Medical Mycology Case Reports 13 (2016) 9–11

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Evidence of delayed dissemination or re-infection with *Blastomyces* in two immunocompetent hosts

Jennifer L. Anderson[a], Jennifer K. Meece[a,*], Matthew C. Hall[b], Holly M. Frost[a,c]

[a] *Marshfield Clinic Research Foundation, Marshfield Clinic, Marshfield, WI, USA*
[b] *Department of Infectious Diseases, Marshfield Clinic, Marshfield, WI, USA*
[c] *Department of Pediatrics, Marshfield Clinic, Minocqua, WI, USA*

ARTICLE INFO

Keywords:
Blastomycosis
Blastomyces
Re-infection
Relapse

ABSTRACT

Relapse or recurrence of blastomycosis in patients is rare. Re-infection of a patient with blastomycosis has not been previously reported. In this report, we describe relapse or reinfection with *Blastomyces* in 2 immunocompetent patients. This is the first study in which genetic typing was performed on paired *Blastomyces* isolates from the same patient obtained months apart.

1. Introduction

Blastomycosis typically presents as a non-specific, febrile illness and is frequently treated as a bacterial pneumonia before *Blastomyces* is identified either by fungal culture, KOH staining, or urine antigen testing. Infections are primarily pulmonary, but can disseminate to skin, bone, central nervous system, and other organ systems in 20–50% of cases [1,2].

Relapse or recurrence of blastomycosis in patients is rare, and varies by the therapeutic agent, length of treatment, and immune capacity of the patient [3,4]. Successful blastomycosis treatment in patients, without death or relapse, is achieved in 80–95% of cases [3,5]. Re-infection of a patient with blastomycosis has not been previously reported. Little is understood regarding acquired immunity to blastomycosis, but it seems likely that exposure may provide future protection against the disease [6,7].

Recent advances in genotyping of *Blastomyces* by microsatellite typing and ITS2 sequencing have demonstrated that there are two unique clades, or species, of *Blastomyces* with *B. dermatitidis* infection being more prevalent in patients with comorbidities and more likely to cause disseminated infection and *B. gilchristii* more likely to cause isolated pulmonary disease [1,8,9]. The mechanism by which organism genetics play a role in ability to disseminate remains unclear.

2. Cases

2.1. Patient 1

A 54 year old male with history of coronary artery disease, prior coronary artery bypass grafting, and cirrhosis, presented to his primary care physician for routine cardiac follow up (day 0). He was noted to be febrile to 101.5 °F (38.6 °C) and have hyperglycemia. He was admitted for further management of new onset type 2 diabetes mellitus. One month prior the patient had been evaluated in the emergency department for cough, facial pressure, and headache. He was diagnosed with a viral respiratory infection that resolved 2 weeks prior to admission.

On admission the patient was persistently febrile with c-reactive protein elevated to 8.1. Chest x-ray (CXR) obtained on day 1 showed a right middle lobe soft tissue density and fluid in the minor fissure. Subsequent computed tomography (CT) chest scan (day 2) showed a right infrahilar mass in the middle lobe. Sputum smear stained with potassium hydroxide (KOH) showed broad budding yeast consistent with *Blastomyces* and sputum culture grew *Blastomyces* when cultured on brain-heart infusion (BHI) agar with blood at 25 °C. CT scan of the sinuses was normal. He was started on itraconazole 200 mg twice daily on day 3 and had resolution of fevers on day 7. He was discharged on day 10. Itraconazole dose was reduced to 100 mg twice daily at discharge after trough level was found to be supra-therapeutic.

The patient remained asymptomatic and was followed up at 2, 3, and 4 weeks after discharge with gradual improvement in CXR and therapeutic itraconazole levels. He was reevaluated at 5.5 months post diagnosis. CXR showed a small right pleural effusion, but was otherwise negative. Itraconazole was discontinued. Monitoring CXR completed at 8 months post diagnosis was normal.

