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ORIGINAL ARTICLE

Diagnostic accuracy of fungal identification in histopathology and cytopathology specimens

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Abstract Tools to diagnose fungal infection are microscopic examination, antigen or antibody-based detection tests, molecular diagnostics, and culture, with culture being the "gold standard" for species-level identification. Although these methods are commonly used in concert and yield concordant results, in some cases tissue is not available for culture, and/or different methodologies yield discrepant results. These discrepancies may be clinically significant, causing confusion and inappropriate or delayed initiation of antifungals. This study evaluates the correlation between microscopic examination and the results of laboratory studies, and identifies clinical scenarios and specimen characteristics associated with tissue sent for microscopic examination without concomitant laboratory studies. We performed an 18-year retrospective review at a tertiary-care, academic medical center in the Midwest United States of all fungal infection diagnoses made by microscopic examination. Only 16% of samples with fungal infection diagnosed by microscopic examination had a concomitant sample submitted for laboratory studies. Of these cases, 36% had no growth on culture and/or had a negative laboratory study. Among cases in which fungal infections were diagnosed and laboratory studies were positive, the accuracy of histopathologic identification was 95%. The most common cause for incorrect morphologic diagnoses was misidentification of *Aspergillus* spp. and *Mucorales*. Our results underscore the importance of educating pathologists with regard to appropriate terminology and increasing knowledge of mycology, particularly in relation to organisms forming hyphae in tissue. Species-level diagnosis of fungi cannot be made by microscopic examination of tissue alone. Anatomic pathology reports should recommend correlation with laboratory studies, and provide a differential diagnosis based on morphology.

Keywords Culture · Fungal · Fungi · Histology · Identification · Morphology

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Introduction

Invasive fungal infections are a cause of significant morbidity and mortality, and in light of increasingly aggressive immunosuppressive therapies, are becoming more common in both the United States and worldwide [1–4]. The diagnosis and appropriate treatment of fungal diseases is dependent on the rendering of rapid and accurate species-level identification by anatomic pathologists and clinical microbiologists. The pathologist's primary diagnostic tools for species-level identification of fungi are histology and laboratory tests, which include antigen detection or serologic tests, molecular diagnostics studies, and culture, each of which has both strengths and limitations. Microscopic examination allows for rapid and cost-effective, presumptive identification of fungal infection. Additionally, by demonstrating the tissue context and host response, histologic sections can aid in distinguishing between colonization and infection, and provide information on invasion and chronicity. Morphologic diagnoses, however, have limited sensitivity and specificity, and a species-level identification can rarely be made on histopathology alone. Next, antigen detection or serologic tests are a non-invasive approach to predict invasive fungal infection, but similar to microscopic examination, may suffer from limited sensitivity and specificity, and often do not provide species-level resolution [5–7]. Molecular diagnostics are rapid and can make

species-level identifications; however, currently there are only a limited number of molecular diagnostic tests available for direct detection of fungi in clinical specimens [8]. Finally, fungal culture is the "gold standard" for species-level identification, but can be slow, taking from days to weeks, depending on the growth rate of the fungal species.

In the majority of cases, histology and laboratory tests are used together as complementary techniques, and produce concordant results. In some cases, though, tissue is sent for microscopic examination without concomitant antigen detection or serologic tests, molecular diagnostics, and/or culture, or the results of microscopic examination and antigen detection or serologic tests, molecular diagnostics, and/or culture are inconsistent. Such discrepancies have the potential to result in the inappropriate or delayed initiation of antifungal therapy.

Our objective was to evaluate the diagnostic accuracy of histologic identification of fungal infection in a large academic medical center in the Midwest United States. Additionally, we evaluate the frequency with which tissue specimens with histologic evidence of fungal infection are submitted for concomitant laboratory tests, and identify clinical scenarios and specimen characteristics affecting this ratio.

Materials and methods

Patient population and setting

Following approval from the Washington University Institutional Review Board, we performed a retrospective analysis of specimens submitted from adult and pediatric patients at a tertiary-care, academic medical center, including Barnes–Jewish Hospital and Saint Louis Children's Hospital, in Saint Louis, MO, USA.

