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Outcomes of persons with blastomycosis involving the central nervous system

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ABSTRACT

Blastomyces dermatitidis is a dimorphic fungus which is potentially life-threatening if central nervous system (CNS) dissemination occurs. Sixteen patients with proven or probable CNS blastomycosis are presented. Median duration of symptoms was 90 days; headache and focal neurologic deficit were the most common presenting symptoms. Magnetic resonance imaging (MRI) consistently demonstrated an abnormality, compared to 58% of computed tomography scans. Tissue culture yielded the pathogen in 71% of histology-confirmed cases. All patients who completed treatment of an amphotericin B formulation and extended azole-based therapy did not relapse. Initial nonspecific symptoms lead to delayed diagnosis of CNS blastomycosis. A high index of suspicion is necessary if there is history of contact with an area where *B. dermatitidis* is endemic. Diagnostic tests should include MRI followed by biopsy for tissue culture and pathology. Optimal treatment utilizes a lipid-based amphotericin B preparation with an extended course of voriconazole.

1. Introduction

The thermally dimorphic fungus *Blastomyces dermatitidis*, which exists in the soil of moist wooded areas (Bakerspigel et al., 1986; Denton et al., 1961), can cause blastomycosis, a local or systemic infection in humans and animals. Primary infection almost always occurs via inhalation of aerosolized conidia released from disrupted soil (Saccente & Woods, 2010) but may rarely result from direct soft tissue inoculation through a break in the skin. *B. dermatitidis* is endemic to parts of the United States and Canada, including the Canadian province of Manitoba and the Kenora region of Ontario where the incidence rates are 0.62 and 7.1 cases per 100,000, respectively (Crampton et al., 2002). Both regions fall within the catchment area of 2 tertiary care hospitals in Winnipeg, Manitoba.

Pulmonary blastomycosis is the most common form of the infection. Subclinical pulmonary blastomycosis has been reported in 30% of at-risk forestry workers in areas where *B. dermatitidis* is endemic (Vaaler et al., 1990). If the host response is inadequate to control the initial infection, *B. dermatitidis* can disseminate via the lymphohematogenous route, the most frequent sites being skin, bone, and genitourinary tract (Saccente & Woods, 2010). Among persons diagnosed with extra-pulmonary blastomycosis, involvement of the central nervous system (CNS) is estimated in 5–10% (Gonyea, 1978; Saccente & Woods, 2010), and in up to 33% of cases in an older autopsy series (Fetter et al., 1967).

The diagnosis of pulmonary and extra-pulmonary blastomycosis can be made rapidly on histopathologic examination, but microbiologic cultures are considered the gold standard (Patel et al., 2010). Blastomycosis of the CNS may present with leptomeningitis, encephalitis, or as solitary or multiple brain or spinal cord abscesses (Bariola et al., 2010; Wylen & Nanda, 1999). *B. dermatitidis* on routine hematoxylin and eosin stain (H&E) is a double-walled, broad-based budding yeast in a granulomatous host-reaction with or without micro-abscess formation and central necrosis. Silver impregnation such as Grocott's methenamine silver (GMS) can increase screening sensitivity while periodic acid-Schiff (PAS), in situ hybridization, and other histochemical stains can help differentiate *B. dermatitidis* from other fungi (Mukhopadhyay & Gal, 2010). Electron microscopy reveals nuclear material in viable yeast and shows cytoplasmic retraction in degenerate forms (Guccion et al., 1996; Jay et al., 1991).

To date, fewer than 125 cases of CNS blastomycosis have been reported, the majority representing single case reports or small series or published before the modern era of extended-spectrum azole therapy and MRI CNS imaging. Cases reported prior to 1965 have been previously summarized (Fetter et al., 1967). More recently described are 27 CNS biopsy specimens, 3 ventricular cytology specimens, 3 CNS autopsy specimens, 8 cerebrospinal fluid (CSF) cultures, and 19

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probable CNS blastomycosis cases based on proven *B. dermatitidis* in an extra-CNS site (Bakleh et al., 2005; Borgia et al., 2006; Chander et al., 2007; Chowfin et al., 2000; Cook, 2001; Cooper et al., 1988; Friedman et al., 2000; Gershan et al., 1994; Gonyea, 1978; Harley et al., 1994; Kravitz et al., 1981; Mangham et al., 1983; Mohazab et al., 1997; Panicker et al., 2006; Raftopoulos et al., 1986; Szeder et al., 2007; Taillan et al., 1992; Ward et al., 1995; Wu et al., 2005; Wylen & Nanda, 1999). A case series of 22 patients presenting to 6 different tertiary care centers in the United States has also recently been reported (Bariola et al., 2010). Two separate guidelines published within the past 5 years have addressed the management of CNS blastomycosis (Chapman et al., 2008; Limper et al., 2011). The body of literature supporting current recommendations, however, remains limited.

