Case report

Rare fatal simultaneous mould infection of the lung caused by Aspergillus flavus and the basidiomycete Coprinus sp. in a leukemic patient

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The basidiomycete Coprinus sp. was isolated repeatedly from bronchial secretions and bronchoalveolar lavage of a 40-year-old woman suffering from a relapse of acute lymphoblastic leukemia 5 years after she underwent autologous bone marrow transplantation. Post-mortem microbiological investigation of lung tissue revealed simultaneously growing Coprinus sp. and Aspergillus flavus. Histopathological examination of the lung demonstrated septate hyphae characteristic of both Aspergillus and Coprinus. The basidiomycete Coprinus sp. should be considered as a potential opportunistic pathogen because of its excellent growth at 37 °C.

Keywords Aspergillus flavus, Coprinus, fatal infection, leukemia

Introduction

Recent advances in the treatment of leukemia have contributed to prolonged life spans, but these have been offset by a considerable increase in the frequency of opportunistic infections caused by viral, bacterial, protozoan and fungal pathogens. Invasive fungal infections may be responsible for up to 30% of deaths among patients with acute leukemia [1]. Candida and Aspergillus spp. remain the two most common fungal pathogens among immunocompromised patients. Pannuti et al. [2] recently found Aspergillus spp. to cause 36% (20 of 55) of cases of proven nosocomial pneumonia in bone marrow transplant recipients. Aspergillus fumigatus is the most frequent isolated species causing invasive aspergillosis. Aspergillus flavus is the second most common species isolated from invasive pulmonary aspergillosis [3].

Several species of basidiomycetes have been isolated from humans and lower animals [4-13]. It has not been clear whether these fungi are capable of causing mycoses [12,13].

Here we report a case involving a woman suffering from relapse of acute lymphoblastic leukemia more than 5 years after bone marrow transplantation who developed a lethal pulmonary infection caused by a basidiomycete and an Aspergillus sp. The basidiomycete Coprinus sp. was repeatedly isolated from bronchial secretions and bronchoalveolar lavage. Post-mortem cultivation of lung tissue revealed simultaneously growth of Coprinus sp. and A. flavus.

Case report

A 40-year-old woman (body weight 51 kg) was diagnosed with acute lymphoblastic leukemia (FAB L 2) in March 1987, and was treated with aggressive chemotherapy resulting in complete remission. In October 1987, she underwent autologous bone marrow transplantation. Stable remission was maintained for over 5 years until July 1993. When admitted to hospital (Department of Internal Medicine, University of Leipzig) on 27 July 1993, the woman had been complaining of severe cough for the previous 4 weeks, and general fatigue and night sweats for 1 week. Auscultation of the lung revealed humid, non-ringing rattle sounds on both basal sides. The body temperature was 38 °C. Her mucous membranes of the...
oral cavity showed thrush. She received ciprofloxacin 250 mg day$^{-1}$, clindamycin 1800 mg day$^{-1}$, gentamycin 240 mg day$^{-1}$ and ceftazidim 4 g day$^{-1}$. For antimycotic therapy of the thrush, an amphotericin B suspension and 100 mg fluconazole were administered orally. Chest radiography showed a homogenous shadow of the right lung as defined by a plain middle lobe syndrome. Bone marrow puncture revealed a B-acute lymphoblastic leukemia with blast cells carrying CD 19, CD 10, CD 22, and HLA-DR. In addition, CD 34, CD 38, CD 13 and partially CD 33 were co-expressed.

On day 3, the patient’s temperature was normal, and remission induction was initiated with prednisolone, vincristinesulphate, daunorubicin hydrochloride and cytarabine. Repeated chest radiography revealed regression of the middle right lobe syndrome with further improvement after stopping ciprofloxacin and fluconazole on day 7. Two days after the end of the first cycle of chemotherapy, on day 29, the patient became neutropenic (white blood cell count 0·1 mm$^{-3}$) and developed dyspnoea at rest without fever. Radiographs showed marked pulmonary infiltrates of the right upper field and of the left middle field (Fig. 1). In addition, a questionable melting process of the right upper field was suggested. Despite broad-spectrum antibiotic and antimycotic therapy with teicoplanin 400 mg day$^{-1}$, ceftazidim 12 g day$^{-1}$, erythromycin 4 g day$^{-1}$ and amphotericin B 50 mg day$^{-1}$ the patient’s condition did not improve. From day 30, the condition of the woman deteriorated with spiking fever over 38 °C. Blood cultures remained sterile throughout. Candida and Aspergillus antigen (Pastorex-Aspergillus, Sanofi Diagnostics Pasteur, Marnes la Coquette, France) and antibodies (Passive haemagglutination, Roche Serologic, München, Germany) could not be detected in patient’s sera. On day 32, the woman was transferred with progressive dyspnoea at 235 min$^{-1}$ to the intensive care unit. After intravenous administration of ajmalin the patient developed sinus bradycardia, and later asystolia. Immediately reanimation was successfully carried out with repeated defibrillations and xylotoxin. Gentamycin 600 mg day$^{-1}$, heparine 10 000 IE day$^{-1}$ and neomycin were administered additionally. To control the underlying disease, immunosuppressive therapy with antithymocyte globulin and granulocyte colony-stimulating factor (G-CSF, filgastrim) were started.

Fibre bronchoscopy and bronchoalveolar lavage (BAL) from the right upper lobe was performed. Methylene blue staining of smears of bronchial secretions and BAL fluid demonstrated septate, dichotomously branching hyphae. Aspergillus antigen detection was reactive both in bronchial secretions (1:512), and BAL fluid (1:64). Treatment with AmBisome (liposomal amphotericin B) 150 mg day$^{-1}$ was begun immediately. Despite supportive care, the woman died on day 33 from circulatory failure.

