Use of Bronchoalveolar Lavage To Detect Galactomannan for Diagnosis of Pulmonary Aspergillosis among Nonimmunocompromised Hosts

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Pulmonary aspergillosis in nonimmunocompromised hosts, although rare, is being increasingly recognized. The diagnosis of pulmonary aspergillosis is difficult, since the recovery of Aspergillus from respiratory samples cannot differentiate colonization from invasion. We assessed the role of bronchoalveolar lavage (BAL) in detecting galactomannan (GM) for diagnosing pulmonary aspergillosis in 73 nonimmunocompromised patients with pulmonary infiltrates for whom the test was ordered. Six patients had pulmonary aspergillosis, two each with acute invasive pulmonary aspergillosis, chronic necrotizing pulmonary aspergillosis, and aspergilloma. All six patients had a BAL GM level of ≥1.18. The sensitivity, specificity, and negative predictive value (NPV) for a BAL GM level of ≥1.0 were 100%, 88.1%, and 100%, respectively. Notably, the positive predictive value (PPV) was only 42.9%, likely reflecting the low prevalence of pulmonary aspergillosis among nonimmunosuppressed patients. The combination of BAL microscopy and culture had a sensitivity and NPV similar to those of BAL GM detection but a higher specificity and PPV (92.5% and 54.6%, respectively). Moreover, a BAL GM test did not identify any cases that were not diagnosed by conventional methods like microscopy and culture. In conclusion, there was no conclusive benefit of determining BAL GM levels in the diagnosis of pulmonary aspergillosis among nonimmunocompromised hosts. Given the likelihood of false-positive results, a BAL GM test should not be ordered routinely in this population.

Invasive infections of lung parenchyma by Aspergillus are rare diseases outside of patients with hematologic malignancies and those who have undergone hematopoietic stem cell transplantation (HSCT) and solid-organ transplantation (SOT) (4, 10, 20). At present, lung biopsies are the diagnostic “gold standard” but are limited by sensitivity and complications (13). While the isolation of Aspergillus species from the respiratory tracts of high-risk patients is predictive of pulmonary aspergillosis (6, 21), culture is limited by poor sensitivity.

A commercially available double-sandwich enzyme-linked immunosorbent assay that detects galactomannan (GM), a cell wall polysaccharide of Aspergillus (Platelia ELISA; Bio-Rad), has been introduced to improve the diagnosis of invasive aspergillosis (5). The sensitivity of a serum GM test is 61% to 71% among HSCT recipients and patients with hematologic malignancies (14) and 30 to 56% among SOT recipients (3, 7, 9). A few studies have assessed the diagnostic utility of bronchoalveolar lavage (BAL) GM testing. Among HSCT recipients and patients with hematologic malignancies, BAL GM testing added to the sensitivity of both BAL culture and serum GM testing (12, 15–18). We recently found similar results among SOT recipients, although false-positive tests due to airway colonization with Aspergillus were common among patients with lung transplants (2).

Over the past decade, invasive aspergillosis has been increasingly recognized in nonimmunosuppressed patients. In these patients, the types of pulmonary aspergillosis include acute invasive pulmonary aspergillosis (IPA), a rapidly progressive infection similar to that of profoundly immunosuppressed hosts (2, 11), chronic necrotizing pulmonary aspergillosis (CNPA), a slowly progressive infection often seen among patients with underlying lung disease (8, 19), and mycetoma or fungus ball. The diagnosis of IPA or CNPA is particularly difficult since the presence of Aspergillus in culture or by microscopy cannot differentiate colonization from invasive disease. The objective of this study was to assess the utility of the BAL GM test in the diagnosis of pulmonary aspergillosis among nonimmunosuppressed patients.

MATERIALS AND METHODS

Identification of patients. We reviewed all cases at the Shands Teaching Hospital at the University of Florida in which BAL fluid was tested for GM between September 2004 and August 2006. We excluded patients who were immunosuppressed, which was defined as neutropenia (absolute neutrophil count of <1,000 neutrophils/mm³), histories of hematologic malignancy, HSCT, SOT, human immunodeficiency virus infection, and the receipt of corticosteroids or other immunosuppressive agents within 6 months of the diagnosis of pulmonary aspergillosis.

