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As Galactomannan Disappoints, Our Quest for a Feasible Diagnostic Standard for Invasive Aspergillosis Continues

Since invasive pulmonary aspergillosis (IPA) was acknowledged as a life-threatening complication in patients with hematologic malignancies in the 1970s (1), clinicians have found themselves confronted with the dilemma of a disease that is difficult to diagnose but is associated with poor outcome when diagnosed too late (2). At this time, there is no diagnostic gold standard that can be used for the majority of patients at risk, as cultural methods lack sensitivity and specificity (3) and histopathology is rarely obtained.

In this issue of the *Journal*, Affolter and colleagues (pp. 309–317) describe the largest prospective cohort at this time of hematologic patients undergoing bronchoalveolar lavage (BAL) with galactomannan testing for diagnosis of IPA (4). They report a sensitivity of 50% and specificity of 73%, which means that for a patient with a baseline risk for IPA of 10%, the positive posttest probability would be 18%, whereas the negative posttest probability would be 7%, which is hardly a meaningful difference. This result was robust to detailed subgroup and sensitivity analysis, excluding patients with or without second BAL, antifungal pretreatment, or hematopoietic stem cell transplantation, and using different cutoffs for positivity. Given the high diagnostic uncertainty and the potentially severe consequences of misclassification, the authors rightfully ask whether there is sufficient diagnostic gain in performing BAL galactomannan sampling.

Indeed, despite an abundance of published trials (5, 6), opinion among clinicians remains divided for two main reasons. First, landmark clinical trials on treatment of IPA have included patients in the absence of positive microbiological tests (7–9), and most clinicians will always treat a high-risk symptomatic patient, independent of other test results (10). Without clinical consequence, some argue, there is low value in determining BAL galactomannan.

Second, definition of cases and controls for diagnostic studies on IPA is almost impossible in absence of a reliable and feasible comparator test. When, for example, Bianchi and colleagues investigated screening tests for prenatal aneuploidy (11), they could rely on almost 100% identification of the investigated disease on delivery of the child. Observed disease was then correlated with predicted disease, and physicians received reliable estimates of the test's performance.

For IPA, the case could hardly be more different. The majority of hematological patients undergoing BAL have a mix of more or less specific lung infiltrates and different host factors, with or without alternative microbiological markers, all of which may or may not be temporally related to the BAL. This is also what Affolter and colleagues encountered in their study (4): 54% of the patients had lung infiltrates, 62% had received prior antifungals, and 51% were tested for galactomannan in serum, while biopsies were performed in about 5%. Only six patients in the study had proven IPA.

Most studies on potential biomarkers of IPA try to get around this vague diagnostic situation by using the classification of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) (12), an approach often reinforced by experts, reviewers, and regulators. This classification performs well as inclusion criterion for clinical studies, where it guarantees that a noteworthy share of observed patients indeed had IPA. However, consider a patient with 8 days

of neutropenia, mold growth from sputum, and halo sign on chest computed tomography. Consider a similar patient with 12 days of neutropenia, but absent microbiological tests. Both would be rated as false-positives because of lack of host or microbiological criteria, although in fact, both patients probably have IPA. In addition, both are likely to receive antifungals (10), leaving almost no chance of either upgrading the patient to a proven diagnosis or observing treatment success without antifungals. Definition of the control group is another pitfall: the EORTC/MSG criteria do not differentiate between patients with negative sputum culture, patients with positive or negative *Aspergillus* polymerase chain reaction, and even patients not receiving any microbiological test. They all accumulate in the "possible" group if clinical signs and a host factor are present. Can such a heterogeneous group be used for inference on a candidate biomarker?

If we use EORTC/MSG criteria for assessing the value of a diagnostic test, we do not learn about the test's ability to diagnose IPA, but merely assess the chance that the test correctly classifies a patient as a member of a high-prevalence group.

There is no simple solution to this issue. At first glance, comparing patients with proven disease only with those who have absolutely no signs of IPA may seem attractive. However, physicians usually order a BAL if they have a clinical suspicion, meaning even patients not fulfilling EORTC/MSG criteria probably belong to a clinically defined high-prevalence group of IPA. In addition, results from highly selected control patients probably cannot be translated into clinical practice in a population with very heterogeneous underlying infections and disorders.

One strategy would be to leave behind the EORTC/MSG criteria and to enforce a combined diagnostic effort under the controlled conditions of an interventional clinical trial. In such a trial, all patients would have to undergo the same standardized set of diagnostic tests at predefined visits, which should include as many potentially useful alternative tests as possible, including polymerase chain reaction. Patients negative in all biomarkers would be classified as true negatives, whereas patients with ambiguous results would be evaluated as their own group. Assessment would be stratified by prior mold-active treatment. Although this strategy would not eliminate bias, interdependence of modestly performing tests would be reduced as far as possible. However, given the low number of patients in whom diagnostic certainty can be established, this would be a task for a large consortium.

Until such a study is made, Affolter and colleagues provide us with the most comprehensive analysis of galactomannan from BAL to date. This work will have considerable influence on future meta-analyses and may lead to reevaluation of the current recommendation for BAL in diagnosis of IPA outside of academic settings, although BAL may still be of value for the differential diagnosis of neutropenic pneumonia syndrome (13, 14). ■

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

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ORIGINAL ARTICLE

Galactomannan in Bronchoalveolar Lavage for Diagnosing Invasive Fungal Disease

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Abstract

Rationale: Invasive fungal disease (IFD) is a significant cause of morbidity and mortality in immunocompromised patients.

