JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1994, p. 224–227
0095-1137/94/$04.00+0

Vol. 32, No. 1

Osteomyelitis and Intervertebral Discitis Caused by *Blastoschizomyces capitatus* in a Patient with Acute Leukemia

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Received 21 June 1993/Returned for modification 31 July 1993/Accepted 1 October 1993

We describe the first known case of vertebral osteomyelitis and discitis caused by *Blastoschizomyces capitatus* in a leukemic patient and the results of therapy. We also reconfirm the microbiological characteristics which differentiate this species from other yeastlike pathogens.

Invasive fungal infections are occurring with increasing frequency in immunocompromised patients, particularly those with hematologic malignancies (4).

Although most invasive fungal infections are usually caused by *Candida*, *Aspergillus*, or *Rhizomucor* species, several reports have documented the increasing clinical role of *Blastoschizomyces capitatus*. The pathogenicity of this fungus has been documented in animal models (10), and human infections have involved the lungs (8, 9), endocarditis (2, 5, 22), and disseminated infections (11, 13, 15, 17, 19), the last occurring especially in immunocompromised patients. Although this organism was originally known as *Trichosporon capitatum* and then *Geotrichum capitatum*, a recent review recommended the retention of the species in a separate genus, *Blastoschizomyces* (24). These authors reported that a larger number of systemic infections had been observed in Europe (85% of reported cases) than in the United States or North America (10% of all cases).

We describe here the first known case of vertebral osteomyelitis and discitis caused by *B. capitatus* in a leukemic patient and the results of therapy. We also reconfirm the microbiological characteristics which differentiate this species from other yeastlike pathogens.

Case report. A 14-year-old girl was admitted to our institution in April 1991 and diagnosed with acute lymphoblastic leukemia. Prior to intensive chemotherapy, a central venous catheter (CVC) was positioned for infusion of antimicrobial agents. During the neutropenic phase (<1,000 neutrophils per μl), the patient developed erythema (from 2 to 3 cm) of the skin exit of the CVC, along with fever (38.2 to 39°C) unresponsive to amikacin (15 mg/kg of body weight per day), ceftazidime (6 g/day), and vancomycin (30 mg/kg/day) therapy. After 7 days of antibiotic therapy, intravenous fluconazole (400 mg/day) was administered for 6 weeks. At the end of the fluconazole treatment, nine separate blood cultures (of three and six consecutive samples from the CVC and a peripheral venipuncture, respectively) revealed a yeastlike fungus morphologically and physiologically consistent with *B. capitatus*. A new central line was placed; the removed CVC was cultured, and yeastlike strains identified as *B. capitatus* were isolated. The patient was then placed on amphotericin B (50 mg/day) for 2 months. In August 1991, she developed fever (38.5 to 39.2°C), and physical examination revealed a marked paravertebral contracture. Magnetic resonance imaging of the lumbar spine showed lytic lesions in the body of L_3 and L_4. Surgical debridement of L_4 was performed. Material from the vertebral body revealed no microorganism after Gram and Ziehl-Neelsen stains, but periodic acid-Schiff (PAS) stain showed fungal elements. Cultures for aerobic and anaerobic bacteria and for mycobacteria were all negative, whereas cultures for fungi yielded *B. capitatus*. The patient was treated with itraconazole (200 mg twice daily). With this therapy, her body temperature normalized, her back and abdominal pain decreased, and her neurological abnormalities disappeared. One year after her follow-up examination, the patient was in little discomfort and without any laboratory, clinical, or radiologic signs of infection.

Microbiological studies. Both the six peripheral cutaneous venipuncture specimens and the three CVC specimens were obtained aseptically after treatment of sites with a 10% povidone-iodine solution, which was allowed to dry. The blood samples were used to inoculate NR-16A, NR-17A, and FUNGAL BACTEC blood culture bottles (BACTEC NR-660 system; Becton Dickinson Italia, Milan, Italy), which were incubated at 37°C. BACTEC blood culture bottles were checked for microbial growth every 8 h for CO_2 production (14). Once a growth index was obtained, portions of the broth from the positive BACTEC bottles were Gram stained for microscopic observation and inoculated onto sheep blood agar (BA) (Unipath S.p.A., Garbagnate Milanese, Milan, Italy) and Sabouraud glucose agar (SGA) (Difco Laboratories, Detroit, Mich.) plates, the latter containing 4% glucose, 0.5 g of cycloheximide per liter, 20,000 IU of penicillin, and 40,000 IU of streptomycin. BA and SGA plates were then incubated in a humidified chamber at 30 and 37°C in ambient air for 36 to 48 h.

