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ORIGINAL ARTICLE

Novel Tests for Diagnosis of Invasive Aspergillosis in Patients with Underlying Respiratory Diseases

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Abstract

Rationale: Invasive pulmonary aspergillosis has been increasingly reported in nonneutropenic patients, including those with underlying respiratory diseases.

Objectives: We compared the diagnostic performances of galactomannan, 1,3-β-D-glucan, and *Aspergillus*-specific lateral-flow device tests with that of conventional culture by using bronchoalveolar lavage fluid samples from patients with underlying respiratory diseases.

Methods: We analyzed 268 bronchoalveolar lavage samples from 221 patients with underlying respiratory diseases (and without hematologic malignancy or previous solid organ transplantation) that were collected for routine microbiological workup between February 2012 and May 2014 at the University Hospital of Graz, Austria. Invasive pulmonary aspergillosis was defined according to European Organization of Research and Treatment of Cancer/Mycoses Study Group criteria modified for patients with respiratory diseases.

Measurements and Main Results: Thirty-one patients (14%) had probable or proven, 25 possible, and the remaining 165 patients no invasive pulmonary aspergillosis. Probable/proven aspergillosis was associated with a significantly higher ($P = 0.034$) 30-day mortality rate of 32%. Sensitivities, specificities, and diagnostic odd ratios differed markedly between galactomannan (cut-off 0.5: optical density index, 0.97, 0.81, 124.4; cut-off 1.0: 0.97, 0.93, 422.1; cut-off 3.0: 0.61, 0.99, 109.8), β-D-glucan (cut-off 80 pg/ml: 0.90, 0.42, 6.57; cut-off 200 pg/ml: 0.70, 0.61, 3.7), lateral-flow device tests (0.77, 0.92, 41.8), and mycological culture (0.29, 0.97, 14).

Conclusions: Probable or proven invasive pulmonary aspergillosis was diagnosed in 14% of our study population and associated with significantly higher 30-day mortality rates. Although the performance of β-D-glucan was limited by low specificity and that of mycological culture by low sensitivity, the *Aspergillus* lateral-flow device seems to be a promising alternative to galactomannan testing, which remains the diagnostic gold standard for aspergillosis. Clinical trial registered with www.clinicaltrials.gov (NCT 02058316).

Keywords: galactomannan; *Aspergillus* lateral-flow device test; 1,3-β-D-glucan; mycological culture; bronchoalveolar lavage

Invasive pulmonary aspergillosis (IPA) is a life-threatening disease caused by the fungus *Aspergillus fumigatus* and other *Aspergillus* species and represents the leading cause of invasive fungal infection (IFI)-related morbidity and mortality in patients with hematological malignancies (1–4). Over recent years, IPA has been increasingly reported in nonneutropenic patients, including those with underlying diseases of the lung (e.g., steroid-treated chronic obstructive pulmonary disease [COPD], asthma, lung cancer, or autoimmune diseases with pulmonary involvement) (4–7). Early recognition and appropriate treatment of IPA

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At a Glance Commentary

Scientific Knowledge on the Subject: Invasive pulmonary aspergillosis (IPA) has been increasingly reported in nonneutropenic patients, including those with underlying respiratory diseases. The performance of a newly available test (the *Aspergillus*-specific lateral-flow device [LFD]) for diagnosis of IPA using bronchoalveolar lavage fluid has not been studied in these patients.

What This Study Adds to the Field: This study supports the clinical use of the *Aspergillus* LFD as an adjunct test for IPA alongside the galactomannan enzyme-immunoassay, the current gold standard test for disease detection. The *Aspergillus* LFD allows rapid and accurate diagnosis of the disease, facilitating earlier initiation of appropriate antifungal therapy and aiding clinical practice in the diagnosis and management of IPA in patients with underlying respiratory diseases.

in patients with COPD is crucial, as mortality rates greater than 90% have been reported (8), and early initiation of systemic antifungal therapy may lead to improved survival rates of up to 80% (9, 10). Diagnosis of IPA in patients with underlying respiratory diseases is difficult, however, as clinical presentation is typically characterized by nonspecific symptoms such as dyspnea, cough, or hemoptysis, and early radiological signs are also unspecific. Typical radiological signs for IPA (e.g., halo or air-crescent sign) are particularly rare in early stages of the disease in nonneutropenic patients (8, 11). Mycological culture—the previous gold standard for IPA diagnosis—is limited by low sensitivity and long turnaround time (10). Therefore, diagnostic tools based on fungal antigen detection have been developed within the last decade and have demonstrated their huge potential in IPA diagnosis (1, 12).

