Diagnostic Value of Conidia Associated With Pulmonary Oxalosis: Evidence of an *Aspergillus niger* Infection

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Bronchoalveolar lavage (BAL) material is commonly received in cytopathology for the exclusion of microorganisms. When crystalline material suggestive of calcium oxalate is present in the specimen, a search for fungal elements should be undertaken. Aspergillus niger *is the hyaline mold associated with the presence of oxalate crystals. Commonly fragments of hyphae and occasionally entire conidiophores may be present in BAL specimens from patients with aspergillosis.*

We report a case of a patient with saprophytic colonization of a bullous/cavitary lesion. The BAL consisted of abundant acute inflammation, crystalline material suggestive of oxalate, and darkly pigmented conidia. Although an extensive search was undertaken, hyphal fragments could not be found. The suspicion of an A. niger *infection was confirmed by culture.*

We believe that even in the absence of hyphal fragments, darkly pigmented, occasionally rough-walled conidia are sufficient evidence to be highly suspicious of an A. niger *infection in patients with pulmonary oxalosis.* Diagn. Cytopathol. 1997;17:292–294.

Key Words: Aspergillus niger; pulmonary oxalosis; Conidia

Aspergillus niger is the etiologic agent of both invasive and noninvasive aspergillosis, associated with pulmonary oxalosis.[1,2,4–13] Therefore, when crystalline material suggestive of calcium oxalate is identified in respiratory specimens, fungal elements should be sought. The fungal elements most commonly identified are hyphal fragments. We report a case of pulmonary oxalosis caused by *A. niger,* which failed to disclose hyphal fragments in the cytologic preparation. The etiology was suggested based on the presence of pigmented, often rough-walled or echinuate conidia.

Materials and Methods

A bronchoalveolar lavage specimen from a patient with severe chronic obstructive pulmonary disease and a cavitary/bullous lung lesion was submitted for cytopathologic examination. The bronchial brush was submitted to the microbiology laboratory for culture and Gram stain. The cavitary lesion radiographically disclosed an air-fluid level suggestive of infection.

Twenty-five milliliters of blood-tinged, cloudy, mucoid fluid was received in the cytopathology laboratory. The specimen was centrifuged for 5 minutes at 2,500 rpm. The supernatant was decanted, and the pellet was resuspended in 5-10 ml of normosol. Approximately 2 ml was applied to a 5-µm membrane filter. The cellularity of the remaining specimen was evaluated via wet preparation with toluidine blue staining. Based on the cellularity, the residual specimen was prepared using the Shandon 2 cytospin (Shandon, Inc., Pittsburgh, PA). The membrane filter and the cytospin preparations were fixed in 95% ethanol, stained with the Papanicolou method, and examined by light microscopy.

The bronchial brush, received in the microbiology laboratory, was vortexed in eight to ten drops of sterile water. The specimen was subdivided. One to two drops of the specimen were air-dried, heat-fixed to a glass slide, stained by the Gram method, and examined by light microscopy. Bacterial, fungal, and mycobacterial cultures were performed on the remaining specimen.

Results

The specimen submitted for cytopathologic examination consisted principally of abundant acute inflammation admixed with crystalline material. The crystalline material consisted of sheaves of birefringent crystals, suggestive of calcium oxalate (Fig. 1).[14] Because of the association of *A. niger* and pulmonary oxalosis, fungal hyphae were sought. Hyphal elements could not be found; however darkly pigmented, occasionally echinuate conidia were identified (Fig. 2). Of the *Aspergillus* species with darkly pigmented conidia, only *A. niger* has both echinuate conidia and an association with pulmonary oxalosis.

The Gram stain disclosed abundant acute inflammation; no organisms were identified. The mycobacteria culture was negative. The fungal and bacterial cultures grew *A. niger* (Fig. 3).

