: Polymerase chain reaction; TAFC: Triacetylufurine C.

Table 3: Diagnostic accuracy of the existing and novel sputum-based tests for invasive pulmonary aspergillosis.

Items	Sensitivity (95% CI; n/N)	Specificity (95% CI; n/N)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Diagnostic OR (95% CI)
Existing tests					
BAL GM ≥0.5 ODI	86% (67–96%; 24/28)	88% (84–91%; 352/402)	6.89 (5.10–9.30)	0.16 (0.07–0.40)	42.2 (14.7–121.1)
BAL GM ≥1.0 ODI	86% (67–96%; 24/28)	94% (91–96%; 377/402)	13.78 (9.16–20.74)	0.15 (0.06–0.38)	90.5 (30.2–268.5)
BAL GM ≥2.0 ODI	57% (37–76%; 16/28)	97% (95–98%; 390/402)	19.14 (10.06–36.41)	0.44 (0.29–0.68)	43.3 (17.1–110.1)
Serum GM ≥0.5 ODI	38% (29–48%; 39/102)	95% (94–96%; 2130/2248)	7.28 (5.38–9.86)	0.65 (0.56–0.76)	11.2 (7.2–17.3)
Serum BDG ≥70 pg/mL	33% (23–43%; 31/95)	89% (88–91%; 1878/2106)	3.01 (2.20–4.13)	0.76 (0.66–0.87)	4.0 (2.6–6.2)
Fungal culture	33% (27–39%; 84/253)	98% (97–98%; 4936/5055)	14.10 (10.99–18.09)	0.68 (0.63–0.75)	20.6 (15.0–28.3)
Sputum-based tests					
Sputum GM ≥1.0 ODI	92% (79–98%; 35/38)	62% (51–72%; 55/89)	2.41 (1.82–3.19)	0.13 (0.04–0.38)	18.9 (5.7–62.0)
Sputum GM ≥2.0 ODI	84% (69–94%; 32/38)	87% (78–93%; 77/89)	6.25 (3.63–10.76)	0.18 (0.09–0.38)	34.2 (12.0–97.1)
Sputum GM ≥3.0 ODI	63% (46–78%; 24/38)	92% (85–97%; 82/89)	8.03 (3.79–17.02)	0.40 (0.26–0.61)	20.1 (7.4–54.4)
Sputum LFD	63% (46–78%; 24/38)	91% (83–96%; 81/89)	7.03 (3.47–14.21)	0.40 (0.27–0.62)	17.4 (6.6–45.7)
Sputum PCR ≥15 copies/μL	92% (79–98%; 35/38)	34% (24–45%; 30/89)	1.39 (1.17–1.66)	0.23 (0.08–0.72)	5.9 (1.8–19.5)
Sputum PCR ≥300 copies/μL	40% (24–57%; 15/38)	92% (85–97%; 82/89)	5.02 (2.23–11.31)	0.66 (0.50–0.86)	7.6 (2.8–20.5)

BAL: Bronchoalveolar lavage; BDG: (1,3)- β -D-glucan; CI: Confidence interval; GM: Galactomannan; LFD: Lateral-flow device; ODI: Optical density index; OR: Odds ratio; PCR: Polymerase chain reaction.

Post hoc analysis

Redefining probable IPA according to the EORTC/MSG clinical criteria (less-circumscribed infiltrate was excluded from the diagnostic framework) did not change the sensitivity of BAL GM, serum GM, BDG, and fungal culture [Table 4]. Sensitivities of the existing tests were also not significantly changed when BAL GM, serum GM, or fungal culture were sequentially removed from the mycological criteria. In all circumstances, BAL GM had significantly higher sensitivity (range 86%–100%) than serum GM (19%–66%), BDG (31%–37%), and fungal culture (23%–48%), with the exception of serum GM alone as the mycological criterion for which the 95% CIs between BAL GM and serum GM overlapped. The limited sample size for the sputum subcohort precluded us to evaluate the influence of diagnostic criteria modification on sputum-based tests.

Discussion

To our knowledge, this is the largest prospective cohort study to comprehensively assess and compare the diagnostic and clinical utility of existing mycological tests and novel sputum-based assays for the detection of IPA in patients with respiratory diseases. There were three major findings. First, BAL GM had significantly higher sensitivity (86%) than serum GM, BDG, and fungal culture, while maintaining high specificity (94%). Second, sputum GM showed similar diagnostic performance as BAL GM. Finally, the sputum *Aspergillus* LFD test had improved sensitivity (63%) compared to serum GM, BDG, and fungal culture.

