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Fulminant blastomycosis with blastomycotic infection of a cerebral glioma

Light microscopic and ultrastructural observations

V. Jay[1], N. Laperriere[2], and R. Perrin[3]

[1] Department of Pathology, Ontario Cancer Institute/Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto M5G 1X8, Ontario, Canada
[2] Department of Radiation Oncology, Ontario Cancer Institute, Toronto, Canada
[3] Department of Neurosurgery, Wellesley Hospital, Toronto, Canada

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Summary. Except for isolated case reports, blastomycosis has not been identified as a significant problem in immunosuppressed patients. We describe an unusual case with blastomycotic infection of a cerebral glioma in a 56-year-old man who underwent radiotherapy for his tumor and died of fulminant blastomycotic pneumonia. This is believed to be the first reported case of *Blastomyces dermatitidis* infection of a cerebral glioma. The light microscopic and ultrastructural features of *B. dermatitidis,* the giant forms of which were encountered in our patient, are described, and the role of immunosuppression due to steroid therapy in the pathogenesis of this fulminant infection are reviewed.

Key words: Blastomycosis – North American Blastomycosis – Brain tumor – Opportunistic infection – Ultrastructure

Invasive fungal infections are common when the immune function is depressed [7]. The most common fungi encountered are Candida and Aspergillus species which rarely if ever cause invasive disease in a normal host. Except for a handful of individual case reports, blastomycosis has not been a significant problem in immunosuppressed patients [2, 5, 11, 22, 26, 31]. We describe presentation of blastomycosis as a fatal pneumonitis in a 56-year-old man who underwent radiation therapy for a cerebral glioblastoma multiforme. The unusual clinical and pathological findings with involvement of the tumorbed itself and the role of immunosuppression due to steroid therapy are discussed.

Case report

This 56-year-old white male, a business executive, presented to the Wellesley Hospital with a 3-month history of decreasing sensation in the right half of his body. There was no other significant past medical history. There was no history of drug abuse. He denied homosexual contact. The patient was a resident of Toronto and there was no history of recent travel.

Clinical examination revealed decreased sensation on the right side, visual field defect with a right inferior quadrantanopsia, and some difficulties in word finding. A CT scan of the head revealed an irregular enhancing mass deep in the left parietal lobe with surrounding edema (Fig. 1). A stereotactic biopsy revealed a

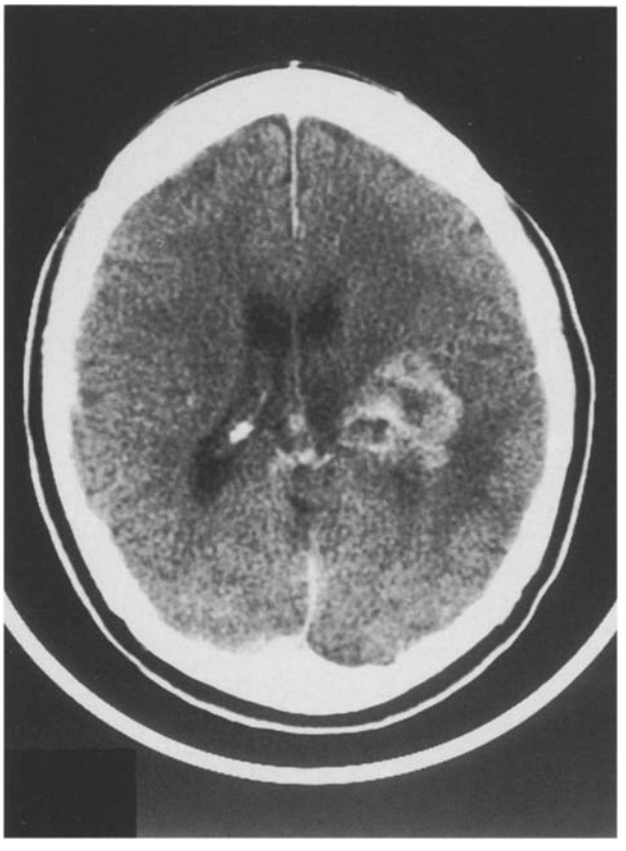

Fig. 1. Contrast-enhanced CT scan of head demonstrating an irregularly enhancing deepseated tumor in the left pareital lobe with surrounding edema

Offprint requests to: V. Jay (address see above)

high-grade astrocytoma with necrosis and endothelial vascular hyperplasia, consistent with the diagnosis of glioblastoma multiforme.