The aim of this study is to correlate clinical manifestations, diagnostic techniques (imaging, microbiology, histopathology), and therapy of CNS blastomycosis. We present the largest series of patients with proven or probable CNS blastomycosis evaluated by a single infectious disease referral service.

2. Methods

2.1. Data collection

The study was approved by the Research Ethics Board, Faculty of Medicine, University of Manitoba. We identified and reviewed medical records of patients diagnosed with blastomycosis, with clinical or radiographic evidence of CNS involvement who received care at the Health Sciences Centre or Saint Boniface General Hospital, Winnipeg, Manitoba, from January 1988 through November 2011. These 2 facilities are Manitoba's tertiary care hospitals. Cases of CNS blastomycosis were found by searching the medical records database of the 2 hospitals using the *ICD-9* code of 116.0 and *ICD-10* code of B40.9. The pathology and microbiology department databases were also searched. A standardized medical record review included demographics, past medical history, history of presenting illness, laboratory and pathology reports, imaging reports, treatment, and clinical outcomes.

We defined CNS blastomycosis as "proven" or "probable" based on previously described criteria (De Pauw et al., 2008) as follows. A "proven" diagnosis requires: a) compatible clinical or radiographic findings, and b) culture or histopathologic demonstration of *B. dermatitidis* obtained from cerebrospinal fluid (CSF) or CNS tissue. "Probable" CNS blastomycosis requires compatible clinical and radiographic findings in conjunction with proven non-CNS blastomycosis (culture or histopathology).

2.2. Microbiology analysis

Fungal CSF cultures were incubated for four weeks at 30 °C using Sabhi agar. Fungal tissue cultures were plated to Sabhi, inhibitory mold agar, and brain heart infusion with sheep blood agar and incubated at 30 °C for 4 weeks.

2.3. Histopathology analysis

Pathology specimens were reviewed by two experienced neuropathologists (MDB, SK). The features evaluated were site of specimen, density of fungal forms, intra- or extracellular location of fungal forms, wall thickness and type of budding, yeast diameter, and inflammatory response. Histochemical staining with GMS, periodic acid–Schiff (PAS), PAS-Alcian blue (PAS-Ab), and Congo red was used in addition to standard H&E. Inflammation was characterized with immunohistochemistry markers including CDs 3, 4, 8, 45, 68, and HLA-DR. Tissue was fixed in 2.5% glutaraldehyde followed by 1% osmium tetroxide for electron microscopy studies.

3. Results

3.1. Clinical

Sixteen patients with probable or proven CNS blastomycosis were evaluated (Table 1), with the majority of cases diagnosed after the year 2000, and one case each diagnosed in 1988, 1994, and 1997. Nine cases (56%) were proven by culture and/or direct histopathologic

Table 1
Summary of clinical manifestations.

Patient #	Age (years), sex	Year of diagnosis	Comorbidities	Presenting symptoms	Symptom duration (days)	Localization of CNS disease	CNS diagnosis	Other organ involvement	Other relevant diagnostics
1	13, F	2001	None	Back pain, FND	30	T12-L1	Proven	Pulmonary	Sputum +
2	37, M	2006	None	H/A, blurred vision, nausea, vomiting	60	Meninges	Proven	Pulmonary	BAL -
3	68, M	2005	DM	H/A, FND	90	Cerebellar parenchyma	Proven	Pulmonary	BAL +
4	37, M	1988	None	H/A, FND	105	Cerebellum	Probable	Bone and joint	Skin nodule Bx pathology +
5	37, M	2005	None	H/A, fever and chills	115	Epidural extension from skull	Proven	Pulmonary, skin, bone	Scalp Bx culture +
6	16, M	2004	None	H/A, FND, seizure	90	Cerebrum	Proven	Pulmonary	None
7	3, M	2001	None	Scalp lesion	60	Epidural extension from skull	Probable	Bone, skin	Scalp Bx pathology +
8	9, M	2006	None	H/A, fever and chills	150	Epidural space and meninges	Probable	Bone, skin	Scalp Bx pathology and culture +
9	59, M	1997	None	FND	28	Meninges	Probable	Pulmonary	None
10	57, M	1994	MPD	Altered mental status	180	Cerebrum and cerebellum	Probable	Pulmonary	BAL +
11	12, M	2005	None	H/A, fever and chills	21	Epidural space and cerebrum	Proven	Scalp	Scalp aspirate culture +
12	63, M	2002	ESRD	Seizure, FND	90	Cerebrum	Proven	Pulmonary, skin, bone	None
13	52, M	2002	DM	H/A, FND, partial seizure	60	Meninges and cerebellum	Proven	None	None
14	12, M	2005	None	H/A, Fever, hypopituitarism, FND	168	Meninges and pituitary stalk	Proven	None	Serum antibody +
15	3, M	2006	None	FND, fever	210	Cerebrum	Probable	None	Serum antibody +
16	63, M	2010	None	H/A, FND	900	Meninges and cerebrum	Probable	Pulmonary	None

