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Prolonged Fever and Splenic Lesions Caused by *Malassezia restricta* in an Immunocompromised Patient

Annabelle de St Maurice, MD¹, Haydar Frangoul, MD¹, Alice Coogan, MD², and John V. Williams, MD^{1,2}

¹Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN

²Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN

Abstract

Malassezia species are commonly found on human skin as commensals but can cause invasive infections in premature infants and immunocompromised hosts. Due to their fastidious growth, diagnosis of *Malassezia* infections can prove challenging. Molecular techniques can aid in diagnosis and treatment of invasive infections. We describe the case of a pediatric oncology patient with splenic lesions secondary to *Malassezia restricta*.

Keywords

malassezia; Hepatosplenic candidiasis; immunodeficiency

INTRODUCTION

Malassezia species can be found on normal human skin as commensals but also are associated with a spectrum of cutaneous diseases including tinea versicolor, seborrheic dermatitis, folliculitis, and eczema in adults and children.(1) In immunocompromised patients, including premature infants, patients with AIDS or malignancy, patients on peritoneal dialysis, and solid organ transplant recipients, disease associated with *Malassezia* species can cause invasive disease and infect other organ systems.(1, 2) In premature neonates, catheter-related fungemia is the most widely reported systemic infection.(3) Sinusitis, mastitis, septic arthritis, malignant otitis externa, skin abscesses, and peritonitis have also been reported.(1) Because most of the species in the *Malassezia* genus require an external lipid source for growth *in vitro*, culture and identification are challenging, as most traditional media do not yield growth of *Malassezia* species in the laboratory. Molecular techniques such as PCR are efficient and sensitive methods for diagnosing invasive *Malassezia* infections. To our knowledge, this is the first reported case of a child with splenic lesions caused by *Malassezia restricta*.

Corresponding author: John V. Williams, MD, Pediatric Infectious Diseases, Vanderbilt University Medical Center, D-7235 Medical Center North, 1161 21st Avenue South, Nashville TN 37232-2581. Phone (615) 936-2186, Fax (615) 343-9723, john.williams@vanderbilt.edu.

CASE REPORT

A 10-year-old Caucasian girl with a history of multiple relapsed neuroblastoma and central venous catheter placement was admitted for prolonged fever for two weeks in the setting of neutropenia at the Monroe Carell Jr. Children's Hospital at Vanderbilt (VCH) in Nashville, TN. Five years before admission, she had undergone autologous stem cell transplant at VCH. In the six months before her admission for fever and neutropenia, she had also received two doses of MIBG therapy. One month prior to admission, she developed profound neutropenia and was started on granulocyte colony stimulating factor (G-CSF). Despite treatment with G-CSF, she remained neutropenic (ANC 0). During the two weeks before admission, she had daily fevers to 39°C. She presented to an outside facility where blood cultures were obtained and empiric broad-spectrum antibiotics and voriconazole (6 mg/kg Q12h IV) were administered. Prior to admission she had not been on anti-fungal prophylaxis. Her fever persisted and she was transferred to VCH for further evaluation and management.

On admission, her temperature was 37.7°C and she was slightly tachycardic. She was noted to have significant pallor but was otherwise well appearing, with no focal findings on physical examination. During the admission, she had daily fevers >39°C. Computed tomography (CT) of the sinuses, chest, abdomen, and pelvis was performed because of persistent fever and neutropenia. Sinus CT demonstrated mild thickening of the paranasal sinuses. Chest CT demonstrated a new small 7mm nodular opacity in the left lower lobe and a 2mm nodule in the right upper lobe. Abdominal CT demonstrated a 4mm hypo-intense lesion in the spleen. The final interpretation was concerning for metastatic disease versus inflammatory process.

The white blood cell count on admission was 200/μL (lymphocytes 89%). The hemoglobin was 8g/dL and the platelet count was 17,000/μL. Histoplasma urine and serum antigens, serum aspergillus antigen, and serum beta-D-glucan were negative. Triglycerides were slightly elevated at 223mg/dL. Bacterial and fungal blood cultures remained negative.

On hospital day 6, she underwent infusion of stored autologous stem cells to improve her neutrophil counts. In addition, on hospital day 7, micafungin (4 mg/kg Q24h IV) was administered to broaden her antifungal coverage. Following the stem cell infusion, she was transferred back to the referring facility on hospital day 10 where her antibiotics were continued. Three weeks after her initial admission, her white blood cell count had increased to $5.4 \times 10^3/\mu\text{L}$, with an absolute neutrophil count of $3.6 \times 10^3/\mu\text{L}$. The empiric antibiotics were discontinued; however, given the CT scan findings and concern for fungal infection, she was discharged home on voriconazole (8mg/kg Q12h PO) and micafungin (4 mg/kg Q24h IV). At discharge on hospital day 24, she was afebrile and in stable condition. Voriconazole levels were within therapeutic range (1.6 mcg/mL). Her liver enzymes were monitored closely and were slightly elevated during therapy (AST 60–100 U/L and ALT 40–98U/L).