Galactomannan (GM) is a circulating polysaccharide component of *Aspergillus* species that is released into the bloodstream by growing hyphae and germinating conidia and can be detected by using a commercial ELISA (Bio-Rad Laboratories, Marnes-la-Coquette, France) (13–15). Although the performance of serum GM testing seems to be promising only in neutropenic patients, who usually develop angioinvasive forms of IPA (1, 16), bronchoalveolar lavage (BAL) fluid (BALF) GM seems the method of choice in nonneutropenic patients who tend to develop airway-invasive forms (17–19). This was confirmed in a study evaluating patients with underlying structural damage of the lung tissue (e.g., COPD, emphysema, bronchiectasis), which found that only one out of 65 patients with COPD with IPA had a positive serum GM test (8). In contrast, GM detection in BAL specimens has been reported to reach sensitivities of about 90% and specificities of 95% (18, 20–22). Limitations of GM testing include potential false-positive test results and the variable turnaround time, with time to results of 3 days or more in many centers (23–25).

These limitations may be overcome by the *Aspergillus* lateral-flow device (LFD) test, a point-of-care test for IPA. The LFD is an immunochromatographic assay using a monoclonal JF5 antibody to detect an extracellular antigen secreted by *Aspergillus* species exclusively during active growth (26). The LFD is easily performed using native BALF and yields results in 15 minutes. In recent studies, the high potential of this new assay in BAL IPA detection was shown in hematological malignancy and solid-organ transplant (SOT) patients (26–31). However, no data on the performance of the LFD test in nonneutropenic patients with underlying respiratory diseases have been published to date.

The 1,3-β-D-glucan (BDG) test is a colorimetric "panfungal" diagnostic assay that was included into mycological criteria in the revised European Organization of Research and Treatment of Cancer/Mycoses Study Group criteria (32). BDG is a cell wall component of most pathogenic fungi, including *Aspergillus*, *Pneumocystis* and *Candida* species, but is not present in the cell walls of the Mucorales. The high negative predictive value (NPV) of approximately 95% (33), when performed with serum specimens, is of particular interest for clinicians to rule out IFIs. In contrast, limited data exist on its diagnostic value in BAL specimens (31, 34).

The aim of this study was to evaluate the diagnostic performances of GM, BDG, and the *Aspergillus* LFD for BAL IPA detection in patients with underlying respiratory disease and to compare these to conventional culture.

Some of the results of these studies have been previously reported in the form of an abstract (35).

Methods

This part-prospective (GM, LFD testing, mycological culture), part-retrospective (BDG testing) cohort study was conducted between February 2012 and May 2014 at the University Hospital of Graz, Graz, Austria. Patients older than 18 years of age with underlying respiratory diseases who underwent routine bronchoscopy and had samples sent for microbiological workup were included. Exclusion criteria were underlying hematological malignancies or previous receipt of SOT. Overall, 268 BAL samples (defined as cases) from 221 patients with underlying respiratory diseases were included. In patients for whom more than one sample was included, time between consecutive sampling was 4 days or more.

IPA was classified according to a slightly modified version of the European Organization of Research and Treatment of Cancer/Mycoses Study Group revised definitions of 2008 that includes modified mycological criteria (single BAL GM $>$ 1.0 optical-density index [ODI] was added) and host factors (preexisting respiratory disease was added). Modifications were necessary because (*1*) publications about the superiority of BAL GM when compared with serum GM were performed after 2008 (20), and (*2*) host factors in these guidelines were originally defined for patients with hematologic malignancies and for severely immunocompromised patients (32, 36), whereas they have not been evaluated in other nonneutropenic patients at risk for IPA.

GM, LFD testing, and conventional culture were performed for all samples and always immediately after sample collection. All samples were then stored at −80°C for retrospective BDG testing (all performed within 6 mo after sample collection).