Patients are numbered and correspond across Tables 1–5.
BAL = Bronchoalveolar lavage, Bx = Biopsy, DM = Diabetes mellitus, ESRD = End-stage renal disease, FND = Focal neurologic deficit, H/A = Headache, MPD = Myeloproliferative disorder (Polycythemia rubra vera).

Table 2
Summary of treatment modalities.

Patient #	Amphoteracin B formulation, duration (days)	Azole type, duration (days)	Peri-operative steroid use
1	AmBd, 7 → LAMB, 120	None given	Dexamethasone
2	AmBd, 11 → ABLC, 90	Voriconazole, 320	Dexamethasone
3	LAMB, 23	Itraconazole, 20	None Given
4	AmBd, 4	Ketoconazole, 365	None Given
5	AmBd, 40	Voriconazole, 365	Dexamethasone
6	AmBd 7 → LAMB, 60	None given	None given
7	AmBd, 63	Itraconazole, 120	None given
8	AmBd, 30	Voriconazole, 180	None given
9	AmBd, 105	Fluconazole, 365	None given
10	AmBd, 100	None given	Dexamethasone
11	AmBd, 53	Itraconazole, 365	None given
12	AmBd, 100	None given	None given
13	AmBd, 42	Itraconazole → Fluconazole, 365	None given
14	AmBd, 32 → ABLC, 19	Voriconazole, 260	Prednisone
15	AmBd, 30	Fluconazole, 260	None given
16	AmBd, 21	Voriconazole	None given

Patient #3 died during treatment. Patient #9 relapsed on ketoconazole, was retreated with Amphotericin B, and then switched to fluconazole. Patient #16 is receiving treatment at the time of manuscript submission.
AmBd = Amphotericin B deoxycholate, LAMB = Liposomal Amphotericin B, ABLC = Amphotericin B Lipid Complex.

evidence on biopsies from within the CNS or a contiguous compartment. Seven cases (44%) were considered probable CNS blastomycosis. The clinical features at time of diagnosis are shown in Table 1. The median age at diagnosis was 37 years (range: 3–63), with seven patients (44%) under 18 years of age. Thirteen patients (81%) had no identifiable underlying medical risk factor for infection with *B. dermatitidis*; 2 persons had diabetes mellitus and 1 had end-stage renal disease. Three patients (19%) had no prior or concurrent (defined as asymptomatic extra-CNS disease revealed by investigations for CNS symptoms) blastomycosis.

The most common symptoms at presentation were headache and focal neurologic deficit in 10 (63%) and 9 (56%) patients respectively. Fevers or chills were present in a minority of cases (5 of 16 cases, 32%). Duration of symptoms at time of diagnosis was variable, with a median of 90 days (range: 21–900).

Head or spinal cord computed tomography (CT) imaging was performed on 13 patients, 7 (54%) of which had magnetic resonance imaging (MRI) prior to any intervention or treatment (Table 3). Three patients had MRI without a CT scan. All 10 MRI investigations demonstrated enhancing lesions or had signal changes. Seven of the 12 (58%) CT scans with contrast demonstrated enhancing lesions; 1 CT scan was performed without contrast. Three patients (43%) had abnormal findings on MRI without enhancing lesions on CT scan.

3.2. Microbiology

The results of CSF analysis were available in eight patients (Table 4). All specimens were lymphocyte-predominant and five of eight demonstrated pleocytosis (range: 20–304 cells/μL). CSF protein levels were elevated in four of these 8 patients (50%). Fungal CSF cultures were submitted in 3 patients prior to treatment, none of whom demonstrated *B. dermatitidis*. The results of tissue culture were available in seven patients who had proven CNS blastomycosis by histology (Table 5). Microorganisms were recovered from 5 (71%) CNS samples, all of which had histologic evidence of the fungus.