The three EORTC/MSG recommended tests (BAL GM, serum GM, and fungal culture) showed sufficient high specificity (range 94%–98%) to act as reliable rule-in

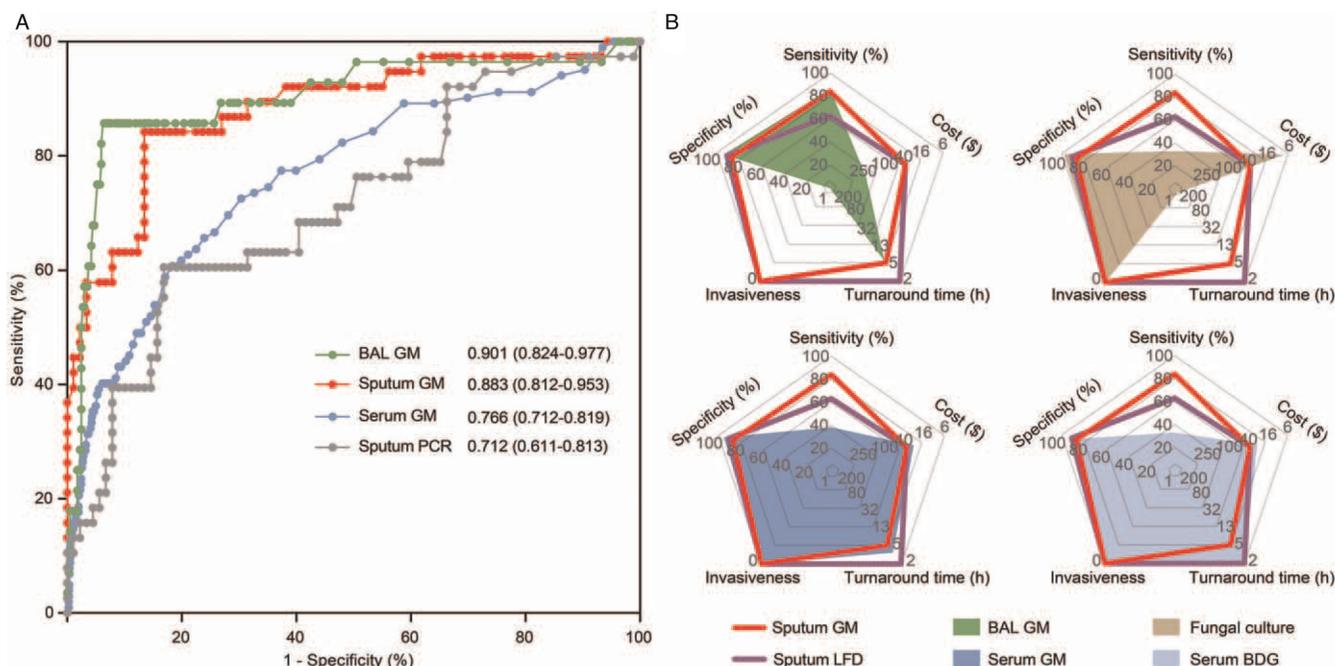


Figure 2: Comparison of clinical utility between existing and sputum-based tests. (A) Comparison of overall diagnostic performance between BAL GM, serum GM, sputum GM, and sputum PCR by ROC curve analysis. The area under the curve values (95% CI) are shown behind each test. (B) Radar charts were used to contrast the clinical utility of sputum GM and LFD tests with that of BAL GM, fungal culture, serum GM, and serum BDG respectively on five dimensions: sensitivity, specificity, invasiveness, turnaround time, and cost. Test costs were calculated according to local (Chengdu) list prices as of 2019 and regular costs on sample collection, processing, and commercial kits. Turnaround time only referred to the minimal time for sample collection and laboratory procedures indicated in the commercial kits regardless of setting-dependent time for specimen transportation or results reporting, etc. Invasiveness scale: 0 = noninvasive, 1 = invasive. BAL: Bronchoalveolar lavage; BDG: (1,3)- β -D-glucan; CI: Confidence interval; GM: Galactomannan; IPA: Invasive pulmonary aspergillosis; LFD: Lateral-flow device; PCR: Polymerase chain reaction; ROC: Receiver operating characteristic.