Objectives: We hypothesize that galactomannan (GM), a component of fungal cell wall, as measured in bronchoalveolar lavage (BAL) might be a diagnostic adjunct in hematologic malignancies.

Methods: A total of 568 hematologic cases undergoing diagnostic bronchoscopy because of respiratory symptoms and/or suspected IFD between 2009 and 2013 at a tertiary care center in Switzerland were included in this prospective, observational cohort study. We compared accuracy of the BAL GM ELISA determination in predicting IFD as classified by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/MSG) definition.

Measurements and Main Results: BAL GM was positive in 155 cases (29.2%). According to the EORTC/MSG criteria, IFD was classified as possible in 182 (34.3%), probable in 45 (8.5%), and proved in six (1.1%). BAL GM provided 50% sensitivity, 73.0% specificity, 16% positive predictive value, and 93% negative predictive value for diagnosing proven+probable IFD. Results were similar when antifungal treatment and radiologic suspicion of IFD were used as the gold standard. The area under the curve of the receiver operating characteristic curve for the diagnosis of proven+probable IFD was 0.716 (95% confidence interval, 0.638–0.794; $P < 0.001$).

Conclusions: GM in BAL had modest agreement with EORTC/MSG criteria for diagnosing IFD in immunocompromised patients with a high degree of antifungal exposure.

Keywords: immunosuppression; bronchoscopy; invasive pulmonary aspergillosis

At a Glance Commentary

Scientific Knowledge on the Subject: Galactomannan, a component of the fungal cell wall measured in bronchoalveolar lavage, might be a diagnostic adjunct for invasive fungal infection in hematologic malignancies.

What This Study Adds to the Field: This large study including a well-characterized cohort of hematologic patients suggests that galactomannan in bronchoalveolar fluid has only a modest accuracy and thus limited clinical usefulness for diagnosing invasive fungal disease in immunocompromised patients with hematologic malignancies. Thus, we recommend caution in using this assay for treatment guidance in immunocompromised patients.

Invasive bronchopulmonary aspergillosis accounts for 30–50% of invasive fungal diseases (IFD) among immunocompromised patients with hematologic malignancies. The lung is the most affected organ by *Aspergillus* species infections and the mortality and morbidity among immunocompromised patients, despite the use of newer antifungal agents, reaches 20% (1–4). Even though most patients at highest risk for IFD never develop this condition, IFD carries

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a devastating prognosis (5, 6). Therefore, it is crucial to improve early and correct diagnosis, thus identifying patients who really need treatment and avoiding unnecessary toxicity and costs (7, 8).

The diagnosis of IFD remains challenging and is based on a combination of clinical factors, host factors, and microbiologic criteria (9). Currently, the gold standard for diagnosis of IFD is the direct examination and culture of pulmonary tissue (10). Unfortunately, routine histology via transbronchial or open lung biopsy is hampered by the common presence of thrombocytopenia in the hematologic cohort. Herein, the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/MSG) developed a classification of the probability of an IFD, which was revised in 2008 (11). Galactomannan (GM), a component of fungal cell wall, can be detected by a sandwich-type ELISA in serum, plasma, cerebrospinal fluid, or bronchoalveolar lavage (BAL) fluid. It was approved by the US Food and Drug Administration at a serum cutoff of 0.5 nmol/ml as a diagnostic adjunct for IFD (7, 12). Reported sensitivity and specificity of serum GM ranged between 0 and 100% and 38 and 98%, respectively (13–16). As compared with serum GM, BAL GM seems to have improved diagnostic performance with corresponding sensitivity and specificity ranging between 57 and 100% and 89 and 99% (13, 17–21). However, most studies infer on a small sample size and recruited a heterogeneous population.

The purpose of our study was to evaluate the accuracy of BAL GM for the diagnosis of IFD in a large, well-characterized, homogenous cohort of hematologic, immunocompromised patients undergoing diagnostic bronchoscopy caused by respiratory symptoms. Some of the results of this study have been previously submitted in the form of an abstract (22).

Methods

This was a prospective, observational cohort study conducted in the University Hospital Basel, a 784-bed tertiary care hospital located in Basel, Switzerland. The primary goal of the study was to analyze the diagnostic performance of BAL GM for IFD. All hematologic patients undergoing diagnostic bronchoscopy with BAL because of respiratory symptoms and/or radiologic abnormalities between January 2009 and July 2013 were included in this analysis. Patients were allowed to be included in the study more than once if episodes were judged to be independent by the treating physician. The study was approved by our institutional ethics committee and subjects provided written informed consent.

Patients

Immunocompromised hematologic patients reporting and/or depicting respiratory symptoms and/or signs, such as cough, sputum production, fever, and/or dyspnea, were considered at risk for respiratory infection, respectively IFD, and underwent flexible, diagnostic bronchoscopy at the discretion of the attending hematologic physician. Neither fever nor previous use of antibiotics was required for establishing the indication for bronchoscopy.

Inclusion criteria were (*1*) age greater than 18 years, (*2*) immunocompromised state as defined by hematologic malignancy, (*3*) decision by the pulmonary consultant to perform bronchoscopy because of suspected pulmonary infection, and (*4*) informed consent by the patient to undergo flexible bronchoscopy and related data analysis. Exclusion criteria were pregnancy and inability to provide informed consent.

Diagnostic Work-up

Flexible bronchoscopy was performed with the patient under conscious sedation using hydrocodon and disoprivan. BAL was performed by three installations of 50 ml each of a pyrogen-free, sterile, 0.9% NaCl solution over the working channel of the bronchoscope according to standard guidelines (23). BAL fluid was recovered by suction. The samples were analyzed for cytology; Gram and appropriate stains and cultures for mycobacteria, bacteria, fungi, and viruses were performed according to the standard procedures (24). Cell differentiation in BAL fluid was reported as absolute numbers and as a percentage of the total cell count (24).