Post-mortem examination revealed an invasive mould infection of the lung. Multiple fungal granuloma up to 5 cm in diameter were seen in all lobes of the lung. Microscopically, widespread alveolar haemorrhage and necrotizing fungal lesions were seen, with invasion of pulmonary vessels by septate hyphae and consequent thrombosis and infarction. There was no evidence of dissemination of the fungal infection. Apart from the lungs, no hyphae could be recovered and no hyphae were seen in the tissues.

**Mycology**

Unfortunately, results of the mycologic studies were available only. Cultivation both of bronchial secretions and BAL fluid on Sabouraud 4% glucose agar (SDA) and Sabouraud glucose broth (Sifin, Berlin, Germany) resulted in growth of identical pure colonies at 26 and 37 °C. White, floccose colonies were observed after 3 days incubation (Fig. 2). The isolates from the clinical material had the following characteristics: when subcultivated on SDA in plates, they grew rapidly and formed white, sparse to dense, and lanose to floccose colonies on the agar surface. The reverse of the colonies was dull-yellow to
Infection by *Aspergillus flavus* and *Coprinus* sp.

**Fig. 2** *Coprinus* sp. Three-day-old colony grown on Sabouraud glucose agar at 37 °C, showing a white, downy-to-fluffy colony with a weak yellow reverse side.

**Fig. 3** *Coprinus* sp. and *Aspergillus flavus*. Three-day-old colonies simultaneously grown post mortem from lung tissue on Sabouraud glucose agar at 37 °C. Typical yellow colonies of *Aspergillus flavus* and smaller white colonies of *Coprinus* sp.

White. Microscopic examination of the original isolates and their subcultures revealed branched, hyaline and septate hyphae bearing few conidia. Production of conidia could be stimulated by subcultivation on yeast nitrogen base agar in plates (Difco, Detroit, USA). After 2 weeks on SDA, large numbers of dark brown, irregularly rounded to ovoid sclerotia appeared; they were filled with closely packed cells having extremely thick cell walls. The fungus failed to grow on Mycosel agar because of its sensitivity to cycloheximide. After unsuccessful attempts by various authorities to identify the fungus, it was sent to Dr R. A. Samson of the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, who identified it as a *Coprinus* sp.

Cultures taken at autopsy from both the left and right lungs grew *Coprinus* sp. Surprisingly, from several pieces of lung tissue a second fungus was grown both on SDA and Sabouraud glucose broth at 37 °C that produced a dense yellow felt of aerial mycelium. Microscopic examination revealed rough-walled conidiophores and yellow green conidial heads with double series of metulae and phialides. The isolates were identified as *A. flavus*. This second mould showed the same intenseness of growth as the *Coprinus* sp. (Fig. 3).

**Discussion**

Previously, it was assumed that basidiomycetes were not infectious [13]. Recently, some well documented reports on filamentous basidiomycetes responsible for mycoses in humans and animals have been reported [14]. *Schizophyllum commune* occasionally has been described as a causative agent of sinusitis [15–18]. This basidiomycete has been reported to be involved in cases of allergic bronchopulmonary mycosis [19], meningitis [20] and onychomycosis [21]. A case of ulceration in the palate in an infant was described [22] with *S. commune* being demonstrated in tissue and isolated in culture. This strain was shown to produce granulomatous lesions and death in mice [23]. In addition, Salfelder *et al.* [12] and Salfelder...
& Schwarz [13] investigated the possible role of ten and 15, respectively, species of basidiomycetes to serve as infectious agents. The basidiomycetes were inoculated intravenously, intraperitoneally and subcutaneously into mice and hamsters. Tissue lesions were observed in lungs, omentum and skin after 1 and 2 months. The fungi were viable in tissue for up to 2 months after inoculation.

The genus *Coprinus* contains both the ‘ink cap’ mushroom and other species having smaller, non-deliquecent fruiting bodies [24]. A number of coprinis are coprophagous, but this is not a universal feature; some may grow on decaying vegetation, and species have been described growing on plaster [25].

There is only one report of infection caused by members of this genus. Speller & MacIver [24] described a case of endocarditis in a 53-year-old man caused by the conidial stage of a *Coprinus* sp. The fungus was isolated from the normal aortic valve of a patient who had undergone a mitral valve replacement procedure. Blood cultures prior to surgery were negative. Aortic valve and adjacent aortic wall removed during surgery revealed massive fungal vegetations. Histopathological examination of the vegetations showed the growth of septate hyphae in association with an acute inflammatory reaction. Identification of this isolate was also carried out at the Centraalbureau voor Schimmelcultures in Baarn, The Netherlands, where the fungus was identified as *Coprinus lagopus*.

Basidiomycetes have rarely been implicated in human or animal disease, presumably mainly because of their inability to grow at 37 °C. The genus *Coprinus* was singled out by Cooney & Emerson [26] in their discussion of thermophilic fungi as the only genus in the basidiomycetes known to contain thermotolerant members.

The *Coprinus* sp. isolated from the present case was able to grow at 37 °C somewhat better than at 26 °C. The ability to grow at 37 °C is believed to account for its pathogenicity.

Histopathological examination of lung tissue revealed only hyphal forms. The hyphae seen in PAS and Grocott stained tissue sections were characteristic of *Aspergillus*. However, *Coprinus* has septate hyphae with similar morphological features. There were no structures like clamp-connections or conidia seen in the tissue. Some of the hyphae without clamp-connections may have been *Coprinus* hyphae. Both *A. flavus* and *Coprinus* hyphae may have been growing in lung tissue. The simultaneous growth of *A. flavus* and *Coprinus* sp. in culture indicates they both may have been contributing to the patient’s infection and subsequent death.

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