Invasive Mucormycosis Management: Mucorales PCR Provides Important, Novel Diagnostic Information

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INTRODUCTION

Early diagnosis and treatment of invasive mucormycosis (IM) affects patient outcomes. In immunocompromised patients, timely diagnosis and initiation of appropriate antifungal therapy are critical to improving survival and reducing morbidity (Chamilos et al., 2008; Kontoyiannis et al., 2014; Walsh et al., 2012).

Differentiating diagnosis between IM and invasive aspergillosis (IA) affects patient outcomes. High mortality and morbidity of IM remain significant healthcare issues due in part to confusion of IM with IA and failure to initiate appropriate therapy (Chamilos et al., 2008; Walsh et al., 2012). Optimal therapies for IA and IM differ, so the ability to distinguish between the two is necessary to provide early treatment with appropriate antifungals.

Because it is less common than IA, some IM diagnoses may be missed. Published studies have not definitively determined the frequency of patients for whom pulmonary IA is suspected but IM is present. We aimed to determine the frequency of *Mucorales* (MUC) PCR positivity in bronchoalveolar lavage (BAL) samples submitted for *Aspergillus* (ASP) PCR panel testing.

We report 1) the results of a 9-month study testing BAL samples for MUC PCR submitted to a clinical laboratory for Aspergillus testing and 2) a case involving a 12 year-old boy with multiply relapsed pre-B cell acute lymphoblastic leukemia.

MATERIALS AND METHODS

We collected DNA eluates from bronchoalveolar lavage (BAL) specimens originally submitted to a clinical laboratory for *Aspergillus* PCR testing. These samples were first tested clinically using a validated *Aspergillus* PCR Panel (Luong et al., 2011). These eluates were then tested using a commercially-available real-time MUC PCR assay (M-227, ICAAC, 2013) that detects known pathogens from seven *Mucorales* genera (*Apophysomyces, Cunninghamella, Lichtheimia* [previously *Absidia*], *Mucor, Rhizomucor, Rhizopus and Saksenaea*). The specificity of this assay as determined in validation has been shown below in **Table 1**.

Unique patient identifiers were used to collect the clinical results of all testing performed at the clinical laboratory. The first result during the testing period for each patient was used for analysis.

During the test period, we identified and reported a clinical case of IM diagnosed by BAL MUC PCR.

Table 1. Assay Specificity – Inclusivity and Cross-reactivity (# of species tested)

Detected[1]		Not Detected	
▪ *Absidia (2)*	▪ *Rhizomucor (2)*	▪ *Alternaria (1)*	▪ *Fusarium (1)*
▪ *Apophysomyces (1)*	▪ *Rhizopus (4; 9 isolates)*	▪ *Aspergillus (6)*	▪ *Irpex (1)*
▪ *Cunninghamella (2)*	▪ *Saksenaea (1)*	▪ *Blastomyces (1)*	▪ *Penicillium (1)*
▪ *Lichtheimia (1)*	▪ *Trichosporon (2)*	▪ *Candida (2)*	▪ *Pneumocystis (1)*
▪ *Mucor (7; 14 isolates)*		▪ *Cladosporium (1)*	▪ *Pseudallescheria (1)*
		▪ *Cryptococcus (1)*	▪ *Syncephalastrum (1)*

Note. The amount of DNA tested for each species was normalized using UV spectroscopy.
[1] The detection of *Mucorales* genera ranged from quantification cycle threshold (Ct) from 9 to 29, while that of the 2 *Trichosporon* spp. was later (Ct 36 to 37).

RESULTS

MUC PCR results of BAL submitted for *Aspergillus* testing. The proportions of samples positive for *Mucorales* and *Aspergillus* in BAL specimens submitted for IA testing are compared in **Table 2**. Out of 869 cases, 12 (1.4%) had POS MUC PCR, of which only two had been ordered for MUC PCR. *Aspergillus* was positive in 56/869 (6.4%) of patients, with 5/869 (0.6%) positive for *Aspergillus fumigatus* and 50/869 (5.8%) positive for *Aspergillus terreus*.

Table 2. Fungal Positivity of Samples Ordered for *Aspergillus* PCR

Test	Result		
	Positive	Negative/Inhibited	% Positivity
Pan-*Aspergillus*	56	813	6.4
Aspergillus fumigatus	50	819	5.8
Aspergillus terreus	5	864	0.6
Mucorales	12	857	1.4

The results of the *Aspergillus* and *Mucorales* testing are compared in **Table 3**, below. Two patients (16.7%) tested positive for both *Aspergillus* and *Mucorales*.