Table 4: Diagnostic sensitivity of the existing tests by different reference standards for the definition of probable invasive pulmonary aspergillosis (95% CI; n/N).

Reference standards	BAL GM ≥ 1.0 ODI	Serum GM ≥ 0.5 ODI	Serum BDG ≥ 70 pg/mL	Fungal culture
Clinical criteria				
Modified EORTC/MSG radiological signs*	86% (67%–96%; 24/28)	38% (29%–48%; 39/102)	33% (23%–43%; 31/95)	33% (19%–30%; 62/253)
Typical EORTC/MSG radiological signs	86% (64%–97%; 18/21)	37% (24%–51%; 19/52)	35% (23%–49%; 19/54)	28% (20%–37%; 32/116)
Mycological criteria				
EORTC/MSG mycological criteria†	86% (67%–96%; 24/28)	38% (29%–48%; 39/102)	33% (23%–43%; 31/95)	33% (27%–39%; 84/253)
BAL GM ≥ 1.0 ODI or serum GM ≥ 0.5 ODI	86% (67%–96%; 24/28)	47% (36%–58%; 39/83)	33% (23%–45%; 24/72)	31% (24%–38%; 54/175)
BAL GM ≥ 1.0 ODI or fungal culture	89% (71%–98%; 24/27)	32% (21%–44%; 23/72)	35% (24%–47%; 26/75)	39% (33%–46%; 84/214)
Serum GM ≥ 0.5 ODI or fungal culture	93% (68%–100%; 14/15)	45% (34%–56%; 39/87)	34% (24%–45%; 29/85)	39% (32%–46%; 84/217)
BAL GM ≥ 1.0 ODI alone	89% (71%–98%; 24/27)	19% (8%–38%; 6/31)	31% (14%–52%; 8/26)	23% (14%–34%; 16/71)
Serum GM ≥ 0.5 ODI alone	89% (52%–100%; 8/9)	66% (53%–78%; 39/59)	37% (25%–50%; 22/60)	38% (29%–47%; 47/124)
Fungal culture alone	100% (69%–100%; 10/10)	34% (22%–48%; 18/53)	37% (25%–50%; 24/65)	48% (41%–56%; 84/174)

* Modified EORTC/MSG radiological signs included typical EORTC radiological signs (dense, well-circumscribed lesion, cavity, and air-crescent sign) and less-circumscribed infiltrate. † EORTC/MSG mycological criteria for probable IPA were BAL GM ≥ 1.0 ODI, serum GM ≥ 0.5 ODI, or a positive fungal culture. BAL: Bronchoalveolar lavage; BDG: (1,3)- β -D-glucan; CI: Confidence interval; EORTC/MSG: European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM: Galactomannan; IPA: Invasive pulmonary aspergillosis; ODI: Optical density index.

tests for IPA in patients with respiratory diseases. However, the sensitivity of serum GM, BDG, and fungal culture was exceedingly low (<40%), relative to that in immunocompromised patients of pooled 78%,^[26] range 64% to 81%,^[27] and range 32% to 50%,^[28,29] respectively, which meant approximately two-thirds of IPA diagnoses would be missed if only a single test was performed. In contrast, BAL GM had a markedly higher sensitivity of 86% (≥ 1.0 ODI), similar to that in immunocompromised (pooled 78%)^[30] and non-immu-

nocompromised (range 65%–97%)^[3,13] patients. The huge sensitivity gain was essentially not affected by applying different clinical or mycological criteria for case definition in the *post hoc* analysis. Besides, the low negative likelihood ratio of 0.15 for BAL GM (≥ 1.0 ODI) [Table 3], which means a negative test result would reduce the odds of IPA post-test by a clinically meaningful factor of 6.7 times compared with pre-test, could help to rule-out IPA and reduce unnecessary use of antifungals.