GM Determination

The GM enzyme immunoassay was performed on uncentrifuged BAL specimens according to the manufacture's specifications (Platelia Aspergillus; BioRad Laboratories, Hercules, CA) (25). GM test results were interpreted as positive when an optical density index of greater than or equal to 0.5 was demonstrated (12).

Diagnostic Criteria

Clinical information, laboratory results, and radiologic reports were collected at the bronchoscopy day and up to discharge from hospital and/or up to vanishing of acute pulmonary clinical symptoms. Clinical history and suspicion of IFD was communicated in writing to the radiologist in all cases. Two independent radiologists (one board certified, one in training) reviewed computed tomography (CT) scans and provided a radiologic assessment based on EORTC/MSG findings. Additionally, retrospective chart review, including CT scans and autopsy results, was performed by two respiratory physicians (one board certified, one in training) in view of the EORTC/MSG classification for data related to hospitalization and/or ambulatory visit requiring diagnostic bronchoscopy.

Use of antifungal agents was defined as (*1*) antifungal prophylaxis (use of antifungal agents to avoid the development of fungal disease, including fluconazole and mold-active agents); (*2*) mold-active antifungal prophylaxis (excluding fluconazole); and (*3*) empirical antifungal treatment (use of antifungal agents active against *Aspergillus* spp. because of a high suspicion but no proof of IFD). Each patient was classified as having proven, probable, possible, or no IFD according to the revised definitions of IFD from the EORTC/MSG Consensus Group (11). Serum GM was included as a criterion to classify disease according to the EORTC/MSG if determined, at the discretion of the attending physician, within 7 days of diagnostic bronchoscopy. GM detection in BAL fluid was not included as a mycologic criterion for IFD to avoid an incorporation bias. If neither mycologic criterion (i.e., direct and indirect) was positive, the case was classified as possible IFD. Disagreements between reviewer physicians were settled by consensus.

Statistical Analysis

Differences in dichotomous variables were evaluated using the chi-square test or Fisher exact test, as appropriate. Normally distributed parameters were analyzed using the Student *t* test for equality of means. All other continuously nonnormally distributed parameters were evaluated

using the nonparametric Mann-Whitney *U* test or Kruskal-Wallis test, as appropriate. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios for BAL GM were calculated by using the EORTC/MSG classification as the gold standard for the diagnosis of IFD.

For explorative purposes, the diseased group was defined differentially as solely proven, proven+probable, and proven+probable+possible IFD cases, and respectively, compared with the remaining categories (i.e., proven versus probable+possible+no IFD, proven+probable versus possible+no IFD, and proven+probable+possible IFD vs. no IFD). In addition, the test performance was reevaluated in the group proven+probable disregarding serum GM as a mycologic criterion to define probable disease. In a further attempt to infer the diagnostic property of BAL GM, clinical judgment (i.e., empirical antifungal treatment and the suspicion of IFD on radiologic studies) was used as the gold standard for IFD. The area under the curve of the receiver operating characteristic curve was calculated for the performance of the binary classifier system because its discrimination threshold is varied. Herein, the combination of proven+probable IFD was compared with possible+no disease.

Univariate and multivariable logistic regression analysis was performed with BAL GM positivity as the dependent variable. Parameters included in the univariate and multivariate analysis were selected *a priori* based on their clinical relevance. We refrained from using automated regression procedures to avoid overfitting. All tests were two-tailed; a P value of less than 0.05 was considered significant. Results were expressed as mean (standard deviation) or median (interquartile range) unless otherwise stated. Data analysis was conducted according to the Statistical Package for Social Sciences (SPSS Statistics, version 21 for Windows; IBM, Zurich, Switzerland) program.

Results

Out of 568 cases, 530 had a complete data set and were included in the present analysis. Figure 1 depicts the study design according to the CONSORT guidelines. Patients excluded because of lack of radiology data (n = 15) or missing BAL GM (n = 23) did not differ from the analyzed population (data not shown). A total of 125 patients were included more than once. From those, 110 cases underwent bronchoscopy a second time within 12 months. For patients included more than once, median time between inclusions was 65 days (95% confidence interval [CI], 26–203). Patient demographics are shown in Table 1. The most common causes of hematologic diseases were acute myeloid leukemia (160 cases, 30.2%), malignant lymphoma (89 cases, 16.8%), and multiple myeloma (six cases, 11.9%). Most of the population had undergone allogeneic hematopoietic stem cell transplantation (336, 63.4%) and about 18% cases were included within 100 days after hematopoietic stem cell transplantation. The most common indication for diagnostic bronchoscopy was fever and cough. In addition, infiltrates were present in most cases (54%) and were considered suspicious of IFD by the radiologists in about 30% of the examinations. Median duration of hospitalization before bronchoscopy was 2 days (95% CI, 1–19). Empirical therapeutic antibiotic use at bronchoscopy was reported in 34% of the cases. Antibiotics were prescribed for a mean of 10.2 days (SD ± 13.2). Most patients were on either empirical antifungal treatment or antifungal prophylaxis at the time of bronchoscopy (n = 329; 62%). Out of these 329 cases, 89 cases (16.8%) were on mold-active antifungal prophylaxis (65 [73%] on voriconzole, 10 [11.2%] on caspofungin, 8 [9%] on posaconazole, 5 [5.6%] on amphotericin B, and 1 [1%] on anidulafungin) (Table 2).