After removal, the catheter tip was cultured on SGA plates by a semiquantitative technique as described previously (1). Catheter tip colonization was defined as the presence of more than 15 CFU after incubation at 37°C for 48 h.

Portions of the bone sample from the L_4 body were cultured for aerobic and anaerobic bacteria and for myco-

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bacteria by conventional methods (3). Moreover, aliquots of the L_4 vertebral body debris were inoculated onto Emmons' modified SGA (Difco) and incubated in ambient air at 30 and 37°C. In addition, a histopathologic examination was performed on tissue sections of the same surgically debrided L_4 body as that used in the microbiological studies by use of the PAS reaction.

Since the yeastlike fungi grown on the original SGA plates were all positive, they were subcultured onto SGA slants and incubated at 30, 37, and 45°C and onto Mycosel (BBL Microbiology Systems, Cockeysville, Md.) slants and incubated at 30°C to evaluate the cycloheximide resistance of the fungi. Conidial morphology and ontogeny were observed by use of 7- to 10-day-old potato dextrose agar (Unipath) plates and cornmeal agar (Unipath) slide cultures.

Biochemical tests were performed by use of ATB 32 C (API System, bioMerieux Italia, Rome, Italy) strips (12, 23), and the results were verified with the AutoMicrobic system yeast biochemical card (Vitek System, bioMerieux Italia). Potassium nitrate (KNO_3) assimilation was determined with a nitrate test medium as reported by others (21). Urease formation was tested by use of Christensen urea agar (Unipath) slants incubated at 28°C and examined for 4 days.

Finally, tests for MICs and minimal fungicidal concentrations (MFCs) of amphotericin B, fluconazole, 5-fluorocytosine, itraconazole, and ketoconazole against our *B. capitatus* strain were done by a microdilution methodology as described by others (6). Moreover, the protocol concerning the preparation of the twofold drug dilutions, the *B. capitatus* inoculum density used (ca. 10^3 CFU/ml), and the step-by-step procedure for susceptibility testing included the National Committee for Clinical Laboratory Standards Subcommittee recommendations for the broth macrodilution testing of yeast cells (20). The lowest concentration of antimycotic drug that completely inhibited visible growth was considered the MIC. For determination of the MFCs, subcultures were made on SGA plates by transferring 10 μl of medium from each microtiter well without fungal growth. The lowest concentration that did not allow fungal growth after incubation at 30°C for 48 h was recorded as the MFC.

The microscopic observation of PAS-stained sections of vertebral lamina debris revealed the presence of small fragments of septate hyphae and a conidium with a tapered end, consistent with the morphology of an annelloconidium of *B. capitatus* (Fig. 1 and 2). Branched hyphae were observed occasionally. In addition, the microscopic observation of a small amount of growth on BA and SGA revealed arthroconidiumlike structures and round to oval, budding yeastlike cells, which were examined further to ascertain the absence of a capsule. Although arthroconidiumlike structures were observed during the initial colony development, we have noted, as have Polacheck and coworkers (22), that annelloconidia form at the apex of a conidiogenous cell during its proliferation. The smooth and oblong annelloconidia divided, successively, by schizogony.

Cream-colored yeastlike colonies were grown on BA and SGA cultures previously inoculated with broth from the nine positive BACTEC bottles. Semiquantitative cultures of the CVC tip revealed a fungal presence of more than 15 CFU after 48 h at 37°C. These colonies were macroscopically similar to that isolated from the peripheral blood samples and the bone specimen cultures. Furthermore, cultures of the bone specimen on Emmons' modified SGA were negative for bacterial growth. Colony features and microscopic morphology at initial examination were compatible to those of *B. capitatus*. More detailed observations of fungi developing on potato dextrose agar plates and on cornmeal agar slide cultures confirmed this preliminary identification. Subcultures on SGA slants and Mycosel slants grew at all working temperatures. Isolated yeastlike organisms were unable to utilize KNO_3 as the sole nitrogen source or to hydrolyze urea. Moreover, when the ATB 32 C and AutoMicrobic system yeast biochemical card systems were used, the isolates were identified correctly as *T. capitatum* (*G. capitatum*), now known as *B. capitatus* (24).

