Performance Characteristics of the Platelia Aspergillus Enzyme Immunoassay for Detection of Aspergillus Galactomannan Antigen in Bronchoalveolar Lavage Fluid

S. Husain,1 C. J. Clancy,1 M. H. Nguyen,1 S. Swartzentruber,3 H. Leather,2 A. M. LeMonte,3 M. M. Durkin,3 K. S. Knox,4 Ç. A. Hage,5 C. Bentsen,6 N. Singh,1 J. R. Wingard,4 and L. J. Wheat1*

University of Pittsburgh, Pittsburgh, Pennsylvania1; University of Florida College of Medicine, Gainesville, Florida2; MiraVista Diagnostics, Indianapolis, Indiana3; Southern Arizona VA Health Care System-University of Arizona, Tucson, Arizona4; Roudebush VA Medical Center-Indiana University School of Medicine, Indianapolis, Indiana5; and Bio-Rad Laboratories, Redmond, Washington6

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We have evaluated the Platelia Aspergillus enzyme immunoassay for detection of galactomannan in bronchoalveolar lavage (BAL) specimens in solid organ transplant patients with aspergillosis. The precision and reproducibility in serum or BAL to which galactomannan was added were similar. Sensitivity was 81.8% in patients with aspergillosis, and specificity was 95.8% in lung transplant patients who underwent BAL for surveillance for infection or rejection. Among transplant controls, positive results were more common in patients (i) who underwent diagnostic BAL performed for evaluation of symptoms or chest computed tomographic abnormalities, (ii) who had undergone lung transplantation, or (iii) who were colonized with Aspergil-

Invasive aspergillosis (IA) is common in hematopoietic stem cell and solid organ transplant patients, and the diagnosis often is determined based on bronchoalveolar lavage (BAL) (11). Galactomannan (GM) antigenemia detection is a useful method for the early diagnosis of IA (7, 11, 12). Several studies in hematologic patients show that detection of Aspergillus GM in BAL can be a sensitive method for the diagnosis of IA. The sensitivity was 89% or higher, and the specificity was 87 to 100% (1, 9, 10). In two studies that compared BAL and serum, sensitivity was lower in serum than in BAL: 47% versus 100% (1) and 44% versus 89%, respectively (9). False-positive results occurred in patients receiving piperacillin-tazobactam, in whom antigenemia also was present (9). False-positive results have also been reported when Plasmalyte was used to perform BAL (4). Positive BAL results may eliminate the need for additional invasive procedures in some patients (10).

We reported previously the detection of Aspergillus GM in BAL from solid organ transplant patients with IA (2, 6). We have further analyzed here those studies and provide additional support for use of BAL testing for diagnosis of IA, including a more detailed analysis of specificity, precision, and reproducibility.

MATERIALS AND METHODS

Clinical materials. The BAL specimens from patients with IA (n = 11) and controls (n = 185) have been previously described (2, 6). Four patients had proven and seven had probable IA, according to published criteria (3). BAL specimens from which Aspergillus or other mold was isolated were considered to be colonized if the patient failed to meet these criteria (i.e., if cultures were not associated with a mold resembling Aspergillus by cytology or histopathology of lung tissue, were not obtained from a sterile site, or were not associated with compatible abnormalities on chest computed tomography [CT] if isolated from a nonsterile site) (5).

Transplant controls included 119 lung transplant patients who underwent bronchoscopy to monitor for infection or rejection (surveillance BAL) and 66 patients with a variety of transplant types who underwent bronchoscopy for evaluation of symptoms or CT abnormalities (diagnostic BAL). The transplantation types included the lung in 26 patients, the kidney in 15 patients, the liver in 13 patients, and the heart in 12 patients (2, 6). Additional healthy control BAL specimens were collected from 56 asymptomatic individuals, including 44 with human immunodeficiency virus (HIV) infection and 12 with no underlying disease, which were obtained through a protocol approved by the Indiana University institutional research board.