3.3. Histopathology

Microscopic findings are presented in Table 5. Eight patients had CNS parenchyma sampled; one was part of a complete autopsy (Patient #3). One patient had tissue biopsied from the spinal cord and three patients had the cranial leptomeninges biopsied.

Double-walled yeast consistent with *B. dermatitidis* was seen by histology in 9 of 12 CNS specimens, while broad-based budding was seen in seven specimens (Fig. 1). Electron microscopy was performed on patients #13 and #15 (Fig. 2). Patient #13 demonstrated a 4-layer cell wall that was compatible with *B. dermatitidis*. Patient #15 had features of probable, not proven, blastomycosis (because of a small diameter and thin double wall).

Table 3
Diagnostic imaging results (summarized) of the central nervous system.

Patient #	CT findings	MRI findings
1	–	Gadolinium enhancing intradural, intramedullary lesion at vertebral level T12 and L1
2	Ventriculomegaly	Abnormal enhancement of leptomeninges in basal cisterns and cerebral convexities
3	Inhomogeneous, enhancing cerebellar lesion with mass effect on fourth ventricle; mastoiditis	Right irregular enhancing cerebellar lesion
4	No lesion in skull/orbits	–
5	Soft tissue swelling and osteomyelitis of the calvarium with communication to the subgalea and epidural area	–
6	Ring enhancing lesion in corona radiata; midline shift; effacement of left lateral ventricle	–
7	Multiple skull lytic lesions	Scalp lesion with underlying bony defects and extradural extension
8	–	Enhancing mass in temporal bone with extra and intracranial components; dural and epidural enhancement
9	No spinal cord lesion; multiple old lacunar infarcts	Diffuse meningeal enhancement along spinal cord; multiple lacunar infarcts
10	Multiple small peripherally enhancing centrally lucent lesions throughout cerebellum and cerebral hemispheres	–
11	Heterogonous enhancing abscess over right coronal suture extending into the epidural space	MRI not performed (post-op MRI showed no residual disease)
12	Ring enhancing lesions throughout hemispheres with cerebral edema	–
13	Enhancing lesion in cerebellum; leptomeningeal enhancement around brainstem	Widespread leptomeningeal enhancement in posterior fossa; small focus of enhancement in cerebellum.
14	1.4×1.2 cm enhancing pituitary mass with thick ring enhancement in suprasellar region	Enhancement of pituitary stalk, meninges. Prolonged signal changes in left and right hypothalamus
15	(Uninfused) – multifocal hemorrhage at junction of white & grey matter felt to represent vasculitis	Prolonged widespread hemispheric white matter signal changes with; vasogenic edema
16	–	Enhancement of the dura in the area of the right tentorium

Table 4
Summary of cerebrospinal fluid analysis for those patients who had lumbar punctures.

Patient #	Cerebrospinal fluid WBC count (cells/μL)	Cerebrospinal fluid protein level (g/L)	Cerebrospinal fluid glucose (mmol/L)
1	3 (87% lymphocytes, 13% monocytes)	0.37	3.1
2	304 (45% lymphocytes, 38% neutrophils, 17% monocytes)	2.13	2.6
4	20 (95% lymphocytes, 5% neutrophils)	0.25	3.3
9	100 (all lymphocytes)	High (value not provided)	Low (value not provided)
13	169 (55% lymphocytes, 29% neutrophils)	2.05	1.1
14	129 (77% lymphocytes, 14% monocytes, 9% neutrophils)	0.9	1.8
15	3 (86% lymphocytes, 11% monocytes, 3% neutrophils)	0.24	3.6
16	3 (83% lymphocytes, 15% monocytes, 2% neutrophils)	Not done	Not done

Histologic features of inflammation varied somewhat. Of the 9 CNS specimens, all had lymphocytic infiltrate, 8 (89%) had granulomas, 6 (67%) had neutrophils, and 4 (44%) had necrosis (Fig. 1). Two of the 9 (22%) CNS specimens had granulomatous inflammation combined with necrosis and neutrophils. All but 3 (67%) specimens demonstrated yeast in both the extracellular matrix and within macrophages.

