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Dermatopathologist accuracy in classifying angioinvasive fungal infections using virtual microscopy

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Abstract

Background: Due to perceived difficulty in the categorization of angioinvasive fungal infections based on histopathology, variation exists in dermatopathology reporting.

Methods: This study characterized the diagnosis of angioinvasive fungal infections by light microscopy at a single academic institution over an 11-year period. Subsequently, the accuracy of blinded reclassification by virtual microscopy was measured.

Results: Seventy-six specimens with hematoxylin–eosin slides were obtained from 33 patients. The mean diagnostic accuracy of dermatopathologists in differentiating mucormycosis, hyalohyphomycosis, and phaeohyphomycosis based on blinded reclassification via virtual microscopy was 74%, with a range of 65%–91%.

Conclusions: While there was a range in diagnostic accuracy, the highest score of 91% and the identification of common sources of error suggest that histopathologic categorization of angioinvasive fungal infections can frequently be performed. However, accurate identification is not always possible given common pitfalls in diagnosis. In addition, standardized and clinically useful reporting should be considered.

KEYWORDS
angioinvasive fungal infections; dermatopathology; hyalohyphomycosis, phaeohyphomycosis; mucormycosis

1 | INTRODUCTION

Angioinvasive fungal infections result in significant morbidity and mortality.[1] The rising number of immunocompromised patients with hematologic malignancies, diabetes, and iatrogenic immunosuppression has led to an increase in angioinvasive fungal infections. In addition, the increase in antifungal resistance patterns has contributed to deaths from angioinvasive fungal infections since 2010.[1,2] Prompt clinical recognition and diagnosis are paramount for rapid treatment to improve outcomes and avoid adverse events.[3] As susceptibilities to antifungal agents vary, correct categorization is helpful to ensure optimal treatment.[3]

Histopathologic examination is the most rapid initial diagnostic tool for preliminary identification of angioinvasive fungal infections. Histopathology can help identify mucormycosis, hyalohyphomycosis, or phaeohyphomycosis based on morphological features such as septation, hyphal diameter, angle branching, and pigmentation (Figure 1).[3] Preliminary diagnoses are typically correlated with tissue cultures or molecular testing, namely polymerase chain reaction (PCR), to provide speciation.[3] However, fungal cultures can provide variable yield or be negative due to sampling error, organism nonviability, or tissue homogenization.[3] Confirmatory tests take several days and cannot provide the rapid diagnostic information needed for targeted therapy.[4] Consequently, much weight is often placed on histopathologic examination.[5] However, well-documented challenges to histopathologic diagnosis exist due to inconsistencies in the histomorphology of fungal species and distortion of hyphae secondary to fragmentation, swelling, and pseudoseptation from folding (Table 1).[3,4]

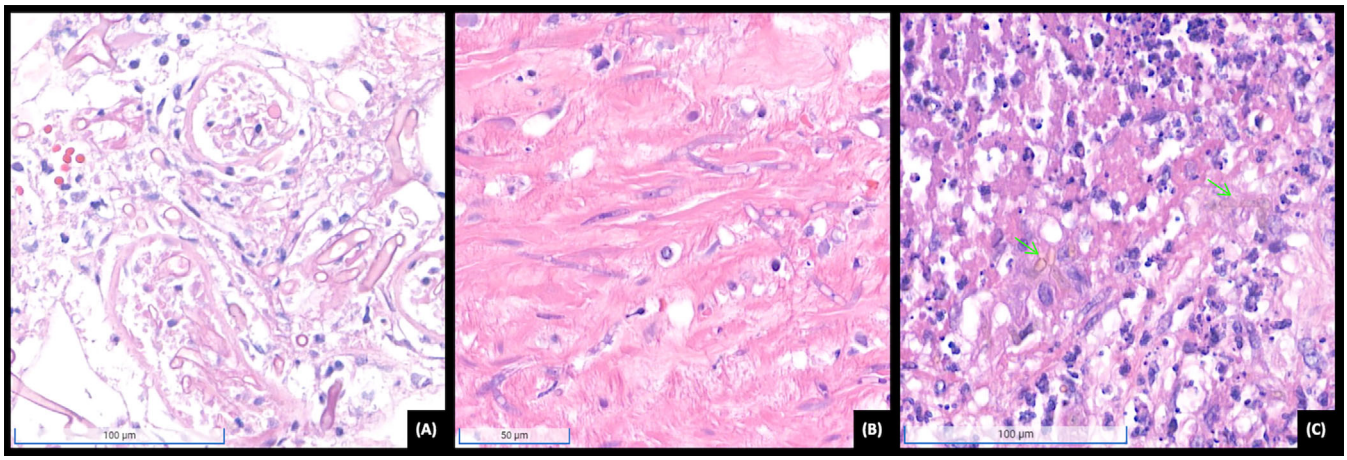

FIGURE 1 Typical characteristics of (A) mucormycosis, (B) hyalohyphomycosis, and (C) phaeohyphomycosis (hematoxylin–eosin, 140× magnification).