The patient was started on Dexamethasone and Ranitidine and his condition stabilized. He underwent a radical course of cranial irradiation with isocentric high energy linear accelerator (25 megavolts) to a dose of 50 Gy in 25 daily fractions in 5 weeks via a parallel pair of fields to a regional volume incorporating the primary tumor plus a 3-cm margin. The patient tolerated this quite well and developed the expected side effects of alopecia and scalp erythema, and remained on dexamethasone 8 mg/day.

Approximately 8 months after his initial symptoms, the patient was admitted to hospital with a 1-week history of increasing dyspnea, mild fever, and generalized weakness. On examination, he appeared quite unwell and confused with a respiratory rate of 40/min, pulse 120/min and temperature of 37.5 °C. He was disoriented to time and place. There was 2+ pedal edema. Examination of the chest revealed bronchial breathing in the left lower lung field with coarse crepitations bilaterally. There were no new neurological abnormalities as compared to the previous examinations.

A chest X-ray revealed bilateral pulmonary changes involving all lobes of the lungs with diffuse interstitial changes consisting of edema, nodules and interstitial infiltrates. Arterial blood gases revealed PO_2 30 mmHg, PCO_2 23 mmHg, pH 7.54, and HCO_3 20 mmol/l. Oxygen and morphine were administered. There was no sputum production. Blood cultures for both aerobic and anaerobic bacteria were made and revealed no growth after 7 days. Empirically, the patient was started on intravenous Keflex, Tobramycin, Erythromycin, and Septra.

The patient's condition continued to deteriorate and he died 36 h after admission to the hospital. The autopsy was limited to examination of the brain, liver and lungs.

Pathological findings

Both lungs were consolidated with a nodular hemorrhagic appearance, the right lung weighing 1080 g and the left lung weighing 1570 g. On cut section, all lobes of both lungs revealed extensive consolidation with firm nodules and multiple abscesses. Microscopic examination of lungs revealed a florid necrotizing pneumonia with abscess formation. Numerous *Blastomyces dermatitidis* organisms were seen within the alveolar spaces, which appeared packed with fungi (Fig. 2). They revealed the characteristic single broad based budding. The sizes were quite variable, being on average about 20 μm with many larger fungi in the range of 28–35 μm. The double-contoured cell walls and the cytoplasmic mass were stained by the periodic acid-Schiff (PAS) stain, with the cytoplasm being often retracted from the cell wall (Fig. 2b). Multiple nuclei were present. The cell walls were also stained by the Gomori methenamine silver stain but not by mucicarmine. There was also severe diffuse alveolar damage and severe edema. Hyperplastic type 2 pneumocytes with moderate nuclear hyperchromatism and atypia were present. The lungs were virtually replaced by fungal growth, with plugging of bronchi and bronchioles with fibrinous and mucoid material abounding in fungi. In rare blood vessels, intraluminal organisms were seen, indicating hematogenous dissemination. Fibrinopurulent pleuritis with *B. dermatitidis* organisms on the pleural surface was also observed. Focally, there was a

