Morphologic Criteria for the Preliminary Identification of *Fusarium, Paecilomyces,* and *Acremonium* Species by Histopathology

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Nontraditional human pathogenic fungi, including *Fusarium, Paecilomyces,* and *Acremonium* species, have been increasingly documented as agents of infection in immunocompromised patients and, occasionally, in normal hosts. Although definitive identification of these fungi requires culture, they often can be identified provisionally in tissue sections by a combination of histologic features, including hyaline septate hyphae and characteristic reproductive structures known as phialides and phialoconidia. These morphologic characteristics, although familiar to mycologists, are easily overlooked by histopathologists; as a result, *Fusarium* species and *Paecilomyces lilacinus* are frequently misidentified in tissue sections as *Aspergillus* or *Candida* species. We identified 19 culture-proved cases of infection with species of *Fusarium, Paecilomyces,* or *Acremonium;* retrospectively reviewed histologic specimens stained by routine hematoxylin and eosin, Gomori methenamine silver, and/or periodic acid-Schiff stains; and delineated morphologic criteria that will help pathologists make a preliminary identification of these fungi by histopathology. Adventitious sporulation was found in 9 of 9 infections caused by *Paecilomyces* species, 7 of 10 infections caused by *Fusarium* species, and in the single case of infection caused by *Acremonium strictum.* Histologic recognition of these morphologies may help clinicians select appropriate initial antifungal treatment and manage the infection. (Key words: *Fusarium; Paecilomyces; Acremonium;* Hyalohyphomycosis; Histopathology; Adventitious sporulation) Am J Clin Pathol 1998;109:45-54.

Fusarium, Paecilomyces, and *Acremonium* species have been documented as etiologic agents in cases of ocular, subcutaneous, pulmonary, endocardial, and disseminated infections.[1–9] These infections occur most frequently in neutropenic and immunocompromised patients, including those with burns, leukemia, or aplastic anemia, patients undergoing chemotherapy, and transplant recipients.[4,6,7,10–14] Immunocompetent hosts also may be affected, however, as demonstrated by reported cases of maxillary sinusitis, keratitis, endophthalmitis, and disseminated infection.[6,8,15,16]

The term *hyalohyphomycosis* denotes infections characterized by the formation of colorless, septate fungal hyphae in host tissue.[17] Because these infections are quite heterogenous both clinically and etiologically, the term is particularly useful for making a histologic diagnosis in the absence of culture results. Aspergillosis can be considered a particular kind of hyalohyphomycosis, but as a practical matter the name more often is used to refer to infections caused by less-common opportunistic pathogens, including species of *Fusarium, Paecilomyces,* and *Acremonium.* The procedure for identifying these species in culture is based largely on morphologic study of their reproductive structures, including fertile cells known as phialides, and the conidia (spores) that arise serially from the apical orifice of each phialide (Fig 1). These reproductive structures can occur within infected human tissue as well, a phenomenon that has been termed *adventitious sporulation.*[18]

In addition to providing an important histologic basis for the identification of *Fusarium, Paecilomyces,* and *Acremonium* species, adventitious sporulation may explain certain aspects of the biological behavior of these organisms in vivo. As with species of *Aspergillus* and *Rhizopus,* species of *Fusarium, Paecilomyces,* and *Acremonium* can invade vascular structures, resulting in occlusion, infarction, and necrosis.[3–6] In contrast to aspergillosis and zygomycosis, however, there is an increased frequency of positive blood cultures, with recovery rates as high as 50%

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for *Fusarium* species *vs* less than 5% for *Aspergillus* species during disseminating disease.[3,19,20] Findings in *Acremonium strictum, Paecilomyces lilacinus, Fusarium moniliforme,* and *Scedosporium prolificans* infections showed that in vivo sporulation can occur in conjunction with positive blood culture results. Accordingly, it was hypothesized that the sustained release of fungal spores into the bloodstream, via angioinvasion and adventitious sporulation, may explain the higher prevalence of positive blood culture results and rapid rate of dissemination associated with these fungi.[4,18]

The accurate identification of *Fusarium* species, *Paecilomyces lilacinus*, and *Acremonium* species is of practical importance, because they often are resistant to several of the available antifungal agents. Selection of initial antifungal drug therapy and possible inclusion of surgical treatments may depend on the identity of the fungus,[21–23] and early specific antifungal therapy is more likely to lead to a positive clinical outcome. Definitive diagnosis of etiology in hyalohyphomycosis requires culture for the identification of the offending organism. Indeed, on tissue biopsy specimens, the causative agents are often misidentified as *Aspergillus* or *Candida* species. An initial presumptive identification often can be made, however, by detecting adventitious sporulation through careful histologic examination of appropriately stained biopsy sections. We retrospectively studied the cases of 19 patients with culture-documented infections with *Fusarium, Paecilomyces,* and *Acremonium* species. The study was undertaken to determine the frequency, histologic characteristics, and clinical circumstances of adventitious sporulation and to catalogue histologic findings that are helpful in distinguishing *Fusarium, Paecilomyces,* and *Acremonium* species from other fungi in tissue biopsy specimens.