Inclusion and exclusion criteria

Querying over an 18-year period (January 1996 to January 2014), all surgical and cytologic specimens from which a pathologist diagnosed a fungal infection were selected from a complete electronic database of all pathology reports generated at our institution (CoPath, Cerner Corporation, Kansas City, MO, USA). A search strategy was designed to contain common morphologic descriptors and terms for fungi, and was applied to the "final diagnosis line," "microscopic description," and "diagnosis comment" of pathology report texts. Search terms used were as follows: Aspergillosis, *Aspergillus*, Blasto, *Blastomyces*, Blastomycosis, Budding, *Candida*, Candidal, Candidiasis, *Coccidioides*, Coccidio idomycosis, *Cryptococcus*, Cryptococcal, Dematiaceous, Dermatophyte, Fungal, Fungi, Fungus, *Fusarium*, Histo, *Histoplasma*, Histoplasmosis, Hyphae, Hyphal, Mold, Mucor, *Mucorales*, Mucormycosis, *Paracoccidioides*, *Penicillium*, Phaeohyphomycosis, *Pneumocystis*, Pseudohyphae, *Rhizopus*, *Scedosporium*, *Sporothrix*, Yeast, Yeast-like, and *Zygomycetes*. Specimens obtained from autopsy cases and cases with fungal infection already documented in the clinical history supplied on the requisition accompanying the specimen were excluded, because of the likelihood of bias in the pathology diagnoses rendered. For all remaining specimens, patient age, gender, ordering hospital service, and the use of special stains was recorded. Our hospital electronic medical record was then used to identify all histology specimens for which a concomitant sample was taken for fungal culture, molecular diagnostics, and/or antigen detection or serologic tests, which we will collectively refer to as laboratory tests, and to determine patient immune status. Antigen detection and serologic tests included were *Aspergillus* galatomannan antigen, direct fluorescent-antibody detection for *Pneumocystis*, *Cryptococcus neoformans* antigen, *Blastomyces dermatitidis* antibody and antigen, and both urine and blood *Histoplasma* antibody and antigen. Molecular diagnostics included were *Histoplasma capsulatum* DNA probe and PCR and *Blastomyces dermatitidis* DNA probe and PCR.

Analysis

All discrepancies between histology and laboratory test results were recorded. Available slides from discrepant cases were re-reviewed by both CAB and RDC, who were blinded to both the prior morphologic diagnoses and culture and/or serologic or molecular diagnostics study results. A root cause analysis for misidentification was performed by both CAB and RDC, using a modified Eindhoven classification model. Recognizing that the distinctions between technical, organizational, and human errors are blurred in practice, categories of misidentification used were sampling error, morphologic mimics, and interpretive error. Fisher's exact test was used to assess statistical significance.

Results

Overview of case selection

We identified a total of 3164 cases where a morphologic diagnosis was rendered. Consistent with the known prevalence of fungal infections and reflective of geographic location, in this investigation, the three most commonly diagnosed fungi based on morphology were *Candida* spp. (n = 2327), *Histoplasma capsulatum* (n = 228), the most common endemic mycosis in the Ohio and Mississippi River valleys, and *Aspergillus* (n = 217), the most common invasive mold (Fig. 1a). Of the 3164 cases, 519 (16%) had a concomitant sample taken for laboratory studies. Of these cases, 186 (36%) had no growth on culture and/or had a negative antigen detection test,

serologic tests, or molecular diagnostics study result. Of the 333 cases for which fungi were recovered in culture and/or antigen detection tests, serologic tests, or molecular diagnostics studies were positive, 318 (95%) were concordant and 15 (5%) were discrepant with the morphologic diagnoses (Fig. 1b).

Clinicopathologic features of discrepant cases

The 15 discrepant cases all involved discordances between culture diagnoses and the diagnosis lines or comment sections of the final pathology reports. Incorrect morphologic diagnoses most commonly involved misidentification of *Aspergillus* (n = 7), followed by misidentification of *Mucorales* (n = 3), *Blastomyces* (n = 2), *Histoplasma* (n = 2), and *Candida* (n = 1) (Table 1). Discrepant cases were frequently from the lung or bronchoalveolar lavage fluid of immune incompetent patients.