Sputum GM test (≥ 2.0 ODI) showed comparable diagnostic sensitivity of 84% as BAL GM (≥ 1.0 ODI). The large AUC value of 0.883 for sputum GM, similar to that for BAL GM (0.901), indicated the good capability of this test in the differentiation between proven/probable IPA vs. no IPA. The low negative likelihood ratio of 0.13 at the threshold of ≥ 1.0 ODI [Table 3] also suggests its clinical value in rule-out of IPA. Given the non-invasiveness, short turnaround time, and low cost, the sputum GM test could therefore act as a useful tool for prompt diagnosis of IPA in respiratory care units. To our knowledge, none of the currently available tests could fulfill this role [Figure 2B]. Serum GM, BDG, and culture are limited by the aforementioned low sensitivity. Although BAL GM has improved sensitivity, bronchoscopy is costly, invasive, and occasionally contraindicated. More importantly, the utility of bronchoscopy in the early phase of IPA diagnosis can be quite limited, as early IPA manifesting atypical radiological signs (eg, pulmonary infiltrate) can hardly be distinguishable from community-acquired pneumonia for which bronchoscopy is generally recommended for non-response to initial treatment or intensive care unit admission.^[31] Collectively, the sputum GM test may promote early diagnosis of IPA in respiratory care units by increasing diagnostic sensitivity and reducing time to diagnosis.

We also firstly evaluated sputum LFD assay for IPA diagnosis in our study population, showing a sensitivity of 63% and specificity of 91%. The sensitivity of the LFD test on sputum is slightly lower than that reported on BAL (around 80%) for diagnosis of IPA in non-hematological patients,^[3,32] but was still considerably higher than that of serum-based tests and fungal culture in our study. The sputum LFD test thus has great potential for early diagnosis of IPA in clinics.

Sputum PCR failed to simultaneously achieve satisfactory sensitivity and specificity when different cut-offs were designated. The poor capability of sputum PCR in discriminating proven/probable from no IPA in patients with respiratory diseases might be attributed to frequent airway colonization of *Aspergillus*, which was indicated by the high positivity of sputum PCR (ranging 47%–74%) in patients with respiratory diseases and no evidence of IPA in previous studies.^[23,33]

TAFC and bmGT are secondary metabolites of *Aspergillus* and associated with fungi toxicity.^[18,19] We firstly measured the two compounds in sputum for IPA diagnosis in non-neutropenic patients, the results of which, however, were disappointing. The sensitivity of TAFC assay in serum (28%) or BAL (40%) among hematology patients was also very low in previous reports.^[18,34] Although an earlier study reported a sensitivity of 62% for serum bmGT assay in hematological patients,^[19] two recent replicate studies showed conflicting results with bmGT barely detected in the serum of IPA cases.^[35,36] Taken together, TAFC and bmGT seem not reliable biomarkers for IPA diagnosis.

Strengths of our study include the comprehensive comparison on the diagnostic accuracy and clinical utility

of existing and novel tests for IPA in a large prospective and consecutive cohort. The rigorous definition of no IPA, including observing clinical improvement without receiving antifungal therapy at discharge to further exclude IPA, allowed an accurate estimation of test specificity. Finally, diagnostic accuracy assessment was not affected by antifungal treatment.

Our study also has several limitations. First, the single-center design may limit the generalizability of the study findings although a large amount of data were collected. Second, the reference standard was modified from the EORTC/MSG criteria, with pulmonary infiltrate, in particular, added as a radiological criterion according to recent proposals.^[4,6] However, our *post hoc* analysis showed that diagnostic sensitivities of the existing tests were not changed when pulmonary infiltrate was removed from the clinical criteria. Third, sensitivities of BAL GM, serum GM, and fungal culture might be overestimated as they were part of the reference standard. However, when these tests were sequentially removed from the mycological criteria, their sensitivities were not decreased correspondingly. Fourth, the clinical utility of sputum GM and LFD tests required further validation, particularly through randomized controlled trials (allocating patients into either conventional diagnostic group or sputum tests guided diagnostic group) to confirm the effects of their clinical use on patient outcomes and economy.

In summary, serum GM, serum BDG, and fungal culture have insufficient sensitivity for the diagnosis of IPA in patients with respiratory diseases. Sputum-based GM and LFD tests hold promise as rapid, sensitive, economical, and non-invasive alternatives to BAL GM in clinical practice.

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Conflicts of interest

JJF received grant funding from the Thousand Talents Program of Sichuan Province and the National Natural Science Foundation of China. LLC reports a grant from the Sichuan Science and Technology Program. PGG reports grants from AstraZeneca and GlaxoSmithKline and has received speaker fees from AstraZeneca, GlaxoSmithKline, and Novartis, outside the submitted work.