Table 3. *Mucorales* Positivity of BAL Samples Ordered for *Aspergillus* PCR

MUC PCR Result	Total	ASP Detected	ASP Not Detected	ASP Inhibited*
Positive	12	2/12 (16.7%)	8/12 (66.7%)	2/12 (16.7%)
Negative	857	54/857 (6.3%)	743/857 (86.7%)	60/857 (7.0%)

*Inhibited *Aspergillus* results indicate that the internal control was not detected at a satisfactory level during testing.

Other pneumonia-related testing ordered for the *Mucorales*-positive patients is summarized below in **Table 4**. Among these 12 patients a total of 52 pneumonia-related testing was performed, with four positive test results. Nine of the 12 patients had no test results return positive.

Table 4. Other Pneumonia-Related Tests Ordered for MUC Positive Patients

Total Tests	Average per Patient	Range per Patient	Positive Results	% Positive Results	Patients with no POS Results
52	5	0-8	4	7.7	9/12

Case study of IM confirmed by MUC PCR. A 12 year-old boy with multiply relapsed pre-B cell acute lymphoblastic leukemia, despite extensive chemotherapy, two allogeneic hematopoietic stem cell transplants, and CAR T-cell therapy, presented with febrile neutropenia (0 cells/mm^3), cough, and right shoulder pain while on fluconazole prophylaxis. Chest CT revealed a right lung cavity, which ultimately became 5.6 x 6.2 x 5.9 cm (**Figure 1**). BAL MUC PCR was positive at ~40-fold above the limit of detection. Microbiologic investigations for other pathogens (*Aspergillus, Histoplasma, Nocardia*, nontuberculous *Mycobacterium),* were negative. BAL cultures were negative, and biopsy was declined. Treatment was changed from voriconazole to amphotericin b-based therapy, with controlled infection while continuing salvage chemotherapy.

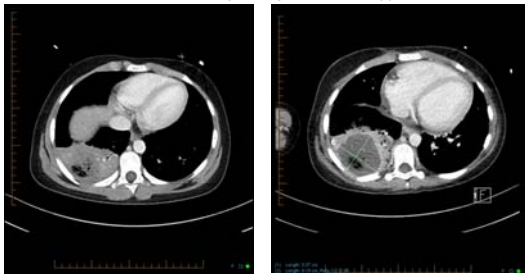

Figure 1: Chest CT of Case Study Patient Positive for MUC PCR. Chest CT shows RLL consolidation in a 5.6 x 6.2 x 5.9 cm area. A thick wall surrounding the cavitation is observed suggesting necrosis and/or abscess formation.

CONCLUSIONS

The observed 5:1 (ASP:MUC) ratio approximates published literature on invasive mold incidence (Wattier et al., 2015; Webb et al., 2018). The observed MUC positive results concurrent with negative results for other pneumonia-related pathogens suggest potential missed opportunities for MUC early diagnosis and treatment.

The described case study illustrates the potential value of *Mucorales* testing in suspected invasive fungal disease. MUC PCR on BAL was the only mycological evidence to support the diagnosis and altered the antifungal treatment strategy.

The observed results of both the screening of suspected IA BAL and the clinical case suggest that MUC PCR may provide actionable diagnostic information for treatment of IM.

References

1. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. Clinical Infectious Diseases 2008; 47: 503-9.
2. Kontoyiannis DP, Azie N, Franks B, Horn DL. Prospective antifungal therapy (PATH) alliance((R)): focus on mucormycosis. Mycoses 2014; 57: 240-6.
3. Luong ML, Clancy CJ, Vadnerkar A, Kwak EJ, Silveira FP, Wissel MC, Grantham KJ, Shields RK, Crespo M, Pilewski J, Toyoda Y, Kleiboeker SB, Pakstis D, Reddy SK, Walsh TJ, Nguyen MH. 2011. Comparison of an Aspergillus real-time polymerase chain reaction assay with galactomannan testing of bronchoalvelolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients.Clin Infect Dis.;52,10.
4. Walsh TJ, Gamaletsou MN, McGinnis MR, Hayden RT, Kontoyiannis DP. Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary, and disseminated mucormycosis (zygomycosis). Clinical Infectious Diseases 2012; 54 Suppl 1: S55-60.
5. Wattier RL, Dvorak CC, Hoffman JA, et al. A Prospective, International Cohort Study of Invasive Mold Infections in Children. Journal of the Pediatric Infectious Diseases Society 2015; 4: 313-22.
6. Webb BJ, Ferraro JP, Rea S, Kaufusi S, Goodman BE, Spalding J. Epidemiology and Clinical Features of Invasive Fungal Infection in a US Health Care Network. Open Forum Infectious Diseases. 2018 Jul 31;5(8):ofy187, doi: 10.1093/ofid/ofy187.