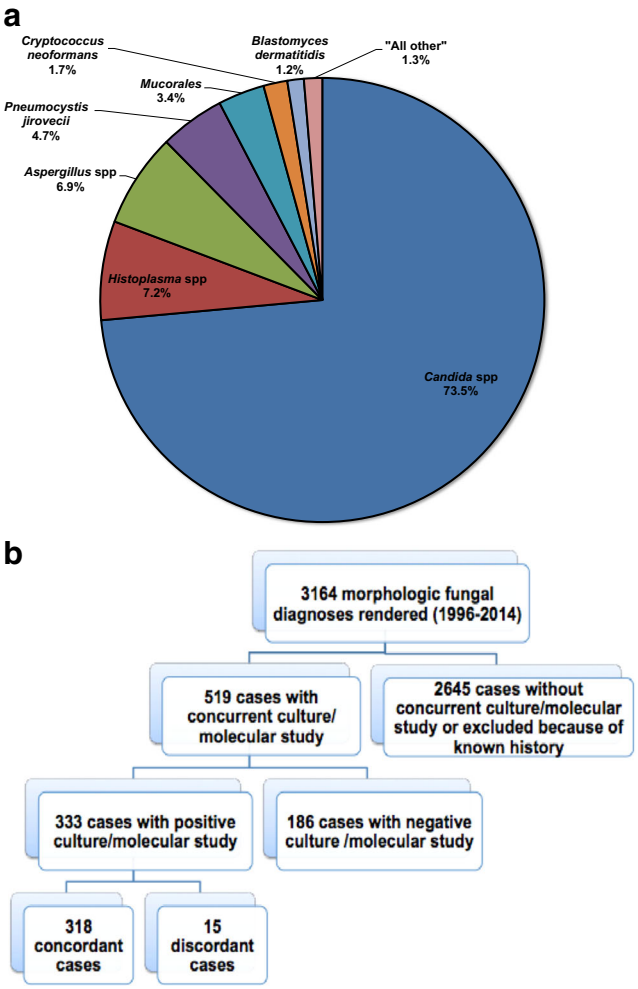

Fig. 1 **a.** Frequency of morphologic diagnoses. Grouped under Mucorales genera are *Rhizopus* and *Mucor*. Grouped into "all other" are dermatophytes (*Trichophyton*), hyalinohyphomyces (*Fusarium* spp, *Scedosporium*, and *Penicillium* spp), and dematiaceous molds. **b** Schematic overview of case selection

Root cause analysis of likely sources of error in discrepant cases

For the 12 cases for which slides were available, re-review showed that most frequently, the probable causes of discrepancies were interpretative errors between *Aspergillus* species and *Mucorales* (Fig. 2). These misinterpretations were bidirectional, with cases of septate hyphal elements called "mucormycosis" (case 5) as well as cases of ribbon-like hyphae called *Aspergillus* (case 7). In these cases, either the presence of septations and rare foci of dichotomous branching, characteristic of *Aspergillus*, was overlooked, or the presence of rare, ribbon-like hyphae and right angle branching, characteristic of *Mucorales*, was overlooked. Notably, there was also a case where septate hyphae were called *Aspergillus*, without mention of other hyaline septate molds and hyaline hyphomycetes, potentially highlighting a lack of knowledge that not all septate filamentous fungal forms are *Aspergillus* (case 12). An additional *Aspergillus*-related interpretive error involved the dual misinterpretation of tangentially sectioned hyphae as yeast forms, and of *Aspergillus* hyphae, which lack constrictions, as *Candida* pseudohyphae (case 3).

In addition to interpretive errors, other likely sources of errors identified could be attributed to morphologic mimics and/or sampling. As has been previously described [9], adjacent empty spherules of *Coccidioides* are a morphologic mimic of broad-based budding yeast, and rare *Coccidioides* endospore containing spherules may be easily overlooked (case 1). Finally, in four cases, both pathologists on re-review remained in agreement with the initial morphologic diagnosis rendered and found no evidence of the organism that grew on culture. These discrepancies were thus attributed to error in sampling. It should be noted that for one of the sampling error cases (case 11), a diagnosis of "changes consistent with allergic bronchopulmonary aspergillosis" was made based on the presence of allergic mucin despite the absence of any organisms on routine sections or GMS stain. While *Aspergillus* is a common etiology of fungal pulmonary hypersensitivity and allergic mucin, it is not the only fungal agent known to do so, and thus the term "allergic bronchopulmonary mycosis" is preferable [10].

