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Banu BİLEZİKÇİ
Beyhan DEMİRHAN
Asuman Nihan HABERAL
Ünser ARIKAN

Invasive Pulmonary Aspergillosis in Solid-Organ Transplant Recipients: Postmortem Histopathologic Findings

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Department of Pathology, Faculty of Medicine, Başkent University, Ankara - TURKEY

Abstract: Invasive aspergillosis is often lethal in transplant recipients. The mortality associated with this disease after organ transplantation approaches 100%, partly because it is difficult to recognize and diagnose early on. The clinical course and histological findings in invasive pulmonary aspergillosis in immunocompromised hosts differ from those seen in patients who develop classic aspergillomas. In immunosuppressed patients, the predominant pathologic abnormalities are tissue invasion, abscess formation, and angioinvasion with or without infarction.

In this study, we examined cases of invasive pulmonary aspergillosis in one kidney recipient and three liver transplant recipients. All the patients were male, and their mean age was 41 years. The mean time to onset of infection was 37 days after transplantation. We discuss the pathologic findings of angioinvasion, infarction and bronchial invasion that were observed in these immunosuppressed individuals.

Key Words: Invasive pulmonary aspergillosis, transplantation

Introduction

Invasive aspergillosis is a well-known serious complication of organ transplantation (1-3). Research has identified several risk factors for invasive *Aspergillus* spp. infection in transplant recipients, including treatment with high-dose corticosteroids, OKT3 therapy, retransplantation, multiple abdominal operations, prolonged surgery, high serum creatinine levels and cytomegalovirus (CMV) infection (4-5).

Although *Aspergillus* can be isolated from patients' respiratory secretions, diagnosis of invasive disease requires histopathologic confirmation, and a definitive diagnosis can only be established through immunochemical studies (6). In this report, we describe the various histopathologic features of invasive pulmonary aspergillosis in one kidney recipient and three liver transplant recipients, and underline the differences between this form of the disease and classic aspergillomas. Two autopsies and two postmortem biopsies are discussed.

Case Descriptions

All four of the infected recipients were male, and their mean age was 41 years. Some of the clinical features of each case are summarized in the Table. The patients had been on various antibiotics within a month of the onset of aspergillosis infection. They were also taking cyclosporine A and prednisone as part of the standard post-transplantation protocol, but none of the four had been treated with OKT3. The mean time to onset of infection was 37 days after transplantation.

Invasive pulmonary aspergillosis was tentatively diagnosed at autopsy in two of the liver recipient cases, and by postmortem needle biopsy in the other two patients. One patient was diagnosed with disseminated invasive pulmonary aspergillosis, in which the lung, kidney and transplanted liver were affected. None of the cases featured any CMV infection-related histopathologic findings.

In both of the liver recipient cases diagnosed at autopsy, we found that the lungs were heavier than normal (normal: 350-425 g).

Gross examination of the lungs revealed consolidation and lobar pneumonia with red hepatization. The tissue had a granular texture, and contained white nodular lesions of variable size. These lesions were most striking at the base of the lung lobes, and some were hemorrhagic (Figure 1). The trachea and bronchi also showed

Table. Patient clinical features.

Case	Gender	Age (Years)	Renal or Liver tx	Etiology
1	M	45	Renal	Nephrolithiasis
2	M	53	Liver	HCV* + Chronic Hepatitis
3	M	24	Liver	Wilson's Disease
4	M	42	Liver	HBV** + End Stage Liver Disease

*HCV- Hepatitis C virus
**HBV- Hepatitis B virus

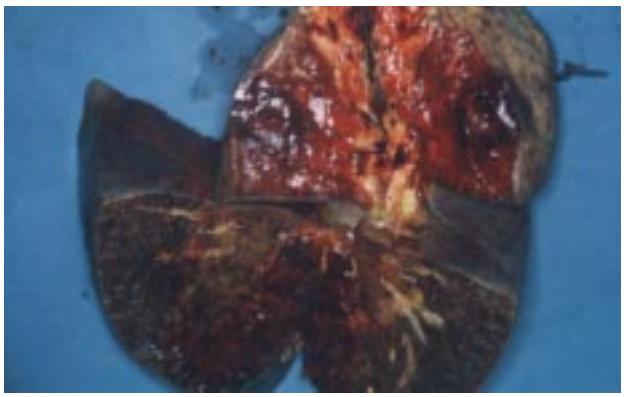

Figure 1. Gross examination of the lungs.

hemorrhage and mucosal erosions. The kidneys in both of these patients contained numerous well-demarcated lesions with distinct borders. The center of these lesions was white-yellow, and the periphery was bright red (Figure 2).