Mycological cultures and GM testing (by using the commercially available Platelia *Aspergillus* EIA/Ag assay [Bio-Rad, Munich, Germany]) were performed at the Institute of Hygiene, Microbiology, and Environmental Medicine, Medical University of Graz, as described previously (1). LFD testing was performed prospectively at the

Microbiology Laboratory, University Hospital of Graz, according to the method described elsewhere (28).

BDG testing was performed at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, using the commercially available Fungitell assay (Associates of Cape Cod, Falmouth, MA) adapted for automation on a BCS-XP coagulation analyzer allowing single-sample testing with fast turnaround time, as described previously (37). BDG testing did not yield results in 14 of 268 (5%) samples due to significant hemolysis. BDG results were therefore available for 254 samples.

For statistical analysis, SPSS 21 (SPSS Inc., Chicago, IL) was used. We calculated sensitivity, specificity, NPV, positive predictive value (PPV), and diagnostic odds ratio (DOR), including 95% confidence intervals (CIs), to compare the diagnostic performance among GM, BDG, LFD, and conventional culture. Receiver operating characteristics analysis was performed and area under the curve (AUC) values (including 95% CI) displayed for GM and BDG values. For all these tests, we performed three calculations to compare (*1*) proven/probable IPA versus no IPA, (*2*) proven/probable IPA versus possible/no IPA, and (*3*) proven/probable/possible IPA versus no IPA.

Our study was conducted in accordance with the Declaration of Helsinki, 1996, Good Clinical Practice, and applicable local regulatory requirements and law. The study protocol was approved by the local ethics committee, Medical University Graz, Austria (EC-number 25-221 ex 12/13) and registered at ClinicalTrials.gov (identifier NCT02058316).

Results

Demographic characteristics and underlying respiratory diseases of the study population are shown in Table 1. The majority of patients were on broad-spectrum antibiotics, and eight patients were on mold-active empirical antifungal therapy at the time bronchoscopy was performed. In six of eight patients, empirical therapy was initiated within 24 hours before bronchoscopy.

A total of 4 patients (4 cases) had proven IPA, 27 patients (27 cases) had probable IPA, 25 patients (26 cases) had possible IPA, and the remaining 165 patients (211 cases) had no evidence for IPA. Overall, 14% of patients (11.5% of cases) had probable or proven IPA. COPD was the most common underlying respiratory disease among patients with proven or probable IPA, and COPD had the highest incidence rate, as 20.9% (14 of 67 patients) of patients with COPD undergoing diagnostic bronchoscopy were diagnosed with proven/probable IPA. High rates of IPA were also found among patients with lung carcinoma (6 of 35 patients; 17.1%). Some 41.9% (13 of 31) of patients with proven/probable IPA were treated at general wards at the time of BAL sampling and 58.1% (18 of 31) at the respiratory care unit (RCU) or intensive care units (ICUs). A trend, although not significant, toward higher incidence rate of proven/probable IPA was found in patients from RCU/ICU (18/95; 18.9%) compared with patients from general wards (13/126; 10.3%, $P = 0.067$). Detailed results for cases treated at general wards, RCU, or ICU at the time of bronchoscopy as well as patients with and without proven/probable IPA are shown in Table 1.

Thirty- and 90-day mortality for patients with probable or proven IPA were 32% (10 of 31 patients) and 38.7% (12 of 31 patients), respectively. For possible IPA, 16% of patients died within 30 days (4 of 25 patients [5 of 26 cases]), and 28% died within 90 days (7 of 25 patients [8 of 26 cases]). For patients with no evidence for IPA, 16% of patients died within 30 days (26 of 165 patients [35 of 211 cases]) and 21% within 90 days (35 of 165 [49 of 211 cases]). The 30-day mortality rates were significantly lower both for patients ($P = 0.027$) and cases ($P = 0.039$) with possible/no IPA versus those with proven/probable IPA. Also significantly lower 90-day mortality was found for patients with possible/no IPA versus those with proven/probable ($P = 0.031$). No significant difference in 90-day mortality was observed for patients with proven/probable IPA treated on ICUs, RCUs, or general wards ($P = 0.588$).

