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Evaluation of the Diagnostic Accuracy and Clinical Utility of Fungal Profile Plus Polymerase Chain Reaction Assay in Pulmonary Infections

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Background. Pulmonary infections due to *Aspergillus*, Mucorales, and *Nocardia* have high morbidity and mortality, in part due to diagnostic challenges. Commercially available molecular assays on bronchoalveolar lavage fluid (BALF) may have increased sensitivity over currently available diagnostic options. Our aim was to characterize the diagnostic performance of assays for each of these pathogens in our patient population.

Methods. The medical records of patients whose BALF was tested by polymerase chain reaction (PCR) for *Aspergillus*, Mucorales, and *Nocardia* between 2019 and 2021 were reviewed in a cross-sectional manner. European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) definitions of "proven," "probable," and "possible" infection were used, including histopathology, serology, and culture. We used (1) "proven" or "probable" infection by EORTC criteria, (2) improvement or stabilization on targeted antimicrobial therapy, and (3) absence of a more likely diagnosis as the reference standard.

Results. The *Aspergillus* PCR assay demonstrated the highest agreement with the diagnostic reference standard, with 31.25% (10/32) sensitivity and 97.17% (206/212) specificity. Positive and negative predictive values were 62.50% (10/16) and 90.35% (206/228), respectively. No Mucorales or *Nocardia* infections were identified by the diagnostic reference standard, so the sensitivity could not be calculated. The specificity of Mucorales and *Nocardia* targets was 98.35% and 96.69%, respectively.

Conclusions. Our data demonstrated relatively poor clinical sensitivity for all 3 constituent PCR assays in our patient population, suggesting a limited role for this test in the diagnosis of *Aspergillus*, Mucorales, or *Nocardia*.

Keywords. invasive fungal infection; bronchoscopy; immunocompromised; pulmonary infection.

Invasive pulmonary infections due to *Aspergillus*, Mucorales, and *Nocardia* are uncommon but cause significant morbidity and mortality. Invasive aspergillosis has an incidence of 1–2 cases per 100 000 in the general population, and invasive mucormycosis has a yearly rate of 0.17 cases per 100 000, with greater prevalence among the immunocompromised [1–4]. Invasive nocardiosis occurs at a rate of 500–1000 cases yearly in the United States, with 60% in patients with immune deficiency [5–7].

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The morbidity and mortality of these infections are attributed to difficulty in diagnosis due to vague symptomatology, the immunocompromised state of many patients, and a lack of high-performing diagnostic tools [3, 6]. For example, detection of *Aspergillus* galactomannan in bronchoalveolar fluid (BALF) has a reported sensitivity 85%–90% and specificity of 90%–95% and is known to cross-react with other fungal species [8–11]. (1,3)-Beta-D-Glucan is a component of the fungal cell wall, which can be detected serologically but lacks specificity and does not identify mucormycosis. The yield of direct microscopy and culture from BALF is variable and inadequate, with sensitivity ranging from 5% to 100% for *Aspergillus* species, and is poorly characterized for Mucorales and *Nocardia* [4, 12, 13].

Consensus definitions for assessing diagnostic tests have been developed by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG), and the 2019 update included polymerase chain reaction (PCR) testing on BALF (S1, S2) [1, 15]. However, the use of PCR to diagnose invasive fungal infection (IFI) remains largely undefined and somewhat controversial [16]. PCR for *Aspergillus* species is currently the most well

studied, with sensitivity and specificity of 77% and 94%, respectively, when performed in parallel with other tests such as *Aspergilllus* galactomannan and culture [17].

Our institution has utilized a reference laboratory's PCR panel called "Fungal Profile Plus" for pan-*Aspergillus* species, *A. fumigatus, A. terreus,* Mucorales, and *Nocardia* species. Manufacturer in vitro data indicate good performance; however, clinical performance has not been reported [16]. We have noted discordance between PCR results and results from other tests and have observed improvement on targeted therapy in the setting of negative PCR results. Therefore, the purpose of this study was to evaluate the clinical performance of the Fungal Profile Plus panel in our patient population.

METHODS

Following institutional review board approval, a retrospective analysis was performed on all patients ≥18 years of age with a BALF specimen sent to Eurofins Viracor Laboratories (Lee's Summit, MO, USA) for *Aspergillus,* Mucorales, and/or *Nocardia* PCR (panel or individual assay components) between January 2019 and June 2021. Specimens were stored at 4°C for up to 2 days or −20°C for up to 4 days before testing.

Informed consent for bronchoscopy was obtained and performed by accepted guidelines for fiberoptic bronchoscopy with bronchoalveolar lavage by pulmonary and critical care physicians [19]. The lobes with the highest disease burden based on imaging and intraprocedural findings were irrigated with 3–60-mL aliquots of normal saline and suctioned into sterile containers.

