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]) (χ² = 19.83, P < 0.001), serum BDG (≥70 pg/mL: 33% [31/95]) (χ² = 24.65, P < 0.001), and fungal culture (33% [84/253]) (χ² = 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 (≥0.2 ODI) had similar specificity (84% [32/38]) (Fisher’s exact P = 1.000) to and slightly lower specificity (87% [77/89]) (χ² = 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]) (χ² = 0.89, P = 0.345) to and lower sensitivity (63% [24/38]) (χ² = 4.14, P = 0.042) than BAL GM (≥1.0 ODI), but significantly higher sensitivity than serum GM (≥0.5 ODI) (χ² = 6.95, P = 0.008), BDG (χ² = 10.43, P = 0.001), and fungal culture (χ² = 12.70, P < 0.001).

Conclusions: Serum GM, 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).\[1-3\] 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),\[1,4\] diagnostic assessment of suspected IPA has been commonly and indispensively 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.\[6-9\] 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 (TAFc),\[18\] 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, TAFc, 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. 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.

Induced sputum samples were collected and processed as previously described for GM, LFD, PCR, TAF, 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., showing an intra-assay coefficient of 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. PCR result, that is, Aspergillus load, was expressed as numbers of copies per μL of the specimen. Sputum TAF and bmGT were simultaneously detected by using the method of high-performance liquid chromatography-tandem mass spectrometry. The method has a limit of detection of ≤1 ng/mL and a lower limit of quantitation of 1.56 ng/mL for both TAF and bmGT.

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 ± standard deviation and compared with the t-test or Mann-Whitney U test where appropriate. Categorical variables were compared by χ² 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 χ² 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 Cleve’s method.[23] Cut-offs for GM, BDG, and PCR tests were determined according to respective ROC curves, kit instructions, and previous literature.[5,11,20] All determine 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 IPA (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].
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 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]) ($\chi^2 = 29.38$, $P < 0.001$). 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/μL; 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 | 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 (65) | 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 | 1.321 | 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 | 0.001 |
| Air-crescent sign                        | 7 (0)         | 7 (11)                  | 0 (0)     | –      | <0.001 | 4 (3)      | 4 (11)      | 0 (0)       | 0.007 | 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 (×10⁶ 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          | 0.12                    | 0.05      | 4.669  | <0.001 | 0.06       | 0.12        | 0.05        | 1.924 | 0.010 |
|                                            | (0.03–0.11)   | (0.05–0.25)             | (0.03–0.11) |        |        | (0.03–0.15) | (0.05–0.23) | (0.03–0.13) |        |        |
| 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)     | 0.264 | 0.001 |
| Serum BDG test                           | 1923 (54)     | 61 (92)                 | 1862 (54) | 39.053 | <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. †χ² 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.
Sputum-based tests for pulmonary aspergillosis; IQR: Interquartile range; LFD: Lateral-flow device; PCR: Polymerase chain reaction; TAFC: Triacetylfusarinine C.

### 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...
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-immunocompromised (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. 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, 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. TAFC and bmGT are secondary metabolites of Aspergillus and associated with fungal toxicity. 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. Although an earlier study reported a sensitivity of 62% for serum bmGT assay in hematological patients, two recent replicate studies showed conflicting results with bmGT barely detected in the serum of IPA cases. 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. 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

JRF 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. JJJ, WX, DYG, and BM report a patent (ZL 2017 1 0428772.9) on simultaneous detection of TAPC 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.

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