Characteristics of Patients According to the EORTC/MSG Classification

According to the EORTC/MSG classification, proven, probable, possible, and no IFD was present in 6 (1.1%), 45 (8.5%), 182 (34.3%), and 297 (56.0%) of the cases, respectively. Histologic examination of surgical lung biopsy specimen was available in 26 patients. A total of 144 (27%) of the cases were on empirical antifungal treatment. Thereby, antifungal treatment was administered to 5 of 6 (83.3%) of the cases with proven, 22 of 45 (48.9%) of the cases with probable, and 67 of 182 (36.8%) of the cases with possible IFD, respectively.

There were significant differences in circulating leukocyte and neutrophil cell counts and in C-reactive protein levels among diagnostic groups ($P < 0.01$ for all). Similarly, cytologic analysis of BAL revealed differences in absolute cell counts ($P = 0.036$) and the percentage of lymphocytes ($P = 0.009$) among patients classified according to the EORTC/MSG classification.

Diagnostic Performance of BAL GM

BAL GM was positive in 155 cases (29.2%). From these there were 3 of 6 (50%) in proven, 22 of 45 (48.9%) in probable, 55 of 182 (30.2%) in possible, and 75 of 297 (25.2%) in no IFD. The diagnostic performance of BAL GM according to the EORTC/MSG classification, radiologic, and clinical suspicion of IFD is presented in Table 3. BAL GM provided 50% sensitivity, 73.0% specificity, 16% PPV, 93% NPV, 1.861 positive likelihood ratio, and 0.70 negative likelihood ratio for proven+probable IFD; corresponding values for proven IFD were 50%, 71%, 2%, 99%, 1.72, and 0.70. The test performance improved its sensitivity when serum GM was disregarded as a criterion to define probable IFD. There was no difference in the test performance when excluding second episodes of the disease. The area under the receiver operating characteristic curve for the diagnosis of proven+probable IFD was 0.716 (95% CI, 0.638–0.794; $P < 0.001$).

There was no significant difference in BAL GM positivity between allogeneic and autologous stem cell transplant recipients (28.8% vs. 40%; $P = 0.484$). Patients receiving empirical antifungal treatment for recent (≤48 h) suspicion of IFD presented more commonly positive BAL GM results (41.4% on treatment vs. 24.5% no treatment; $P < 0.001$) and higher median BAL GM levels (0.4 [0.2–0.6] vs. 0.3 [0.2–0.4]; $P < 0.001$). However, when both antifungal prophylaxis (including mold-active and inactive drugs) and empirical antifungal treatment were considered as an antifungal treatment, neither BAL GM positivity (31.7% on antifungal therapy vs. 24.6% no antifungal therapy; $P = 0.08$) nor median BAL GM levels (0.3 [0.2–0.5] vs. 0.3 [0.2–0.4]; $P = 0.121$) differed significantly between both groups.

Patients on antibiotic therapy at the time of bronchoscopy had more commonly positive BAL GM results (34.8% on treatment vs. 25.2% no treatment; $P < 0.021$) and had higher median BAL GM levels (0.3 [0.2–0.6] vs. 0.3 [0.3–0.5]; $P = 0.002$). There was no difference in BAL GM

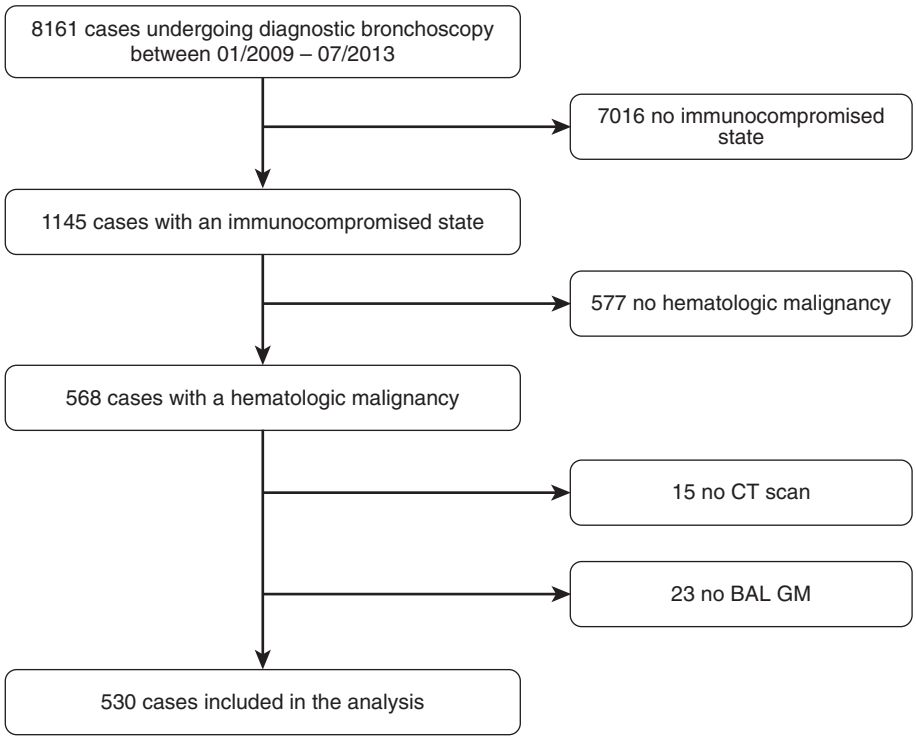

Figure 1. Study design according to the CONSORT guidelines. BAL = bronchoalveolar lavage; CT = computed tomography; GM = galactomannan.

positivity and levels between patients treated with tazobactam/piperacillin and other antibiotics including or not those on antibiotic prophylaxis with trimethoprim/sulfamethoxazol (P = ns for all).