Special stain use

Use of special stains (Grocott–Gomori's-methenamine silver stain (GMS), periodic acid–Schiff (PAS), Fontana–Masson,

Table 1 Clinicopathologic features of discrepant cases

Case	Age	Gender	Immune status	Specimen type	Morphologic diagnosis*	Culture diagnosis	Special stains	Likely source of error
1	74	M	Competent	Wedge lung biopsy	Consistent with Blastomycosis	*Coccidoides immitis*	GMS	Morphologic mimic
2	66	M	Incompetent (T-cell lymphoma)	Lung FNA	Consistent with *Blastomyces*	*Cryptococcus neoformans*	GMS and Mucicarmine	Slides not available
3	59	M	Incompetent (lung transplant)	Endobronchial lung biopsy	Consistent with *Candida* species	*Aspergillus fumigatus*	GMS	Interpretive
4	66	M	Competent	Transbronchial lung biopsy	Consistent with Mucormycosis	*Aspergillus species*	GMS	Interpretive
5	54	F	Competent	Sinus contents	Consistent with Mucormycosis	*Aspergillus flavus*	GMS	Interpretive
6	56	F	Incompetent (AML)	Maxillary sinus biopsy	Mucormycosis	*Aspergillus fumigatus*	GMS	Interpretive
7	63	M	Incompetent (BMT)	Transbronchial lung biopsy	Consistent with *Aspergillus*	*Rhizopus* species	GMS	Interpretive
8	66	M	Incompetent (AML)	Skin punch biopsy	Consistent with *Aspergillus*	*Rhizopus* species	None	Interpretive
9	75	F	Competent	Bronchoalveolar lavage	Organisms suggestive of *Aspergillus*	*Dactylaria* species	None	Slides not available
10	56	M	Incompetent (CLL)	Bronchoalveolar lavage	Consistent with *Aspergillus*	*Candida glabrata*	None	Sampling
11	59	M	Competent	Transbronchial lung biopsy	Consistent with allergic bronchopulmonary Aspergillosis	*Paecilomyces* and *Penicillium*	GMS	Sampling
12	30	F	Incompetent (AML)	Skin punch biopsy	Suggestive of *Aspergillus* species	*Fusarium* species	None	Interpretive
13	76	M	Competent	Bronchial washing	Suggestive of *Aspergillus*	*Penicillium* and *Alternaria* species	None	Sampling
14	70	F	Incompetent (CLL)	Bronchoalveolar lavage	Consistent with *Histoplasma*	*Cryptococcus neoformans*	GMS	Slides not available
15	48	M	Competent	Wedge lung biopsy	Consistent with *Histoplasma*	*Cryptococcus neoformans*	GMS	Sampling

AML — acute myelogenous leukemia, AFB — acid-fast bacillus stain, BMT — bone-marrow transplant, CLL — chronic lymphocytic leukemia, FNA — fine needle aspiration, GMS — Grocott–Gomori's methenamine silver stain, *derived from diagnosis line of pathology report