Sensitivity, specificity, NPV, PPV, and DOR plus 95% CI for proven/probable IPA versus no IPA are shown in Table 2. Results for proven/probable IPA versus possible/no IPA are shown in Table 3, and for possible/probable and proven IPA versus no IPA in Table 4.

Receiver operating characteristics analysis revealed an AUC value of 0.965 (95% CI, 0.935–0.996) for GM and 0.752 (95% CI, 0.662–0.842) for BDG for differentiation between proven/probable IPA versus no IPA (Figure 1). For differentiation between proven/probable IPA versus possible/no IPA, AUCs were as follows: GM 0.966 (95% CI, 0.934–0.998), BDG 0.743 (95% CI, 0.652–0.833). For differentiation of proven/probable/possible IPA versus no IPA, GM AUC was 0.819 (95% CI, 0.746–0.893) and BDG AUC was 0.676 (95% CI, 0.594–0.758).

GM levels between 0.5 and 1.0 ODI were found in 11 of 26 (42.3%) cases with possible IPA and 27 of 211 (13%) cases without evidence for IPA.

In 68 patients (81 cases), BAL cultures grew *Candida* species and all were interpreted as colonization. Ninety-three percent of samples (75 of 81 cases) had BDG levels greater than 80 pg/ml, and 72% (58 of 81 samples) had BDG levels greater than 200 pg/ml. Forty-four of these 81 (54.3%) cases were hospitalized at ICU, 14 (17.3%) cases at RCU, and 23 (28.4%) on general wards. In one patient, BAL culture grew *Penicillium* species (another mold), but GM, BDG, and LFD remained negative.

Discussion

We assessed the diagnostic performance of GM, BDG, and the *Aspergillus* LFD test as well as conventional culture for IPA in BAL samples in a cohort of patients with underlying respiratory disease. There were three major findings. First, proven or probable IPA was diagnosed in 14% of patients with underlying respiratory diseases where BAL was sent for microbiological workup and was associated with significantly higher 30- and 90-day mortality rates. Second, *Aspergillus* LFD testing of BAL specimens seems a promising tool for IPA diagnosis, especially if GM results are not rapidly available. Third, performances of BDG (specificity $< 50\%$) and conventional culture (sensitivity around 30%) were less convincing.

COPD was the most frequently observed underlying respiratory disease in both our overall study population (30%) and those with proven/probable IPA (45%). Patients with COPD undergoing bronchoscopy also had the highest IPA rate (20.9%), followed by patients with lung cancer (17.1%). Previously reported IPA incidence in patients with chronic lung diseases varies widely from 1.9% in patients with acute exacerbation of COPD (38) to 22% in patients with COPD with isolation of *Aspergillus* species from the upper respiratory tract (7). In patients with

Table 1. Demographic Data and Underlying Diseases of Patients and Cases Admitted to General Wards, the RCU, or ICU as well as Patients and Cases without or with Possible IPA and Those with Probable or Proven IPA