Results of the logistic univariate and multivariate regression models are depicted in Table 4. In the univariate analysis, antibiotic and empirical antifungal treatment and probable IFD (as compared with no IFD) were significantly associated with a positive BAL GM test. In the multivariate analysis including all variables selected *a priori*, only empirical antifungal treatment demonstrated an independent, significant association with a positive GM determination in the BAL. A further model exploring only variables significantly associated with BAL GM positivity in the univariate analysis confirmed the association of empirical antifungal treatment (odds ratio, 1.943; 95% CI, 1.2–3.147; P = 0.007) and probable IFD (as compared with no IFD; odds ratio, 2.366; 95% CI, 1.203–4.653) with BAL GM positivity, whereas antibiotic therapy at bronchoscopy lost significance after adjustment (odds ratio, 1.061; 95% CI, 0.672–1.676; P = 0.798).

In 170 (32%) of the cases, there was a strong clinical suspicion of IFD in the chest CT scan according to the EORTC/MSG classification and from those, 58 (34%) had a positive BAL GM. Detailed information on radiologic findings is depicted in Table 5.

Aspergillus culture in the BAL was positive in 26 cases (4.9%). These cases presented more often BAL GM positivity (76.9% vs. 26.7%; $P < 0.001$) and higher BAL GM levels (4.7 [0.48–8.0] vs. 0.3 [0.2–0.5]; $P < 0.0001$). Aspergillus was identified as *A. fumigatus* in 15 cases (13 probable, 2 proven IFD), *A. niger* in two cases (possible IFD), *A. glaucus* in one case (probable IFD), and *A. nidulans* in one case (probable IFD).

Serum GM was assessed in 271 (51.1%) cases. In 33 (12.2%) cases, GM was considered positive (⩾0.5 ng/ml), from which 10 (30.3%) had a positive BAL GM. There was a weak correlation between serum and BAL GM (r = 0.265; $P < 0.001$), suggesting that only 7% of the variability of BAL GM depends on serum GM. The diagnostic performance of BAL GM was similar in the subgroup of patients undergoing serum GM testing and in the overall population. Accordingly, for the group proven+probable (n = 47) versus possible+no (n = 224) IFD, sensitivity was 45% (30–60%), specificity 71% (64–76%), PPV 24% (16–35%), NPV 86% (80–91%), positive likelihood ratio 1.52 (1.04–2.21), and negative likelihood ratio 0.78 (0.60–1.03).

Discussion

In this analysis we report the diagnostic performance of BAL GM for predicting IFD in a large, well-characterized cohort of hematologic patients. We found that, as compared with the EORTC/MSG classification, BAL GM has only a modest accuracy for the diagnosis of IFD. Accordingly, BAL GM results were poorly associated with radiologic suspicion of fungal disease or serum GM. Finally, and in contrast to previous reports, empirical antifungal treatment at the time of bronchoscopy seems not to decrease BAL GM results positivity. Taken together, BAL GM seems to have limited clinical usefulness for diagnosing and excluding IFD.

This is the largest study evaluating the diagnostic accuracy of BAL GM in hematologic patients with pulmonary symptoms undergoing diagnostic bronchoscopy. Our results stand in contrast to several and in accordance with a few previous studies. Luong and coworkers (20) report the sensitivity and specificity of BAL GM to be 100% and 87%, respectively. Interestingly, the sensitivity of the BAL GM remained 100% regardless of which cutoff has been used. In that study, BAL GM was significantly superior to BAL fungal microscopy (sensitivity, 58%), fungal culture (sensitivity, 75%), and serum GM (sensitivity, 58%). The authors proposed, thus, that a negative BAL GM strongly supports the absence of IFD. Similarly, Guo and coworkers (18) and Avni and coworkers (26) reported corresponding sensitivities and specificities of 90% and 94% and 77% and 93%, respectively. In contrast to these previously reported data, we obtained a much poorer overall performance of the BAL GM with a sensitivity of 50% and specificity of 73%. In line with our results, Racil and coworkers (21) reported similar findings in an observational study including 230 cases with hematologic disease. Moreover, Bergeron and coworkers (17) also reported a limited sensitivity (57%) but higher specificity (95%).

Table 1. Demographics of 530 Immunosuppressed Patients Undergoing BAL for Suspicion of IFD Classified According to the EORTC/MSG