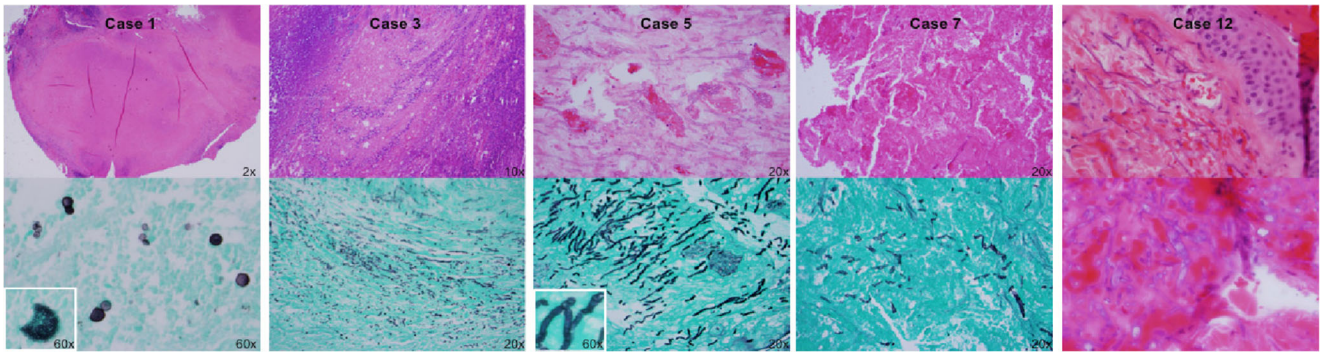

Fig. 2 Select micrographs from discrepant cases. *Case 1.* Adjacent yeast-like forms misdiagnosed as Blastomycosis. Cultures grew *Coccidioides*. *Case 3.* Misinterpretation of tangentially sectioned hyphae as *Candida* yeast forms. Cultures grew *Aspergillus*. *Case 5.* Misinterpretation of hyphal elements as mucormycosis. Cultures grew *Aspergillus*. *Case 7.* Misinterpretation of hyphal elements as *Aspergillus*. Cultures grew *Rhizopus*. *Case 12.* Misinterpretation of nonpigmented, branched, uniform, septate hyphae as *Aspergillus*. Cultures grew *Fusarium*

mucicarmine, and/or Alcian Blue) was variable. Pathologists more frequently ordered special stains in cases with *Histoplasma*, *Pneumocystis*, *Mucorales*, or *Blastomyces* (Table 2). The most commonly used stain was GMS (43.58%, 1379/3164). PAS was the second most commonly used stain (5.97%, 189/3164), and it was often ordered in combination with GMS (43.39%, 82/189) in cases with *Candida* diagnoses. The Fontana–Masson, mucicarmine, and Alcian Blue stains were employed infrequently, and were not preferentially employed in cases of *Cryptococcus*. Special stains were employed significantly more frequently in discordant (66.67%, 10/15) than in concordant (54.09%, 172/318) cases ($P < 0.01$).

Laboratory utilization patterns by medical service

The hospital services that most frequently submit samples for histologic examination are reflective of the body sites commonly infected by fungi (Table 3). Consistent with an inhaled route of infection and the lung as a primary infection site, cardiothoracic surgery and pulmonary/critical care were common ordering services for specimens containing *Histoplasma*, *Blastomyces*, *Pneumocystis*, *Aspergillus*, *Mucorales*, and *Cryptococcus*. For *Mucorales* and *Aspergillus*, submissions from otolaryngology indicate rhinocerebral infections as an additional common primary manifestation. Dermatology was a common ordering service for many fungal groups, consistent with a combination of disseminated infection and more rarely, primary cutaneous infection. *Cryptococcus* central nervous system dissemination was manifested in neurology service submissions. Finally, ophthalmology submissions in the "all other" group were predominantly cases of *Fusarium* keratitis.

Factors affecting the ordering of concomitant laboratory tests

We determined the frequency with which laboratory tests were performed as well as the frequency with which these studies were positive, and found wide variability between different fungi (Fig. 3). With an

Table 2 Use of special stains. Results are displayed as *n* (%) where *n* is the number of cases with stain performed and *N* is the total number of cases

Fungus (*N*)	GMS: *n* (%)	PAS: *n* (%)	Fontana–Masson: *n* (%)	Mucicarmine: *n* (%)	Alcian Blue: *n* (%)
Candida spp. (2327)	812 (35)	152 (7)	3 (0)	2 (0)	1 (0)
Histoplasma spp. (228)	180 (79)	14 (6)	1 (0)	6 (3)	0 (0)
Aspergillus spp. (217)	77 (35)	2 (1)	0 (0)	0 (0)	0 (0)
Pneumocystis jirovecii (150)	145 (97)	1 (1)	0 (0)	2 (1)	0 (0)
Mucorales (107)	93 (87)	6 (6)	2 (2)	0 (0)	0 (0)
Cryptococcus neoformans (55)	16 (29)	4 (7)	0 (0)	2 (4)	0 (0)
Blastomyces dermatitidis (38)	36 (95)	5 (13)	0 (0)	2 (11)	0 (0)
"All other" (42)	20 (48)	5 (12)	0 (0)	0 (0)	0 (0)