	Overall Study Population	General Ward	RCU	ICU	Patients with No IPA or Possible IPA	Patients with Probable or Proven IPA	*P* Value*
No. of patients	221	126	20	75	190	31	
Sex							
Male	128 (58)	68 (54)	13 (65)	47 (63)	110 (58)	18 (58)	
Female	93 (42)	58 (46)	7 (35)	28 (37)	80 (42)	13 (42)	
Median age (range), yr	64 (18–92)	62 (18–92)	69.5 (51–89)	67 (21–82)	63 (18–89)	69 (24–92)	
Primary underlying respiratory disease per patient							
COPD	67 (30)	31 (25)	9 (45)	27 (36)	53 (28)	14 (45)	
Bacterial pneumonia	48 (21)	15 (12)	7 (35)	26 (35)	42 (22)	6 (19.5)	
Rheumatoid/autoimmune diseases with lung involvement	46 (21)	37 (29)	3 (15)	6 (8)	42 (22)	4 (13)	
Bronchial carcinoma	35 (16)	31 (25)	1 (5)	3 (4)	29 (15)	6 (19.5)	
Viral pneumonia	9 (4)	—	—	9 (12)	9 (5)	—	
Chronic respiratory failure	4 (2)	2 (2)	—	2 (3)	4 (2)	—	
Bronchial asthma	4 (2)	4 (3)	—	—	4 (2)	—	
Bronchiectasis	5 (2)	5 (4)	—	—	5 (3)	—	
AIDS with pneumonia	2 (1)	1 (1)	—	1 (1)	1 (0.5)	1 (3)	
Pulmonary tuberculosis	1 (1)	—	—	1 (1)	1 (0.5)	—	
Care per patient							
General ward	126 (57)	—	—	—	113 (60)	13 (42)	
RCU	20 (9)	—	—	—	14 (7)	6 (19)	0.031
ICU	75 (34)	—	—	—	63 (33)	12 (39)	
Mortality							
30-d mortality	35 (16)	4 (3)	5 (25)	26 (35)	30 (16)	10 (32)	0.027
90-d mortality	48 (22)	8 (6)	7 (35)	33 (44)	40 (21)	12 (39)	0.031
No. of cases (i.e., samples)	268	129	35	104	237	31	
Sex							
Male	149 (44)	70 (54)	21 (60)	58 (56)	131 (55)	18 (58)	
Female	119 (56)	59 (46)	14 (40)	46 (44)	106 (45)	13 (42)	
Median age (range), yr	65 (18–92)	62 (18–92)	69 (51–89)	67.5 (21–82)	64 (18–92)	69 (24–92)	
Primary underlying respiratory disease at the time of BAL sampling							
COPD	83 (31)	32 (25)	15 (43)	36 (35)	69 (29)	14 (45)	
Bacterial pneumonia	63 (24)	15 (12)	12 (34)	36 (35)	57 (24)	6 (19)	
Rheumatoid/autoimmune diseases with lung involvement	51 (19)	38 (29)	4 (11)	9 (9)	47 (19)	4 (13)	
Bronchial carcinoma	36 (13)	32 (25)	1 (3)	3 (3)	30 (13)	6 (19)	
Viral pneumonia	15 (6)	—	—	15 (14)	15 (6)	—	
Chronic respiratory failure	7 (3)	2 (2)	3 (9)	2 (2)	7 (3)	—	
Bronchial asthma	5 (2)	4 (3)	—	1 (1)	5 (2)	—	
Bronchiectasis	5 (2)	5 (4)	—	—	5 (2)	—	
AIDS with pneumonia	2 (1)	1 (1)	—	1 (1)	1 (0.4)	1 (3)	
Pulmonary tuberculosis	1 (0.4)	—	—	1 (1)	1 (0.4)	—	
Care at the time of bronchoscopy							
General ward	129 (48)	—	—	—	116 (49)	13 (42)	
RCU	35 (13)	—	—	—	29 (12)	6 (19)	
ICU	104 (39)	—	—	—	92 (39)	12 (39)	
Mortality							
30-d mortality	50 (19)	4 (3)	8 (23)	38 (37)	40 (17)	10 (32)	0.039
90-d mortality	69 (26)	9 (7)	10 (29)	50 (48)	57 (24)	12 (39)	

Definition of abbreviations: AIDS = acquired immunodeficiency syndrome; BAL = bronchoalveolar lavage; COPD = chronic obstructive pulmonary disease; ICU = intensive care unit; IPA = invasive pulmonary aspergillosis; RCU = respiratory care unit.
Data are presented as n (%) unless otherwise noted.
**P* values were calculated using chi-square test for possible or no IPA versus proven or probable IPA and displayed in case of significance.

Table 2. Sensitivities, Specificities, PPVs, NPVs, and DORs (Including 95% CI) for GM, LFD, BDG, and Conventional Culture for Diagnosing Proven/Probable IPA versus No IPA