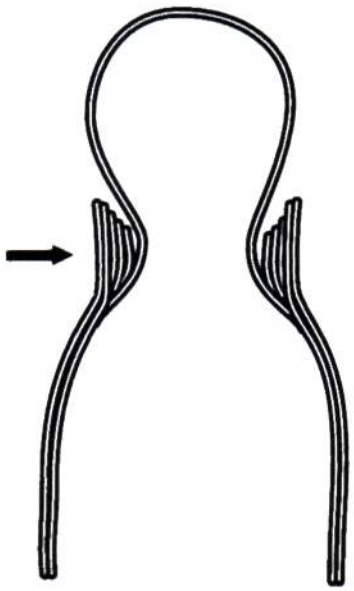

FIG 1. Sectional diagram of phialidic conidiogenesis. Conidia arise serially at the phialide orifice, with each conidium leaving a cell wall remnant on detachment. Accumulation of these remnants results in periclinal thickening (arrow) and is histologically apparent as a broad, darkly stained band.

METHODS

The records of 19 patients, seen at Duke University Medical Center or the Veterans Affairs Medical Center, Durham, North Carolina, during a 12-year period (1985–1996), were reviewed. These patients had cultures positive for *Fusarium, Paecilomyces,* or *Acremonium* species and at least one tissue biopsy specimen or fluid containing demonstrable fungus. All corresponding histologic sections stained by the hematoxylin and eosin (H&E), Gomori methenamine silver (GMS), and periodic acid-Schiff (PAS) methods were reviewed by three observers (KL, DH, WS). All specimens were examined for the presence of hyphae, regularity of hyphal diameter, frequency of hyphal septation, type of hyphal branching, presence of phialides and phialoconidia, and presence of yeast-like (unicellular) budding cells. Tissue response, including acute inflammation, granulomas, necrosis, vascular invasion, and thrombosis, was evaluated. Infections were characterized as occurring in an open space (eg, paranasal sinus or ocular surface, pulmonary cavitary lesion, ulcerated skin) or closed space (ie, surrounded by tissue or fluid). Medical records were examined to determine underlying disease, presence of neutropenia, evidence of dissemination, blood culture results, and patient outcome.

RESULTS

Nineteen patients who had fungal cultures positive for *Fusarium, Paecilomyces,* or *Acremonium* species and tissue or cytologic specimens containing visible fungal cells were identified. One patient had lung lesions, 11 had lesions involving the skin and/or subcutaneous tissue, 1 had both lung and subcutaneous lesions, 4 had eye infections, and 2 had peritonitis (Table 1).

In five cases in which culture results preceded histologic examination, a tissue diagnosis of hyalohyphomycosis was rendered (Table 2). These included two cases of keratitis described as consistent with *Fusarium* sp, one case of keratitis described as consistent with *Paecilomyces* sp, one soft tissue lesion

TABLE 1. CLINICAL FEATURES OF PATIENTS WITH HYALOHYPHOMYCOSIS CAUSED BY *FUSARIUM*, *PAECILOMYCES*, AND *ACREMONIUM* SPECIES*

Case	*Underlying Disease*	*Neutropenic*	*Biopsy/Resection Site*†	*Culture*	*Focal/Disseminated*	*Blood Culture*	*Outcome*
1	CML	Yes	Lung, skin	*A strictum*	Dissem	+	Fatal
2	ALL	No	Lung	*P lilacinus*	Dissem	+	Survived
3	None	No	Eye	*Fusarium* sp	Focal	ND	Survived
4	None	No	Cornea	*F oxysporum*	Focal	ND	Survived
5	None	No	Cornea	*P lilacinus*	Focal	ND	Survived
6	None	No	Cornea	*P lilacinus*	Focal	ND	Survived
7	Renal txp	No	Skin	*F solani*	Focal	ND	Survived
8	Diabetes	No	Skin	*Fusarium* sp	Focal	ND	Survived
9	BM txp	Yes	Skin/sub	*F solani*	Focal	–	Survived
10	AML	Yes	Skin/sub	*Fusarium* sp	Dissem	+	Fatal
11	AML	Yes	Skin/sub	*F solani*	Dissem	–	Fatal
12	Lymphoma	Yes	Skin/sub	*Fusarium* sp	Dissem	–	Fatal
13	CTCL	Yes	Skin/sub	*Fusarium* sp	Dissem	–	Fatal
14	Heart txp	No	Skin/sub	*P lilacinus*	Dissem	ND	Survived
15	Renal txp	No	Skin/sub	*P lilacinus*	Focal	–	Survived
16	ALL	No	Skin/sub	*P lilacinus*	Dissem	–	Fatal
17	AML	Yes	Skin/sub	*P lilacinus*	Focal	–	Fatal‡
18	CRF	No	Per dial	*F moniliforme*	Focal	ND	Fatal‡
19	CRF	No	Per dial	*P variotii*	Focal	ND	Survived

CML = chronic myelogenous leukemia; Dissem = disseminated; ALL = acute lymphocytic leukemia; txp = transplant; BM txp = bone marrow transplant; AML = acute myelogenous leukemia; CTCL = cutaneous T-cell lymphoma; CRF = chronic renal failure; ND = not done; Skin/sub = skin and subcutaneous soft tissue; Per dial = peritoneal dialysate/dialysis catheter; + = culture positive; – = culture negative.

*Two cases discussed in the text are excluded from the table: One was not cultured; the other did not occur within the time period under review.

†All sites were culture positive.