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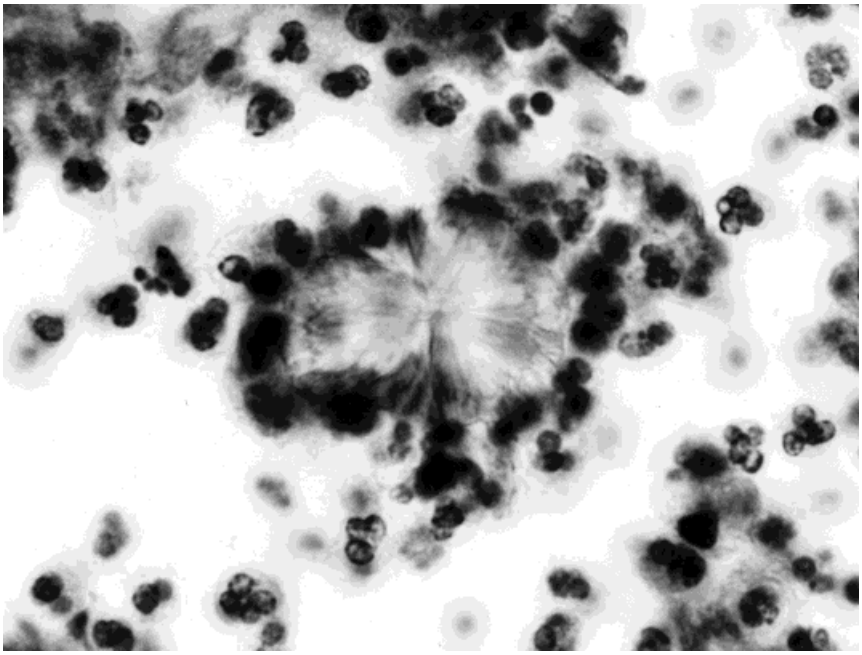

Fig. 1. Crystal suggestive of calcium oxalate in a background of abundant acute inflammation (Papanicolaou stain, ×400).

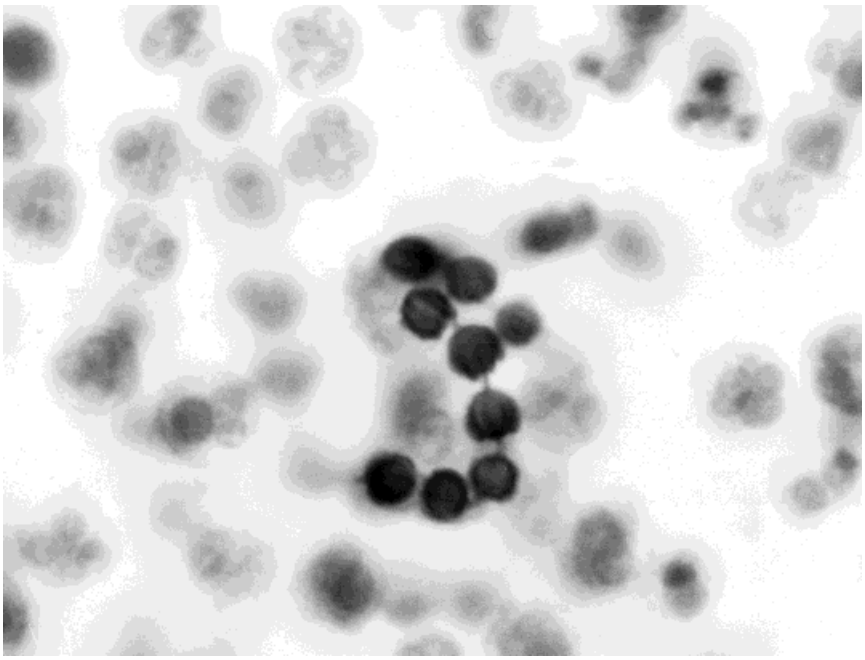

Fig. 2. Darkly pigmented conidia (Papanicolaou stain, ×680).