Patient Characteristics	All	Possible	Probable	Proven	No
Demographics					
Patient number	530 (100)	182 (34.3)	45 (8.5)	6 (1.1)	297 (56.0)
Age, yr	54 ± 13.6	56 ± 15.6	50 ± 13.6	44 ± 10.3	54 ± 13.6
Male sex	353 (66.6)	126 (69.2)	34 (75.6)	0 (0)	193 (64.9)
Hospitalized	352 (66.4)	145 (79.7)	42 (93.3)	6 (100)	159 (53.5)
Symptoms					
Fever	151 (28.5)	67 (36.8)	18 (40.0)	2 (33.3)	65 (21.9)
Cough	274 (51.7)	92 (50.5)	22 (48.9)	3 (50)	157 (52.8)
Dyspnea	129 (24.3)	35 (19.2)	9 (20.0)	3 (50)	82 (27.6)
Sputum	107 (20.1)	46 (25.3)	5 (11.1)	1 (16.7)	55 (18.5)
Chest computed tomography scan					
Infiltrates	286 (54.0)	103 (56.6)	28 (62.2)	3 (50.0)	152 (51.1)
Suspicion of fungal lesion	170 (32)	125 (68.7)	32 (71.1)	5 (83.3)	8 (2.7)
Stem cell transplantation					
Allogeneic	336 (63.4)	106 (58.2)	33 (73.3)	4 (66.7)	193 (64.9)
<100 d after allogeneic HSCT	99 (18.6)	40 (22.0)	20 (44.4)	0 (0)	39 (13.1)
Autologous	17 (3.2)	5 (2.7)	0 (0)	1 (16.7)	11 (3.7)
Immunosuppressive therapy					
Steroids	194 (36.6)	65 (35.7)	25 (55.6)	4 (66.7)	100 (33.6)
Ciclosporine	137 (25.8)	51 (28.0)	14 (31.1)	0 (0)	72 (24.3)
Tacrolimus	82 (15.4)	14 (7.7)	8 (17.7)	0 (0)	60 (20.2)
Mycophenolate	73 (13.7)	22 (12.1)	4 (8.9)	0 (0)	47 (15.8)
Azathioprine	1 (0.1)	0 (0)	0 (0)	0 (0)	1 (0.3)
Chemotherapy	115 (21.6)	51 (28.0)	11 (24.4)	0 (0)	53 (17.8)
Antimicrobial therapy at bronchoscopy including prophylaxis					
Antibacterial	423 (79.8)	142 (78.0)	41 (91.1)	5 (83.3)	234 (79.1)
Antifungal	329 (62.0)	114 (62.6)	31 (68.9)	5 (83.3)	179 (60.2)
Empirical antimicrobial therapy at bronchoscopy					
Antibacterial	181 (34)	72 (39.6)	26 (57.8)	3 (50)	80 (26.7)
Antifungal	144 (27)	67 (36.8)	22 (48.9)	5 (83.3)	50 (16.7)
Disease					
Acute myelogenous leukemia	160 (30.2)	52 (28.6)	15 (33.3)	1 (16.7)	92 (31.0)
Multiple myeloma	63 (11.9)	17 (9.3)	7 (15.6)	1 (16.7)	38 (12.8)
Lymphoma	89 (16.8)	34 (18.7)	6 (13.3)	2 (33.3)	47 (15.8)
Myelodysplasia	60 (11.3)	21 (11.5)	5 (11.1)	1 (16.7)	33 (11.1)
Chronic lymphocytic leukemia	58 (10.9)	29 (15.9)	2 (4.4)	1 (16.7)	27 (9.1)
Acute lymphoblastic leukemia	45 (8.5)	14 (7.7)	3 (6.7)	1 (16.7)	27 (9.1)
Chronic myelogenous leukemia	30 (5.7)	7 (3.8)	3 (6.7))	0 (0)	20 (6.7)
Others	25 (4.7)	8 (4.4)	4 (8.9)	0 (0)	13 (4.4)
Microbiology					
Aspergillus culture BAL	26 (4.9)	3 (1.6)	18 (40.0)	2 (33.3)	3 (1.0)
Bacteriology BAL	103 (19.4)	37 (20.3)	11 (24.4)	1 (16.7)	50 (17.9)
GM					
BAL GM, ng/ml	0.3 (0.20–0.40)	0.30 (0.20–0.50)	0.40 (0.30–4.20)	0.40 (0.25–8.15)	0.30 (0.20–0.50)
Positive BAL GM	155 (29.2)	55 (30.2)	22 (48.9)	3 (50)	75 (25.2)
Blood GM, ng/ml	0.2 (0.20–0.3)	0.2 (0.20–0.30)	0.5 (0.33–0.70)	0.4 (0.25–0.70)	0.2 (0.20–0.30)
Positive blood GM	33 (6.2)	6 (3.3)	25 (55.6)	2 (33.3)	0 (0)
Laboratory					
C-reactive protein, mg/L	47.0 (13.9–115.0)	53.40 (16.2–121.5)	45.00 (9.5–119.0)	90.75 (34.5–344.3)	15.20 (3.5–70.6)
Leukocytes, $\times 10^3$/L	4.1 (0.8–8.1)	3.95 (1.0–8.4)	4.00 (1.4–8.4)	5.90 (1.5–8.3)	6.30 (3.9–9.9)
Neutrophils, $\times 10^3$/L	2.8 (0.47–5.48)	2.80 (0.5–5.4)	2.90 (0.5–6.3)	4.49 (1.4–6.4)	3.90 (2.1–6.0)
Neutropenia, <0.5	111 (20.9)	55 (30)	13 (29.5)	1 (16.6)	42 (14.1)
BAL					
BAL cells, $\times 10^6$/L	126.3 (56.4–246.5)	142.0 (58.15–272.1)	131.3 (71.7–245.0)	212.0 (72.0–371.3)	111.1 (54.0–220.3)
Macrophages, $\times 10^6$/L	74.0 (43.3–90.0)	75.0 (45.0–92.5)	81.5 (53.0–92.2)	55.0 (31.5–78.8)	71.5 (40.0–90.0)
Lymphocytes, $\times 10^6$/L	6 (3.0–15.8)	5.0 (2.0–16.0)	4.0 (1.5–12.0)	2.5 (2.0–4.3)	7.0 (3.0–19.0)
Neutrophils, $\times 10^6$/L	7 (2.0–33.0)	6.00 (2.0–36.0)	6.0 (1.0–36.0)	42.5 (15.0–66.3)	7.0 (2.0–31.0)
Eosinophils, $\times 10^6$/L	0.0 (0.0–0.0)	0.0 (0.0–0.00)	0.0 (0.0–0.3)	0.0 (0.0–1.3)	0.0 (0.0–0.5)

Definition of abbreviations: BAL = bronchoalveolar lavage; EORTC/MSG = European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; GM = galactomannan; HSCT = human stem cell transplantation; IFD = invasive fungal disease.
Data are given as absolute counts (%), means ± SD, or medians (interquartile range).

