

## Infections post transplant

# Pulmonary infection with microsporidia after allogeneic bone marrow transplantation

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### Summary:

**Microsporidia are obligate, intracellular protozoal parasites that can be pathogenic in immunocompromised individuals. The majority of cases of microsporidiosis have been documented in patients with HIV, and only a few case reports exist of infection in solid organ transplant patients. We report the first case of pulmonary microsporidial infection in an allogeneic bone marrow transplant recipient in the US. The patient was a recipient of a T-cell-depleted graft who succumbed to complications from respiratory failure 63 days post transplant. The diagnosis was made post mortem by electron microscopy and confirmed with PCR. Although rare, microsporidial infection should be considered in the differential diagnosis of unexplained pulmonary infection in bone marrow transplant patients.**

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Microsporidia are obligate, intracellular protozoal parasites of the phylum Microspora. The first case of microsporidia causing human disease was reported in 1959,<sup>1</sup> but additional reports were rare until the advent of HIV in the 1980s. In 1985, the first case of infection with microsporidia was described in an HIV patient,<sup>2</sup> and subsequently, numerous publications have described infections in HIV-affected patients.<sup>3,4</sup> Microsporidial infection has been documented in several immunocompetent individuals who had only self-resolving travelers diarrhea.<sup>5</sup> Although the majority of documented infections with microsporidia have been in patients with HIV, patients who are immunocompromised from other diseases are at risk for microsporidial infection. Six reports of microsporidial infection have been

documented in patients following solid organ transplant,<sup>6–10</sup> and one after bone marrow transplant in India.<sup>11</sup> There have been no cases of microsporidial infection reported in bone marrow transplant patients in the US. We, describe the first reported case of microsporidial infection in a bone marrow transplant recipient in the US.

### Patient

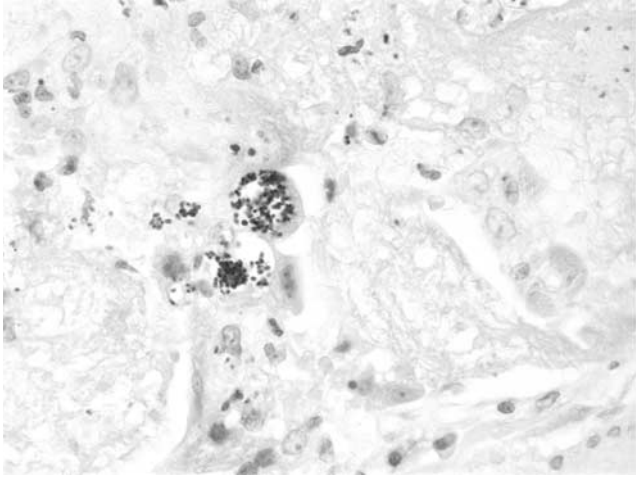
The patient was a 21-year-old female who developed AML in January of 2002 following therapy for stage IIA Hodgkin's disease, which was completed in August 2000. She achieved remission with Mitoxantrone and Cytarabine. She underwent bone marrow transplantation with an unrelated donor, mismatched at the A locus, in May 2002. Her conditioning regimen included Thiotepa, cyclophosphamide, total body irradiation, and antilymphocyte globulin. Cyclosporine was given for GVHD prophylaxis. The marrow was partially T-cell-depleted with OKT3 and complement.<sup>12</sup>

She had myeloid engraftment with an ANC greater than 500/mm<sup>3</sup> on day +22; however, she remained significantly immunocompromised secondary to receiving ALG and a T-cell-depleted graft. On day +49, rising CMV antigenemia to 103 viral nuclei/200 000 cells was noted. She was treated with ganciclovir and her antigenemia declined to zero by day +53. A chest CT at that time was negative for parenchymal disease.

She underwent endoscopy and sigmoidoscopy on day +54 for a markedly distended abdomen with ileus, and both were negative for GVHD or pathogens, except for scant yeast. On day +56, she developed *Candida glabrata* bacteremia, and Ambisome was initiated. Repeat blood cultures were negative for yeast.