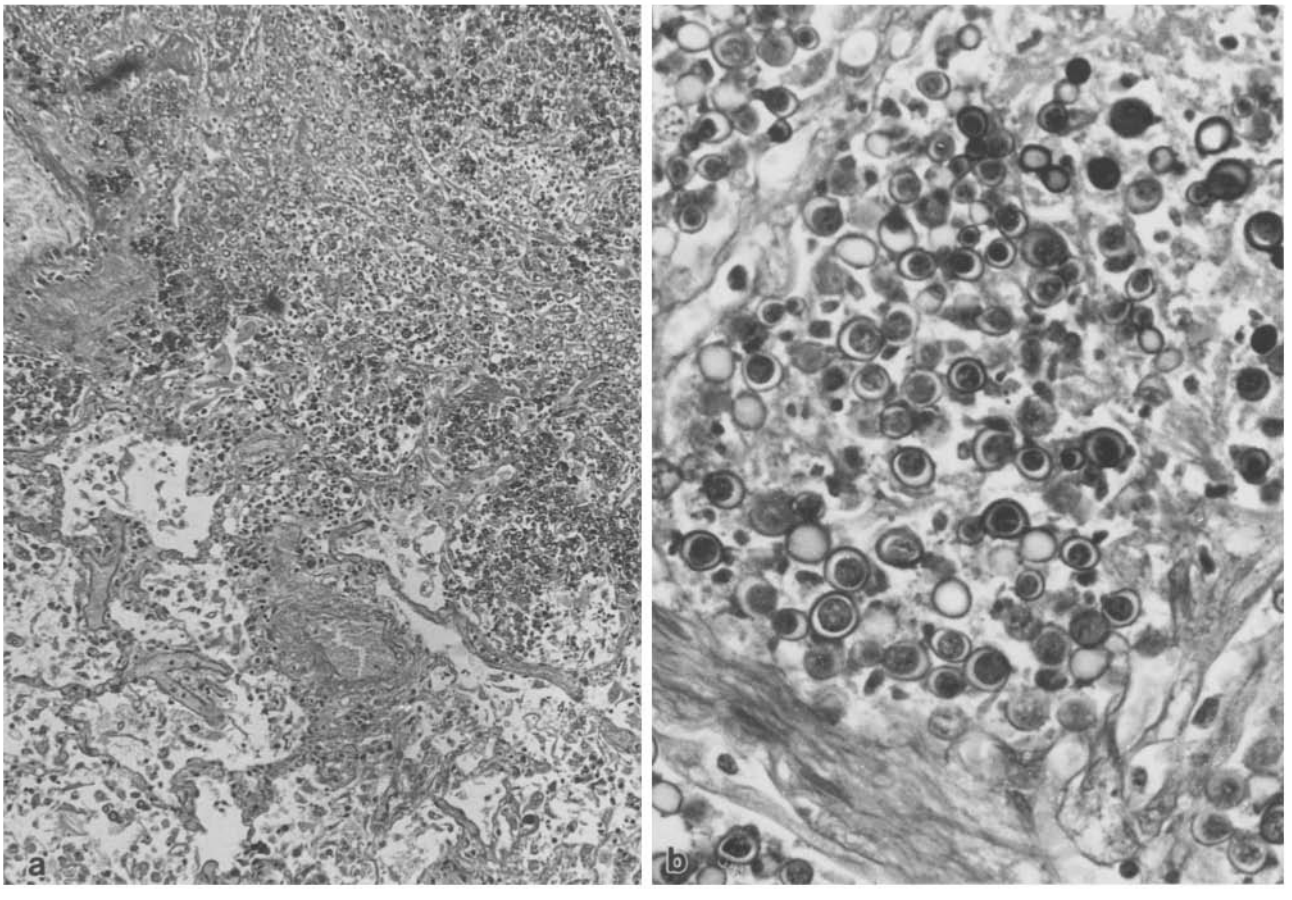

Fig. 2. **a** Low-power micrograph of lung showing extensive fungal pneumonitis with alveoli packed with *Blastomyces dermatitidis* organisms. **b** High-power micrograph illustrating fungal morphology: note the variability in fungal size with giant forms. **a, b** PAS; **a** × 112; **b** × 560

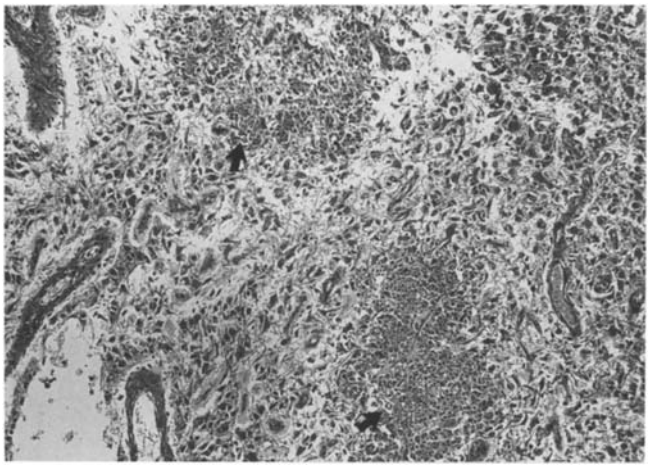

Fig. 3. Low-power micrograph of tumor bed illustrating two foci of fungal infection within the tumor (*arrows*). Hematoxylin-Eosin. × 56

component of chronic inflammation with macrophages and plasma cells.