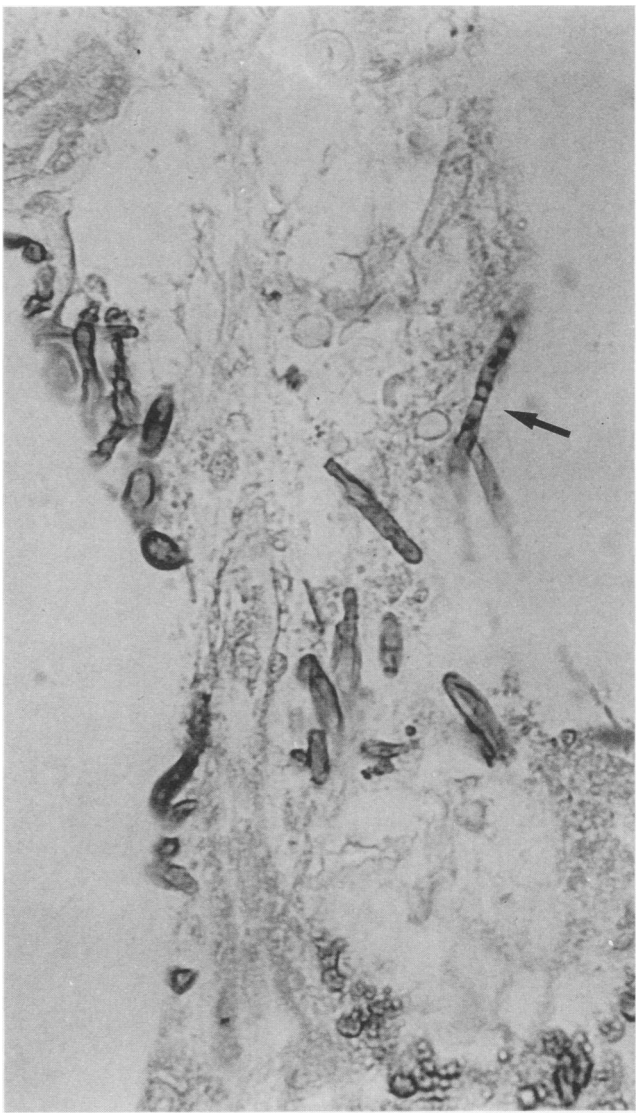

FIG. 1. Section of vertebral lamina showing (arrow) small fragments of septate hyphae, consistent with the morphology of *B. capitatus* (PAS stain; original magnification, ×1,000).

The in vitro antifungal susceptibility testing of five antifungal drugs against our *B. capitatus* isolate showed that the organism was more susceptible to itraconazole than to amphotericin B and ketoconazole, the respective MICs (MFCs) being 0.2 (0.2), 0.78 (1.56), and 1.56 (3.12) μg/ml. In contrast, *B. capitatus* was inhibited or killed by >100 μg of 5-fluorocytosine per ml, whereas fluconazole inhibited and killed the clinical isolate at concentrations of 25 and 100 μg/ml, respectively. The results show that itraconazole is