Test Methods	Sensitivity	Specificity	PPV	NPV	DOR (95% CI)
BAL GM > 0.5 ODI	97 (30/31)	81 (170/211)	42 (30/71)	99 (170/171)	124.4 (16.5–938.9)
BAL GM > 1.0 ODI	97 (30/31)	93 (197/211)	68 (30/44)	99 (197/198)	422.1 (53.5–3,328)
BAL GM > 3.0 ODI	61 (19/31)	99 (208/211)	86 (19/22)	95 (208/220)	109.8 (28.5–423.3)
BDG > 80 pg/ml	90 (27/30)	42 (84/199)	19 (27/142)	96 (84/87)	6.6 (1.9–22.4)
BDG > 200 pg/ml	70 (21/30)	61 (122/199)	21 (21/98)	93 (122/131)	3.7 (1.6–8.5)
LFD	77 (24/31)	92 (195/211)	60 (24/40)	97 (195/202)	41.8 (15.6–111.8)
Conventional culture	29 (9/31)	97 (205/211)	60 (9/15)	90 (205/227)	14 (4.5–43)
BAL GM > 3.0 ODI and/or positive LFD	100 (31/31)	91 (192/211)	62 (31/50)	100 (192/192)	621.9 (36.6–10,563)

Definition of abbreviations: BAL = bronchoalveolar lavage; BDG = 1,3-beta-D-glucan; CI = confidence interval; DOR = diagnostic odds ratio; GM = galactomannan; IPA = invasive pulmonary aspergillosis; LFD = lateral-flow device; NPV = negative predictive value; ODI = optical density index; PPV = positive predictive value.
Data are presented as % (n/total) unless otherwise noted.

underlying respiratory diseases, patients with COPD seem to be most vulnerable for IPA development (7, 39–42). A possible explanation is found in pathophysiology. COPD leads to various changes in the local immune response to *Aspergillus* species. Impairment of polymorphonuclear neutrophils, eosinophils, macrophages, lymphocytes, as well as a decrease of phagocytosis and antigen recognition in lung tissues may result in a shift from fungal colonization to fungal infection (39, 43, 44). This study also found a high IPA rate among patients with lung cancer undergoing bronchoscopy with microbiological work up. Reported IPA incidence rates in patients with bronchial carcinoma vary widely between 2.6 and 40.6% (6, 45) and mortality reaches 71.5% (5). High-dose systemic corticosteroid treatment in cases of central nervous system metastasis, tumor necrosis, poststenotic mucus retention, as well as advanced stage of bronchial carcinoma and recent chemotherapy have been described as the main risk factors in those patients (5, 6, 45).

Overall, the 30-day mortality rate for patients with proven or probable IPA was 32% in this study cohort and was therefore comparable to previously published mortality rates in patients with hematological malignancies of 34% (3).

We also determined that a BAL GM cut-off of 1.0 ODI was the most promising threshold value for IPA detection in this nonneutropenic cohort without antifungal prophylaxis, with a sensitivity of 97% and specificity of 93% for proven/probable IPA compared with no IPA. Lowering the cut-off value to 0.5 ODI would have resulted in a lower test specificity of 81% (i.e., one out of five patients without IPA would have had a false-positive GM result) but also an increase of probable IPA cases (in this study, 42% of patients with possible IPA had GM results between 0.5 and 1.0 ODI). Increasing the cut-off for GM in BAL samples to an ODI of 3.0 would have led to a decrease of sensitivity (when compared with culture results) and a slight increase in test specificity. Combining GM with a cut-off of 3.0 with LFD was, however, promising.

Because GM testing can be limited by a long diagnostic turnaround time, the *Aspergillus* LFD point-of-care test may represent a promising alternative for the rapid and reliable diagnosis of IPA with BAL specimens. This is the first study to investigate the diagnostic performance of the BAL LFD test in patients with underlying respiratory diseases and without hematological malignancies or previous SOT. Previous studies evaluated the diagnostic performance of BAL LFD test in different patient populations: in SOT recipients sensitivity reached 91% and specificity 88% (29), and in patients with underlying hematological malignancies sensitivity reached 85% and specificity 100% when compared with patients

Table 3. Sensitivities, Specificities, PPVs, NPVs, and DORs (Including 95% CI) for GM, LFD, BDG, and Conventional Culture for Diagnosing Proven/Probable IPA versus Possible/No IPA