Patient charts were reviewed for age, sex, and other clinical factors of invasive pulmonary disease as defined by the EORTC/MSG. The demographics of patients with multiple specimens were only counted once. The reference standard used included EORTC/MSG illness defined as proven or probable, improvement or stabilization on targeted antimicrobials, and absence of a more likely etiology as determined by medical record review by the treating physician.

To evaluate for probable invasive infection criteria, host factors considered included immunological status and reason for immunocompromise if applicable. For the purposes of this investigation, computed tomography interpretation by a radiologist or pulmonologist that contained the following keywords or their derivatives were also considered: "tree in bud," "nodularity," "halo sign," "cavitary," "mycetoma," "round consolidation," "fungal," or "air-crescent." Mycological evidence for "probable" invasive disease varies by species according to EORTC/MSG criteria and was extrapolated to include *Nocardia* in this protocol, with findings of filamentous modified acid-fast bacilli required for "proven" infection.

PCR diagnostic yield statistics including sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated, as well as Fisher exact tests for each assay, to evaluate the clinical performance and corresponding 95% confidence intervals. A statistical significance level of .05 was used for all tests. All statistical analyses were conducted using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). A separate comparative analysis was performed excluding 20 patients who underwent multiple bronchoscopies.

RESULTS

Of 251 total BALF specimens, endogenous inhibition was detected in 5 specimens (3 *Aspergillus*, 0 Mucorales, and 2 *Nocardia*), which were excluded from the analysis. Two hundred twenty-one unique patients had BALF specimens submitted, of whom 59.7% (137) were male, with an average age of 58.3 years. One hundred twenty-six (57.0%) patients received targeted antifungal therapy. Two hundred one (90.9%) had significant immune compromise, which is further characterized in Table 1.

Of the 251 individual bronchoscopic procedures included in the analysis, radiographic indications were 111 (44.2%) with multiple nodules, 8 (3.2%) with round consolidation, 29 (11.6%) with halo sign, 30 (12.0%) with cavitary lesions, 54 (21.5%) with multifocal pneumonia, and 19 (7.6%) with other indications. In total, 232/251 (92.4%) patients had radiologic findings that could reasonably be considered to have 1 of the 3 infections in question.

Of 244 specimens submitted for pan-*Aspergillus* assay, 32 satisfied our definition of clinical positivity, with 26 probable and 6 proven (Table 2). Among those 32, we found 31.25% (10/32) sensitivity (95% CI, 15.19%–47.31%). The specificity was 97.17% (206/212; 95% CI, 94.94%–99.40%). Our review also identified 7 cases in which any *Aspergillus* PCR assay was positive and the diagnostic reference standard was not met. In addition to the pan-*Aspergillus* assay, PCR reactions targeting *A. fumigatus* and *A. terreus* were also performed. Of the 16

Table 1. Patient Characteristics

	Unique Patients in Analysis (n = 221)
Gender: male, No. (%)	132 (59.7)
Age, mean ± SD, y	58.3 ± 15.1
Received antifungal therapy, No. (%)	126 (57.0)
Reason for immunocompromise, No. (%)	
Hematologic malignancy	146 (66.1)
Solid organ transplant	17 (7.7)
Solid malignancy	17 (7.7)
Autoimmune disorder on chronic immunosuppression	17 (7.7)
Immunodeficiency (acquired or hereditary)	4 (1.8)
None	20 (9.1)

Table 2. Performance of Constituent PCR Assays Including Patients With Multiple Specimens

	Pan-*Aspergillus* PCR Assay		Mucorales PCR Assay		*Nocardia* PCR Assay	
	Calculated	95% CI	Calculated	95% CI	Calculated	95% CI
Sensitivity, % (No.)	31.25 (10/32)	15.19–47.31	0 (0/1)	N/A	0 (0/0)	N/A
Specificity, % (No.)	97.17 (206/218)	94.94–99.40	98.35 (239/243)	96.75–98.95	98.32 (234/238)	96.69–99.95
Positive predictive value, % (No.)	62.50 (10/16)	38.78–86.22	0.00 (0/0)	N/A	0.00	N/A
Negative predictive value, % (No.)	90.35 (206/228)	86.52–94.18	99.58 (239/240)	98.77–100.40	99.57 (234/235)	98.74–100.41
Positive likelihood ratio	11.04	0.65–21.44	0	N/A	0	N/A
Negative likelihood ratio	0.71	0.54–0.87	1.02	1.00–1.03	1.02	1.00–1.03

Abbreviations: N/A, not available (unable to calculate); PCR, polymerase chain reaction.

specimens positive by species-level PCR, 8 were positive for *A. fumigatus* and 1 was positive for *A. terreus*. The other 7 are presumed to represent other *Aspergillus* species not specifically tested. All specimens that were positive for *A. terreus* or *A. fumigatus* were positive by pan-*Aspergillus* PCR.

Four out of 244 specimens (1.59%) were positive by the Mucorales PCR assay; however, none of these were positive by the reference standard. In 1 patient with negative Mucorales PCR, histopathology of lung tissue demonstrated hyphae morphologically consistent with Mucorales, and culture grew *Mucor indicus*. The specificity was 98.35% (239/244; 95% CI, 96.75–99.95%), and the sensitivity was 0% (0/1).