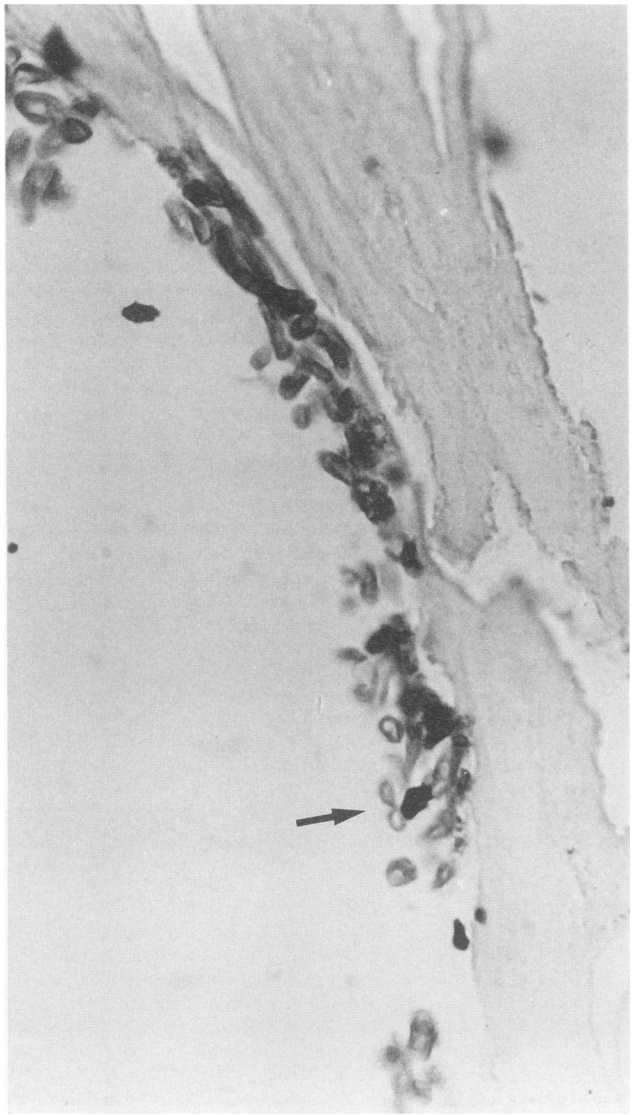

FIG. 2. Section of vertebral lamina showing (arrow) a conidium with a tapered end, consistent with the morphology of an annelloconidium of *B. capitatus* (PAS stain; original magnification, ×1,000).

highly active as a fungistatic agent but is also active as a fungicidal drug. Unexpectedly, fluconazole was sevenfold to ninefold less active than the lipophilic triazole compound against *B. capitatus*.

Among mycotic agents, *B. capitatus* has been reported as a cause of disseminated infection. This fungus is not a rare organism, being present in the soil, normal human skin, and digestive and respiratory tracts. Infections do not seem to differ from other fungal infections. Usually, the clinical picture is characterized by unresponsive fever or fever recurring after antimicrobic treatment in the setting of severe neutropenia. Previously published cases of *B. capitatus* (*T. capitatum*) invasive or disseminated infection included septicemia (11, 17, 19), respiratory tract infection (2, 5), alimentary tract infection (13), endocarditis (22), kidney and liver involvement (15, 16), and meningitis (18). Our case is unusual because of the aggressive nature of the infection. The portal of entry for *B. capitatus* could be traced to a contaminated CVC, with consequent hematogenous dissemination to cause metastatic osteomyelitis. Our experience confirms the observation of Gathe et al. (7) that fungal osteomyelitis is a slowly progressive infection with periods of dormancy. Osteomyelitis is sometimes difficult to diagnose, particularly when the causative agent or the localization is atypical. The final diagnosis can only rely on a microbiological examination of specimens obtained from the inflamed tissue by surgery. The isolation of multiple colonies of *B. capitatus* from portions of laminectomy samples and the observation of fungal elements in PAS-stained sections clearly established its causative role in our patient's infection and the association of this yeastlike fungus with osteomyelitis.

Our observations confirm previous data (22, 24) indicating that *B. capitatus* may be differentiated from other morphologically similar yeastlike pathogens by (i) the annelloconidia that undergo schizogonic division to form abundant arthroconidiumlike structures, (ii) the capability to grow at 45°C on SGA and to be cycloheximide resistant, and (iii) the incapability to produce the enzyme urease.

Prophylaxis and treatment of *B. capitatus* infection are not yet well established.

Although our in vitro antifungal susceptibility studies were conducted with only one clinical isolate, the results showed that itraconazole was the most active drug against *B. capitatus*. This result correlated with our in vivo observation of the patient failing on fluconazole and amphotericin B but responding to itraconazole, thus demonstrating a good relationship between susceptibility results and therapeutic outcome. Although there is a suggestion that the susceptibility results have some predictive value for large groups of isolates, with respect to a given patient with a given isolate, the data are limited. Nevertheless, itraconazole represents a significant step forward in antifungal therapy, the impact of which is yet unknown.

This work was partly supported by Associazione Donatori Sangue, Pescara, Italy. R.P. was supported 40% by an MURST grant.

We thank Marcello Piccirilli for technical assistance during the histopathologic examination.

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