Six weeks after hospital discharge, repeat CT of the chest, abdomen, and pelvis revealed multiple low-density lesions in the liver, spleen, and kidneys. She underwent a percutaneous

needle biopsy of a splenic lesion by interventional radiology. Bacterial culture and gram stain of the lesion were negative, as were acid-fast stain and mycobacterial culture. Fungal culture was negative; however, Gomori methenamine silver staining revealed elongated ellipsoid-shaped cells attached to each other at constricted septation sites, consistent with fungal pseudohyphae, and scattered yeast forms in a background of granulomas, lymphocytes, and neutrophils, with no evidence of malignancy (Figure 1). Tissue specimen was tested by qualitative fungal PCR at the University of Washington Molecular Microbiology laboratory. *Aspergillus*-specific PCR was negative, while 28S rDNA primers yielded a PCR product. Direct sequencing of this PCR product identified the specimen as *Malassezia restricta*.(4) Based on these results, the patient's micafungin was discontinued and she was continued on PO voriconazole. There were no recurrent acute respiratory illnesses or positive fungal cultures. Nine months after starting treatment with voriconazole, the patient ultimately expired due to complications from metastatic neuroblastoma.

DISCUSSION

Persistent fever and abdominal pain in an immunocompromised patient should prompt further investigation, including imaging. Nodular splenic lesions should prompt consideration of invasive fungal disease. The most common fungi isolated in patients with malignancy and splenic lesions are *Candida* species.(5, 6) Other fungi that have been implicated include *Curvularia* (7), *Aspergillus*, *Blastomyces* (6), *Penicillium* (8) and *Rhizopus*.(9) *Malassezia furfur* has been implicated in splenic lesions in premature infants (10) but has not been reported to cause hepatosplenic infection in immunocompromised patients. Because each of these fungal pathogens requires specific antimicrobials, accurate diagnosis is key to therapeutic success. Obtaining a biopsy of splenic lesions and sending tissue for evaluation by pathology, microbiology, and molecular testing can assist in making an accurate diagnosis for improved therapeutic success.

Malassezia yeasts are single-celled eukaryotic organisms that are common microbial flora of human skin.(2) Lipid dependence for *in vitro* growth is a common feature among all *Malassezia* species with the exception of *M. pachydermatis*. Phenotypic properties including temperature tolerance, enzyme reactions, and ability to metabolize lipid supplements vary across *Malassezia* species. While phenotypic differences can be useful in a laboratory setting to confirm the diagnosis and further speciate the fungus, the fastidious nature of these organisms makes differentiating different species using culture difficult.(1, 2) *M. restricta* and other *Malassezia* spp. can form pseudohyphae both in culture and in clinical specimens, which may aid morphologic diagnosis (2, 11–13). More recently, molecular techniques that detect DNA and RNA have aided with species identification.(14–16) Given the difficulty with culturing fungal pathogens, particularly *Malassezia* species, molecular techniques should be considered in the workup of immunocompromised patients with a history concerning for fungal infection in order to provide the most appropriate treatment.

Treatment of cutaneous *Malassezia* infections is generally with topical therapy; infections are difficult to eradicate because *Malassezia* frequently colonizes the skin.(1) Systemic and serious infection is treated with systemic antifungals. The requirement of *Malassezia* species for *in vitro* lipid supplementation makes susceptibility testing technically challenging and

has produced varied results.(17) Advances in technique have led to improved results and some guidelines for antifungal therapy. Minimum inhibitory concentrations (MIC) of itraconazole, fluconazole, voriconazole, ketoconazole, posaconazole, and terbinafine against *M. restricta* are all low and within an achievable range with usual dosing.(1, 17–19)

Given that the management of fungal abscesses differs by causative organism and that culture of fungal pathogens is often challenging, molecular methods for the diagnosis of causative agents may be an important tool for the treatment and diagnosis of infections in immunocompromised patients.

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A. H&E stain of splenic biopsy demonstrating poorly formed granuloma (40X).

B. Gomori methenamine silver stain of organisms showing pseudohyphae and a few yeast forms (40X).

C. Gomori methenamine silver stain of organisms showing pseudohyphae (60X).

Figure 1. Histology images of biopsy specimens

A. H&E stain of splenic biopsy demonstrating poorly formed granuloma (40X).

B. Gomori methenamine silver stain of organisms showing pseudohyphae and a few yeast forms (40X).

C. Gomori methenamine silver stain of organisms showing pseudohyphae (60X).