Test Methods	Sensitivity	Specificity	PPV	NPV	DOR (95% CI)
BAL GM > 0.5 ODI	97 (30/31)	78 (184/237)	36 (30/83)	99 (184/185)	104.5 (13.9–781.7)
BAL GM > 1.0 ODI	97 (30/31)	94 (222/237)	66 (30/45)	99 (222/223)	444 (56.6–3,483)
BAL GM > 3.0 ODI	61 (19/31)	99 (234/237)	86 (19/22)	95 (234/246)	123.5 (33.1–475.8)
BDG > 80 pg/ml	90 (27/30)	42 (93/224)	17 (27/158)	97 (93/96)	6.4 (1.9–21.7)
BDG > 200 pg/ml	70 (21/30)	59 (133/224)	19 (21/112)	94 (133/142)	3.4 (1.5–7.8)
LFD	77 (24/31)	85 (202/237)	41 (24/59)	97 (202/209)	19.8 (7.9–49.4)
Conventional culture	29 (9/31)	97 (231/237)	60 (9/15)	91 (231/253)	15.8 (5.1–48.4)
BAL GM > 3.0 ODI and/or positive LFD	100 (31/31)	84 (199/237)	45 (31/69)	100 (199/199)	326.5 (19.6–5,459)

Definition of abbreviations: BAL = bronchoalveolar lavage; BDG = 1,3-beta-D-glucan; CI = confidence interval; DOR = diagnostic odds ratio; GM = galactomannan; IPA = invasive pulmonary aspergillosis; LFD = lateral-flow device; NPV = negative predictive value; ODI = optical density index; PPV = positive predictive value.
Data are presented as % (n/total) unless otherwise noted.

Table 4. Sensitivities, Specificities, PPVs, NPVs, and DORs (Including 95% CI) for GM, LFD, BDG, and Conventional Culture for Diagnosing Proven/Probable/Possible IPA versus No IPA

Test Methods	Sensitivity	Specificity	PPV	NPV	DOR (95% CI)
BAL GM > 0.5 ODI	72 (41/57)	80 (169/211)	49 (41/83)	91 (169/185)	10.3 (5.3–20.1)
BAL GM > 1.0 ODI	53 (30/57)	93 (196/211)	66 (30/45)	88 (196/223)	14.5 (6.9–30.4)
BAL GM > 3.0 ODI	33 (19/57)	99 (208/211)	86 (19/22)	88 (208/246)	34.7 (9.8–122.9)
BDG > 80 pg/mL	78 (43/55)	42 (84/199)	27 (43/158)	88 (84/96)	6.4 (1.9–21.7)
BDG > 200 pg/mL	64 (35/55)	61 (122/199)	29 (35/112)	86 (122/142)	2.8 (1.5–5.1)
LFD	75 (43/57)	92 (195/211)	73 (43/59)	93 (195/209)	37.4 (17–82.4)
Conventional Culture	16 (9/57)	97 (205/211)	60 (9/15)	81 (205/253)	6.4 (2.2–18.9)
BAL GM > 3.0 ODI and/or positive LFD	88 (50/57)	91 (192/211)	72 (50/69)	96 (192/199)	72.2 (28.7–181.3)

Definition of abbreviations: BAL = bronchoalveolar lavage; BDG = 1,3-beta-D-glucan; CI = confidence interval; DOR = diagnostic odds ratio; GM = galactomannan; IPA = invasive pulmonary aspergillosis; LFD = lateral-flow device; NPV = negative predictive value; ODI = optical density index; PPV = positive predictive value.
Data are presented as % (n/total) unless otherwise noted.

without IPA (27, 31). In this study, the sensitivity of the LFD for IPA diagnosis in patients with underlying respiratory diseases was 77%, and the specificity was 92%, for proven/probable disease (vs. no IPA) and was therefore comparable to previous results in other patient populations, indicating the high diagnostic potential of this test when combined with BAL samples. Of interest is also the high NPV of the BAL LFD test, which was 97% in this study and also between 95 and 100% in previous studies (27, 29, 31). Azoulay and colleagues previously reported that up to two-thirds of patients receiving antifungal therapy in the ICU setting have no evidence for IFIs (46). Overtreatment with antifungals is not only associated with a huge financial burden but also bears the risk of side effects, drug–drug interactions, and even development of resistance (47, 48). Thus, using the LFD test may be an effective way to reduce unnecessary antifungal treatment.