BAL GM determination. A BAL GM test was ordered at the discretion of physicians caring for the patients. The BAL fluid was sent on dry ice via overnight mail to MiraVista Diagnostics (Indianapolis, IN) for GM assay using a Platelia Aspergillus enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA). The BAL GM results were made available to the patients’ physicians. Decisions about the institution of antifungal therapy were made by the patients’ physicians and required approval by the infectious disease consultation team.

Case definitions. The classification of invasive aspergillosis into categories of proven, probable, and possible IPA using modified European Organization for Research and Treatment of Cancer-Mycoses Study Group criteria cannot be
<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Underlying disease(s)</th>
<th>Reason(s) for BAL</th>
<th>Antibiotic(s) used prior to or at the time of BAL</th>
<th>CXR/CT scan result</th>
<th>BAL GM level(s)</th>
<th>Serum GM level(s)</th>
<th>Microscopy result, TBBX result</th>
<th>Culture</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Outcome at follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>66 (M)</td>
<td>CAD, DM, and obesity; admitted with acute pneumococcal sepsis</td>
<td>Fever, septic shock</td>
<td>CFP, Levo, Vanc</td>
<td>Bil parenchymal opacity, diffuse GGO, nodule</td>
<td>1.18</td>
<td>1.41 (+1 day)</td>
<td>Inflammatory cells (yeasts), not done</td>
<td>BAL, <em>A. flavus</em>; sputum (~1 day), <em>A. flavus</em></td>
<td>Proven IPA</td>
<td>No antifungal</td>
<td>Died, 4 days</td>
</tr>
<tr>
<td>61 (M)</td>
<td>Previously healthy; admitted with severe upper GI bleed</td>
<td>Shock</td>
<td>CFP, Metro, Vanc, Tim</td>
<td>Nodular infiltrates</td>
<td>8.44</td>
<td>2.58 (~4 days), 2.69 (~2 days)</td>
<td>Not done, not done</td>
<td>BAL, <em>A. fumigatus</em>; sputum (~3 days), <em>A. fumigatus</em></td>
<td>Presumed IPA</td>
<td>VORI until death</td>
<td>Died, 10 days</td>
</tr>
<tr>
<td>67 (F)</td>
<td>COPD; cavitary lung lesion was found on admission CXR</td>
<td>Respiratory symptoms for 4 mo and cavitary lung lesion</td>
<td>Cipro, Metro</td>
<td>Consolidation with cavitary infiltrates</td>
<td>2.0; after antifungal treatment, –0.62 (+19 days)</td>
<td>0.05 (+5 days)</td>
<td>Hyphae, inflammation and necrosis; hyphae seen in tissue</td>
<td>BAL, <em>A. fumigatus</em>; <em>A. fumigatus</em></td>
<td>Proven CNPA</td>
<td>VORI and antibiotics until death</td>
<td>Died, 2 mo</td>
</tr>
<tr>
<td>56 (M)</td>
<td>Healthy, admitted after a stroke; cavitary lung lesion was found on admission CXR</td>
<td>Wt loss, cough, hemoptysis, abnormal CXR</td>
<td>None</td>
<td>Cavitary lesion with surrounding consolidation</td>
<td>7.41, 7.34</td>
<td>0.98 (~2 days)</td>
<td>Hyphae, chronic granulomatous inflammation, presence of eosinophils (no hyphae)</td>
<td>BAL, <em>A. fumigatus</em>; sputum (~3 days), <em>A. fumigatus</em></td>
<td>Probable CNPA</td>
<td>VORI for 1.3 yr</td>
<td>Lived (F/u, 1.3 yr)</td>
</tr>
<tr>
<td>51 (M)</td>
<td>Lung cancer, in remission for 5 yr; admitted for hemoptysis</td>
<td>Fever, respiratory complaints, and hemoptysis</td>
<td>Gati</td>
<td>Cavitary lesion with fungus ball; surrounding consolidation</td>
<td>1.43, 1.15</td>
<td>0.08 (~3 days), 0.11 (~4 days), 0.08 (~5 days)</td>
<td>No hyphae, not done</td>
<td>BAL, <em>A. fumigatus</em>; <em>A. fumigatus</em></td>
<td>Proven aspergillosis; MAI pneumonia and cavitary lesion</td>
<td>VORI for 1 yr and Rx for MAI</td>
<td>Lived (F/u, 1 yr)</td>
</tr>
<tr>
<td>46 (M)</td>
<td>Crohn’s colitis, in remission, MAI pneumonia on treatment; admitted with hemoptysis</td>
<td>Fever, respiratory complaints, and hemoptysis</td>
<td>Mero and meds against MAI</td>
<td>Cavitary lung lesion with fungus ball</td>
<td>8.89 (LLL), 8.64 (RL)</td>
<td>Not done</td>
<td>Inflammatory cells (no hyphae), chronic and acute inflammation (no hyphae)</td>
<td><em>A. fumigatus</em></td>
<td>Proven aspergillosis; MAI cavitary lung lesion</td>
<td>VORI for 6 mo and Rx for MAI</td>
<td>Lived (F/u, 6 mo)</td>
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Abbreviations: TBBX, transbronchial biopsy; M, male; F, female; CAD, coronary artery disease; DM, diabetes mellitus; COPD, chronic obstructive lung disease; AAA, abdominal aortic aneurysm; CXR, chest X ray; Bil, bilateral; GGO, ground glass opacification; MAI, *Mycobacterium avium*-*Mycobacterium intracellulare*; RLL, right lower lobe; LLL, left lower lobe; RL, right lung; F/u, follow-up time; Rx, treatment; meds, medications; CFP, cefipime; Levo, levofloxacin; Vanc, vancomycin; Cipro, ciprofloxacin; Gati, gatifloxacin; Metro, metronidazole; TBBX, transbronchial biopsy; Mero, meropenem; VORI, voriconazole.