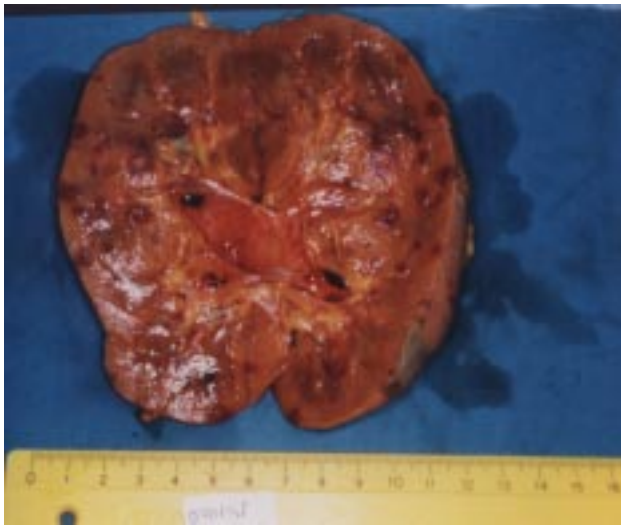

Figure 2. Gross examination of the kidney.

All specimens were fixed in 10% formaldehyde and embedded in paraffin. The tissue blocks were cut in serial 4-5 µm-thick sections, and the sections were deparaffinized and then stained with hematoxylin-eosin, methanamine silver and periodic acid-Schiff (PAS).

Microscopic examination of the autopsy sections of lung, kidney and graft liver revealed numerous nodules filled with masses of hyphae. Some of the hyphal masses showed central necrosis. Typical of *Aspergillus* spp., the hyphae were uniform in size, narrow, septate and basophilic. Necrotic hyphae were often eosinophilic. The hyphal branches tended to arise at 45° angles. Characteristic hyphal morphology was demonstrated better with methanamine silver and PAS stains than with hematoxylin-eosin (Figures 3-4). In addition to the nodules, the specimens showed suppurative inflammatory infiltrate, parenchymal hemorrhage and tissue necrosis, and the hyphae were invading the walls of the veins and the bronchi. One patient's kidney showed microabscess formation.

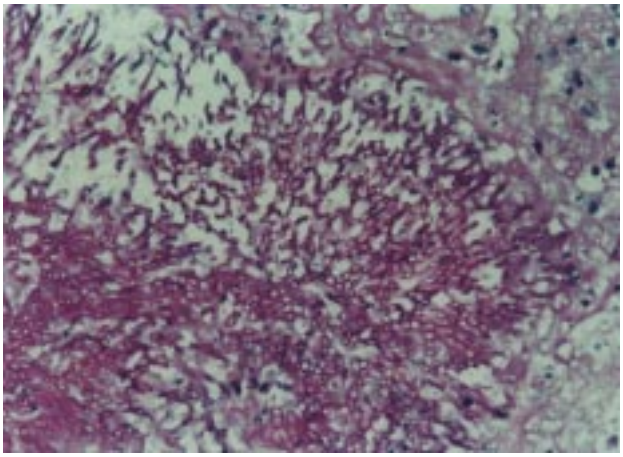

Figure 3. Characteristic hyphal morphology (PAS X 100).

In the two cases diagnosed by postmortem needle biopsy, specimens of liver, lung and kidney were collected from the kidney recipient, and samples of spleen, lung, liver, kidney and bone marrow were collected from the liver recipient. In both these patients, only the lungs showed typical *Aspergillus* hyphal morphology.

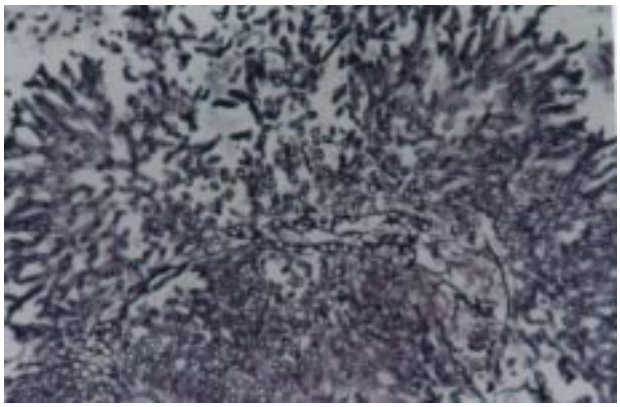

Figure 4. Characteristic hyphal morphology (Methanamine Silver X 100).