Platelia Aspergillus enzyme immunoassay (EIA). The Platelia Aspergillus test was performed according to the manufacturer’s recommendations for testing serum (Bio-Rad Laboratories, Marnes-la-Coquette, France). BAL specimens were handled in a biosafety cabinet and were treated with EDTA at 104°C for 4 min, followed by centrifugation prior to testing. Serum samples were not processed in a biosafety cabinet. Specimens were tested real-time as they were received as part of clinical care for one of the studies (2) and in batch for the other study, which included consecutive lung transplant recipients who underwent BAL for surveillance for suspected rejection or infection or for evaluation of symptoms or CT abnormality (6). Results of >0.5 were verified by repeat testing the following day.

RESULTS

Precision and reproducibility were compared in serum and BAL by testing normal human serum or BAL that had been shown to be negative in the Platelia Aspergillus EIA, into which the positive kit control and cutoff control were reconstituted. In serum, the averages ± one standard deviation and the coefficient of variation (indicated in parentheses) of five aliquots of the positive control were 2.93 ± 0.35 (0.12) on day 1 and 3.23 ± 0.25 (0.08) on day 2. For the cutoff control, the
results were 0.61 ± 0.12 (0.19) on day 1 and 0.59 ± 0.06 (0.10) on day 2. In BAL, the average of five aliquots of the positive control were 3.40 ± 0.20 (0.06) on day 1 and 3.61 ± 0.09 (0.02) on day 2, while the averages of the cutoff control were 0.86 ± 0.05 (0.06) on day 1 and 0.91 ± 0.03 (0.04) on day 2.

Using the 0.5 or 1.0 cutoff, the sensitivity was 81.8% among patients with IA (Fig. 1). Only one sample per patient was used to calculate the sensitivity and specificity. The two false-negative results occurred in lung transplant patients who were receiving mold-active antifungal agents, itraconazole and voriconazole in one patient each, while the results were positive in all nine IA patients who were not receiving mold-active antifungal agents (P = 0.018; Fisher exact test). The effect of mold-active antifungal agents (amphotericin B administered intravenously or aerosolized, itraconazole, and voriconazole) also was assessed in patients who underwent diagnostic BAL for evaluation of symptoms or chest CT abnormalities or in whom Aspergillus or other mold was isolated from BAL but in which IA was not diagnosed. Among patients whose BALs were classified as diagnostic, GM was detected in 4 of 7 (57.1%) patients who were receiving mold-active antifungal agents compared to 5 of 59 (8.5%) who were not receiving them (P = 0.005). Among patients colonized with mold, GM was detected in 3 of 16 (18.8%) patients who were receiving mold-active antifungal agents compared to 5 of 40 (12.5%) who were not (P = 0.676). GM antigenemia was detected in one of four (25%) IA patients with paired BAL and serum.

Specificity in lung transplant patients who underwent surveillance BAL to monitor for rejection or infection was 95.8% using the 0.5 cutoff and 96.6% using the 1.0 cutoff (Table 1). Among the surveillance controls, GM was detected more often in specimens deemed to be colonized with Aspergillus (15.4%) or other mold (10%) than in specimens with negative cultures (1.2%) (Table 2). Most of the patients were receiving mold-active antifungal prophylaxis at the time of surveillance BAL, as they received prophylaxis for 3 months after transplantation.

In the diagnostic BAL group, positive results were noted in 27.3% at the 0.5 cutoff and 13.6% at the 1.0 cutoff. Positive results were more common after lung transplantation (46.2%) than after transplantation with other organs (15.0%; P = 0.010) (Table 2). In the lung transplant group, positive results were more common in specimens colonized with Aspergillus (60%) than in noncolonized specimens (38.5%; P = 0.414) or those colonized with other mold (33.3%; P = 0.559). Three of nineteen (15.8%) BALs containing Penicillium spp. and one containing Fusarium were positive. The results were negative in all four specimens containing Paecilomyces spp. Four of the eighteen (22.2%) lung transplant patients with positive BALs subsequently developed IA. Positive results were not observed in healthy subjects, none of whom were neutropenic or receiving immunosuppressive medications.

Four patients among the diagnostic controls were receiving piperacillin-tazobactam, of whom GM was detected in the BAL in three. These four were lung transplant patients, and two had positive cultures for Aspergillus or other mold (Paecilomyces). Serum was not tested for GM in these patients.