Date given relative to the initial BAL (e.g., +1 day indicates 1 day after BAL, and –1 day indicates 1 day before BAL).

Fungal hyphae were found within the lung parenchyma and blood vessels upon autopsy.

BAL was performed on several lung specimens.
Proven aspergilloma. In the absence of Aspergillusious causes that might account for the pulmonary findings had to be ruled out. Re- demonstrated within the tissue specimen; in this setting, other infectious or noninfectious causes that might account for the pulmonary findings had to be ruled out. The diagnosis was presumed acute IPA when invasive hyphae were not demonstrated within the tissue specimen and accompanied by the growth of Aspergillus spp. were isolated from the sputum or BAL fluid, the diagnosis was mycetoma, presumably aspergilloma. Subacute was defined as a duration of pulmonary symptoms of 1–3 months, and acute was defined as a pulmonary infection (duration of ≤ 1 month) associated with the demonstration of septated hyphae invading lung parenchyma on biopsy specimens and accompanied by the growth of Aspergillus spp. (3). The two investigators independently reviewing the cases.

Proven CNPA was defined as a subacute infection associated with the demonstration of septated hyphae invading lung parenchyma on biopsy specimens and accompanied by the growth of Aspergillus in culture. For our purposes, subacute was defined as a duration of pulmonary symptoms of >1 month. The diagnosis was presumed CNPA when invasive hyphae were not demonstrated within the tissue specimen; in this setting, other infectious or noninfectious causes that might account for the pulmonary findings had to be ruled out by the treating physicians using standard diagnostic approaches such as BAL cultures and microscopy with or without transbronchial biopsy, other respiratory cultures, blood cultures or cultures from other sites, and treatment and response to nonfungal therapy. Alternative diagnoses to pulmonary aspergillosis were based on the consensus opinions of the physicians managing the patients as well as the two investigators independently reviewing the cases.

Proven acute IPA was defined as a pulmonary infection (duration of ≤ 1 month) associated with the demonstration of septated hyphae invading lung parenchyma on biopsy specimens and accompanied by Aspergillus culture growth. The diagnosis was presumed acute IPA when invasive hyphae were not demonstrated within the tissue specimen; in this setting, other infectious or noninfectious causes that might account for the pulmonary findings had to be ruled out.

Data analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated on a per-patient basis for BAL GM testing, serum GM testing, BAL microscopy, and culture. The optimal cutoff for BAL GM testing was determined using Fisher’s exact test and expressed in two-by-two contingency tables; P values of ≤0.05 were considered to be significant.