‡Death not attributable to fungal infection.

described as consistent with *Fusarium* sp, and one pulmonary lesion described as consistent with *Acremonium* sp. Two lesions of skin and soft tissue were identified histologically as consistent with hyalohyphomycosis before culture-based identification. Of the remaining cases, two were originally determined to be consistent with *Aspergillus* species, and four others to be candidiasis; in five cases, the presence of fungal elements was reported without further characterization. One case was initially identified as negative for organisms.

On review, specimens from all patients contained septate, hyaline hyphae. The hyphae in nearly all cases were irregular, with considerable variation in diameter (Fig 2). True hyphal branching was identified in 17 of the 19 specimens. Six contained 45-degree hyphal branching, two had 90-degree branching, and nine had both 45- and 90-degree branching (Table 2).

Phialoconidia were present in 16 cases, including all 12 cases involving closed tissue spaces (Table 2). Phialoconidia were unicellular spores varying in shape from subglobose (almost spherical) (Fig 3, A), ellipsoidal (Fig 3, B), or oval (Fig 3, C) to cylindrical (Fig 3, D) and sometimes curved (Fig 3, E). Rare examples of multicellular *Fusarium* conidia (Fig 3, F) were present in one case of colonized necrotic skin and one case of peritonitis. Common to most of these conidial shapes was the presence of a rounded apex and a flat basal scar representing the point of detachment from the parent phialide. Unicellular phialoconidia of *Fusarium* species ranged in size from 2.2 to 4.4 × 5.3 to 19.1 μm (average, 3.0 × 9.4). Phialoconidia of *P lilacinus* ranged from 1.4 to 3.5 × 2.5 to 15.4 μm (average 2.3 × 5.0).

Phialides, from which phialoconidia arose, were seen in 15 of 19 cases, including 11 of 12 closed-space

FIG 2. Case 13: Irregular hyphae with 45- and 90-degree branching (Gomori methenamine silver, ×200).

TABLE 2. HISTOLOGIC FEATURES OF HYALOHYPHOMYCOSIS CAUSED BY *FUSARIUM*, *PAECILOMYCES*, AND *ACREMONIUM* SPECIES

			Fungal Features			*Tissue Reaction*					
Case	*Original Diagnosis*	*Culture*	*Branch Angle*	*Phialides*	*Conidia*	*Closed/ Open Space*	*Granulomas*	*Necrosis*	*Acute Inflammation*	*Vascular Invasion*	*Thrombosis*
Lung											
1	c/w *Acremonium**	*A strictum*	45, 90	+	+	Open†	NA	NA	NA	NA	NA
2	Filamentous septate fungi	*P lilacinus*	‡	+	+	Closed	+	+	–	+	–
Cornea/eye											
3	c/w *Fusarium**	*Fusarium* sp	45	–	–	Both	–	–	+	‡	NA
4	c/w *Fusarium**	*F oxysporum*	90	–	–	Both	–	–	+	NA	NA
5	Fungi	*P lilacinus*	‡	+	+	Open	–	+	+	NA	NA
6	c/w *Paecilomyces**	*P lilacinus*	45, 90	+	+	Both	–	+	+/–	NA	NA
Skin											
1	Invasive mycosis	*A strictum*	45, 90	+	+	Closed	–	+§	+/–	+	–
7	Candidiasis	*F solani*	45, 90	+	+	Open	–	+§	+	–	–
8	Hyphae	*Fusarium* sp	45	+	+	Open	–	+	–	–	–
Skin and soft tissue											
9	Aspergillosis	*F solani*	45, 90	–	–	Both	–	–	–	+	–
10	Septate fungi	*Fusarium* sp	90	–	+	Closed	–	–	–	+	+
11	Aspergillosis	*F solani*	45	+	+	Closed	–	+	+/–	+	–
12	c/w *Fusarium**	*Fusarium* sp	45, 90	+	+	Closed	+	–	–	+	+
13	Hyalohypho-mycosis	*Fusarium* sp	45, 90	+	+	Closed	–	–	+/–	+	+
14	Candidiasis	*P lilacinus*	45	+	+	Closed	+	–	+	–	–
15	Candidiasis	*P lilacinus*	45	+	+	Closed	+	+	–	–	–
16	Candidiasis	*P lilacinus*	45	+	+	Closed	–	+	+/–	+	+
17	c/w hyalohypho-mycosis	*P lilacinus*	45, 90	+	+	Closed	–	+	–	+	+
Peritoneal dialysate											
18	Fungi	*F moniliforme*	45, 90	+	+	Closed	NA	NA	NA	NA	NA
19	Negative	*P variotii*	45, 90	+	+	Closed	NA	NA	NA	NA	NA

NA = Not applicable; + = observed; – = not observed.
*Culture results preceded histologic examination.
†Histologic material consisted of mucus, cellular debris, and a small amount of fibrous tissue.
‡Insufficient fungal cells for evaluation.
§Infarct present.

infections (Table 2). Phialides usually appeared as tapering structures laterally or terminally on hyphae (Fig 4, A–D). Infrequently, the phialidic orifices arose directly on the hyphal axis (Fig 4, E). With *P lilacinus* infections, phialides sometimes arose from isolated fungal cells, which suggests that conidia can germinate to form phialides directly. In profile, phialides frequently appeared in the shape of a flask or bottleneck. Additional clues for their identification included the presence of a terminal orifice from which conidia were elaborated and periclinal wall thickening. Periclinal thickenings within the phialide apex consist of cell-wall remnants from the synthesis of multiple conidia (Fig 1). These stain more intensely than other portions of the phialide, resulting in the appearance of a broad, dark band at the neck of the phialide (Fig 4). Because the amount of periclinal thickening is proportional to the number of conidia produced, it may not be apparent on all phialides. Phialides with phialoconidia still attached were detected occasionally.