TABLE 1 Characteristics of angioinvasive fungal infections (mucormycosis, hyalohyphomycosis, or phaeohyphomycosis).

Group	Mucormycosis	Hyalohyphomycosis	Phaeohyphomycosis
Species	*Mucor*, *Rhizopus*	*Fusarium*, *Aspergillus*, *Paecilomyces*, *Scedosporium*	*Curvularia*, *Exophiala*
Diameter	Broad, hollow "ribbon-like" or "sausage-like" hyphae	Narrow, nonpigmented **Caveat: dead organisms may swell**	Narrow
Septation	Aseptate **Caveat: compression may appear as pseudoseptations**	Septate	Septate
Wall Color	Refractile Hollow/pink wall	Non-refractile Blue cytoplasm	Brown wall (hallmark feature)
Angle branching	Broad 90°	Narrow	Narrow

There are minimal data on the accuracy of dermatopathologists in categorizing angioinvasive fungal infections based on histopathology. Furthermore, there is considerable interrater variability in reporting.[6] Some dermatopathologists routinely suggest either mucormycosis, hyalohyphomycosis, or phaeohyphomycosis, while others will not provide this information due to concern over the reliability of histomorphology. The rise in angioinvasive fungal infections, discordance between histopathology and microbiology, variability in reporting, and rapid mortality of untreated or inadequately treated infections signal a need for study and improvement in histopathologic classification of angioinvasive fungal infections.[4,5]

This study undertook an 11-year review at a single academic institution to identify skin biopsy or excision specimens with angioinvasive fungal infections that had confirmatory tissue culture or PCR. Dermatopathologists subsequently performed a blinded review of these specimens and recorded presumptive diagnoses. Diagnostic accuracy was measured, and common sources of error were identified. Results may influence practices in histopathologic categorization of angioinvasive fungal infections and reporting.

2 | MATERIALS AND METHODS

A retrospective 11-year review of all skin and soft tissue histopathological specimens with a diagnosis relating to angioinvasive fungal infections was performed using Integrated Data Repository (IDR) Services between May 1, 2011 and August 1, 2022. Initial results were filtered using Beaker laboratory information system search terms and IDR filters (Table S1). Patient electronic medical records were then reviewed to identify histopathological specimens with speciation by tissue or blood culture, PCR, or fluorescence in situ hybridization. Only confirmed cases of mucormycosis (*Rhizopus*, *Mucor*), hyalohyphomycosis (*Fusarium*, *Aspergillus*, *Paecilomyces*, *Scedosporium*), and phaeohyphomycosis (*Curvularia*, *Exophiala*) were included. Cases with confirmed multiple fungal co-infections caused by more than one category of mold (mucormycosis, hyalohyphomycosis, or phaeohyphomycosis) or non-mold infections, such as *Cryptococcus*, *Histoplasmosis*, and *Blastomycosis*, were excluded. Original hematoxylin–eosin (H&E) stained slides and, if available, Gomori methenamine silver (GMS) or periodic acid—Schiff (PAS) stained slides were obtained and numerically labeled. Cases with unusable quality (i.e., due to fading over time

or containing only a minuscule fragment of fungal elements) were excluded.

Three fellowship-trained and board-certified dermatopathology faculty members and a dermatopathology fellow at a single academic institution were invited to participate in a blinded reclassification of the cases. Informed consent was obtained prior to study participants receiving a brief educational training in the form of a self-directed online PowerPoint presentation on the diagnostic histopathologic features of angioinvasive fungal infections. The blinded participants then navigated to an online survey (Qualtrics) with links to corresponding whole slide images (WSIs) stored in a web-based repository (Concentriq, Proscia). Participants evaluated each slide and recorded their presumed fungal diagnosis (mucormycosis, hyalohyphomycosis, or phaeohyphomycosis) through the survey. Slides were randomized and presented without any identifying information. Each participant was prohibited from discussing the cases or consulting any resources until all participants had completed the survey. At the completion of the evaluation, the diagnostic accuracy was calculated as the percent of correctly categorized fungi, with categorization based on speciation by microbiologic or molecular methods. For statistical analysis, data were analyzed by Fisher's one-tailed test with a significance set at $p < 0.05$. The Fisher test compared the mean diagnostic accuracy of the two-group versus three-group categorizations.