The BDG assay, when tested in BAL, was limited by a low specificity varying from 42 to 61%. Even increasing the cut-off from 80 pg/ml to 200 pg/ml did not lead to a definite increase in specificity. The high frequency of positive BDG results with BAL specimen is not surprising, however, as BDG is also produced by *Candida* species, and colonization of the respiratory tract by *Candida* species occurs frequently, whereas pneumonia due to *Candida* species is an absolute rarity (49). In this study, the BDG test resulted positive in 93 and 72% of patients with *Candida* colonization, when detection thresholds of 80 pg/ml and 200 pg/ml were used, respectively. Although specificity has been described as the major limitation of the BDG assay in recent smaller studies, specificity was markedly lower in this study (31, 34). Differences in specificity (a previous study reported a specificity of 76% for the 80 pg/ml cut-off) may be explained by the fact that different proportions of the study populations were treated at ICUs, which is associated with higher *Candida* colonization rates of the respiratory tract (49). In this study, 38.8% of all patients were treated at ICUs, whereas only 6% of patients in the previous study required ICU treatment (31). Potential false positivity due to *Candida* colonization is therefore a major limiting factor for BDG diagnosis of IPA when using BAL. However, the high NPV (up to 97% using the 80 pg/ml cut-off) may be useful to rule out clinical suspicion of IPA.

In this study, we observed a low sensitivity (29%) of mycological culture for proven/probable IPA, with a PPV of 60%. This is in accordance with previously published data reporting sensitivities of 30% and less (10). However, there are several factors warranting the use of mycological culture. First, culture is necessary for susceptibility testing, which is of vital

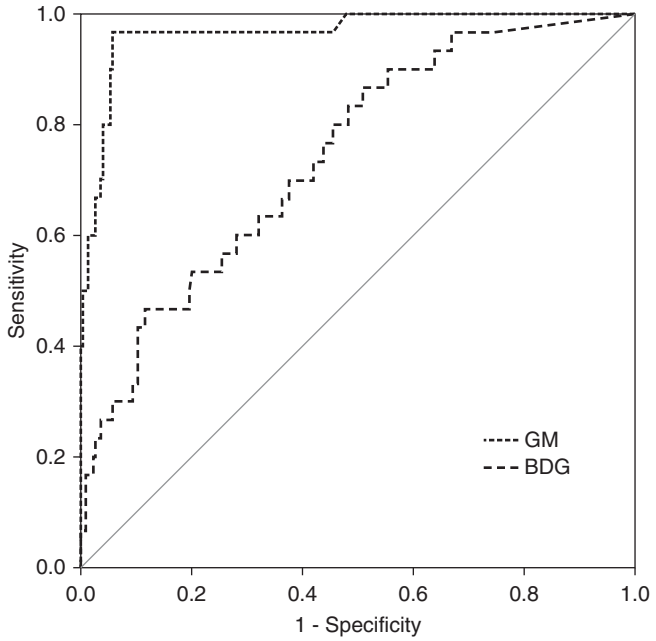

Figure 1. Receiver operating characteristics curve analysis for proven/probable invasive pulmonary aspergillosis (IPA) versus no IPA. BDG = 1,3-β-D-glucan; GM = galactomannan.

importance because azole resistance in *Aspergillus* species has emerged (50). Second, culture may also detect other molds (e.g., Mucorales) that give negative biomarker results.

Our study has several limitations, including its single-center design, which meant that numbers, particular those of proven IPA cases, were low. Another limitation is the absence of reliable definitions of possible and probable IPA in nonneutropenic patients, which necessitated modifications to the definitions used for neutropenic patients. Retrospective BDG testing may also be considered another limitation of our study, as it was reported recently that reproducibility of BDG evaluations using frozen BAL samples may be poor, although no trends in sensitivity have been found (34). Finally, only patients in whom BAL was routinely obtained and sent for microbiological workup were included, which likely resulted in an overestimation of IPA rates.

In summary, the GM-ELISA and *Aspergillus* LFD showed the best diagnostic performance for IPA diagnosis when used with BAL specimens in a cohort of nonneutropenic patients with underlying respiratory diseases. The performance of the BDG test was limited by low specificity and that of mycological culture by low sensitivity. Our results show that the *Aspergillus* LFD test may be a promising alternative to the GM test, which is the current the gold standard for IPA diagnosis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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