The patient remained in his usual state of health until 18 months post initial diagnosis when he presented to his primary care physician for mental status changes. Per the family, the patient had become apathetic and disoriented. He had been otherwise asymptomatic. CT

* Corresponding author.
E-mail address: frost.holly@marshfieldclinic.org (H.M. Frost).

http://dx.doi.org/10.1016/j.mmcr.2016.09.002
Received 17 June 2016; Received in revised form 18 August 2016; Accepted 13 September 2016
Available online 14 September 2016

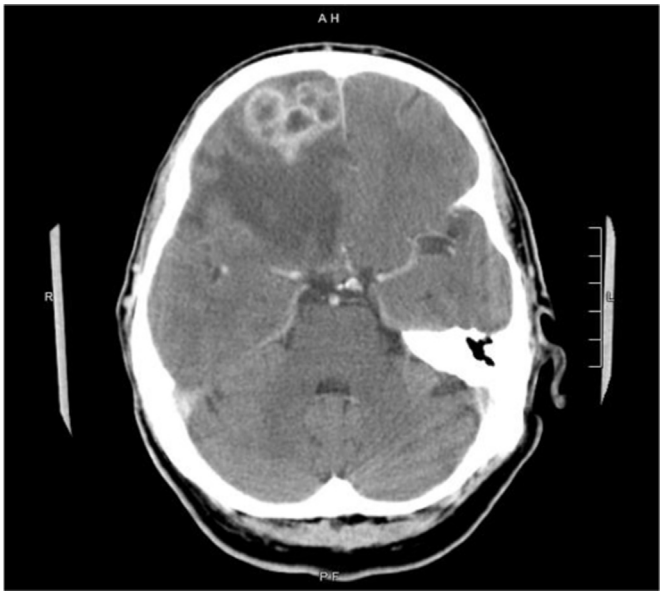

Fig. 1. Head CT of Case Patient1 obtained 18 months after first being diagnosed and treated for blastomycosis.

head revealed a 3.5 cm enhancing mass with multiple ring enhancement and nodular like appearance in the right inferior frontal lobe (Fig. 1). The patient underwent craniotomy and resection one week later. Methenamine silver stain of the biopsy revealed fungal microorganisms morphologically consistent with *Blastomyces*. Subsequent culture of the biopsy on BHI agar with blood at 25 °C confirmed *Blastomyces* infection.

He was started on liposomal amphotericin B, 425 mg daily, which was transitioned to every other day after development of azotemia. Follow up magnetic resonance imaging (MRI) obtained 4 weeks post craniotomy showed 80% improvement in edema and resolution of mass effect. After completion of 6 weeks of amphotericin B therapy he was changed to fluconazole, 400 mg daily. MRI obtained 6, 9, and 12 months post craniotomy showed mild edema with stable postoperative changes. Fluconazole was decreased to 200 mg daily 9 months post craniotomy and discontinued 12 months post craniotomy. MRI obtained 22 months post craniotomy showed complete resolution of edema with stable postoperative changes. The patient remained free of blastomycosis for the subsequent 13 years.

Fungal cultures of sputum, from March of 2000, and brain biopsy, from September of 2001, were previously bio-banked and available for pathogen genetic analysis. Deoxyribonucleic acid (DNA) was extracted from mold-form clinical specimens, genotyped with 27 polymorphic microsatellite marks as previously described [10], and species-typed by sequencing a portion of the ITS2 of rDNA [9]. Both isolates, obtained 18 months apart, were found to be *Blastomyces dermatitidis*, microsatellite Group 2 organisms [10] and were genetically identical at all 27 microsatellite loci.

2.2. Patient 2

A 48-year-old male smoker, with history of coronary artery disease with stenting, and two year history of chronic right upper lobe infiltrates and bronchiectasis, was hospitalized for chest pain (day 0). A stress test showed no evidence of coronary ischemia. CXR and subsequent CT scan showed a new soft tissue mass in the mediastinum and right hilar region. He reported no fever, chills, or night sweats, though he had mild cough attributed to smoking. He underwent resection of lymph nodes and pulmonary nodules from the right hilum and right upper lobe. KOH stain of biopsy revealed broad budding yeast consistent with *Blastomyces* and fungal cultures on BHI agar with blood at 25 °C confirmed the diagnosis. The patient was discharged (day 2) on itraconazole 200 mg by mouth, twice per day.

The patient returned for follow up 5 weeks later where mild cough and intercostal neuralgia were noted. He was started on carbamazepine and continued on itraconazole. At 2 month follow-up, the patient's itraconazole level was < 0.05 μg/ml and treatment was changed to 10 ml of itraconazole solution, twice daily (10 mg/ml). No subsequent itraconazole levels were obtained. Four months post diagnosis, the patient complained of weight loss, loss of appetite, and headaches. CT scan of the head was obtained to rule out dissemination of *Blastomyces* to the central nervous system, and was negative. He continued with itraconazole treatment until 8 months of therapy was completed.

Two months after completion of itraconazole the patient presented with chest pain. CT scan revealed pulmonary infiltrates and subsequent bronchoscopy with bronchoalveolar lavage (BAL) showed no evidence of *Blastomyces*. The patient was treated for pneumonia with resolution of symptoms.