3 | RESULTS

Based on chart review, 76 specimens (42 mucormycosis, 29 hyalohyphomycosis, and 5 phaeohyphomycosis) with H&E, GMS, and/or PAS-stained slides were obtained from 33 patients. Classification based on light microscopy was attempted in 54 of these 76 specimens; 52 (96%) of these 54 specimens were classified correctly as either mucormycosis, hyalohyphomycosis, or phaeohyphomycosis (Table 2). Only two cases of phaeohyphomycosis were misclassified as hyalohyphomycosis at the time of original diagnosis (Table 2). When considering all specimens with and without attempted classification, the mean diagnostic accuracy at the time of diagnosis [correctly classified/(cases with attempted classification + cases without attempted classification)] was 68% (Table 3). For the 22 specimens in which classification was not attempted, cases were signed out descriptively (Table 4).

For the blinded reclassification of specimens, the mean diagnostic accuracy of dermatopathologists in differentiating mucormycosis, hyalohyphomycosis, and phaeohyphomycosis in the three-group analysis was 74% with a range of 65%–91% (Table 5). Sub-analysis was performed to determine if accuracy improved when participants were evaluated on the ability to distinguish mucormycosis from mycoses not due to Mucorales (hyalohyphomycosis or phaeohyphomycosis). For this analysis, the responses by survey participants were reclassified as correct if either hyalohyphomycosis or phaeohyphomycosis was selected for infections due to non-Mucorales organisms. The two-group analysis resulted in an insignificant increase in mean diagnostic accuracy (Table 6).

4 | DISCUSSION

The classification was attempted at the time of original diagnosis in 54 of the 76 specimens obtained from the chart review. Of those 54 specimens, 52 specimens were correctly classified as either

TABLE 2 Three-group analysis of accuracy in differentiating angioinvasive fungal infections at the time of original diagnosis calculated as [correctly classified cases/(total cases where classification was attempted)].

	Correctly identified number of cases	Percentage of correctly identified cases
Mucormycosis ($n = 39$)	39	100%
Hyalohyphomycosis ($n = 12$)	12	100%
Phaeohyphomycosis ($n = 3$)	1	33%
Overall ($n = 54$)	52	96%

TABLE 3 Three-group analysis of accuracy in differentiating angioinvasive fungal infections at the time of original diagnosis calculated as [correctly classified cases/(total cases where classification was attempted + cases where no classification was attempted)].

	Correctly identified number of cases	Percentage of correctly identified cases
Mucormycosis ($n = 42$)	39	93%
Hyalohyphomycosis ($n = 29$)	12	41%
Phaeohyphomycosis ($n = 5$)	1	20%
Overall ($n = 76$)	52	68%

TABLE 4 Descriptive reporting without attempted classification at the time of original diagnosis.

Description provided	Number of cases
Angioinvasive fungal infection	2
Deep fungal infection	11
Fungal organisms present	5
Vasoinvasive fungal hyphae	1
Invasive fungal hyphae	1
Deep fungal infection with angioinvasion	1
Septate fungal hyphae	1
Broad, branching, and pauci septate hyphae	1
Total	22

TABLE 5 Three-group analysis of dermatopathologist accuracy in differentiating angioinvasive fungal infections.

	Correctly identified number of cases	Percentage of correctly identified cases	*p* Value
Mucormycosis ($n = 42$)	28 (18–41)	66% (43%–98%)	–
Hyalohyphomycosis ($n = 29$)	26 (25–28)	89% (86%–97%)	–
Phaeohyphomycosis ($n = 5$)	2.5 (1–3)	50% (20%–60%)	–
Overall ($n = 76$)	56 (49–69)	74% (65%–91%)	0.08

Note: *p* Value comparing overall mean of two- and three-group analysis.

TABLE 6 Two-group analysis of dermatopathologist accuracy in differentiating mucormycosis versus non-Mucorales mycoses (hyalohyphomycosis and phaeohyphomycosis).

	Correctly identified number of cases	Percentage of correctly identified cases	*p* Value
Mucormycosis ($n = 42$)	28 (18–41)	66% (43%–98%)	–
Non-Mucorales mycoses ($n = 34$)	32 (30–34)	93% (88%–100%)	–
Overall ($n = 76$)	60 (52–71)	79% (68%–93%)	0.08

Note: *p* Value comparing overall mean of two- and three-group analysis.

mucormycosis, hyalohyphomycosis, or phaeohyphomycosis. The two cases misclassified at the time of original diagnosis were also misclassified during later blinded recharacterization. The discordance between the 96% accuracy at the time of original diagnosis and the 74% accuracy on reclassification is likely multifactorial. Classification was not attempted in 22 of 76 specimens at the time of original diagnosis. Multiple previously unclassified cases were subsequently misclassified during the blinded reclassification. When considering cases with and without attempted classification, diagnostic accuracy was 68% at the time of original diagnosis. Dermatopathologists may be more likely to classify cases with classic morphologic features compared to cases with overlapping or equivocal features.