In 14 of 17 cases, necrosis and/or acute inflammation were present; in 4 cases, granulomas were seen (Table 2). An infarct was identified in two cases. The presence of both vascular invasion and thrombosis was identified in five patients, four of whom had disseminated disease and died. Phialoconidia were present within blood vessel lumens in four infections caused by *Fusarium* species (cases 10–13), and phialides were seen in three of these (cases 11–13); blood culture results were positive for one of the four patients. Biopsy specimens from patients with neutropenia exhibited little or no inflammatory response to the fungi.

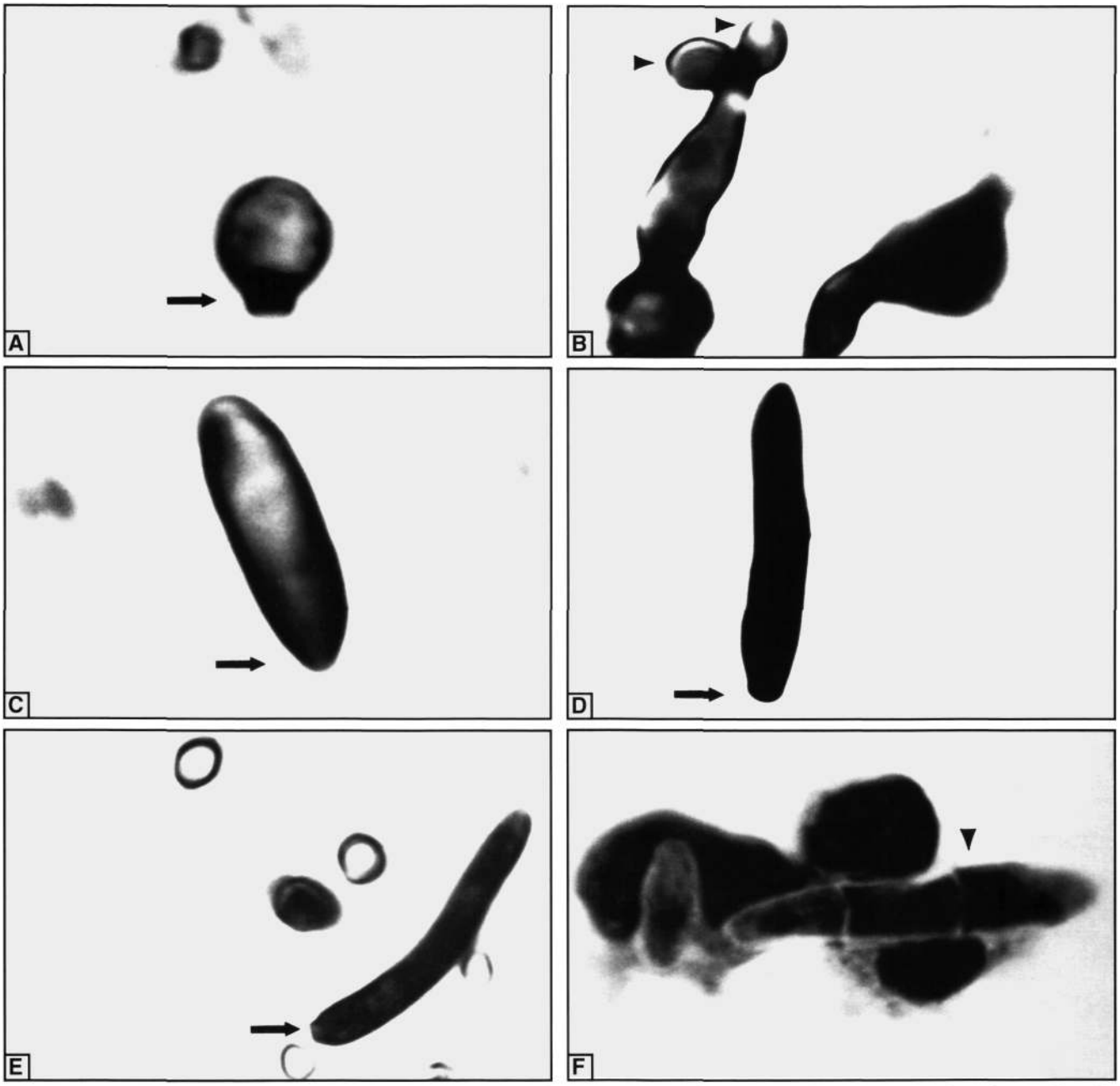

FIG 3. Variation in size and shape of conidia (Gomori methenamine silver, ×1,500). A, Subglobose, *Paecilomyces variotii* endocarditis. B, Case 14: ellipsoidal (arrowheads). C, Case 5: oval. D: Case 17: cylindric. E, Case 11: curved (periodic acid–Schiff, ×1,250). F, Case 18: three-celled macroconidium (arrowhead) of *Fusarium moniliforme*. Conidia often are distinguished by a rounded apex, and a tapered base with truncate scar (arrows).