On day +58, she developed respiratory distress. A CT scan revealed interstitial pneumonitis. She was treated with multiple broad-spectrum antimicrobials. In addition, CMV antigenemia increased to 44 (44 nuclei/200 000 cells), and therapy was changed to Foscarnet. She subsequently required intubation for impending respiratory failure. Broncho-alveolar lavage on day +60 was negative for CMV and PCP. Thoroscopic lung biopsy on day +62 was presumptively negative for CMV, but was suggestive for

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**Figure 1** Microsporidia in multiple cytoplasmic supranuclear vacuoles of bronchial epithelial cells. Brown-Brenn Gram stain,  $\times 600$ .

toxoplasmosis by light microscopy (Figure 1). Therapy with Pyrimethamine and sulfadiazine was instituted. On day +63, she developed a left frontal intracranial hemorrhage with midline shift and blood in her third and fourth ventricles. Ventilatory support was withdrawn, and she died quickly thereafter. Permission for autopsy was not obtained.

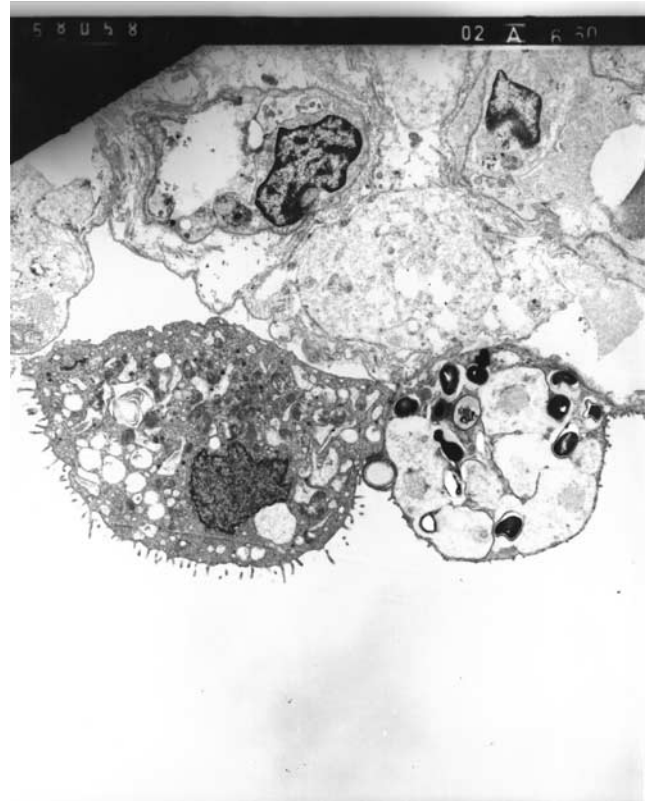
## Results

Studies on the lung biopsy were completed post mortem. Immunohistochemical staining for Toxoplasmosis was negative. Electron microscopy confirmed microsporidiosis (Figure 2), which by molecular analysis, utilizing PCR and DNA sequencing, subsequently was revealed as *Encephalitozoon cuniculli* type III species.

In addition, rare budding yeast was visualized on Gram stain of her lung tissue, but fungal culture of the lung tissue was negative. Also, scant CMV was noted with further immunohistochemical analysis of the lung tissue, but no evidence of CMV vasculitis was found. The tissues obtained from endoscopy and sigmoidoscopy were reinvestigated and revealed no evidence of microsporidia by light microscopy.

## Discussion

Microsporidia are obligate eucaryotic intracellular protozoal parasites of the Phylum Microspora. Microsporidiosis (infection with microsporidia) has been demonstrated in a large number of vertebrate and invertebrate species. In humans, eight of the 150 genera have been shown to cause disease (*Brachiola*, *Enterocytozoon*, *Encephalitozoon*, *Nosema*, *Pleistophora*, *Septata*, *Trachipleistophora*, and *Vittaforma*).<sup>3,4</sup> Both ultrastructural and molecular techni-



**Figure 2** One of two alveolar lining cells demonstrating multiple electron-dense intravacuolar spores. Transmission electron microscopy,  $\times 7000$ .

ques are utilized to classify the greater than 1000 species in the phylum.<sup>3</sup>

The mode of transmission of microsporidia is not known. Microsporidia are released into the environment in stool, urine, and respiratory secretions by infected animals. It is not known whether humans obtain infection from person to person contact, the environment, other mammals, or insects.<sup>3</sup> It is unclear whether infection in immunocompromised individuals is a newly acquired infection or a reactivation of latent infection acquired prior to immunosuppression.<sup>13</sup> Of note, microsporidium species *Encephalitozoon cuniculli III* is found in dogs, and our patient had dogs, as well as cats and rats.