3.4. Treatment

Initial treatment data was available for all sixteen patients (Table 2). All received antifungal treatment as follows. Fifteen patients were initially treated with amphotericin B deoxycholate while one patient received liposomal amphotericin B. Of these 15 patients, 6 (40%) developed acute kidney injury (AKI), defined as a twofold increase in serum creatinine above baseline. Five of these 6 patients were switched to lipid-based formulations of amphotericin B. The other patient diagnosed with AKI remained on the deoxycholate formulation and was closely monitored with no progression of renal disease. Renal function stabilized or improved in all patients and none required renal replacement therapy. Treatment with amphotericin B continued for a median duration of 41 days (range: 4–105 days). This was followed by an oral azole-based agent in 12 (75%) patients, most commonly voriconazole, for a median of 342 days (range: 120–365 days; one patient remains on therapy at the time of writing). One of these 12 patients (#12) had end-stage renal disease and was receiving intermittent hemodialysis prior to the diagnosis and treatment of blastomycosis. This patient developed leukopenia while on treatment with the deoxycholate formulation, which was stopped after 60% of the planned course. The remaining four patients did not receive azole-based therapy for the following reasons: i) 1 died before finishing amphotericin B treatment, ii) 2 had resolution of symptoms after completing amphotericin B treatment, and iii) 1 had resolution of symptoms after completing amphotericin B treatment and was lost to follow up after transfer to the referring community hospital.

Three patients died during treatment course. One died of a sudden cardiac arrest while receiving amphotericin B treatment. At autopsy, *B. dermatitidis* was identified in the respiratory and central nervous systems, but the cause of death was not determined. This was not considered failed therapy as his CNS symptoms had resolved and he had not completed his full therapy course. Another patient died of progressive myelodysplastic syndrome. A third patient, who presented with altered level of consciousness and became quadriplegic post-biopsy due to brainstem edema, died of an unknown cause (no autopsy performed) while on voriconazole after being transferred in stable condition to a peripheral hospital.

All surviving patients had clinical resolution of neurological symptoms while on amphotericin B and none was re-admitted with a diagnosis of CNS blastomycosis after completing the combination therapy.

4. Discussion

The clinical manifestations of our patients parallel previously described observations in persons with CNS blastomycosis (Bariola et al., 2010; Ward et al., 1995). The most common presentations include meningitis and mass effects (intracranial, epidural, or spinal cord). A significant proportion (7 of 16, 44%) of patients described in this study were under the age of 18. This raises the question whether the risk of developing blastomycosis and subsequent dissemination to the CNS is

Table 5
Tissue pathology and microbiology results.

Patient #	Site of biopsy	Histological features	Fungal density	Tissue culture
1	Intramedullary tumor of T12-L1	Necrosis, granuloma, MNGC, neutrophils	12	+
2	Dura, leptomeninges, cerebrum	Granuloma, MNGC, neutrophils	6	+
3*	Dura, cerebellum	Chronic lymphoplasmacytic infiltrate only	>20	Not performed
4	Elbow soft tissue	Granuloma, MNGC	>20	Not performed
5	Cerebrum	Necrosis, granuloma, MNGC, neutrophils	>20	+
6	Cerebrum	Granuloma, MNGC, neutrophils	3	+
7	Scalp tissue	Necrosis, granuloma, MNGC, neutrophils	2	-
8	Scalp tissue	Granuloma, neutrophils	2	+
9	Meninges of lumbar spine	Focal clusters of lymphocytes	0	-
10	Bone marrow biopsy	Not applicable	0	Not performed
11	Temporalis muscle, dura	Granuloma, MNGC, neutrophils	2	+
12	Cerebrum	Coagulative necrosis	>20	Not performed
13	Dura, leptomeninges	Necrosis, granuloma, MNGC	15	Not performed
14	Dura, cerebrum, ventricular nodule	Granuloma, MNGC, neutrophils	7	-
15	Cerebrum	Granuloma, MNGC	2	-
16	Dura, leptomeninges, cerebrum	Chronic lymphoplasmacytic infiltrate only	0	-

Chronic inflammation consisting of lymphocytes was identified in all biopsy specimens (except in Patient #10) along with the specific features listed for each patient. Fungal density was determined by the area of highest organism number in one 400× magnification field (0.249 mm^2). Immunosuppresion was present in three cases (60%) where the fungal density was 15/0.249 mm^2 or higher.
* = Autopsy with respiratory and CNS blastomycosis identified, + = *B. dermatitidis* recovered in fungal culture, - = *B. dermatitidis* not recovered in fungal culture. MNGC = multinucleate giant cell, N/A = not applicable.