Table 2. Mold-Active Antifungal Prophylaxis at the Time of Bronchoscopy in 530 Cases of Immunocompromised Patients with Hematologic Malignancies

Mold-Active Antifungal Prophylaxis	Possible (*n = 182*)	Probable (*n = 45*)	Proven (*n = 6*)	No (*n = 297*)
Voriconazole	36	10	0	19
Posaconazole	3	2	1	2
Caspofungin	3	2	0	5
Amphotericin B	2	0	1	2
Anidulafungin	0	0	0	1

One potential explanation for the poor sensitivity of BAL GM could be mold active treatment. Of interest, we found rather more commonly positive BAL GM results and higher median BAL GM levels in patients with acute suspicion of IFD receiving antifungal drugs during less than 48 hours. So far, the role of antifungal prophylaxis and treatment in the performance of BAL GM remains unclear. Although most data show a significant decrease of the performance of BAL GM (27, 28), there is also some evidence that empirical antifungal treatment does not affect BAL GM (21, 29). Our results suggest that acute (≤48 h) antimold therapy does not diminish detection of BAL GM. Indeed, empirical antifungal treatment was independently associated with an increased risk of a positive GM test in the multivariate analysis. Nevertheless, the paucity of untreated cases with proven and probable IFD further hampers conclusions on the influence of drug therapy on test performance. Accordingly, all patients with proven IFD and negative BAL GM were on empirical active antifungal treatment (one posaconazol, two voriconazol). One of the three cases with positive BAL GM and proven IFD was not on active antifungal treatment at the time of bronchoscopy, whereas both other cases were on treatment (caspofungin and amphotericin B).

It is also tempting to hypothesize that the selection of patients considered "at risk" for fungal disease and undergoing BAL might have affected the overall performance of the test (selection bias). However, per definition, the prevalence of the disease does not affect sensitivity and specificity of the test. We persuade a liberal approach, offering diagnostic lavage to all immunosuppressed patients fulfilling host-criteria, which might have diminished the pretest probability for the disease. Conversely, most of the previous reports did not provide information on the number of screened cases considered for inclusion (CONSORT guidelines), thus precluding inferences on PPVs and NPVs of the test. Additionally, some studies restricted inclusion to patients with suspected radiologic lesion on conventional chest radiograph and/or did not report on findings of the CT scans. Finally, although BAL GM was examined on its diagnostic accuracy, it has been misleadingly included as diagnostic criterion for the EORTC/MSG classification as well, thus artificially increasing the number of probable cases of IFD.

In this study, the diagnostic performance of BAL GM was calculated based on the suggested cutoff of greater than or equal to 0.5, as previously described (12). We have also examined whether the use of a different cutoff, for instance 1, could improve the clinical usefulness of the test. Whereas sensitivity remained similar (proven 50%, proven+probable 41%, proven+probable+possible 19%), specificity improved (proven 86%, proven+probable 88%, proven+probable+possible 88%). Thus, the cutoff 1 allows excluding IFD with a higher accuracy as compared with the cutoff 0.5.

The performance of BAL GM improved in regards to its sensitivity when serum GM was disregarded for defining probable disease. This means that the probability of a patient with positive BAL GM determination to present IFD was higher in cases with a positive mycologic examination of the BAL as compared with

Table 3. Diagnostic Performance of Galactomannan Determination in the Bronchoalveolar Lavage in 530 Cases of Immunocompromised Patients with Hematologic Malignancies

	Sensitivity	Specificity	PPV	NPV	PLR	NLR
As compared with EORTC/MSG						
Proven	0.50 (0.12–0.88)	0.71 (0.67–0.75)	0.02 (0.04–0.06)	0.99 (0.98–1.0)	1.72 (0.77–3.88)	0.70 (0.32–1.57)
Proven and probable	0.49 (0.35–0.63)	0.73 (0.69–0.77)	0.16 (0.11–0.23)	0.93 (0.90–2.48)	1.81 (1.32–2.48)	0.70 (0.53–0.92)
Proven and probable*	0.73 (0.55–0.87)	0.74 (0.70–0.78)	0.16 (0.10–0.22)	0.98 (0.96–0.99)	2.76 (2.14–3.56)	0.37 (0.21–0.65)
Proven and probable and possible	0.35 (0.29–0.41)	0.75 (0.70–0.80)	0.52 (0.44–0.60)	0.60 (0.54–0.65)	1.40 (1.07–1.82)	0.87 (0.77–0.97)
As compared with clinical judgment						
Receiving empirical antifungal treatment	0.42 (0.34–0.50)	0.75 (0.71–0.80)	0.39 (0.31–0.47)	0.78 (0.73–0.82)	1.69 (1.30–2.20)	0.77 (0.67–0.90)
Suspicion of IFD on radiologic studies	0.34 (0.27–0.42)	0.73 (0.68–0.78)	0.37 (0.30–0.46)	0.70 (0.65–0.75)	1.27 (0.97–1.67)	0.90 (0.79–1.02)

Definition of abbreviations: EORTC/MSG = European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; IFD = invasive fungal disease; NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV = positive predictive value.
*Classification disregarding serum galactomannan as a mycologic criterion for defining probable disease according to the EORTC/MSG.