RESULTS

Description of patient population. Over a 2-year period at our university medical center, a BAL GM test was ordered for 73 patients who were not known to be immunosuppressed. Twenty-seven patients were previously healthy, and 14 patients had underlying lung diseases (chronic obstructive pulmonary disease, asthma, or bronchiectasis [nine patients]; primary pulmonary hypertension [two patients]; chronic cough [two patients]; and cystic fibrosis [one patient]). Six patients had pulmonary aspergillosis (Table 1); two of these patients had acute IPA, two patients had CNPA, and two patients had aspergilloma.

Performance of BAL GM testing. Twenty-two patients had a BAL GM level of ≥0.5, and 15 patients had BAL GM levels of
TABLE 3. Description of patients with BAL GM levels of ≥1 but with no evidence of pulmonary aspergillosis

| Age (yr) | Underlying disease(s) | Reason(s) for BAL | Antibiotic(s) prior to or at the time of BAL | Chest X-ray/ct scan result | BAL GM level(s) | Serum GM level(s) | Microscopy result, TBBX result | Culture result | Diagnosis | Treatment (duration) | Outcome |
|---------|-----------------------|-------------------|---------------------------------------------|-----------------------------|------------------------|-----------------|---------------------|-------------------------------|---------------|-----------|----------------------|---------|
| 52 (M)  | Healthy               | Fever, respiratory symptoms | CTX, AZI | Micronod IF, air space disease, med LN | 1.04 | Not done | Hyphae, chronic inflammation | Aspergillus fumigatus, Aspergillus niger | CAP | No antifungal, antibiotics | Lived (F/u, 6 mo) |
| 48 (F)  | COPD, pulmonary HTN   | SOB               | CTX, AZI | Multiple nodules, diffuse GGO, hilar LN | 1.33, 0.11 | 0.11 (+3 days), 0.05 (+2 m) | No hyphae, bronchiolitis (interstitial lung disease) | No fungi | Pulmonary HTN | No antifungal, antibiotics and steroid | Died (2 mo) |
| 22 (F)  | Healthy with miliary TB | Respiratory symptoms | CTX, AZI | New cavity lung lesion and tub | 0.32 (+12 days), 1.57 | 0.07 (+12 days), 0.11 | No hyphae, necrotizing granuloma | No fungi | Miliary TB | VORI, 5 days, TB medications | Died (19 days) (from disseminated tuberculosis) |
| 77 (M)  | HTN                   | Fever, respiratory symptoms | CTX, AZI | Airspace disease, hilar and med LN | 1.58 | 0.06 (+3 days) | No hyphae, BOOP | Candida | BOOP | No antifungal, steroid | Lived (F/u, 1 yr) |
| 52 (M)  | ICM                   | Respiratory symptoms | None | New cavity lesion with surrounding infiltrate, hilar LN | 2.38, 0.15 | 0.15 (+10 days), 0.09 (+12 days), 0.07 (+13 days) | No hyphae, nondiagnostic | No fungi | Lung cancer (adenocarcinoma) | VORI, 3 mo (based on BAL GM), antibiotics | Died (4 mo) (lung cancer) |
| 57 (M)  | CAD, HTN              | Respiratory symptoms | CTX, AZI | New cavity lesion, med LN | 2.67 | Not done | No hyphae, acute and organizing pneumonia | Aspergillus terreus | CAP, TB | No antifungal, antibiotics | Lived (F/u, 3 mo) |
| 2 (M)   | Healthy               | ARDS, fever       | CTX, Vanc | Consolidation | 2.89, 0.98 | 0.09 (+3 days), 0.14 (+5 days) | No hyphae, not done | No fungi | MRSA pneumonia on viral pneumonia | No antifungal, antibiotics | Lived (alive at d/c; no F/u available) |
| 64 (M)  | COPD                  | Chest pain, s/p falls | None | Mass with necrotic center | 4.98, 6.13 | 0.12 (+4 days) | Yeast, not done | Candida albicans | Disseminated community-acquired MRSA infection | No antifungal, antibiotics | Died (9 days) |
| 54 (F)  | Hepatitis C virus with ESLD | Weakness, encephalopathy, respiratory failure | Tim, Vanc | Consolidation (focal), diffuse GGO | 1.26, 0.37 | Not done | Hyphal elements and yeasts, not done | No fungi | Diffuse alveolar hemorrhage | No antifungal | Died (8 days) (hepatorenal syndrome and sepsis) |

