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Role of Antifungal Susceptibility Testing in Non-*Aspergillus* Invasive Mold Infections

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No clinical breakpoints are available to delineate antifungal drug efficacy in non-*Aspergillus* invasive mold infections (NAIMIs). In this analysis of 39 NAIMI episodes, the MIC of the first-line antifungal drug was the most important predictor of therapeutic response. For amphotericin B, an MIC of ≤0.5 μg/ml was significantly associated with better 6-week outcomes.

Molds other than *Aspergillus* spp. account for an increasing proportion of invasive fungal infections in the expanding population of immunosuppressed patients (1). *Mucorales*, *Fusarium* spp., and *Scedosporium* spp. are the most frequently seen non-*Aspergillus* mold pathogens and are associated with a high mortality rate, while *Paecilomyces* spp. and *Scopulariopsis* spp. are emerging opportunistic pathogens (1–5). Antifungal agents often have variable activity against these organisms, many of which are notoriously resistant to multiple antifungal drug classes. The utility of *in vitro* susceptibility testing in this setting is controversial, as clinical breakpoints are lacking and the correlation between drug MICs and outcomes has, to our knowledge, never been demonstrated. The aim of this study was to investigate factors influencing the outcome of non-*Aspergillus* invasive mold infections (NAIMIs) with a focus on the association between MICs and response to therapy.

Retrospective analysis of patient medical records where a non-*Aspergillus* mold was isolated from a clinical specimen between 2009 and 2013 at Duke University (Durham, NC, USA) led to the identification of 39 proven or probable NAIMI cases, according to European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG) definitions (6). Antifungal susceptibility testing (amphotericin B, voriconazole, posaconazole, micafungin) of these samples was performed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 procedure (7). The odds ratio (OR) and 95% confidence interval (CI) for predictors of the 6-week response were reported. Fisher's exact test was used for the comparison of categorical variables. This study was approved by the institutional review board of Duke University.

Data regarding underlying conditions, fungal species, first-line antifungal drugs, MICs, surgical procedures, and outcomes of all 39 cases are listed in Table S1 in the supplemental material. Twenty-two patients (56%) had hematological malignancies, 13 patients (33%) were solid-organ transplant recipients, and 3 patients (8%) had diabetes mellitus as the only risk factor for mucormycosis (Table 1). The remaining patient underwent allogeneic bone marrow transplantation for panscleroticmorphea. *Mucorales* and *Fusarium* spp. accounted for 49% and 31% of cases, respectively. *Scedosporium apiospermum*, *Scedosporium prolificans*, *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*), *Paecilomyces variotii*, and *Scopulariopsis* spp. were isolated in the remaining cases. Mortality at week 4 was 46%, and response to therapy, defined as a complete or partial recovery at week 6 according to EORTC-MSG definitions (8), was 31%. Multiple antifungal drugs were administered during the course of infection, and the analysis was restricted to the drugs used as first-line treatment (i.e., first antifungal drug administered as empirical or targeted therapy and maintained for at least 72 h). Overall, lower MIC values of the first-line antifungal drugs were associated with better success rates, with 86% response at week 6 for an MIC of ≤0.5 μg/ml, 25% for an MIC of 1 to 4 μg/ml, 20% for an MIC of >4 μg/ml, and 0% in the absence of antifungal therapy. In univariate analysis, having received a first-line antifungal drug for which the MIC was ≤0.5 μg/ml was the strongest predictor of therapeutic response (OR, 26.0; 95% CI, 2.62 to 258.20; $P = 0.005$), followed by infection limited to a single site (OR, 7.27; 95% CI, 1.33 to 39.9; $P = 0.02$) and having received surgical intervention (OR, 6.0; 95% CI, 1.30 to 27.77; $P = 0.02$); while underlying disease, neutropenia, and type of fungal pathogen did not have a significant impact on therapeutic responses (Table 2).

We performed subanalyses for individual drugs and pathogens. Amphotericin B was the first-line treatment in 10 cases (8/10 mucormycosis) and was associated with a significantly better 6-week response when the pathogen MIC was ≤0.5 μg/ml versus >0.5 μg/ml (83% versus 0%; $P = 0.05$) (Table 3). For patients with mucormycosis, there was a trend toward higher response rates for those having received amphotericin B versus another drug as initial therapy (63% versus 18% response at week 6; $P = 0.07$). Voriconazole was the first-line treatment of fusariosis in 67% of cases despite consistently high MICs (≥16 μg/ml) and was associated with failure in all cases but one (87.5%).