The liver was enlarged weighing 1600 g and grossly was not very remarkable except for occasional whitish specks on the cut surface. On microscopy, it was studded with microabscesses and fungal organisms could be identified within Kupffer cells. There was mild steatosis involving the hepatocytes. In a few portal triads, a few macrophages and occasional plasma cells were present. Special stains did not demonstrate bacterial, fungal, or mycobaterial infection in sections of the lung and liver. Immunostaining for cytomegalovirus antigen revealed no immunopositivity in sections of the lung and the liver.

The brain weighed 1460 g. There was slight thickening and opacification of the leptomeninges, but no evidence of meningeal exudates. Coronal sections of the brain showed an irregular partially necrotic highly vascular tumor in the left temporal, parietal and occipital white matter with involvement of the thalamus and basal ganglia and extension to the ventricular surface. Microscopic examination revealed a high grade astrocytoma (glioblastoma multiforme) with extensive radiation necrosis and radiation vasculopathy. The tumor itself was composed of plump gemistiocytic astrocytes with moderate nuclear pleomorphism. Extensive hemosiderin accumulation indicated old hemorrhage. Besides the radiation-associated changes, there were no other unusual features in the tumor which had relatively few mitoses.

The most noteworthy finding was that of *B. dermatitidis* organisms within the tumor (Fig. 3) and the brain. These were seen in several patterns: well-organized microabscesses with fungi, well-organized epithelioid granulomas with fungal forms within Langhan's giant cells, microglial nodules or scattered phagocytes containing fungal organisms. In the cerebellum, focal meningeal involvement was noted; in one area, the microabscess was seen to rupture into the subarachnoid space with overlying meningeal inflammation. Fungi were not seen within reactive or neoplastic astrocytes, but rather within microglia or multinucleated giant cells. They were not identified in the pituitary, pineal, or choroid plexus. There was evidence of recent hypoxic-ischemic encephalopathy with eosinophilic Purkinje cells in the cerebellum. There was no evidence of any other opportunistic infection in the brain and immunostaining of several sections failed to disclose cytomegaloviral infection.

Ultrastructural studies

Methods. Pieces of formalin-fixed tissue from the lung and liver were submitted for electron microscopic studies. Tissues were

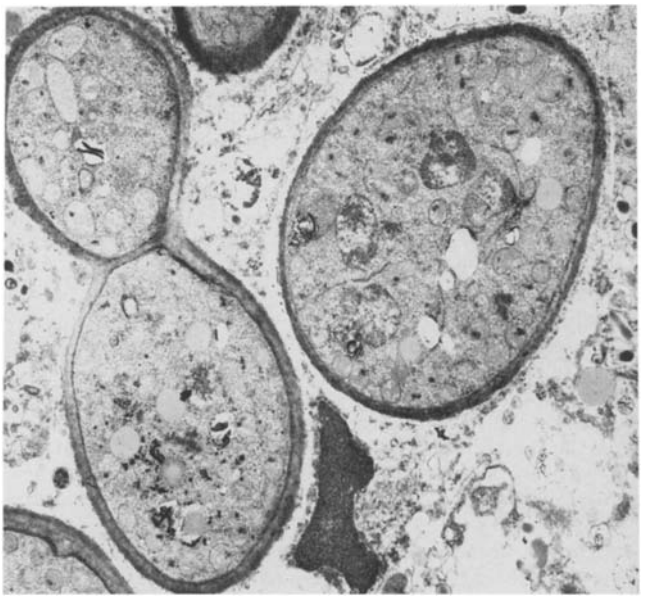

Fig. 4. Electron micrograph from lung sample reveals budding organisms of *Blastomyces dermatitidis* with broad-based budding, cell walls with outer and inner layers separated by electron-lucent zone, multiple nuclei, lamellar or cleft-like structures, tubular structures, numerous mitochondria, and lipid droplets. × 8300