XMD has received conference speaker fees paid by Hanzhong Medical Association, outside the submitted work. JYQ has received speaker fees from Pfizer Pharmaceuticals Ltd and Chengdu Medical Association, outside the submitted work. JFF, WX, DYG, and BM report a patent (ZL 2017 1 0428772.9) on simultaneous detection of TAFC and bmGT in sputum licensed to West China Hospital of Sichuan University by the State Intellectual Property Office in China. All other authors declare no competing interests.

References

- Cornillet A, Camus C, Nimubona S, Gandemer V, Tattevin P, Belleguic C, *et al.* Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. *Clin Infect Dis* 2006;43:577–584. doi: 10.1086/505870.
- Xu H, Li L, Huang WJ, Wang LX, Li WF, Yuan WF. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: a case control study from China. *Clin Microbiol Infect* 2012;18:403–408. doi: 10.1111/j.1469-0691.2011.03503.x.
- Prattes J, Flick H, Prüller F, Koidl C, Raggam RB, Palfner M, *et al.* Novel tests for diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. *Am J Respir Crit Care Med* 2014;190:922–929. doi: 10.1164/rccm.201407-1275OC.
- Schauvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, *et al.* Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018;6:782–792. doi: 10.1016/S2213-2600(18)30274-1.
- Bartoletti M, Pascale R, Cricca M, Rinaldi M, Maccaro A, Bussini L, *et al.* Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study. *Clin Infect Dis* 2021;73:e3606–e3614. doi: 10.1093/cid/ciaa1065.
- Guinea J, Torres-Narbona M, Gijón P, Muñoz P, Pozo F, Peláez T, *et al.* Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. *Clin Microbiol Infect* 2010;16:870–877. doi: 10.1111/j.1469-0691.2009.03015.x.
- Hishe V, Rajan SS, Sittig DF, Morgan RO, Chaudhary P, Singh H. Defining and measuring diagnostic uncertainty in medicine: a systematic review. *J Gen Intern Med* 2018;33:103–115. doi: 10.1007/s11606-017-4164-1.
- Winters B, Custer J, Galvagno SM Jr, Colantuoni E, Kapoor SG, Lee H, *et al.* Diagnostic errors in the intensive care unit: a systematic review of autopsy studies. *BMJ Qual Saf* 2012;21:894–902. doi: 10.1136/bmjqs-2012-000803.
- Delsuc C, Cottereau A, Frealle E, Bienvenu AL, Dessein R, Jarraud S, *et al.* Putative invasive pulmonary aspergillosis in critically ill patients with chronic obstructive pulmonary disease: a matched cohort study. *Crit Care* 2015;19:421. doi: 10.1186/s13054-015-1140-1.
- 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;46:1813–1821. doi: 10.1086/588660.
- Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, *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;71:1367–1376. doi: 10.1093/cid/ciz1008.
- Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vandenschueren S, *et al.* Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008;177:27–34. doi: 10.1164/rccm.200704-606OC.
- Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, *et al.* Diagnostic value of galactomannan antigen test in serum and bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. *J Clin Microbiol* 2017;55:2153–2161. doi: 10.1128/JCM.00345-17.
- Bergeron A, Porcher R, Sulahian A, de Bazelaire C, Chagnon K, Raffoux E, *et al.* The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. *Blood* 2012;119:1831–1956. doi: 10.1182/blood-2011-04-351601.
- Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. *Lancet Infect Dis* 2004;4:349–357. doi: 10.1016/S1473-3099(04)01045-X.
- Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vaccine Immunol* 2008;15:1095–1105. doi: 10.1128/CVI.00068-08.
- Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect Dis* 2009;9:89–96. doi: 10.1016/S1473-3099(09)70019-2.
- Carroll CS, Amankwa LN, Pinto LJ, Fuller JD, Moore MM. Detection of a serum siderophore by LC-MS/MS as a potential biomarker of invasive aspergillosis. *PLoS One* 2016;11:e0151260. doi: 10.1371/journal.pone.0151260.
- Vidal-García M, Domingo MP, De Rueda B, Roc L, Delgado MP, Revillo MJ, *et al.* Clinical validity of bis(methylthio)gliotoxin for the diagnosis of invasive aspergillosis. *Appl Microbiol Biotechnol* 2016;100:2327–2334. doi: 10.1007/s00253-015-7209-6.
- White PL, Price JS, Posso RB, Barnes RA. An evaluation of the performance of the Dynamiker Fungus (1-3)- β -D-Glucan Assay to assist in the diagnosis of invasive aspergillosis, invasive candidiasis and *Pneumocystis pneumonia*. *Med Mycol* 2017;55:843–850. doi: 10.1093/mmy/myx004.
- Engel TGP, Slabbers L, de Jong C, Melchers WJG, Hagen F, Verweij PE, *et al.* Prevalence and diversity of filamentous fungi in the airways of cystic fibrosis patients - a Dutch, multicentre study. *J Cyst Fibros* 2019;18:221–226. doi: 10.1016/j.jcf.2018.11.012.
- Xiao W, Gong DY, Mao B, Du XM, Cai LL, Wang MY, *et al.* Sputum signatures for invasive pulmonary aspergillosis in patients with underlying respiratory diseases (SPARED): study protocol for a prospective diagnostic trial. *BMC Infect Dis* 2018;18:271. doi: 10.1186/s12879-018-3180-z.
- Baxter CG, Dunn G, Jones AM, Webb K, Gore R, Richardson MD, *et al.* Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol* 2013;132:560.e10-566.e10. doi: 10.1016/j.jaci.2013.04.007.
- Walsh TJ, Wissel MC, Grantham KJ, Petraitiene R, Petraitis V, Kasai M, *et al.* Molecular detection and species-specific identification of medically important *Aspergillus* species by real-time PCR in experimental invasive pulmonary aspergillosis. *J Clin Microbiol* 2011;49:4150–4157. doi: 10.1128/JCM.00570-11.
- Cleves MA. From the help desk: comparing areas under receiver operating characteristic curves from two or more probit or logit models. *Stata J* 2002;2:301–313. doi: 10.1177/1536867X0200200307.
- Leefflang MM, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJ, Hooft L, *et al.* Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* 2015;2015:CD007394. doi: 10.1002/14651858.CD007394.pub2.
- White SK, Schmidt RL, Walker BS, Hanson KE. (1 \rightarrow 3)- β -D-glucan testing for the detection of invasive fungal infections in immunocompromised or critically ill people. *Cochrane Database Syst Rev* 2020;7:CD009833. doi: 10.1002/14651858.CD009833.pub2.
- Lass-Flörl C, Resch G, Nachbaur D, Mayr A, Gastl G, Auberger J, *et al.* The value of computed tomography-guided percutaneous lung biopsy for diagnosis of invasive fungal infection in immunocompromised patients. *Clin Infect Dis* 2007;45:e101–e104. doi: 10.1086/521245.
- Hoeningl M, Prattes J, Spiess B, Wagner J, Pruellner 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;52:2039–2045. doi: 10.1128/JCM.00467-14.
- de Heer K, Gerritsen MG, Visser CE, Leefflang MM. Galactomannan detection in broncho-alveolar lavage fluid for invasive

- aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* 2019;5:CD012399. doi: 10.1002/14651858.CD012399.pub2.
31. Lim WS, Baudouin SV, George RC, Hill AT, Jamieson C, Le Jeune I, *et al.* BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax* 2009;64 (Suppl 3):iii1–iii55. doi: 10.1136/thx.2009.121434.
32. Eigl S, Prattes J, Lackner M, Willinger B, Spiess B, Reinwald M, *et al.* Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. *Crit Care* 2015;19:178. doi: 10.1186/s13054-015-0905-x.
33. Sheehan RL, Niven RM, Ryan DM. Comparison of safety and efficacy of airway clearance techniques, hypertonic saline and bronchoscopy in a severe asthma service. *Thorax* 2016;71 (Suppl 3):A152–A153. doi: 10.1136/thoraxjnl-2016-209333.271.
34. Orasch T, Prattes J, Faserl K, Eigl S, Düttmann W, Lindner H, *et al.* Bronchoalveolar lavage triacetylfusarinine C (T AFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. *J Infect* 2017;75:370–373. doi: 10.1016/j.jinf.2017.05.014.
35. Mercier T, Reséndiz Sharpe A, Waumans D, Desmet K, Lagrou K, Maertens J. Gliotoxin and bis(methylthio)gliotoxin are not reliable as biomarkers of invasive aspergillosis. *Mycoses* 2019;62:945–948. doi: 10.1111/myc.12967.
36. Hammarström H, Stjärne Aspelund A, Christensson B, Heußel CP, Isaksson J, Kondori N, *et al.* Prospective evaluation of a combination of fungal biomarkers for the diagnosis of invasive fungal disease in high-risk haematology patients. *Mycoses* 2018;61:623–632. doi: 10.1111/myc.12773.

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