PAS — periodic acid–Schiff

Table 3 Relationship between submitting service and frequency of concomitant laboratory study. With the exception of *Cryptococcus neoformans* and *Blastomyces dermatitidis*, for which there were two services with the same submission frequency, and "all other," for which three services alone accounted for more than 70% of submissions, the four services most frequently submitting samples for histologic examination are shown for each fungus group. Results are displayed as *n* (%) where *n* is the number of cases with histologic exam requested, concomitant laboratory study performed, or negative laboratory study result per submitting service

Fungus	Service	Histologic exam requested: *n* (%)	Concomitant laboratory study performed: *n* (%)	Negative laboratory study: *n* (%)
Candida spp.	Gastroenterology	605 (26)	4 (1)	4 (100)
	Medicine	271 (12)	34 (13)	10 (29)
	Internal medicine	203 (9)	28 (14)	9 (32)
	Otolaryngology	171 (7)	0 (0)	–
Histoplasma spp.	Cardiothoracic surgery	108 (47)	54(50)	51 (94)
	Dermatology	15(7)	3 (20)	2 (67)
	Medicine	14(6)	5 (36)	3 (60)
	General surgery	13(6)	3 (23)	1 (33)
Aspergillus spp.	Pulmonary/critical care	44 (20)	25 (57)	5 (20)
	Cardiothoracic Surgery	30 (14)	13 (43)	6 (46)
	Otolaryngology	23 (11)	2 (9)	0 (0)
	Medicine	22 (10)	12 (55)	0 (0)
Pneumocystis jirovecii	Medicine	30 (20)	0 (0)	–
	Pulmonary/critical care	28 (19)	20 (71)	0 (0)
	Internal medicine	18 (12)	10 (56)	1 (10)
	Cardiothoracic surgery	12 (8)	9 (75)	1 (11)
Mucorales	Dermatology	30 (28)	15 (50)	6 (40)
	Otolaryngology	19 (18)	13 (68)	8 (62)
	Pulmonary/critical care	16 (15)	8 (50)	2 (25)
	Ophthalmology	12 (11)	8 (67)	2 (25)
Cryptococcus neoformans	Medicine	10 (18)	4 (40)	1 (25)
	Pulmonary/critical care	8 (15)	2 (25)	1 (50)
	Internal medicine	8 (15)	6 (75)	3 (50)
	Neurology	6 (11)	6 (100)	1 (17)
	Cardiothoracic surgery	4 (7)	2 (50)	1 (50)
Blastomyces dermatitidis	Dermatology	12 (32)	10 (83)	1 (10)
	Cardiothoracic surgery	6 (16)	5 (83)	1 (20)
	Pulmonary/critical care	5 (13)	4 (80)	1 (25)
	General surgery	3 (8)	3 (100)	3 (100)
	Medicine	3 (8)	2 (67)	2 (100)
"All other"	Ophthalmology	12 (29)	8 (67)	0 (0)
	Dermatology	10 (24)	3 (30)	1 (33)
	Otolaryngology	9 (21)	0 (0)	–

average of 40.6%, laboratory test rates were not significantly different between *Histoplasma*, *Aspergillus*, *Pneumocystis*, *Mucorales*, *Cryptococcus*, and "all other" fungi; however, laboratory test rates were significantly lower for *Candida* (6.96%) and higher for *Blastomyces* (86.84%) ($P < 0.01$ for both). The low laboratory test ordering rate for *Candida* was largely driven by upper gastrointestinal and oropharyngeal samples submitted from the gastroenterology and otolaryngology services (Table 3).

Factors impacting culture based recovery and antigen, serologic, or molecular detection of fungi

In 36.0% of the cases with concomitant laboratories studies, no fungal organisms were recovered, or antigen, serologic, or molecular diagnostics study results were negative. A particularly high negative laboratory study result rate (74.7%, $P < 0.01$) was observed for *Histoplasma*. Additionally, culture recovery and/or positive laboratory test rates were significantly higher for *Aspergillus* (83.8%), *Pneumocystis jirovecci*