At the time of original diagnosis, dermatopathologists reviewed specimens with light microscopy in real-time and were provided with clinical context (i.e., centrofacial necrosis in a patient with diabetic ketoacidosis suggestive of mucormycosis). In contrast, during reclassification, dermatopathologists retrospectively reviewed WSIs on standard-issue computer monitors without intradepartmental consultation with colleagues or clinical context. One WSI per case was scanned; thus, not all tissue sections available at the time of original diagnosis were reviewed during reclassification. Limitations of virtual microscopy include the inability to adjust fine focus and potential difficulty navigating. These factors may have impacted the accuracy of assessment, including features that require a global/composite assessment of many data points, such as angle branching.[7]

For the original cases, five dermatopathologists signed out 57 of the 76 cases, while seven general pathologists signed out on the remaining 19 cases. For the vast majority of cases, the dermatopathologists who reviewed at the time of original diagnosis were distinct from the dermatopathologists who participated in reclassification. Two of the five dermatopathologists who participated in the reclassification collectively reviewed 7 of the 76 original cases at the time of diagnosis. The remaining 69 cases had not previously been reviewed by any of the dermatopathologists participating in reclassification.

When attempting to differentiate between mucormycosis, hyalohyphomycosis, and phaeohyphomycosis during reclassification, dermatopathologist accuracy averaged 74%. This result is similar to the 79% accuracy of dermatopathologists observed in a 10-year retrospective study of mold and yeast cultures by Sangoi et al.[5] This earlier study included a broader group of fungal infections over multiple organ systems, limiting direct comparison to the cutaneous-restricted study described here.[5] However, the principles of classification should be similar across various organs affected by the same mycoses. Sangoi et al. attributed discrepant diagnoses to morphologic mimics, use of inappropriate terminology, and incomplete knowledge of mycology; discrepant diagnoses were neither operator dependent nor attributed to preceding antifungal therapy or the use of special stains.[5]

Determining the type of fungal infection can guide initial treatment.[4] For instance, mucormycosis requires treatment with liposomal amphotericin B, due to resistance to voriconazole, while *Aspergillus* infection requires treatment with voriconazole and an echinocandin agent.[3,4] However, variation in morphologic features may result in decreased diagnostic accuracy. For example, mucormycosis is aseptate, but twists or compressions of the organisms may appear as "pseudoseptations" and result in a mistaken diagnosis of hyalohyphomycosis.[3,4] Pseudoseptations may also arise from division of the single cell organism in fungi belonging to Mucorales.[3,4] Mucormycetes tend to have broad hyphae in comparison to the narrow hyphae of hyalohyphomycosis and phaeohyphomycosis.[3,4] However, dead organisms may swell such that hyalohyphomycosis or phaeohyphomycosis can display variable size and bubble-like swellings that focally resemble mucormycosis (Figure 2B).[3,4] When hyaline hyphomycetes swell, a pale-staining cell wall can differentiate hyalohyphomycosis from mucormycosis.[3] Broad- and narrow-angle branching can differentiate between mucormycosis and hyalohyphomycosis, respectively, but this characteristic is less reliable.[3,4] Therefore, dermatopathologists must analyze the overall tendency of branching angles within the specimen rather than a single organism, and other characteristic features should