Discussion

Invasive aspergillosis is an opportunistic infection that occurs almost exclusively in immunosuppressed and debilitated hosts. *Aspergillus fumigatus* is the species most often isolated from patients with invasive or disseminated infection. The clinical findings in invasive pulmonary aspergillosis are non-specific. Sputum cultures yield growth in only about one-third of cases (7). The diagnosis is usually made based on the detection of typical *Aspergillus* hyphae, the invading form of the fungus, in tissue specimens collected at postmortem examination (6-8-9-10).

The clinical course and histological findings in invasive pulmonary aspergillosis in immunocompromised hosts are different from those seen in patients who develop classic aspergillomas (11). Colonizing aspergillosis, or aspergilloma, implies growth of the fungus in pulmonary cavities with minimal or no invasion of the tissues. In these classic cases, cavities generally form where there are pre-existing lesions. Proliferation at these sites produces free masses of hyphae inside the cavities. The surrounding inflammatory reaction may be mild, or there may be chronic inflammation and fibrosis (7).

In contrast, in invasive forms of the infection, the predominant pathologic abnormalities are tissue invasion, abscess formation, and angioinvasion with or without infarction (12); thus, the examiner usually sees areas of hemorrhage and infarction superimposed on the necrotizing inflammatory tissue reactions (7). Although the finding of typical hyphae is generally diagnostic, other types of septate hyphae seen in tissue sections can be mistaken for *Aspergillus* spp. These fungi include *Pseudallescheria boydii, Fusarium* spp. and other opportunistic hyalohyphomycetes, and *Candida* spp. Recognizing and checking for the subtle differences in the morphology of these different fungi may help distinguish them from aspergilli. Histopathologic diagnosis of aspergillosis must be considered presumptive unless confirmed by immonohistochemistry or by microbiologic isolation of *Aspergillus* (13). Studies have shown that histopathologic demonstration of hyphal invasion of blood vessels or tissue is associated with poor prognosis (14). All four of our cases exhibited diffuse vascular and bronchial invasion with necrosis.

Disseminated invasive aspergillosis is defined as involvement of two or more non-contiguous organ sites (4). In mice with disseminated aspergillosis, T helper-dependent lung mucosal immunity plays an essential role in host defense against invasive pulmonary aspergillosis (15). The primary host defense against dissemination of *Aspergillus* organisms is professional phagocytes. However, immunosuppressive regimens that prescribe corticosteroids tend to seriously impair phagocytic activity, even in the absence of overt granulocytopenia (1). One of our patients had the disseminated form of the disease, which, in this case, was characterized by lung, kidney and graft liver involvement. In addition to hyphal invasion of tissues and necrosis, this patient's kidneys contained many microabscesses.

Aspergillus organisms are capable of penetrating any host tissue. Secretory proteinases produced by the fungus can facilitate hyphal destruction of fibrillar proteins such as elastin and collagen, but these enzymes may only be of minor importance in the pathogenesis of invasive aspergillosis. One report stated that microscopic examination of aspergilli penetrating vessel walls showed no obvious lysis of wall proteins, thus emphasizing the importance of mechanical disruption of fibrillar proteins by the growing hyphae (16). If elastolysis contributes at all to the invasion of vessel walls by aspergilli, then it seems to be very localized and/or transient in nature (17).

In the lungs of immunosuppressive patients, infection rapidly becomes systemic when there is tissue necrosis and hemorrhage related to vascular invasion. *Aspergillus* lesions tend to be distributed in a generalized pattern in the lung, but in highly vascular tissues, such as the

kidney, the lesions are focal and may form microabscesses. Needle biopsy is less likely to reveal fungal forms in tissues where disseminated infection produces focal lesions. In such cases, repeated needle biopsies are necessary to obtain adequate material for study.

Correspondence author:
Banu BİLEZİKÇİ
Başkent Üniversitesi Patoloji Anabilim Dalı
12. sokak No: 7/1 06490,
Bahçelievler, Ankara - TURKEY

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