Four of 239 specimens (1.59%) submitted for *Nocardia* PCR analysis were positive; however, none of these were positive by the reference standard. In 1 case, lung biopsy tissue demonstrated filamentous bacteria by Grocott's Methenamine silver stain and also grew 6 colonies of *Nocardia farcinia*; however, the corresponding BALF specimen was negative for all targets by PCR and culture, making the sensitivity 0%. The specificity was 98.32% (234/239; 95% CI, 96.09–99.72%).

A sensitivity analysis excluding 20 patients with multiple bronchoscopic specimens submitted was performed, and the results were not significantly different from the initial results (Table 3). The results of the PCR assays were also evaluated according to the EORTC category for likelihood of invasive fungal infection. Both additive analysis and pan-*Aspergillus* assays

demonstrated correlation, with a *P* value <.05. Positive Mucorales and *Nocardia* PCR assays were much less likely to align with the EORTC category, with *P* values of .635 and >.999, respectively (Table 4).

DISCUSSION

Data regarding the performance of PCR assays on BALF are scant. *Aspergillus* molecular testing is the most studied, with reported sensitivities of 64%–87% and specificities of 98%–99% [18, 20–22]. This is compared with *Aspergillus* galactomannan in BALF, with sensitivity and specificity of 85%–90% and 90%–95%, respectively [8–10]. Data on similar Mucorales and *Nocardia* assays are scarce. One study on BALF PCR for Mucorales combined PCR and high-resolution melt analysis for a combined 90% sensitivity [23]. One small study of *Nocardia* PCR reported 100% sensitivity by multiplex PCR in patients with HIV (n = 4), while another study in lung transplant patients detected *Nocardia* in 5 of 29 (17.2%) BALF specimens; however, none were associated with clinical disease [24, 25].

Our data did not mirror these, which is likely partially attributable to the stringency of our reference standard. Stringency of the reference standard may also partially explain the 7 cases that were positive by *Aspergillus* PCR but did not meet the definition for clinical positivity, although it also may be that

Table 3. Performance of Constituent PCR Assays Excluding Patients With Multiple Specimens

	Pan-*Aspergillus* PCR Assay		Mucorales PCR Assay		*Nocardia* PCR Assay	
	Calculated	95% CI	Calculated	95% CI	Calculated	95% CI
Sensitivity, % (No.)	35.29 (6/17)	12.58–58.01	0 (0/1)	N/A	0 (0/0)	N/A
Specificity, % (No.)	97.30 (180/185)	94.96–99.63	98.52 (200/203)	96.86–100.18	98.48 (194/197)	96.77–100.19
Positive predictive value, % (No.)	54.55 (6/11)	25.12–83.97	0.00 (0/0)	N/A	0.00	N/A
Negative predictive value, % (No.)	94.24 (180/191)	90.94–97.54	100 (200/200)	N/A	99.49 (194/195)	98.48–100.49
Positive likelihood ratio	13.06	−1.02 to 27.13	N/A	N/A	0	N/A
Negative likelihood ratio	0.67	0.67–0.43	N/A	N/A	1.02	1.00–1.03

Abbreviations: N/A, not available (unable to calculate); PCR, polymerase chain reaction.

Table 4. PCR Reaction by EORTC Category for Likelihood of Invasive Pulmonary Infection

	EORTC			
	Possible	Probable	Proven	P Value
Pan-*Aspergillus* PCR +	6	6	4	<.001
Pan-*Aspergillus* PCR -	183	40	4	
Mucor PCR +	3	1	0	.635
Mucor PCR -	187	44	9	
Nocardia PCR +	4	0	0	>.999
Nocardia PCR -	183	43	9	
Summative PCR +	13	7	4	.003
Summative PCR -	553	127	23	

Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; PCR, polymerase chain reaction.

colonization created false-positive results. Other limitations are related to the study's retrospective design, including chart review by a single unblinded researcher. Furthermore, low overall case numbers contributed to inherently imprecise estimates of performance. Most patients analyzed had hematological malignancies, which may not be generalizable to other populations. Finally, the study period overlapped with the coronavirus disease 2019 pandemic, which may have biased some of the imaging impressions and/or influenced the overall population's characteristics.

In conclusion, the data generated from this analysis give pause to indiscriminate use of the described PCR assays. The performance is insufficient for a screening modality, given the threat of progression to fulminant disease. Prompt empiric treatment remains crucial, regardless of results, when clinical suspicion is high. Positive assays in the absence of clinical suspicion may misrepresent contamination or colonization as true infection. For this reason, clinical judgment should continue to supersede PCR results, and PCR for Mucorales, *Aspergillus,* and *Nocardia* on BALF should not be routinely used independent of histologic and immunological studies. Further study in additional populations and with larger numbers, particularly of Mucorales and *Nocardia* cases, is needed.

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