Histologic Features of Zygomycosis
Emphasis on Perineural Invasion and Fungal Morphology

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Objective—Invasive zygomycosis is rapidly progressive and is associated with angioinvasion and infarction. Invasive disease requires emergent surgical and medical intervention. Because it is important for surgical pathologists to recognize these fungi and their preferential sites of growth, the objective of this article is to describe the fungal morphology and histopathologic findings in biopsies from patients with zygomycotic disease, with emphasis on preferential sites of fungal growth.

Design.—Medical record and histologic review identified 20 patients with zygomycosis. Inclusion criteria included the presence of typical ribbonlike hyphae and positive culture, a clinical history of invasive zygomycosis, or both. The histologic features of disease and the fungal morphology were assessed.

Results.—Fungus ball (15%), rhinocerebral (55%), and pulmonary (30%) disease were the types of disease represented. The inflammatory responses were predominantly neutrophilic (50%), predominantly granulomatous (5%), pyogranulomatous (25%), or absent (20%). Invasive disease was characterized by prominent infarcts (94%), angioinvasion (100%), and, surprisingly, prominent perineural invasion (90%) in biopsies that contained nerves for evaluation. At least rare hyphal septa were always seen (100%), and most branches (93%) varied from 45° to 90°.

Conclusions.—As known to mycologists, zygomycetes are paucisepolate, rather than asperate, molds. Therefore, the presence of an occasional septum is expected. Perineural invasion is a common finding in invasive zygomycosis, as are angioinvasion and infarcts. Therefore, prior to excluding the presence of these fungi in biopsies suspected to contain zygomycetes, the perineural space should be carefully examined.

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Organisms of the class Zygomycetes, which includes the orders Mucorales and Entomophthorales, are well-known causes of infection in the upper and lower respiratory tract, orbit, meninges, brain, and other sites. These organisms most frequently infect individuals with defects in immunity. They are therefore commonly encountered by medical microbiologists, as well as by surgical and autopsy pathologists. Culture is necessary for speciation, since the tissue forms of these organisms are usually indistinguishable.

In tissue and in culture, clinically significant zygomycete hyphae can often be distinguished from other invasive hyaline molds by their greater width and obtuse branching pattern. A term often used by surgical pathologists and frequently encountered in the literature, asperate, is also ascribed to the zygomycetes. However, this term is a misnomer. The zygomycetes are in fact paucisepolate rather than asperate, having septa at irregular intervals.

The zygomycetes are known to be angiotropic. Indeed, a common feature of the zygomycetes is vascular invasion with subsequent infarction of the surrounding tissue. A lesser known aspect of the histopathology associated with this organism is its neurotropic activity, which has been reported in only 3 instances in the medical literature, but appears to be a frequent finding.

METHODS
A computerized search of the Surgical Pathology files of the Cleveland Clinic Foundation, Cleveland, Ohio, for patients whose biopsies contained organisms consistent with Zygomycetes or Mucor was performed. A computerized search of the files of the Section of Microbiology was then performed for these same patients, and culture results, if present, were noted. For those patients without confirmatory culture results, chart review was performed to confirm the clinical diagnosis of zygomycete infection.

Surgical pathology slides were reviewed separately by a pathology resident (J.L.F.) and an infectious disease pathologist/medical microbiologist (G.W.P.). The type of inflammation (neutrophilic, granulomatous, pyogranulomatous, or absent) was noted. Invasiveness was assessed, including the presence of infarcts, angioinvasion, and perineural invasion. Additionally, the morphology of the hyphae was assessed, with note taken of the presence and amount of septation and the angle of hyphal branching. The number of septa was categorized as few if they were infrequently identified, moderate if several septa were identified in each ×40 high-power field (hpf), and many if abundant septa, similar to those seen in a typical Aspergillus species, were seen in each ×40 hpf. The presence of other fungal species in the same specimen was assessed as well. Angles of branch points were assessed as (a) 45° only, (b) 90° only, or (c) ranging between 45° and 90°.

RESULTS
A total of 20 patients with zygomycotic disease were identified. Three types of disease were identified: rhino-
cerebral disease (Table 1), pulmonary disease (Table 2), and fungus ball (Table 3). The type of diseases included fungus ball (n = 3, 15%), rhinocerebral (n = 11, 55%), and pulmonary (n = 6, 30%). Ten patients (50%) had cultures submitted at the time of surgery that were positive for Zygomycetes (6 Mucor, 3 Rhizopus, and 1 Rhizomucor). The remaining 10 patients had clinical histories consistent with fungal infection.

The inflammatory response to the fungi varied considerably. The most common inflammatory response, occurring in 10 patients (50%), was composed entirely of neutrophils. A pyogranulomatous response, which consisted of an approximate equivalent mixture of neutrophils and granulomas, was seen in 5 (25%) patients. A single patient (5%) had a granulomatous response alone. In the remaining biopsies from 4 patients (20%), no inflammatory response was seen.

Characteristic invasive features were present in nearly all cases, irrespective of tissue of origin. Hyphal invasion of arterial and venous walls (angioinvasion) was seen in all patients with invasive zygomycosis in which vessels were present for evaluation (n = 15, 100%), and associated infarction of surrounding tissues was noted in 16 (94%) of 17 cases.