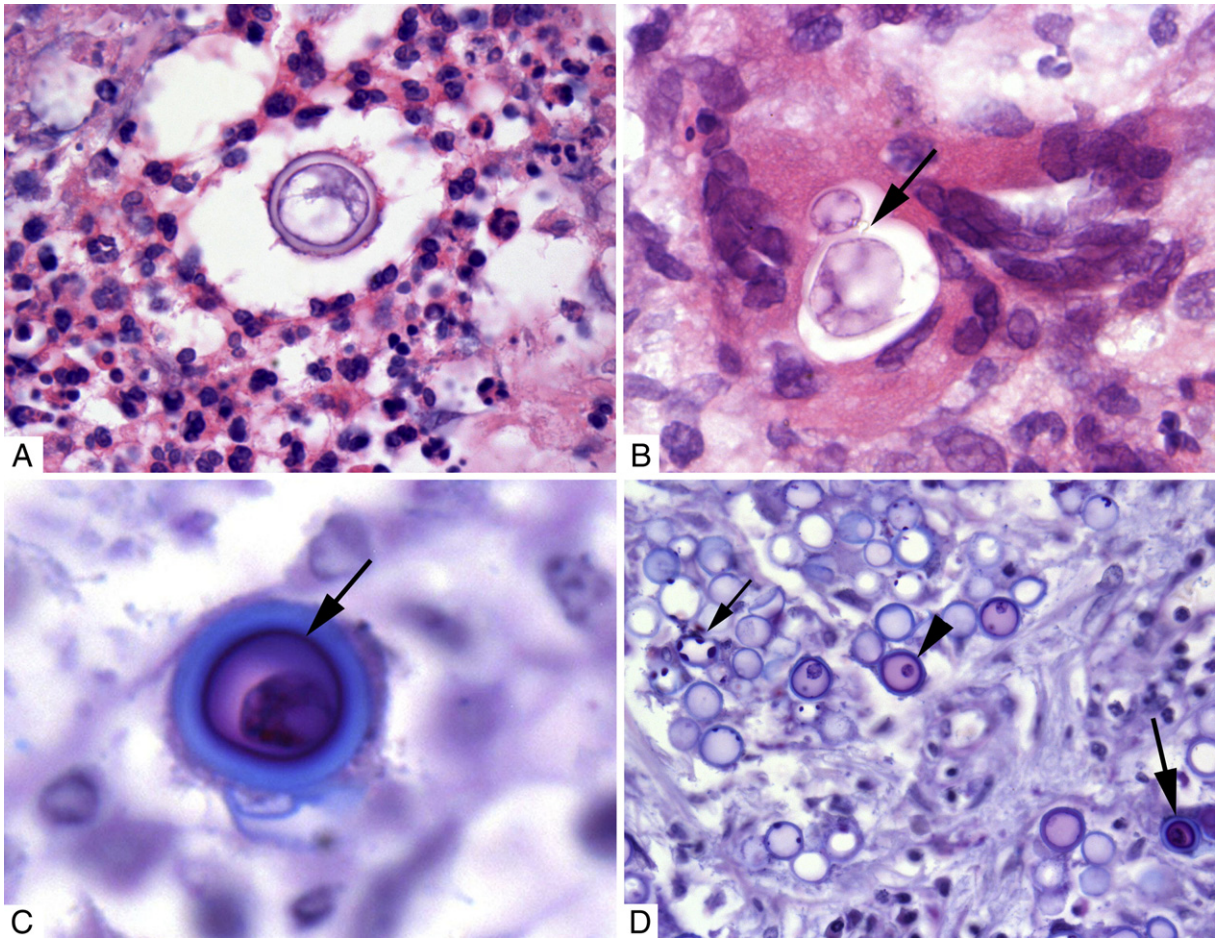

Fig. 1. *B. dermatitidis* in human CNS cortex tissue (1000× original magnification). (A) Yeast with a double wall surrounded by neutrophils and necrosis. (B) Budding yeast with a broad base (arrow) and slightly basophilic nuclear material. The yeast is present within a multinucleated giant cell. (C) Yeast with AB-stained outer wall and PAS-stained inner wall (arrow) and PAS-stained cytoplasm. (D) High density of yeast with variable stages of nuclear degeneration. Viable yeast has a large nucleus (large arrow), early degeneration is associated with a shrunken nucleus (arrowhead), and late degeneration is associated with fragmentation of the nucleus (small arrow). A and B: H&E preparation. C and D: PAS + Alcian Blue (AB) preparation.

related to age of exposure, immaturity of the immune system, or a combination of both. Another question was raised with the finding of a peak incidence of CNS blastomycosis in 2005 and 2006. This peak followed years of above-average rainfall in the Kenora, Ontario region (http://ec.gc.ca/dccha-ahccd/default.asp?lang=en&n=2E5F8A39-1). The timeline is consistent with inoculation in the moist summer months and subsequent development of symptoms after a 1–3-month incubation period (Light et al., 2008). An additional explanation for fewer diagnoses of CNS blastomycosis prior to the peak in 2005–06 may have been a lack of vigilance. Medical suspicion and public awareness were both increased following this peak.