Microsporidia may cause a broad spectrum of disease.<sup>3</sup> The majority of patients have infection limited to the gastrointestinal and biliary tracts. Immunocompromised patients may develop chronic, severe, nonbloody, non-mucoid diarrhea with weight loss and fat malabsorption or hepatitis and peritonitis. Other common manifestations of microsporidial infection include keratoconjunctivitis, sinusitis, urinary tract infection, myositis, and lower respiratory tract infections. Rarely, patients can develop cerebral infections, urethritis, cutaneous infections, osteomyelitis, and abscesses. Pulmonary infections have been found primarily with microsporidia from the genus *Encephalitozoon*. Most patients with respiratory tract infections also have systemic or intestinal microsporidiosis, but isolated

cases of pulmonary involvement do exist.<sup>3,14</sup> Unfortunately, prevalence data of pulmonary microsporidial colonization are not known.

Our patient and the previously described BMT patient differ from most HIV patients in that both had primary pulmonary disease. In HIV patients, gastrointestinal disease is the most common presentation, and isolated pulmonary disease is rare.<sup>3,4</sup> Gastrointestinal involvement has also been found post solid organ transplant. Both our patient and the other reported BMT patient did have gastrointestinal symptoms with paralytic ileus, but gastrointestinal biopsy was negative for microsporidia. In addition, both bone marrow transplant patients were noted to have multiple pathogens in their lungs. It is uncertain as to which organism caused the majority of symptoms in either patient; however, much larger quantities of microsporidia were noted compared to the other pathogens. It is possible that a synergistic or additive effect between the organisms existed, or that one organism served as a portal of entry for another by causing lung parenchymal damage. It is unknown as to whether our patient had microsporidia in other tissues outside the esophagus, sigmoid colon, and lungs as no autopsy was performed.

Transmission electron microscopy (TEM) of tissue specimens should be used to validate the diagnosis of microsporidiosis.<sup>15</sup> Unfortunately, TEM is expensive, labor intensive, and time consuming. TEM is also less sensitive than light microscopy and cytology, if examining stool or urine. Light microscopy of plastic-embedded tissue specimens using a variety of special stains (toluidine blue, modified trichrome, brown brenn, warthin-starry) can yield quite sensitive and specific results when used in combination.<sup>15</sup> Light microscopy and cytology of stool, urine, broncho-alveolar lavage, and cerebrospinal fluid are fairly reliable; however, these are less-sensitive and specific diagnostic tools. The simplest method used to diagnose microsporidiosis in patients with gastrointestinal disease is light microscopic examination of stool specimens, but this requires multiple samples and special techniques including sedimentation and spore concentration.<sup>3</sup> Unfortunately, neither light microscopy nor electron microscopy are helpful in speciation. Molecular analysis is necessary for speciation, but is available only in a research setting.

Of note, our patient was initially presumed to have toxoplasmosis. Toxoplasma and microsporidia are both intracellular organisms of similar size and appearance, but can be distinguished by immunohistochemical and special stains. Specifically, microsporidia stain well with the Brown–Brenn modification of the Gram stain,<sup>15</sup> while toxoplasma do not. Since many pathologists might not consider microsporidia in the differential diagnosis of pulmonary infections in transplant patients, it would be important to suggest special staining for microsporidia if a transplant recipient is diagnosed with toxoplasmosis by biopsy.

The results of treatment of microsporidial disease with antimicrobials are poor. Albendazole has been shown to be beneficial in treating symptoms and only rarely cure disease in patients with gastrointestinal infections caused by *Encephalitozoon intestinalis*.<sup>16</sup> Recently, a double-blinded placebo-controlled trial<sup>10</sup> compared fumagillin to control in

12 immunocompromised patients with intestinal microsporidiosis with *Enterocytozoon bienewsi* species and found a marked improvement of symptoms and elimination of organisms by stool culture. Further studies are warranted to determine if fumagillin is effective in treating patients infected with different microsporidia species or with different organ involvement.

In conclusion, microsporidia may be responsible for infections in bone marrow transplant patients and should be considered in the differential diagnosis of unexplained infection in these patients.

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