Most patients were immunosuppressed, including eight patients with leukemia and/or lymphoma, two with renal transplants, one with a heart transplant, and one with a bone marrow transplant. Of seven patients with neutropenia, five had evidence of disseminated fungal infection. All six patients who died had evidence of disseminated infection. Infections in immunocompetent patients were all cases of keratitis. All infections caused by *P lilacinus* exhibited adventitious sporulation, but only one death was attributed to this species. Of the five *Fusarium* species infections occurring in patients with neutropenia, four were disseminated and showed adventitious sporulation; the fifth patient survived a focal infection of the finger, and adventitious sporulation could not be demonstrated.

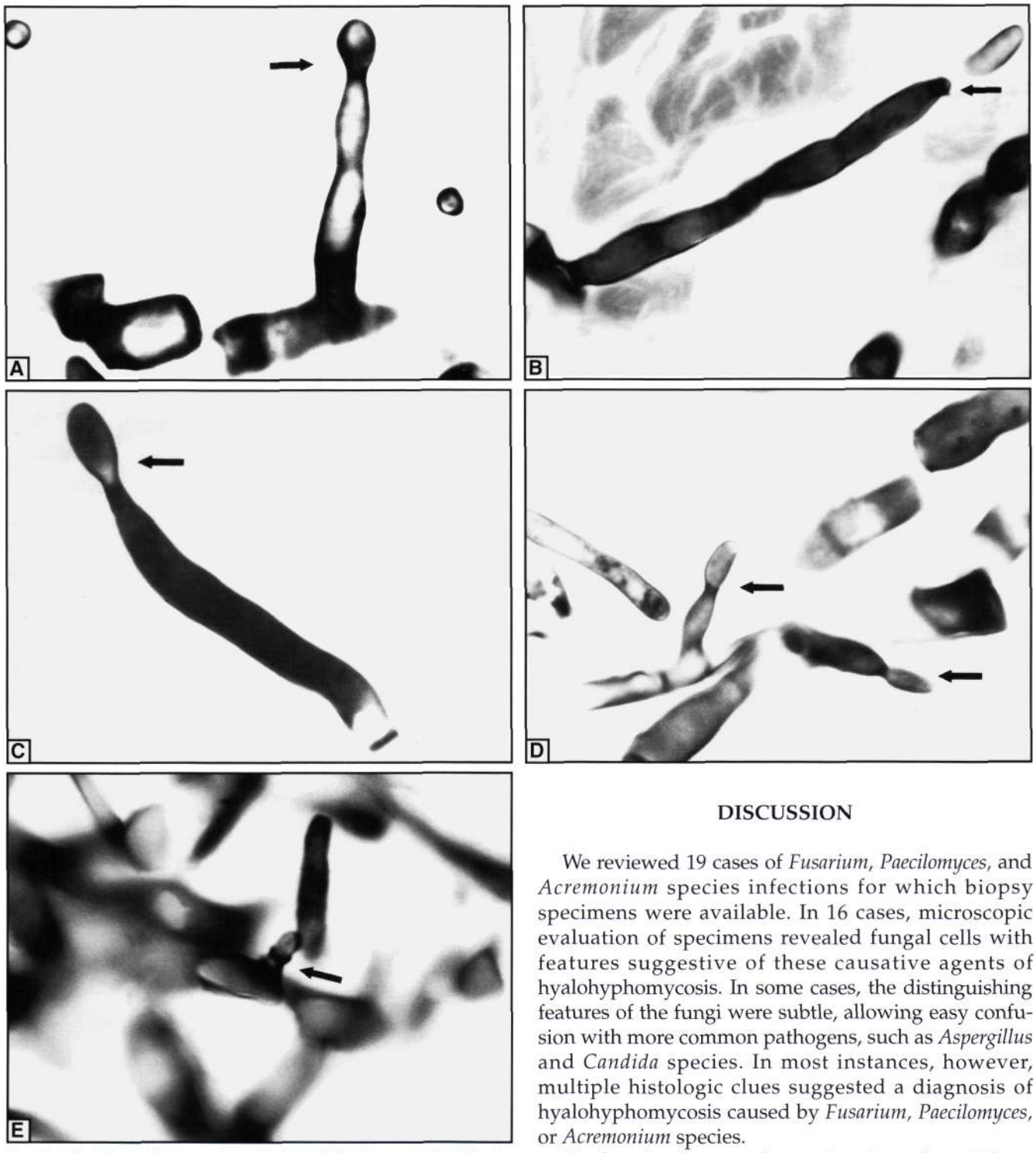

FIG 4. Phialides with emerging phialoconidia (arrows). Periclinal thickenings stain as a broad, dark band (Gomori methenamine silver, ×1,500). A, Case 16. B, Case 12. C, Case 17. D–E, Case 13. D, intravascular.

DISCUSSION

We reviewed 19 cases of *Fusarium*, *Paecilomyces*, and *Acremonium* species infections for which biopsy specimens were available. In 16 cases, microscopic evaluation of specimens revealed fungal cells with features suggestive of these causative agents of hyalohyphomycosis. In some cases, the distinguishing features of the fungi were subtle, allowing easy confusion with more common pathogens, such as *Aspergillus* and *Candida* species. In most instances, however, multiple histologic clues suggested a diagnosis of hyalohyphomycosis caused by *Fusarium, Paecilomyces,* or *Acremonium* species.