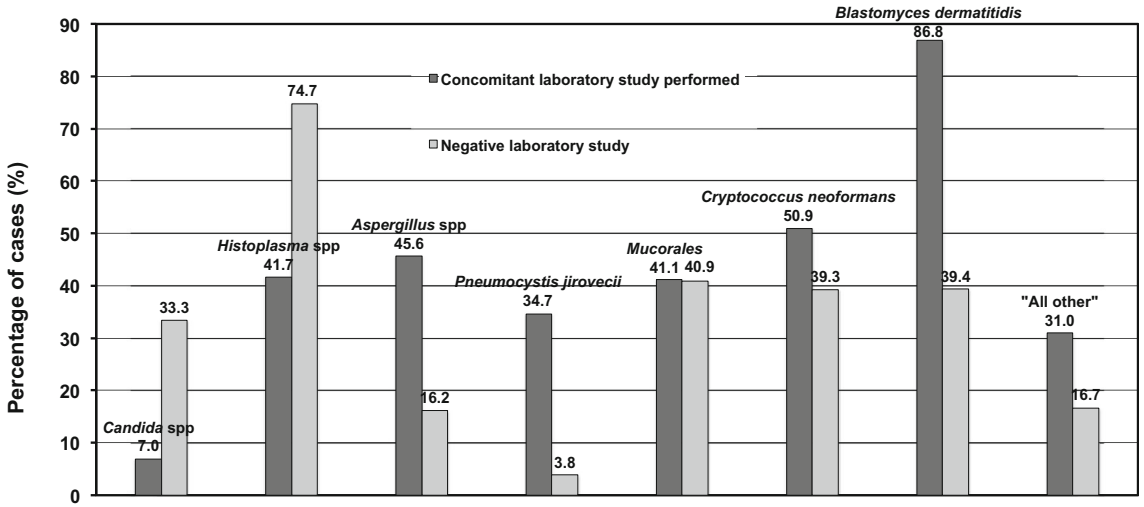

Fig. 3 Frequency at which histologic specimens were sent for laboratory studies, and frequency at which laboratory studies were positive

(96.2%), and dermatophytes, hyaline hyphomycetes, and dematiaceous molds (83.3%) ($P < 0.01$). The high negative laboratory study result rate for *Histoplasma* was largely driven by thoracic lymph nodes with burnt-out granulomas submitted from the cardiothoracic surgery service (Table 3).

Discussion

Paramount to the timely initiation of appropriate therapy in response to invasive fungal infections is rapid and accurate diagnosis, which is best achieved through histology (microscopic examination) in combination with laboratory studies (culture, antigen detection or serologic tests, or molecular diagnostics). This study identifies areas for both pathologist and clinician education that can be targeted to improve 1) the accuracy of morphology-based differential diagnoses, and 2) the frequency with which tissue specimens with evidence of fungal infection are submitted for laboratory studies.

As with previous studies [11–13], in this study, the most common cause for incorrect morphologic diagnoses was interpretive error between *Aspergillus* species and *Mucorales*. This misinterpretation of septate versus non- or pauci-septate hyphae has significant potential for adverse consequences, as invasive aspergillosis and mucormycosis are treated with different classes of antifungals [14]. *Aspergillus* species usually appear as thin, septate hyphae with acute-angle, dichotomous branching. In contrast, *Mucorales* usually appear as wide, ribbon-like, non- or pauciseptate hyphae with right-angle branching, and are relatively poorly stained by GMS. In practice, however, in cases with only rare hyphae, and when hyphae are degenerate or swollen, the distinction between septate and pauciseptate hyphae and the assessment of hyphal width and branch angle may be indeterminate. In such cases, use of GMS stain and evaluating hyphal width at areas of septation, which are less affected by swelling, is recommended [15]. Thus, enhanced pathologist education on fungi and fungal terminology may improve the accuracy of morphology-based differential diagnoses. Specifically, as the distinction between *Aspergillus* species and *Mucorales* can be subtle, education in the form of greater exposure to specimens with different fungi may be necessary to afford pathologists the opportunity to develop an eye for these differences.