a TBBX, transbronchial biopsy; M, male; F, female; Micronod IF, micronodular infiltrates; COPD, chronic obstructive pulmonary disease; SOB, shortness of breath; s/p, status post; HTN, hypertension; TB, tuberculosis; ICM, ischemic cardiomyopathy; CAD, coronary artery disease; ESLD, end-stage liver disease; BOOP, bronchiolitis obliterans with organizing pneumonia; LN, lymphadenopathy; GGO, ground glass opacity; med, mediastinal; CAP, community-acquired pneumonia; d/c, discharge; F/u, follow-up; CTX, ceftriaxone; AZI, azithromycin; Tim, timentin; Vanc, vancomycin; VORI, voriconazole.
b Date given relative to the initial BAL (e.g., +1 day indicates 1 day after BAL, and −1 day indicates 1 day before BAL).
c BAL was performed from several lung segments.
The GM level was increased from 43% to 33% (cutoff, ≥1.0). All six patients with pulmonary aspergillosis had BAL GM testing at various interpretive cutoffs are presented in Table 2. Of note, both patients with BAL GM levels of ≥0.5 concomitantly with a BAL GM level of ≥1.0. Seven patients did not receive antifungal agents; none had any further evidence of aspergillosis (data not shown). In addition, none received piperacillin-tazobactam or amoxicillin-clavulanate.

**DISCUSSION**

A review of the experience at our institution failed to demonstrate a benefit of BAL GM testing in the diagnosis of pulmonary aspergillosis in nonimmunocompromised hosts. A BAL GM test was highly sensitive in this population (100% at a cutoff of ≥1.0), with a good specificity (88.1%) and excellent NPV (100%). The test was limited, however, by a PPV of 43%, which reflects the low prevalence of pulmonary aspergillosis in this population. BAL GM testing was no more sensitive than the combination of BAL microscopy and culture and exhibited a lower PPV than these tests. Moreover, BAL GM testing did not identify any cases of IPA or CNPA that were not diagnosed by conventional methods like microscopy and culture and only increased the likelihood of obtaining false-positive results. Our findings differ from those of previous reports of BAL GM testing among hematologic malignancy, HSCT, and SOT patients, for whom the test generally added to the sensitivity of microscopy and culture and identified cases of pulmonary aspergillosis that were not diagnosed by these methods (2, 12, 15, 16, 18).

This review reiterates that pulmonary aspergillosis is a rare but often unrecognized cause of serious lung disease outside of high-risk groups (1). It is notable that both patients with IPA in our series had serum GM levels of >0.5 concomitantly with a BAL GM level of ≥1.18. These findings suggest that the combination of BAL and serum GM might be useful in subsets of nonimmunocompromised patients for whom IPA is a serious diagnostic consideration. We reported two nonimmunocompromised patients, for example, who developed biopsy-proven IPA following acute pneumonia due to influenza virus (2) and Streptococcus pneumoniae (this report). It is difficult to make definitive recommendations about specific patients who might benefit from the use of the BAL GM test. Nevertheless, clinicians might consider judicious GM testing among certain patients who have ongoing pneumonias of unclear etiology that have not responded to standard therapies.

Unlike IPA and CNPA, the cases of aspergilloma in our series were readily evident upon CT scan and BAL culture. Clearly, BAL GM testing does not have a diagnostic role in routine cases of mycetoma. Moreover, we did not demonstrate an association between elevated BAL GM levels and invasion from mycetomas into local parenchyma, as a tissue biopsy was not performed in one case and failed to demonstrate invasive hyphal elements in the second.

In conclusion, physicians should exercise restraint in ordering and interpreting BAL GM tests for nonimmunocompromised hosts in order to avoid overdiagnosing pulmonary aspergillosis and subjecting patients to unnecessary treatment. The observations from this study should be corroborated in prospective studies.

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L. J. Wheat is the President and Director of MiraVista Diagnostics, which performs BAL GM testing as a commercial reference service. The remaining authors do not report any conflict of interest.

**REFERENCES**