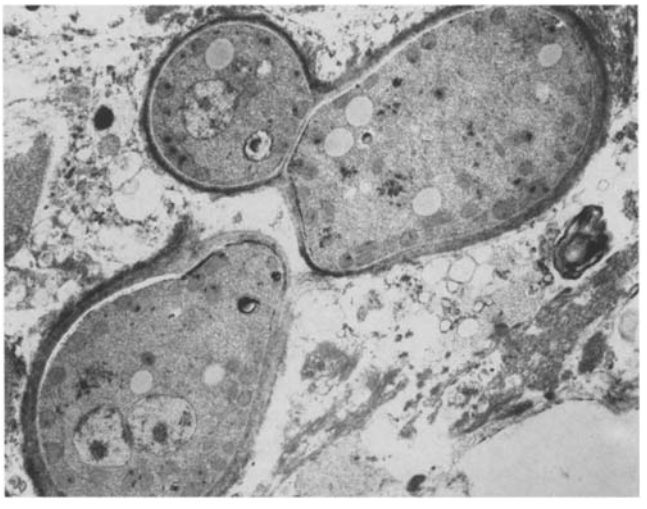

Fig. 5. Electron micrograph from lung sample illustrates initiation of budding with shearing of outer layers of the cell wall in one cell; in the other, the inner layer of the cell wall contributes to the septum. × 6590

postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Spurr's embedding media. Ultrathin sections were stained with lead citrate and uranyl acetate and studied with a Zeiss 902 electron microscope.

EM findings. Both the lung and liver samples showed relatively well-preserved and viable fungal forms in contrast to the host tissues. Most organisms were extracellular; due to postmortem artifact and poor preservation of host tissues, the detailed morphological appearances of cells harboring the intracellular fungi could not be commented upon. The fungi had lamellated cells walls with two distinct layers separated by a relatively electron-lucent intervening zone (Fig. 4). The outer aspect of the cell wall presented an irregular external surface. The thickness of the cell

walls was variable ranging from 0.3 to 0.8 μm. The organelles included moderate numbers of mitochondria, glycogen and lipid granules, a few freelying as well as membrane-bound structures with electron-dense lamellated profiles, a few tubular structures, and multiple nuclei (Fig. 4). The nuclei had one to two nucleoli and showed some heterochromatin condensations (Figs. 4, 5). A few fungi contained numerous mitochondria. A number of cells in the process of budding were encountered: the initiation of budding was marked by a shearing of the outermost electron-dense lamellated layers of the cell wall (Fig. 5). The inner layers of the cell wall persisted over the protruding bud and covered the daughter cell and the broad-based septum (Fig. 5). Scattered bacteria were also found in the lung sample.

Discussion

Blastomycosis is caused by *B. dermatitidis,* a dimorphic fungus that dwells as a saprophyte in the soil worldwide, but principally in the southeastern, south central, and upper midwestern regions of the USA with new endemic foci being uncovered in other geographical locations [13, 18, 29]. The organism enters the body via the lungs with subsequent dissemination to other organs and patients most often present with cutaneous or pulmonary complaints, although the infection may be asymptomatic [18, 29].

Blastomycosis involving the central nervous system (CNS) has been reported infrequently in the literature almost invariably in association with systemic infection [1, 4, 10, 14, 15, 20, 21, 23–25, 27, 30]. The estimates of CNS involvement range from 3 %–10 % in clinical studies and from 6 %–33 % in autopsy studies [4, 10, 27]. Patients with CNS blastomycosis may present with acute or chronic meningitis or mass lesions of the brain or spinal cord including multiple or single abscesses or granulomas. A clinical diagnosis of CNS blastomycosis is difficult, and in many of the cases a presumptive diagnosis has been based on the identification or isolation of the organism from other sites. Although, the diagnosis has been made on the basis of cerebrospinal fluid (CSF) examination, CSF smears or cultures may be negative [15].