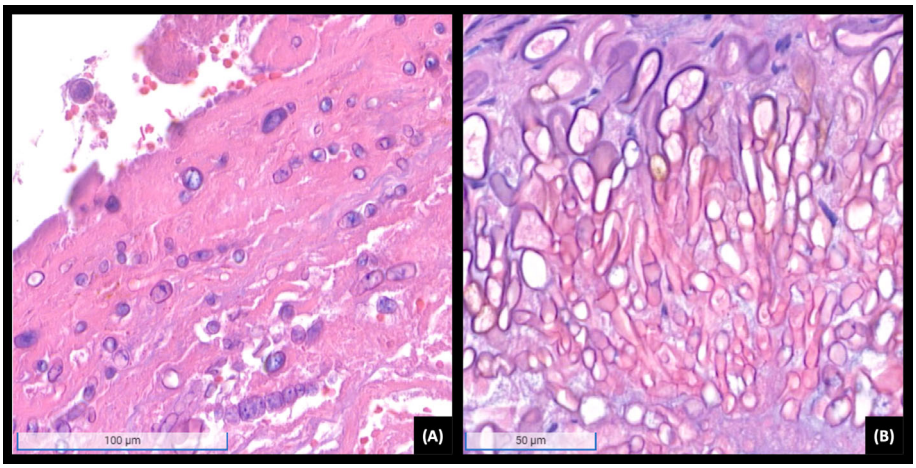

FIGURE 2 Common discrepant morphology of angioinvasive fungal infections. (A) Mucormycosis displaying a boxcar pseudoseptate appearance (hematoxylin–eosin [H&E], ×100 magnification). (B) Phaeohyphomycosis displaying red and pink color with swollen nonpigmented hyphae superiorly that simulate Mucorales (H&E, ×170 magnification).

be considered first.[3,4] Hyalohyphomycosis and phaeohyphomycosis both present with septations and narrow branching. If the hallmark brown pigment of dematiaceous molds is not present, they may be indiscernible from nondematiaceous molds as they are both narrow branching, septate organisms. The improved accuracy of 79% on subgroup analysis comparing mucormycosis versus hyalohyphomycosis/phaeohyphomycosis could be attributed to these overlapping histopathological features (Table 6). However, this improved accuracy was found to be insignificant ($p = 0.08$).

Although the design of the study did not include survey data on why the participants chose particular answers, the post-study review revealed two potential pitfalls that we suspect were responsible for the majority of incorrect answers (Figure 2). In multiple specimens of mucormycosis, fungal elements appeared blue and showed pseudoseptations. Areas of apparent single-cell division also appeared to mimic septation; however, spaces separated the fungal elements rather than bonafide septations (Figure 2A). In two cases of phaeohyphomycosis, the fungal elements were predominantly red in color (Figure 2B). This color is distinct from the lighter pink typically associated with mucormycosis. Careful examination in one of the specimens also revealed focal areas of brown pigment. Awareness of these two pitfalls may increase diagnostic accuracy.

Differential PAS and GMS staining to differentiate between viable and non-viable fungal elements has been described.[8] However, there were no cases meeting the inclusion criteria for which both PAS and GMS were performed. Similarly, the utility of immunostains for fungi was not explored in this study.

Given the variation in histopathological presentation, many dermatopathologists do not provide any clinically useful classification beyond the broad diagnosis of angioinvasive fungal infection on formal reports.[5] The challenges inherent to accurate histomorphologic classification of angioinvasive fungal infections have spurred discussion on best practices in reporting. It has been proposed to include the definitive diagnosis on the diagnosis line with a conditional differential outlined in the comment section.[5] For example, in this study, many pathology reports delineated "angioinvasive fungal infection" or "vasoinvasive fungal infection" on the diagnosis line with a comment section detailing "concerning for hyalohyphomycosis" or "consistent with [Mucorales]." There was variability in the comment section reporting of clinically actionable classification or groups, but all comment sections recommended correlation with culture or PCR. These practices are in line with the standardized template for hyphal fungal organisms outlined by Sangoi et al.[5] While this earlier study found misclassifications resulting in incorrect treatments and adverse patient outcomes, our study only found two misclassifications at the time of original diagnosis without subsequent adverse therapeutic decisions or outcomes.[5] With additional awareness of potential pitfalls, we hope further studies will demonstrate that best practice includes providing classification as well as confidence level at the time of diagnosis. With or without classification, pathology reports should recommend confirmation with culture or PCR.

This study had several limitations. Only four dermatopathologists participated in the study, and there was a wide range of diagnostic accuracy from 65% to 91%. This limits the generalization of results. The structure and format of this study could be reproduced and distributed on a larger scale to include a greater number of participant dermatopathologists across academic and non-academic practices. In addition, this study had only five cases of phaeohyphomycosis. Multiple specimens were also used from some patients. Furthermore, we were unable to assess which specimens were obtained from patients who received prior antifungal treatment.

Ultimately, while there was a wide range in diagnostic accuracy, post-study review revealed new diagnostic challenges. Mucormycosis may appear blue in color with pseudoseptations partly due to single-cell division, while phaeohyphomycosis may appear predominately red, which may mask subtle areas of brown pigment. A larger study with more specimens and participants may be performed in the future to determine if the clinically useful classification of angioinvasive fungal infections can be accurate, teachable, and standardizable in reporting.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

IRB approval status: Reviewed and approved by IRB; approval #IRB202201777, exempt Protocol #ET00018547.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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