In addition to emphasizing *Mucorales* morphology, morphologic-based differential diagnoses may be improved with the knowledge that in practice, most of the septate, hyaline molds cannot be distinguished from *Aspergillus* based on histologic morphology in tissue sections, where typically only hyphae (and not fruiting structures) are present, and morphologies may be mixed due to tissue reaction and antifungal therapy. As highlighted by the case 12 discrepancy, not all nonpigmented, septate hyphae with acute-angle branching are *Aspergillus*. However, the fact that these discrepancies were infrequent highlights the fact that *Aspergillus* is a very common cause of invasive mold infection overall. With new medical advances in immunosuppression and more aggressive chemotherapies, though, "new," "non-*Aspergillus*" septate molds previously thought to be environmental organisms not pathogenic to humans, have emerged as human pathogens which are a diagnostic challenge to clinicians and pathologists alike [16–18].

To provide both maximal morphology-based information and an accurate differential diagnosis, when invasive hyphal elements are encountered, we advocate wording diagnoses using the templates proposed by Sangoi et al. and Guarner et al. [11, 15]. Diagnosis should provide a description of the morphology of the fungal elements followed by a differential of fungi consistent with the observed morphology. Briefly, when hyphal fungal organisms are identified, the pathologist should specify if the

hyphae are septate or pauci-/nonseptate. If septate hyphae are present in the absence of fruiting bodies, it should be noted that *Aspergillus* spp. cannot be morphologically distinguished from dermatophytes, hyalinohyphomyces (such as *Fusarium*, *Scedosporium*, and *Penicillium* spp.), and dematiaceous molds. When yeast-like organisms are identified, the pathologist should specify if pseudohyphae are present. Finally, in all cases, the quantity of organisms and the presence or absence of tissue necrosis and vascular invasion should be noted, and correlation with laboratory studies should always be recommended.

Culture, antigen detection and serologic tests, and molecular diagnostics study rates are largely dictated by clinical utility. The majority of specimens with a morphologic diagnosis of *Candida* were submitted by gastroenterologists performing upper endoscopies and were esophageal biopsies, where there is frequent overgrowth of normal flora. Thus, the significantly lower rate at which tissue specimens with *Candida*, as compared to other fungi, had a concomitant culture probably reflects that in cases of *Candida* esophagitis, it is most cost-effective to treat based on clinical suspicion and morphologic diagnosis alone. Additional specimens with *Candida* organisms on histologic sections but without concomitant laboratory studies were submitted by otolaryngologists. Oropharyngeal colonization by *Candida* is common. The clinical and pathological features of this disease process are straightforward, and routine culture of the oral cavity is discouraged, as normal flora complicates interpretation of culture findings.

The purpose of special stains is to highlight organisms, which can be especially helpful in cases where organisms are rare, and in some cases, to aid in identification based on staining characteristics [19]; however, here we found no evidence that the use of special stains improved the accuracy of morphologic diagnoses. Perhaps reflecting the fact that cases which were ultimately discrepant with cultures presented pathologists with a higher level of diagnostic challenge, and thus necessitated special stains, we found that special stains were actually significantly more frequently ordered in discordant than in concordant cases. A number of factors may contribute to the high frequency of special stain use in cases of *Histoplasma*, *Pneumocystis*, *Mucorales*, and *Blastomyces* diagnoses, and the relatively low frequency of special stain use in cases of *Candida* and *Aspergillus* diagnoses. First, due to their small size, both *Histoplasma* and *Pneumocystis* are difficult to visualize without special stains. Also, our pathologists may be more comfortable/familiar with *Candida* and *Aspergillus* morphology due to the relative ubiquity of these two fungi. In addition to pathologists' preferences, experience, and knowledge, clinician requests, a factor which we were unable to assess in this retrospective study, may also contribute to variability in special stain ordering.

In this 18-year retrospective review of surgical pathology and cytology specimens from which a pathologist diagnosed fungi at a large tertiary care medical center, we show that the histologic identification of fungi in tissues is usually accurate; however, reporting should be standardized to take into account morphologic mimics and the limitations of histology. Discrepancies between morphologic diagnoses and laboratory test results highlight the need for education regarding the morphology of *Aspergillus* and *Mucorales* in tissue. Overall, only 16% of cases with morphologic diagnoses of fungi had a concomitant sample sent for a laboratory test, and of these cases, 36% had a negative laboratory test result.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in relation to this study.

Ethical approval For this type of retrospective study, formal consent is not required.

Informed consent Informed consent was not required for this study.

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