Table 4. Logistic, Multivariate Analysis for Prediction of a Positive Galactomannan Test in the Bronchoalveolar Lavage of 530 Immunocompromised Patients with Hematologic Malignancies

Clinical Criteria	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Length of hospital stay before BAL	1.005 (0.995–1.014)	0.361	1.156 (0.535–2.196)	0.713
Days after HSCT	1.184 (0.714–1.962)	0.513	1.000 (0.985–1.015)	0.985
Neutropenia, yes vs. no	0.704 (0.436–1.139)	0.153	0.407 (0.157–1.055)	0.064
Age, yr	0.999 (0.986–1.012)	0.845	0.990 (0.967–1.013)	0.399
Antibiotic therapy at bronchoscopy	1.565 (1.061–2.310)	0.024	1.423 (0.663–3.052)	0.365
Empiric antifungal treatment at bronchoscopy	2.180 (1.455–3.267)	<0.001	2.165 (1.038–4.518)	0.040
"No" IFD vs. possible IFD	1.282 (0.850–1.937)	0.236	0.849 (0.401–1.796)	0.668
"No" IFD vs. probable IFD	2.831 (1.492–5.372)	0.001	1.626 (0.673–3.928)	0.282
"No" IFD vs. proven IFD	2.960 (0.585–14.981)	0.190	0.645 (0.090–4.607)	0.662

Definition of abbreviations: BAL = bronchoalveolar lavage; CI = confidence interval; HSCT = human stem cell transplantation; IFD = invasive fungal disease; OR = odds ratio.

those with a positive serum GM test, suggesting that the mycologic examination of the BAL (cytology or direct microscopy or culture) is more reliable than serum GM for diagnosing IFD.

Remarkably, we found higher positivity and levels of BAL GM in patients receiving antibiotics at the time of bronchoscopy, with no difference between tazobactam/piperacillin and other antibiotic classes. We are not able to extrapolate on causality but rather assume that sicker and/or febrile patients tended to be treated more often with antibiotics. In this sense, our data do not support the notion that piperacillin-tazobactam is responsible for false-positive results on BAL and serum GM levels (30).

Our study has a few limitations. First, although we included a large cohort of hematologic patients with a thoughtful pulmonary evaluation including clinical, laboratorial, chest CT, and BAL results, this was a monocentric study and therefore our results might not be generalizable to other centers with a different patient population. Second, the number of proven cases in our study was relatively low and histologic examination was available in only 26 cases. We have not performed any other alternative test for diagnosing IFD, such as aspergillus polymerase chain reaction, because aspergillus polymerase chain reaction proved insufficient to diagnose IFD in our previous experience (8). In any case, the current study represents the largest series described so far in a hematologic cohort examining BAL GM.

Third, we have included a selected population fulfilling host criteria and undergoing BAL because of respiratory symptoms and/or radiologic abnormalities. In these patients, suspicion of IFD might have been higher than in patients in whom attending physicians refrained from performing diagnostic bronchoscopy. Fourth, serum GM was considered as a mycologic criterion to define probable disease in those patients, in whom the serum GM determination was performed according to the clinical indication as assessed by the treating physician. Theoretically, patients not undergoing serum GM testing had a lower chance of being upgraded to probable disease, because they would require a positive direct test (either cytology or direct microscopy or culture) to fulfill mycologic criteria. Nevertheless, all patients underwent bronchoscopy with all three direct mycologic tests. They could, therefore, still fulfill criteria for probable disease. Importantly, a positive serum GM "only" upgrades possible to probable disease; therefore, patients classified as no IFD would not become a possible IFD, even if serum GM was positive. Remarkably, specificity of BAL GM did not vary significantly among analyzed populations (overall, considering or disregarding serum GM, and only in patients in whom serum GM has been determined) and sensitivity of BAL GM improved slightly in the whole population when serum GM levels were disregarded.

Fifth, there are difficulties in defining the ideal control group in IFD. In this study, we have considered the whole population classified as nondiseased (either probable+possible+no, possible+no, or no, respectively) as the control group. Interestingly, exclusion of intermediate categories from the analysis (i.e., comparing only proven+probable with no without considering probable cases) would produce a selection bias, potentially generating results evidencing a stronger association between test and disease. Likewise, the selection of "clear cut" cases (only the ones

Table 5. Detailed Radiographic Findings in 530 Cases of Immunocompromised Patients with Hematologic Malignancies

Radiologic Findings	Possible (*n* = 182)	Probable (*n* = 45)	Proven (*n* = 6)	No (*n* = 297)
Infiltrate	103	28	3	152
Nodule	26	6	1	21
Cavern/air crescent	3	4	0	0
Halo sign	25	2	1	0
Tree-in-bud/mucus plugging	2	0	0	9
Ground glass	16	2	1	21

with a clean chest CT, negative serum GM, negative sputum culture, and not receiving mold-active antifungal prophylaxis/treatment) would generate "ideal" control subjects. However, these patients are not found among the population requiring the diagnostic test (in our population, only 18 cases out of 530 [3.4% cases] would qualify according to these criteria) and therefore the performance of the test would be artificially improved. The same rational applies to the comparison with untreated-only patients.

Finally, and most importantly, the study suffers from a main limitation: the lack of a true gold standard for investigating IFD. Thus, although we have compared BAL GM with the EORTC/MSG classification, currently considered the gold standard for diagnostic purposes, it does not necessarily definitely rule out the presence of IFD (31). Nonetheless, this study reflects the "real world" situation that carrying physicians deal with when they use this test. Strengths of the study were the prospective data accrual, complete patient characterization, large sample size, and homogenous patient population.

In summary, at our center, GM in BAL fluid had modest agreement with EORTC/MSG criteria for diagnosing IFD in immunocompromised patients with a high degree of prior antifungal exposure. Thus, we recommend caution in using this assay for treatment guidance in immunocompromised patients. ■

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

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