Among transplant controls from whom Aspergillus or other mold was isolated, 21.4% were positive at the 0.5 cutoff and 14.3% were positive at a 1.0 cutoff (Table 1). Of noncolonized

![FIG. 1. GM detection in BAL in patients with IA and controls. The broken horizontal line at 0.5 is the cutoff for positivity, and the number within the box (n) represents the number of patients with negative results. A dashed line at 1.0 is provided for reference for estimation of the accuracy at a higher cutoff.](image-url)

### TABLE 1. Platelia Aspergillus EIA results and culture and/or histopathology

| Group                        | GM 0.5 cutoff | GM 1.0 cutoff | Culture and/or histopathologya |
|------------------------------|---------------|---------------|--------------------------------|
| Invasive pulmonary aspergillosis | 9/11 (81.8%) (52.3–94.9) | 9/11 (81.8%) (52.3–94.8) | 8/11 (72.7%) (43.4–90.2) |
| Surveillance controlsb       | 5/119 (4.2%) (1.8–9.4) | 4/119 (3.4%) (1.3–8.4) | 13/119 (10.9%) (6.4–17.7) |
| Diagnostic controlsb         | 18/66 (27.3%) (18.0–39.1) | 8/66 (12.1%) (6.2–22.1) | 12/66 (18.2%) (10.7–29.2) |
| Surveillance and diagnostic  | 23/185 (12.4%) (8.4–17.9) | 12/185 (6.5%) (3.7–11.0) | 25/185 (13.3%) (9.3–19.2) |
| Noncolonized controls        | 11/150 (7.3%) (4.1–12.6) | 2/150 (1.3%) (0.4–4.7) | NA NA |
| Colonized controls (Aspergillus or mold) | 12/56 (21.4%) (12.7–33.8) | 8/56 (14.3%) (7.4–25.8) | NA NA |
| Healthy controls with HIV infection | 0/56 (0.0%) (0.0–6.4) | 0/56 (0.0%) (0.0–6.4) | NA NA |

a Surveillance BAL was performed for monitoring for rejection or infection after lung transplantation.
b Diagnostic BAL was performed for evaluation of symptoms or chest CT abnormalities.
c CI, confidence interval.
d NA, not applicable.
controls, BAL was positive at the 0.5 cutoff in 7.3% compared to 1.3% at a 1.0 cutoff. Some patients underwent several bronchoscopies but were evaluated only once per category, explaining why the total for this parameter is 206.

The negative predictive values (NPV) ranged from 97.3 to 99.0%, depending upon the cutoff for positivity and prevalence (Table 3). The NPV for culture or histopathology were slightly lower, ranging from 96.4 to 98.4%. Positive predictive values (PPV) for antigen detection were higher with the 1.0 cutoff than with the 0.5 cutoff, especially if the diagnostic BALs were included in the calculation. The PPV also was higher for antigen detection than for culture or histopathology in all of the comparisons except for diagnostic BALs at the 0.5 cutoff.

The reproducibility was assessed by comparing the results of the first and second test for all positive specimens. The reproducibility was 93.3% for specimens between 0.5 and 0.9 \((n = 15)\) and 100% for those \(\geq 1.0\) \((n = 17)\) (Fig. 2).

### DISCUSSION

Our findings show that precision and reproducibility were comparable in BAL and serum. Sensitivity was 82% in patients with IA, both at the 0.5 and at the 1.0 cutoff. The two false-negative cases both were receiving mold-active antifungal therapy, a known cause for false-negative antigenemia (8). Diagnostic BAL and specimens colonized with \textit{Aspergillus} or other molds were positive more often in patients receiving mold-active therapy, however. The reasons for a higher positivity rate in those receiving mold-active therapy are unknown but could include undiagnosed invasive disease, which led to the initiation of therapy. Antigenemia was detected in only one of four IA patients. The experience during clinical testing at MiraVista Diagnostics supports the higher sensitivity in BAL than serum. For example, antigenemia was detected in only 19.4% of patients in whom a simultaneous BAL specimen was positive. The higher sensitivity of BAL also has been noted by others (1, 9). The sensitivity was higher for antigen detection than was the histopathology or culture of BAL specimens.