In our case, a variation from the typical fungal morphology was encountered with many giant forms with diameters measuring from 28 to 35 μm (normal range 8–15 μm). Excellent preservation allowed for clear documentation of the ultrastructural morphology, especially with reference to the budding characteristics of this pathogen. Our observations confirm the findings reported in the literature with regards to the size and distribution of various organelles [6, 20]. In an ultrastructural study of a cerebellar blastomycoma, Mirra et al. [20], described fungi which were intracellular in membrane-bound compartments within multinucleated giant cells and macrophages. In our case, besides organisms within macrophages in the liver, most yeasts were extracellular, and in an excellent state of preservtion compared to the host tissues which were autolyzed. Organisms in various stages of budding were identified: it is apparent that the outermost layers of the cell wall are sheared off at the initiation of this process with contribution of the inner layers to the septum and the new coat of the daughter cell. The most abundant organelle was the mitochondrion. The mitochondria showed well-preserved cristae and electron-dense particulate material reminiscent of calcium. The lamellated cell walls ranged from 0.3 to 0.8 μm and the outermost layers which were most electron dense was ragged with irregular surface projections. A relatively electron-lucent zone separated the outer layers from the innermost electron-dense layers of the cell wall. Tubular structures with a lumen were seen coursing the cytoplasm with no communication to the cell membrane. Other features included multiple nuclei with prominent nucleoli, membrane-bound lamellated or membranous whorls akin to myelin figures in mammalian cells, electron-dense cleft-like profiles, and moderate numbers of lipid droplets, and diffusely distributed particulate glycogen.

The tissue response to *B. dermatitidis* is a combined granulomatous and suppurative reaction as illustrated in our case. Interestingly, despite extensive sampling, the chronic inflammatory component was minimal in the lungs which showed alveoli packed with organisms. Fatal adult respiratory distress syndrome (ARDS) due to *B. dermatitidis* has been described by Evans and colleagues in a report of two patients [9]. ARDS secondary to blastomycosis has also been reported in pregnancy [19], which has been considered to be related to the pregnancy-related immunosuppression.

To our knowledge, this is the first reported case of blastomycotic infection of a cerebral glioma. The tumor itself showed no unusual features except the anticipated radiation necrosis. The fungi occurred within microglial nodules, epithelioid granulomas or microabscesses within the tumor bed. They were either extracellular or within macrophages and were not contained within the tumor cells. This is in contrast to the report of Ho et al. [12] of cytomegalovirus infection of a glioma in a patient with the acquired immune deficiency syndrome [AIDS], where virus-infected tumor cells were described.

The epidemiology and ecology of blastomycosis are not fully elucidated [13, 18 29]. In this regard, it is of interest that our patient was a city dweller and had no occupational exposure to wood, soil, or animals. Immunosuppression related to steroid therapy was presumably the predisposing factor in our patient for development of fulminant blastomycosis. Although the autopsy was limited to examination of lungs, liver and brain only, it is evident that the patient had hematogenous spread of the disease, with fungal organisms demonstrable within cells of the monocyte-macrophage system and also within vascular spaces in the lungs. There were no visible skin lesions in our patient, thus the portal of entry and original infection is most likely the lung and this fulminant infection perhaps represents endogenous reactivation of a latent infection, which is a well-recognized phenomenon [8, 16].

Blastomycosis is not a common infection associated with immunosuppression. It is rarely reported as an opportunistic pathogen in the immunocompromised host usually in the setting of hematological malignancy

or steroid immunosuppression [2, 5, 22, 26]. Of 78 patients with blastomycosis, Recht et al. [26] found an underlying hematological malignancy in 3 cases and association with glucocorticoid therapy in 3 patients. The clinical picture in these 6 patients was similar to blastomycosis in nonimmunosuppressed patients with chronic pulmonary infiltrates or isolated skin ulcers. Blastomycosis complicating bone marrow transplantation has been described by Winston et al. [31].

The standard therapy advocated for this infection is amphotericin B and ketaconazole [3, 7, 28]. Ketaconazole, which has the advantages of oral administrability and less severe toxicity has the disadvantage of poor penetration of the blood-brain-barrier and treatment failures resulting in death of some patients with severe pulmonary blastomycosis. Pitrak et al. [23] described development of a cerebral mass lesion in a patient treated with oral ketaconazole for pulmonary blastomycosis, with resolution of the lesion after intravenous amphoterecin therapy. Our patient did not have the benefit of therapy due to the rapidly of clinical course and death before definitive diagnosis of the nature of the pneumonitis.

In summary, a case of fulminant blastomycosis with fungal infection of a glioma is described. In our patient, besides steroid immunosuppression, there appears to be no other predisposing factor, although the rapidity of the clinical course precluded a detailed workup of the immunological status. Blastomycosis has been described in the setting of the AIDS [11, 17]; in our patient, there is no evidence to suggest this as a possible predisposing factor; furthermore, besides this uncommon fungal pathogen, no other opportunistic infections are identified.

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