Symptoms were present for a median duration of 90 days prior to diagnosis with a wide range (21–900 days), consistent with the literature (Ward et al., 1995). This range may be due to the vague and nonspecific nature of the initial manifestations. Of note, most patients were afebrile at presentation despite clinical evidence of blastomycosis in another organ. These findings are also consistent with the literature (Bariola et al., 2010; Friedman et al., 2000; Gonyea, 1978). It is important to keep the differential diagnosis broad when patients present with meningitis or focal neurological deficits. No single clinical finding can rule in or out the diagnosis of CNS blastomycosis. It is rather a constellation of symptoms, in conjunction with a history of exposure and compatible findings on CNS imaging studies, which suggests the diagnosis.

CNS tissue culture had a low sensitivity (71%) compared to histopathology, similar to reported sensitivity from other tissues (Patel et al., 2010). This similarity may suggest that culture is no more difficult in the CNS than the more common respiratory and skin infections. CSF analysis was consistent with aseptic or viral meningitis in four proven CNS blastomycosis cases. The combination of tissue culture and CSF results in this study suggests that direct sampling for histopathology of CNS lesions is essential in clinical-radiologic discordant cases or clinically equivocal CNS lesions where infection cannot be differentiated from a neoplastic process. Although CT imaging is typically performed first, MRI consistently demonstrated changes, as in three patients who had no enhancement on infused CT scans. The latter represents a higher CT false negative rate than previously reported (Bariola et al., 2010).

Only a minority of cases in the current study demonstrated the commonly described features of necrotizing granuloma with neutrophils (Mukhopadhyay & Gal, 2010). It is therefore crucial to consider *B. dermatitidis* when granulomatous inflammation is seen on histology of patients who have been in an area where this pathogen is endemic. Although budding was not identified in all cases, when it was seen, all buds were broad-based. The histochemical stains PAS-Ab and Congo red, in addition to GMS, highlight structural features of *B. dermatitidis*. Electron microscopy has a role in helping to define the cell wall and internal structures but is limited by poor preservation of tissue.

Immunosuppresion is a risk factor for development of CNS blastomycosis. Only 19% of the patients in the current study had a comorbid condition which was a risk factor for disseminated blastomycosis, compared to up to 55% reported in the literature

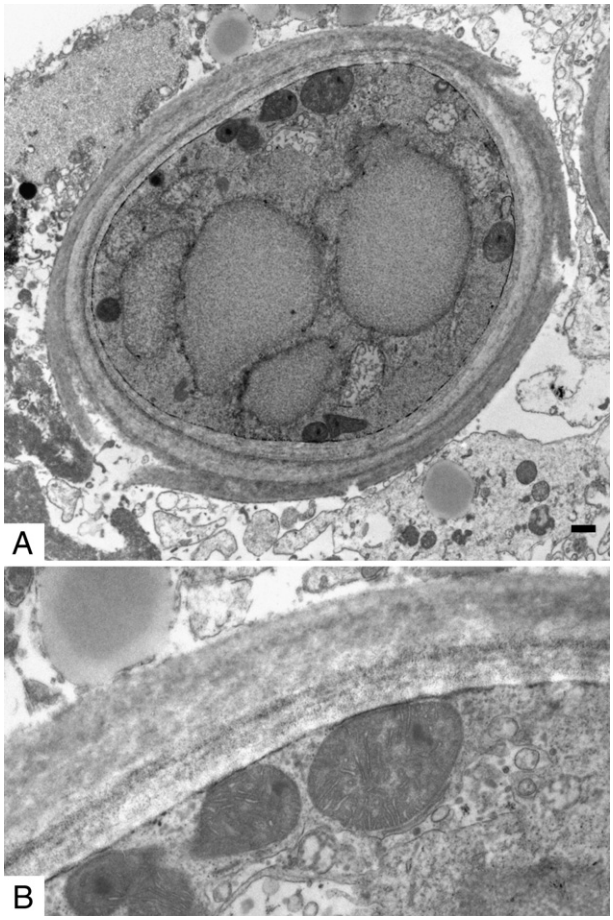

Fig. 2. *B. dermatitidis* in human CNS cortex tissue examined with electron microscopy. (A) Yeast demonstrating the cell wall and mitochondria with cristae. Cytoplasmic retraction corresponds to a "shade-effect" seen on histochemical preparations (Original magnification 10,000×). (B) High-power view of the "double wall" by light microscopy shows 4 layers, from inner to outermost: i) Loose electron-lucent zone, ii) Electron-dense zone, iii) Compact electron-lucent zone, iv) Ill-defined thickened electron-dense zone. Multiple mitochondria are present (Original magnification 30,000×).