Hyaline hyphae are the predominant fungal form in cases of aspergillosis and of hyalohyphomycosis caused by other moulds. It often has been reported that *Fusarium* species and other moulds cannot be differentiated from *Aspergillus* species by routine histopathologic examination.[11,19,20,24,25] Infection with

Aspergillus species frequently is diagnosed when septate hyphae that have 45-degree branching are identified. By comparison, the hyphae of *Fusarium, Paecilomyces,* and *Acremonium* species more often exhibit variations in diameter, and both 45- and 90-degree branching typically are present. This nonuniformity can suggest the possibility of an etiologic agent other than A*spergillus* species, which usually tend to form hyphae of a more consistent diameter and less commonly exhibit 90-degree branching.

Rhizopus species and other zygomycetous moulds also exhibit hyphae of irregular diameter that tend to branch at 90 degrees, and it is possible to confuse these with the hyphae of *Fusarium* and other species.[26] Hyphae of the zygomycetous fungi tend to be of larger diameter, however, and become only sparsely septate as metabolically senescent portions of hyphae are sealed off by septum formation. This lack of regular septation causes an absence of internal support of these broad hyphae and characteristically allows them to become collapsed, twisted, and folded in a ribbon-like appearance. In contrast, hyphae of the agents of hyalohyphomycosis are of smaller diameter, form septa regularly in conjunction with nuclear division, and are less prone to folding as a result. Hyphal elements of *P lilacinus* are particularly likely to be mistaken for *Candida* species, because many of the filaments are markedly constricted at the points of septation. Further, most infections with *P lilacinus* show an abundance of phialides and phialoconidia that superficially resemble the pseudohyphae and budded yeasts seen in candidiasis.

Although hyaline hyphae are the predominant fungal forms seen in hyalohyphomycosis, it is the detection of conidia and phialides within a closed lesion that firmly supports a diagnosis of non-*Aspergillus* hyalohyphomycosis. When fungal stains show both unicellular forms and filaments that appear to be hyphae or pseudohyphae, infection with *Fusarium, Paecilomyces,* or *Acremonium* species should be considered, and a thorough search should be conducted for phialides and phialoconidia. Because each phialide produces multiple phialoconidia, conidia may be relatively numerous in tissue, but phialides will not always be evident. The size and shape of phialoconidia vary considerably, and subglobose to oval forms are easily confused with the yeast cells of *Candida* species. Useful distinguishing features, including a broad truncate basal scar and a rounded apex, can be seen on most conidia when favorably oriented in the tissue section. Identifying a subpopulation of conidia still attached to phialides also is extremely helpful.

Phialides, although always less numerous than phialoconidia, also are key elements in the tissue diagnosis of hyalohyphomycosis caused by species of *Paecilomyces, Fusarium,* and *Acremonium.* Care must be taken to distinguish the tips of phialides from the tapering profiles of hyphae transected as they exit the plane of section at oblique angles. The latter structures lack darkly stained periclinal wall thickening, often terminate in a point rather than an aperture, and stain less intensely toward their end as a result of unequal sectioning (Fig 5, A). Hyphae may terminate in swollen terminal cells that mimic conidia attached to phialides (Fig 5, B). These swellings, however, often are larger than conidia; although their point of attachment may feature a septum, it lacks the broad, darkly stained periclinal thickening typical of a phialide (Fig 1).

The size and shape of conidia and phialides formed in tissue overlap between *P lilacinus* and *Fusarium* species; thus, it often is not possible to suggest one genus over the other in a given case. In several *P lilacinus* infections, however, conidia were markedly smaller than the size range observed for *Fusarium* species. In addition, these conidia often were subglobose to very short ellipsoidal, a finding not observed with *Fusarium* species. Further experience may show these to be reliable differential characteristics in some cases of hyalohyphomycosis caused by *P lilacinus.* Adventitious sporulation also was more abundant in infections caused by *Paecilomyces* species than in most infections caused by *Fusarium* species. Two specimens from *Fusarium* infections required tissue recuts before phialides or phialoconidia could be demonstrated.

Conidia and phialides often can be spotted by using a 40× objective, but a 100× oil-immersion objective allows better detection and rigorous examination of features, such as periclinal wall thickening and conidial scars. Conidia and phialides are not differentiated reliably on routine H&E-stained sections because the fungal cell wall usually does not retain this stain. The GMS stain was the best overall histochemical method for evaluation of adventitious sporulation. In some instances, however, conidia exhibited particularly intense Brown and Brenn or PAS staining, perhaps owing to an increased carbohydrate and lipid content (Fig 6). Obtaining both GMS and PAS stains on each case has the added benefit of providing multiple step sections in cases in which adventitious sporulation is particularly sparse.