Thirty-three months after initial blastomycosis diagnosis the patient underwent endoscopic sinus surgery for chronic sinusitis with sinus washout smear and cultures negative for *Blastomyces*. CXR obtained preoperative demonstrated an incidental small left lower lobe nodule and follow up CXR with CT scan (37 months post initial diagnosis, unavailable for publication) demonstrated multiple left lower lobe nodules in an area measuring 3×4×9 cm. The patient remained asymptomatic. He underwent bronchoscopy with BAL the following month. Sputum and BAL samples were negative by KOH stain, but positive for *Blastomyces* by culture on BHI agar with blood at 25 °C. He was started on itraconazole 200 mg once daily. At the patient's 1-month follow-up post diagnosis, chest x-ray was improved. He completed 6 months of itraconazole therapy as has been free of blastomycosis for the last 10 years.

Fungal cultures of lung tissue, from 2002, and sputum, from 2005, were previously bio-banked and available for pathogen genetic analysis. DNA was extracted from mold-form clinical specimens, genotyped with 27 polymorphic microsatellite marks as previously described [10], and species-typed by sequencing a portion of the ITS2 of rDNA [9]. Both isolates, obtained 38 months apart, were found to be *B. dermatitidis*, microsatellite Group 2 organisms [10], but were genetically different at 12 of 27 microsatellite loci.

3. Discussion

This report describes prolonged or recurrent blastomycosis in two immunocompetent patients and is the first in which genetic typing was performed on paired *Blastomyces* isolates from the same patient obtained months apart.

The first case demonstrates a long time period between initial pulmonary infection and dissemination to the central nervous system with complete resolution of symptoms prior to dissemination. Since we have genetically identical isolates from this patient collected 18 months apart from the sputum and brain, we feel confident that this is a case of relapse. This is particularly concerning given that the patient was immunocompetent and received appropriate treatment with itraconazole, therapeutic monitoring, and follow up imaging according to current guidelines [11]. Though delays in diagnosis have been associated with increased risk for disseminated disease, prolonged time from initial infection to dissemination in the setting of treatment has not been previously described. The ability of this strain of *B. dermatitidis* to evade the human immune system and disseminate makes it an interesting candidate for whole genome sequencing.

In the second case, the patient has resolution of most symptoms and clear imaging studies between diagnoses, however, it could be argued that the presence of chronic mild cough and sinus congestion in the interim may be indicative of chronic *Blastomyces* infection, though cultures were negative from both sites between diagnoses. *Blastomyces* is a fastidious organism and, though culture is the gold standard for

diagnosis, lack of growth with culturing does not guarantee that infection is not present. This case is also complicated by the sub-therapeutic dosing of itraconazole, concurrent treatment with carbamazepine that can interfere with itraconazole absorption, and lack of appropriate follow-up and monitoring, which places him at increased risk for incompletely treated infection.

The second patient had pulmonary blastomycosis diagnoses 38 months apart, which are far longer than what has been previously reported. Though genotyping demonstrated that both isolates were the same species, they differed at 12 of 27 loci and were likely different strains. This is significant as it indicates that re-infection with *Blastomyces* must be considered, even in immunocompetent hosts. No social information regarding the second case patient's hobbies or interests are available to determine possible exposures or risk factors, such as hiking, hunting, or fishing. The patient does reside in a highly endemic area for blastomycosis, associated with high rates of infection. Alternatively, the patient may have been infected with both strains of the organisms simultaneously and host selection may have allowed for development of infection in contralateral lungs at different time points, though this seems less likely, even in the setting of sub-therapeutic treatment. It is also possible during culture; one of the two strains was selected for even if both were present in the clinical specimen.

Re-infection has not been previously reported with *Blastomyces*. Prior murine studies have demonstrated that *Blastomyces* infection likely infers T-cell, rather than humoral, immunity. The organism has several mechanisms to down regulate T-cell response in the host and the effectiveness of these mechanisms likely vary by organism genotypes [7,12–14]. This is an important consideration as most patients with blastomycosis have continued environmental exposures after infection. This is also important for future work looking at host immune response to infection and development of possible vaccine targets.

Both cases show that physicians should maintain a high degree of clinical suspicion for *Blastomyces* infection and dissemination post treatment, even if patients are immunocompetent, treated appropriately, symptoms have abated, and imaging has normalized.

Conflict of interest

There are no conflicts of interest.

Acknowledgements

This work was supported by the Marshfield Clinic Research Foundation.

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