Perineural invasion was a finding in a surprisingly high percentage of patients with invasive zygomycosis whose biopsies contained nerves for evaluation (Figure 1). Nerves were present for evaluation in the biopsies from 10 patients, and 9 of these (90%) had associated perineural invasion. In these lesions, the hyphae appeared to invade the perineurium, the loose fascial sheath surrounding the nerve. The neural tissue itself was also involved in some instances. Occasionally, only rare hyphal elements were present (Figure 2).

In the patients with zygomycetes identified in fungus balls, the hyphae had a compressed distorted appearance. Because the hyphal walls of the zygomycetes appear to be thin relative to those of other fungi, they may be prone to such distortion. They are also susceptible to twisting and folding, producing septalike artifacts. A careful search for true septations revealed at least rare septation in all cases (n = 20, 100%) (Figure 3). These septa were identified in

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### Table 1. Rhinocerebral Zygomycosis*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Infarct</th>
<th>Inflammation</th>
<th>Angioinvasion</th>
<th>Perineural Invasion</th>
<th>Fungal Morphology</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>Rhizopus</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>Mucor</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>Rhizopus</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>Mucor</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>No</td>
<td>Rare sept, 45–90</td>
<td>Mucor</td>
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<tr>
<td>9</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>Yes</td>
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<td>Mucor</td>
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<tr>
<td>10</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>Yes</td>
<td>Mod sept, 45–90</td>
<td>Mucor</td>
</tr>
<tr>
<td>11</td>
<td>No</td>
<td>Gran</td>
<td>NP</td>
<td>NP</td>
<td>Rare sept, 90</td>
<td>Rhizomucor</td>
</tr>
</tbody>
</table>

* Neut indicates acute inflammation with predominance of polymorphonuclear leukocytes; gran, granulomatous inflammation; NP, vessels or nerves are not present for evaluation; Rare sept, rare extent of septation; Mod sept, moderate extent of septation; 45–90, branching between 45° and 90°; and 90° branching only.

### Table 2. Pulmonary Disease*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Infarct</th>
<th>Inflammation</th>
<th>Angioinvasion</th>
<th>Perineural Invasion</th>
<th>Fungal Morphology</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>None</td>
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<tr>
<td>4</td>
<td>Yes</td>
<td>Neut</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Neut, Gran</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>Rhizopus</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
<td>NP</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
</tbody>
</table>

* Neut indicates acute inflammation with predominance of polymorphonuclear leukocytes; Gran, granulomatous inflammation; NP, vessels or nerves are not present for evaluation; Rare sept, rare extent of septation; and 45–90, branching between 45° and 90°.

### Table 3. Fungus Ball*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Inflammation</th>
<th>Mixed Fungi</th>
<th>Hyphal Compression/Distortion</th>
<th>Fungal Morphology</th>
<th>Culture</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Neut</td>
<td>Aspergillus conidiophores, hyaline septate hyphae</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>Mucor</td>
</tr>
<tr>
<td>2</td>
<td>Neut</td>
<td>Aspergillus conidiophores, hyaline septate hyphae</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Neut</td>
<td>Hyaline septate hyphae</td>
<td>Yes</td>
<td>Rare sept, 45–90</td>
<td>None</td>
</tr>
</tbody>
</table>

* Neut indicates acute inflammation with predominance of polymorphonuclear leukocytes; Rare sept, rare extent of septation; and 45–90, branching between 45° and 90°.
areas away from identifiable folds in the hyphae so as to avoid artifacts. In only 1 patient, an individual with rhinocerebral disease, was the septation somewhat more frequent. This organism was shown by culture to be *Mucor*. No other characteristics identified this case as being different from the other examples.

Branching was identifiable in all biopsies. The degree of branching was assessed and separated into 3 categories: 45° only, 90° only, or from 45° to 90°. In the biopsies from 19 patients, the angle of hyphal branching varied from 45° to 90°. In one case, branch points of only 90° were identified. This case was the only example of *Rhizomucor* in the series and was an extremely limited sample; hyphae were limited to 1 microscopic field. The limited extent of disease in this example was the most likely cause of the limited branch variation.

In the fungus balls, admixed zygomycete and non-zygomycte hyphae were seen in all 3 patients reviewed. Two of these contained conidiophores resembling those of *Aspergillus*. Additionally, all 3 contained hyaline septate fungi admixed with the typical ribbonlike pauciseptate hyphae characteristic of the zygomycetes.

**COMMENT**

We present a study of the histologic features present in biopsies from 20 patients with zygomycosis. In addition to the typical, well-described histologic features, at least rare hyphal septa were always seen, and a high preponderance of perineural invasion was identified.