Examination of minced tissue, as a potassium-hydroxide-calcofluor white preparation or a smear stained with GMS or PAS, may reveal adventitious sporulation more readily than examination of a tissue

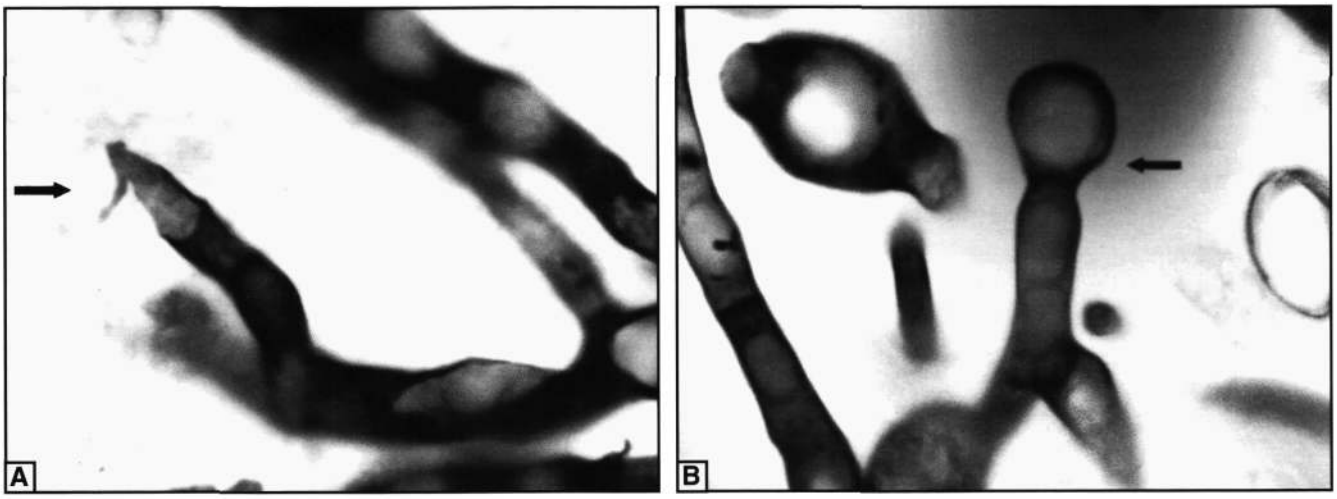

FIG 5. Case 13. A, Unequally transected hypha (arrow) can resemble a phialide (Gomori methenamine silver, ×1,250). B, Terminal hyphal swelling (arrow) can resemble a conidium attached to a phialide (Gomori methenamine silver, ×1,000).

section, because whole phialides and conidia are more easily detected and their important details can be viewed in three-dimensional profile.[4] Histologic sections, in contrast, offer the advantages of permanence and the ability to study fungal cells relative to surrounding tissue, but the alignment and exact longitudinal sectioning required for identification of phialides and phialoconidia is necessarily dependent on chance.

The occurrence of unicellular budding yeast forms (in addition to the formation of phialides and phialoconidia) has been documented for *P lilacinus*.[18,28,29] Examples were found in three of seven cases of infection with *P lilacinus*, very rare examples in the two cases of *A strictum*[4] and *Fusarium moniliforme*[18] infection, and none in the remaining cases. Such yeast-like cells can be distinguished from conidia by demonstrating the stage of development at which holoblastic buds are present but have not yet been delimited from the parent cytoplasm by cell-wall synthesis.

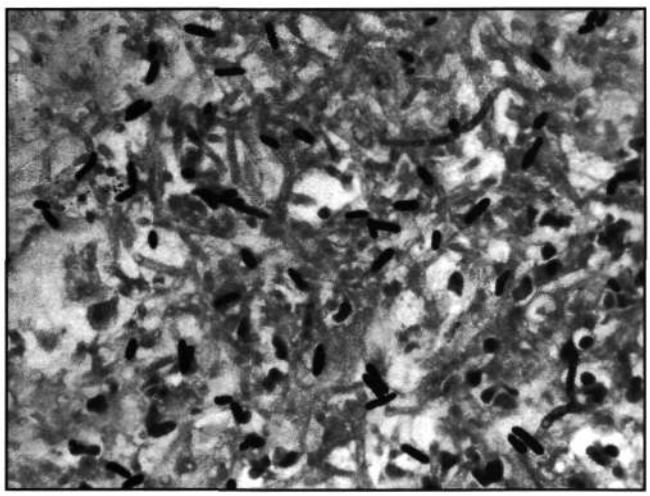

FIG 6. Case 17: Conidia can intensely retain Brown and Brenn stain, whereas vegetative hyphae (background) remain pale (×400).

The most common tissue responses were necrosis and acute inflammation, with an occasional granulomatous host response. As with *Aspergillus* and *Rhizopus* species, *Fusarium, Paecilomyces,* and *Acremonium* species can invade blood vessels and cause thrombosis. In four cases, phialoconidia of *Fusarium* species were present within blood vessel lumens; phialides also were visible in lumens in three of these. Blood culture results were positive for one of the four patients. These findings support the likelihood that the relatively higher rate of positive blood culture results associated with these organisms, and the rapid dissemination of *Fusarium* species, compared with *Aspergillus* species and the zygomycetes, are caused by the occurrence of intravascular adventitious sporulation. In 12 cases, fungal infection occurred within a closed tissue space. Phialoconidia were detected in all of these cases, and phialides were identified in 11 of them. These findings emphasize that adventitious sporulation can occur in tissue without direct exposure to the atmosphere. This was suggested for *Paecilomyces* species as early as 1963[9,29] and shown clearly in 1985,[27] but to our knowledge this observation is newly documented herein for species of *Fusarium.* In situ phialidic sporulation is known to occur with *Aspergillus* species, but it has been reported only from clinical situations in which there was air space present, such as in colonization of airways, pulmonary cavities,

paranasal sinuses, necrotic skin, and the external ear canal. We are aware of no evidence of adventitious phialidic sporulation of *Aspergillus* species within a closed lesion. It is noted, however, that the species *Aspergillus terreus* can form spores, usually solitary, directly attached to vegetative hyphae. A histologic review of this rare agent of aspergillosis is in progress and will be the subject of a later report.