Discussion

Allergic or hypersensitivity pneumonitis, saprophytic colonization, and invasive fungal disease are the three varieties of pulmonary aspergillosis.[1,2] The saprophytic colonization of a distended bronchial lumen or pre-existing cavity may result in the formation of a ''fungus ball,'' without tissue invasion.[1] Alternatively, and most commonly in immunocompromised patients, the fungus may cause tissue destruction and invade underlying tissues, particularly blood vessels.[1] This often results in pulmonary infarction and hematogenous dissemination of the organism.[1,2] In both these instances, the diagnosis of aspergillosis is usually suggested by the pres-

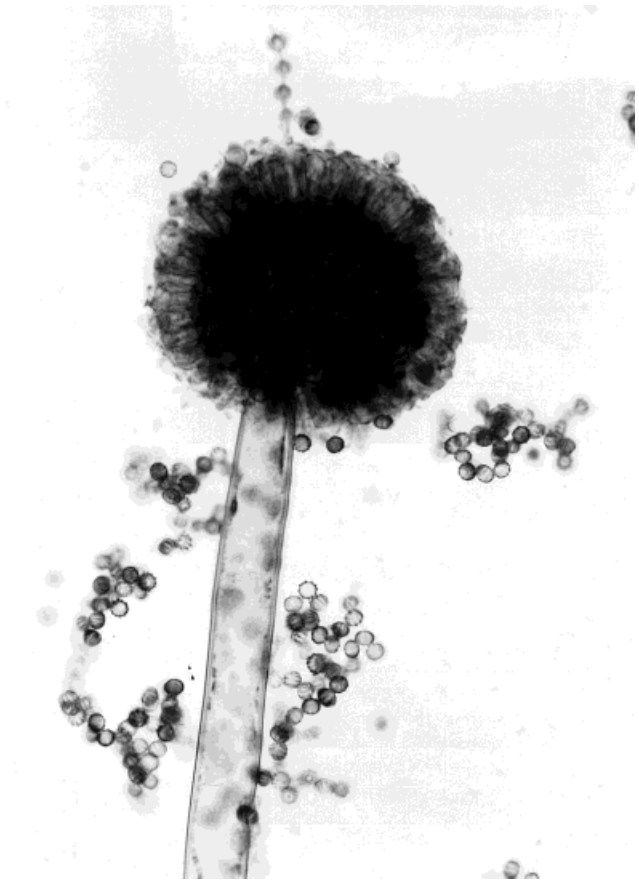

Fig. 3. The conidiophore and conidia of *A. niger* (wet preparation, ×680).

ence of septate hyphal fragments, with acute angle branches, in sputum or bronchoalveolar lavage material.

Sporulation may occur when the fungus grows in an open space, such as a bronchial lumen or a cavity. The conidiophore is the asexual spore-bearing structure of the genera *Aspergillus* and is the principal structure used to differentiate the various species.[1,3] The conidiophore is a specialized portion of hyphae which terminates in a vesicle, upon which the primary sterigmata arise. In some species, secondary sterigmata may arise from the primary. The sterigmata in turn produce the asexual spores or conidia. The distribution of the primary sterigmata on the vesicle, the presence of secondary sterigmata, and the pigmentation of the conidia are all important in the differentiation of the *Aspergillus* species.[3] Occasionally, the entire conidiophore may be present in a respiratory specimen, allowing genus and species identification.[4]

Aspergillus niger is the fungus commonly associated with pulmonary oxalosis. Oxalic acid is a product of *A. niger* fermentation. When present in high concentration, calcium salts precipitate in tissues and body fluids. The localized increase of oxalic acid and the calcium oxalate crystal formation, which has been shown to be important in the generation of local oxidants, are probably important mechanisms of cell injury.[6] Therefore, the identification of oxalate crystals in fluids or tissues specimens should raise the possibility of aspergillosis caused by *A. niger.* Often typical hyphal elements, present in the specimen, assure the diagnosis. Occasionally, the entire conidiophore may be present in the submitted material, allowing speciation using the same criteria employed in the medical mycology laboratory.

In this case, careful examination of the material disclosed darkly pigmented conidia. The conidia of *Aspergillus niger* are darkly pigmented and 4–5 μm in diameter. Conidial characteristics, which are used in the speciation of *A. niger,* may be employed in the clinical specimen. Relatively few species have brown-black conidia, and of these only *A. niger* has been associated with pulmonary oxalosis.

When examining a respiratory specimen which contains oxalate crystals, fungal elements should by sought. In the absence of the more commonly encountered hyphal fragments, conidia, if present, disclose a fungal etiology. If darkly pigmented, echinuate conidia are present in a patient with pulmonary oxalosis, *A. niger* is the etiologic agent.

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