The concept that the organisms of the class Zygomycetes have pauciseptate hyphae is one that is well known to mycologists and medical microbiologists, but is often underappreciated by surgical pathologists. These fungi are often thought to be aseptate, rather than pauciseptate. If the septations are thought not to occur in the zygomycetes, these organisms may be erroneously identified as a septate fungus, such as *Aspergillus*. The most characteristic feature of these molds is the presence of broad ribbonlike hyphae. Nearly all cases had a branching pattern that varied from 45° to 90°. In contrast to the zygomycetes, *Aspergillus* tends to demonstrate an acute branching pattern and has narrower, more uniform septa.145

It is important not to overestimate the number of septa in the zygomycetes. The hyphae of the zygomycetes appear thinner-walled compared to other fungal hyphae, which possibly accounts for their weaker staining with Gomori methenamine silver and periodic acid–Schiff techniques relative to fungi with thicker cell walls. The hyphae of the zygomycetes appear more prone to twisting and folding compared to those of other species. Such folds can be misinterpreted as septa and can cause the affected hyphae to be misinterpreted as those of a septate fungus, such as *Aspergillus*.

The extensive perineural invasion reported here suggests additional modes of invasion and spread. In the most commonly encountered clinical setting, rhinocerebral zygomycosis, it is believed that the fungal hyphae gain access to the meninges, orbit, and brain both by invading local vessels and via direct extension through the cribiform plate into the central nervous system. Retrograde extension of the fungi into the brain by means of the nerves is another plausible mechanism. To our knowledge, this mode of invasion has not been described in the pathology literature before, but this phenomenon has been reported by researchers in ophthalmology and otolaryngology. The rhinocerebral presentation of zygomycosis is the one most associated by earlier researchers with neural and perineural invasiveness, although we noted perineural invasion in tissue from the lung as well.

Stefani and Mehraein4 described a patient with acute rhino-orbito-cerebral mucormycosis in which there was histologic evidence of involvement of the optic nerve. The patient was a 68-year-old man with diabetes mellitus who was admitted for treatment of diabetic coma. He developed acute orbital cellulitis and necrosis with an associated new sinus density. The patient’s clinical course continued to deteriorate, and he died 10 days after admission. On autopsy, new infarcts were noted throughout the patient’s brain, including the right frontal lobe. Microscopic examination demonstrated vascular invasion by zygomycete hyphae with associated infarcts. Examination of the affected globe revealed hyphal invasion of the optic nerve, both in an area where a host giant cell reaction was present and in an area without an associated inflammatory response. The fungi penetrated the perineurium and involved the nerve.8
McLean reported a second example of perineural invasion in 1996. The patient was a 65-year-old with diabetes mellitus who developed unilateral periorbital edema and facial swelling with sinus opacification on computed tomography. The patient ultimately died and an autopsy was performed. Findings included evidence of trigeminal nerve involvement by hyphae resembling Mucor.

Although the presence of perineural invasion is noted in nearly all patients in this study whose biopsies contained nerves, the relative importance of this finding as a means of spread of disease is unknown. The most prominent findings associated with Zygomycetes infection were invasion of blood vessels and tissue infarctions. Furthermore, vascular access appears to allow the fungus to invade contiguous structures. However, perineural invasion may represent another means of spread of disease in this population.7,9,10

The mechanisms of angiotropism or perineural invasion remain unknown. Limited research has been done regarding the role of virulence factors of zygomycotic disease. The role of iron uptake appears to be of importance in the development of the host-parasite relationship, and a number of studies have established the importance of iron acquisition by several of these organisms.11-15 Other host factors, such as the roles of diabetic ketoacidosis and fungistatic properties of serum have been explored.16-19 However, a possible link between the mechanisms of vascular and neural invasion has not yet been drawn. Perhaps the perineural sheath of loose connective tissue merely represents a "path of least resistance" compared to the surrounding tissues. However, different mechanisms must exist for vascular invasion, since blood vessel walls are more dense than fascia.

Although the relative importance of perineural invasion as a means of spread of disease remains unknown, it is an important practical consideration for the surgical pathologist, especially when evaluating small biopsies at frozen section. Because of their characteristic weak staining with hematoxylin-eosin, the hyphae of the zygomycetes may be easily overlooked, particularly in a poorly stained frozen section. In this series, some biopsies contained relatively few hyphae that stained poorly; the pale hyphae may be easily overlooked, particularly in a poorly stained frozen section. In this series, some biopsies contained relatively few hyphae that stained poorly; the pale hyphae were difficult to identify in the normally eosinophilic background of the perineurium. Another important practical consideration is that occasional acute angle branch points and occasional septation do not exclude zygomycosis.

In summary, we report a series of 20 cases of zygomycosis, 10 with confirmatory cultures and the remainder with a history of zygomycosis. All biopsies contained ribonlike hyphae typical of the zygomycetes. Three diseases were present: rhinocerebral, pulmonary, or fungus ball. One histopathologic feature heretofore underreported in the literature, perineural invasion, was noted in the vast majority of patients (90%) whose biopsies contained nerves for evaluation. Angioinvasion and infarcts associated with invasive zygomycotic disease were the most common findings. We reemphasize that septa may be seen in these fungi, since they are pauciseptate rather than aseptate molds. Furthermore, rather than hyphal branch points being 90° only, the angle of branching varies from 45° to 90° and is only rarely 90° only. These findings have implications for those individuals interested in the histopathologic features of infection by the agents of zygomycosis.

References