Our data show that NAIMIs are still associated with unaccept-

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TABLE 1 Characteristics of patients and non-*Aspergillus* invasive mold infections

Characteristic	No. (%) of patients
Patients ($n = 39$)	
Male	24 (62)
Age (median yr [range])	59 (5–76)
Underlying condition	
Hematologic malignancy	22 (56)
Allogeneic hematopoietic stem cell transplantation	12 (31)
Solid-organ transplantation	13 (33)
Immunosuppressive therapy	23 (59)
Neutropenia	16 (41)
Diabetes mellitus	19 (49)
Infection	
Proven/probable	32 (82)/7 (18)
Fungal pathogen	
Mucorales[a]	19 (49)
Fusarium spp.	12 (31)
Other[b]	8 (20)
Primary site of infection	
Lung	23 (59)
Skin/Soft tissue	9 (23)
Sinus	6 (15)
Intra-abdominal	1 (3)
Multiple sites	21 (54)
First-line antifungal therapy	
Amphotericin B	10 (26)
Voriconazole	17 (44)
Posaconazole	1 (3)
Micafungin	8 (21)
No therapy	3 (8)
Outcome	
Response to therapy (week 6)	12 (31)
Mortality (week 4)	18 (46)

[a] *Rhizopus* spp. ($n = 10$), *Cunninghamella* spp. ($n = 4$), *Mucor* spp. ($n = 3$), *Lichtheimia* spp. ($n = 2$).
[b] *Scedosporium apiospermum* ($n = 3$), *Scedosporium prolificans* ($n = 1$), *Purpureocillium lilacinum* ($n = 2$), *Paecilomyces variotii* ($n = 1$), *Scopulariopsis* spp. ($n = 1$).

ably high mortality rates. Unexpectedly, we found the MIC value of the first-line antifungal drug to be the most important factor in predicting response to therapy, an association that was not demonstrated previously (9–12). It should be emphasized that prior studies assessing predictors of NAIMI focused on overall mortality as an outcome, whereas we specifically assessed response to therapy based on clinical and radiological signs according to EORTC-MSG definitions (8).

The limited data set does not allow for the determination of clinical breakpoints for individual mold-antifungal drug combinations, but our findings suggests that a cutoff of 0.5 μg/ml for amphotericin B among non-*Aspergillus* molds (especially *Mucorales*) is associated with better outcomes. Amphotericin B MIC values of *Mucorales* may vary widely (0.125 to 4 μg/ml in this study), and there appears to be a role for antifungal susceptibility testing to guide antifungal therapy in this setting. While all patients in our series received standard amphotericin B dosing of 3 to 5 mg/kg, higher doses (i.e., 10 mg/kg), as suggested by some experts and despite potentially more adverse events (13, 14), may be appropriate for cases involving *Mucorales* with higher MICs, as these infections were associated with poor outcomes using standard dosing.

TABLE 2 Predictors of response to therapy at week 6

Predictor	OR (95% CI)[b]	*P* value
Underlying condition		
Neutropenia	0.36 (0.08–1.62)	0.18
Hematologic malignancy	0.69 (0.18–2.70)	0.6
Solid-organ transplantation	1.70 (0.41–6.98)	0.5
Type of infection		
Mucormycosis	2.91 (0.70–12.09)	0.2
Fusariosis	0. 34 (0.06–1.87)	0.14
Localized infection[a]	7.27 (1.33–39.9)	0.02
Management		
Surgery	6 (1.30–27.77)	0.02
First-line antifungal drug with MIC of ≤0.5 μg/ml	26 (2.62–258.20)	0.005

[a] Invasive fungal infection limited to a single organ.
[b] OR, odds ratio; 95% CI, 95% confidence interval.

The role of antifungal susceptibility testing for *Fusarium* spp. and other rare mold species (e.g., *Scedosporium* spp., *Paecilomyces* spp., and *Scopulariopsis* spp.) remains unclear. Most experts have highlighted the lack of correlation between MICs and outcomes for *Fusarium* spp. and recommended voriconazole as first-line therapy regardless of the MIC values (10, 15, 16). However, in our series, most cases of fusariosis were treated with voriconazole, for which MICs were consistently very high (≥16 μg/ml), and outcomes were very poor. None of the patients with fusariosis received initial therapy with amphotericin B, the only drug displaying relevant *in vitro* activity against these *Fusarium* isolates (MICs, 1 to 4 μg/ml).

Neither CLSI nor the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has defined clinical breakpoints for non-*Aspergillus* molds because of the lack of data correlating MIC values and outcomes. As NAIMIs are relatively rare diseases often with a lack of microbiological documentation, it will be difficult to obtain a sufficiently large data set to address this question in the future. Moreover, multiple confounding factors, such as the degree of immunosuppression or the type and severity of infection, may affect the response to therapy. Although our data have several limitations (retrospective design, limited number of cases), this series of 39 microbiologically documented NAIMI episodes (of which 82% were proven) highlights the crucial role of appropriate initial antifungal therapy in this severe disease. Moreover, it suggests a correlation between lower MICs, particularly for amphotericin B, and better response to therapy and a role for antifungal susceptibility testing of non-*Aspergillus* molds.

TABLE 3 Response to amphotericin B therapy at week 6 for various MIC cutoffs

MIC cutoff (μg/ml)	Response rate (no. [%]) ($n = 10$[a])		
	MIC ≤ cutoff	MIC > cutoff	*P* value
0.25	2/2 (100)	3/8 (38)	0.40
0.5	5/6 (83)	0/4 (0)	0.05
1	5/7 (71)	0/3 (0)	0.17
2	5/7 (71)	0/3 (0)	0.17
4	5/8 (63)	0/2 (0)	0.44

[a] *Rhizopus* spp. ($n = 6$), *Mucor* spp. ($n = 1$), *Cunninghamella* spp. ($n = 1$), *Scedosporium apiospermum* ($n = 1$), *Purpureocillium lilacinum* ($n = 1$).

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