In our series of patients, hyalohyphomycosis occurred most frequently in those who were immunosuppressed. Patients with neutropenia had particularly poor outcomes, and the biopsy specimens typically revealed a minimal inflammatory response to the infecting fungus. Four immunocompetent patients had corneal infections with *Fusarium* species or *P lilacinus*, a condition previously reported.[6,30] Of the four infections caused by *Fusarium* species that did not exhibit adventitious sporulation, three were ocular infections of immunocompetent hosts.

Recognition by clinicians and pathologists of hyalohyphomycosis caused by non-*Aspergillus* species is increasingly important. Many of the causative organisms are resistant to the commonly used antifungal agents, such as amphotericin B and fluconazole.[3,4,7,31–33] Rapid institution of specific antifungal therapy is essential for a successful outcome, and consideration of more aggressive therapies, such as multidrug therapy or surgical debridement, may be influenced by a tissue diagnosis of non-*Aspergillus* hyalohyphomycosis.[22,23,34]

Although culture remains the standard for identification of the causative agents of hyalohyphomycosis, presumptive identification of these fungi in histologic sections is of value for several reasons. Histology results can be obtained in less than 24 hours, whereas a preliminary culture identification may require several days. In case 17, histologic detection of phialoconidia allowed an immediate presumptive diagnosis of hyalohyphomycosis caused by *P lilacinus* or *Fusarium* species and prompted addition of fluconazole therapy to the existing amphotericin B regimen 7 days before preliminary culture results. In case 15, a patient admitted to the hospital for antifungal drug treatment of histologically diagnosed invasive candidiasis was instead discharged for outpatient surgical management when review of the skin biopsy specimen showed adventitious sporulation, consistent with his history of localized *P lilacinus* infection.

When culture is not requested or is unsuccessful for technical reasons, histopathologic examination may be the sole source of information regarding the nature of the infection. In one recent cutaneous biopsy specimen that was not cultured, identification of phialides and distinctively shaped conidia allowed the initial diagnosis of aspergillosis to be amended to a presumptive diagnosis of hyalohyphomycosis caused by *Fusarium* species (Fig 7). In addition, because saproprobic fungi, such as *Fusarium* species, occasionally can contaminate laboratory cultures, correlation of histologic and culture results provides a valuable confirmation of the clinical relevance of the culture isolates. Finally, histologic investigation of hyalohyphomycosis can be applied to archival tissue. Figure 3, A shows one of numerous phialoconidia from a surgical specimen dating from 1974 in which the patient (excluded from tables) had a culture-proved infection with *Paecilomyces variotii*.[5]

Morphologic criteria on GMS and PAS Schiff stains are presented that suggest the presence of hyalohyphomycosis caused by *Fusarium, Paecilomyces,* or *Acremonium* species. Diagnostic clues include the presence of adventitious sporulation consisting of phialides and phialoconidia and the presence of irregular hyphae with both 45- and 90-degree branching. Adventitious sporulation was found in all 7 cases of infection caused by *P lilacinus*, in both cases of infection caused by *P variotii*, and in 7 of 10 cases of infection caused by *Fusarium* species. Because of the high rate of positive blood culture results, the rapid dissemination of infection in certain patients, and the intrinsic antifungal drug resistance of many of these species, it is clinically relevant to make the specific diagnosis of non-*Aspergillus* hyalohyphomycosis as early as possible to help the clinician select an antifungal regimen.

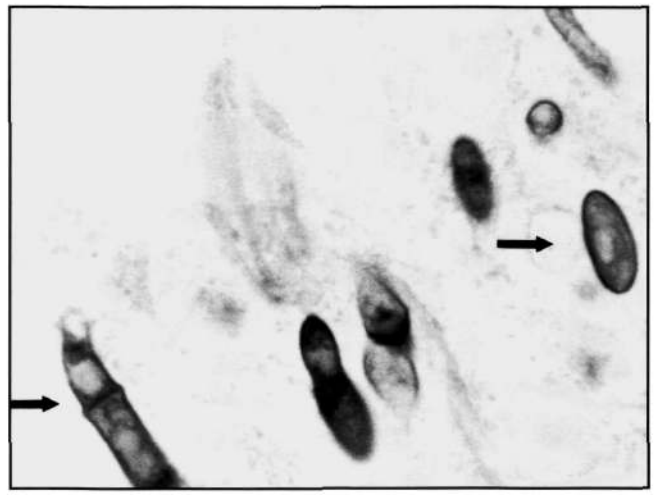

FIG 7. The presence of one- and two-celled conidia (arrows) and phialides (not shown) allowed a presumptive diagnosis of hyalohyphomycosis caused by *Fusarium* species, in the absence of culture (periodic acid–Schiff, ×788).

Adventitious sporulation in closed lesions has been observed for several additional fungal species, including an unpublished case of phaeohyphomycosis caused by *Mycoleptodiscus indicus* and *Lecythophora hoffmannii* (Schell, unpublished data, 1996, 1997). It is likely that the phenomenon of in situ sporulation occurs with many species and that detailed study of these increasingly common infections could offer potential for improved medical management of these patients.

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