The specificity was 95.8% in surveillance BAL specimens from lung transplant patients. Increasing the cutoff from 0.5 to 1.0 increased the specificity by 0.8%. The specificity for culture and/or histopathology was 89.1% in surveillance BALs. The PPV was higher for antigen detection than for culture or histopathology at either cutoff. For example, assuming 5% prevalence, the PPV for antigen detection was 50.6% at the 0.5 cutoff and 55.9% at the 1.0 cutoff compared to 26.0% for culture or histopathology. If the surveillance and diagnostic BALs are combined, the PPV for antigen detection was 25.8% at the 0.5 cutoff and 39.8% at the 1.0 cutoff compared to 22.1% for culture or histopathology. Thus, antigen detection appears to be more accurate than culture or histopathology when BAL is used for the diagnosis of IA. Although a greater accuracy may be achieved using lung biopsy specimens, surgical biopsy is rarely performed for evaluation of symptoms or CT abnormalities in transplant patients.

Positive results were more common in transplant controls who underwent diagnostic BAL (27.3%), those colonized with \textit{Aspergillus} or other mold (21.4%), and especially those who had undergone lung transplantation (46.2%). Raising the cutoff to 1.0 reduced the positivity rate in the diagnostic or colonized groups from 24.5 to 13.9%. Positive results in some of these controls may have been caused by undiagnosed IA or other mold infection, as evidenced by subsequent diagnosis of IA in four of our patients with positive diagnostic BALs. Positive results were not observed in healthy individuals not at risk for IA or airway colonization with mold.

### TABLE 3. Predictive values of GM detection and culture and/or histopathology in BAL

| % Prevalence | Control category | GM | Culture and/or histopathology |
|--------------|------------------|------------------|------------------|------------------|
| % Sensitivity | NPV | Specificity | PPV | % Sensitivity | NPV | Specificity | PPV |
| 0.5 cutoff | 1.0 cutoff | 0.5 cutoff | 1.0 cutoff |
| 5 | Diagnostic | 81.8 | 98.7 | 72.7 | 13.6 | 81.8 | 98.9 | 87.9 | 26.2 |
| Surveillance | 81.8 | 99.0 | 95.8 | 50.6 | 81.8 | 99.0 | 96.6 | 55.9 |
| Both | 81.8 | 98.9 | 87.6 | 25.8 | 81.8 | 99.0 | 93.5 | 39.8 |
| 10 | Diagnostic | 81.8 | 97.3 | 72.7 | 25.0 | 81.8 | 97.8 | 87.9 | 42.9 |
| Surveillance | 81.8 | 97.9 | 95.8 | 68.4 | 81.8 | 98.0 | 96.6 | 72.8 |
| Both | 81.8 | 97.7 | 87.6 | 42.3 | 81.8 | 97.9 | 93.5 | 58.3 |
| 72.7 | 98.27 | 81.8 | 17.37 |
| 72.7 | 98.41 | 89.1 | 25.98 |
| 72.7 | 98.37 | 86.5 | 22.08 |
| 72.7 | 96.42 | 81.8 | 30.74 |
| 72.7 | 96.71 | 89.1 | 42.56 |
| 72.7 | 96.61 | 86.5 | 37.44 |
Piperacillin-tazobactam may have caused a positive result in a few patients. Although three of four lung transplant patients receiving it had positive results, two also had positive cultures for Aspergillus or Paecilomyces. Others have also reported positive results in BAL from patients receiving piperacillin-tazobactam (9). Two of the three BAL specimens from our patients receiving piperacillin-tazobactam also were positive at the 1.0 cutoff.

In conclusion, the performance characteristics of the Platelia Aspergillus EIA in BAL were similar to those in serum, but the sensitivity was higher in BAL. Use of a 1.0 cutoff improved the specificity, but results between 0.5 and 0.9 should not be disregarded without careful consideration of the diagnosis of IA, since some of these patients may have IA or develop it later. Antigen detection should be added to culture and histopathology in the evaluation of pulmonary infiltrates in organ transplant patients.

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