(Bariola et al., 2010). Although most fungal forms were identified within multinucleated giant cells, some may be extracellular (Tang et al., 1984). The majority of cases in the current study had both intra- and extracellular yeast, while one patient with type II diabetes mellitus had innumerable fungal forms exclusively in the extracellular compartment. Another patient diagnosed with type II diabetes mellitus demonstrated a robust inflammatory response with no granuloma formation. The third immunosuppressed patient with known end-stage renal disease did not mount a granulomatous response. These findings suggest that a cell-mediated immune response (Harding, 1991; Taxy, 2007) may be necessary for containing *B. dermatitidis*. Immunosuppresion may pose an especially high risk for fulminant infection and may therefore influence type and duration of treatment. The role of cell-mediated immune response in the dissemination of blastomycosis to the CNS remains unknown.

The Infectious Disease Society of America (IDSA) recommends four to six weeks of a lipid formulation of amphotericin B (5 mg/kg per day) for the initial treatment of CNS blastomycosis. This recommendation is based on a concern of greater toxicity with amphotericin B deoxycholate due to the prolonged duration required, as well as experimental animal data showing higher CNS drug concentrations with liposomal amphotericin B (Groll et al., 2000). Conversely, the American Thoracic Society recommends either liposomal amphotericin B or amphotericin B deoxycholate be used as initial therapy for CNS disease and continued until clinical improvement. There is limited published data (case reports and a case series) surrounding the use of lipid formulations of amphotericin B (Bariola et al., 2010; Chowfin et al., 2000; Panicker et al., 2006).

Regardless of the formulation of amphotericin B chosen, both Societies recommend continued or sequential therapy with an azole for an extended course. The IDSA supports use of fluconazole, itraconazole, or voriconazole for at least 12 months or until resolution of CSF abnormalities. Voriconazole is the most promising azole with good intrinsic activity against *B. dermatitidis,* excellent CSF penetration, and several reports of successful treatment (Bakleh et al., 2005; Bariola et al., 2010; Borgia et al., 2006; Panicker et al., 2006). Itraconazole has the greatest intrinsic activity against *B. dermatitidis,* but low CSF levels are attained, whereas fluconazole has excellent CSF penetration, but relatively poor activity. Both itraconazole and fluconazole were used successfully in the pre-voriconazole era (Chapman et al., 2008; Limper et al., 2011).

Our case series adds to published clinical experience with either amphotericin B deoxycholate or a lipid formulation of amphotericin B as initial therapy (Chapman et al., 2008; Limper et al., 2011). Amphotericin B deoxycholate is used as first line therapy preferentially due to cost limitations. A lipid formulation of amphotericin B may limit renal toxicity and is recommended in patients with pre-existing renal disease or if significant toxicity develops during treatment. We also report on the successful use of voriconazole in five of our patients, supporting the favorable in vitro and pharmacokinetic properties of this agent in the treatment of CNS blastomycosis. Extended azole-based therapy during the continuation phase of treatment is recommended. In our study there were no CNS or non-CNS relapses seen in patients who completed both treatment modalities. Finally, although serum levels of azole medications were not routinely measured in our patients, this practice is now recommended.

Limitations of this study due to its retrospective nature were identified. The first is that not all patients were interrogated on their environmental exposures such as outdoor activities involving soil disruption. Another limitation is that the duration of follow-up was based on data in the chart. All 16 patients were followed to completion of intravenous antifungal therapy, but 6 were lost to follow-up upon discharge or transfer from the tertiary care hospitals.

5. Summary

CNS blastomycosis is a relatively uncommon consequence of *B. dermatitidis* infection and should therefore be in the differential diagnosis of meningitis and masses of the central nervous system in persons who have had contact with areas in which *B. dermatitidis* is endemic. Securing a biopsy is critical. Our findings show that histopathology, in conjunction with MRI, plays an important role in the diagnosis as CSF and tissue culture have a lower sensitivity. Effective treatment of CNS blastomycosis consists of initial amphotericin B followed by an oral azole antifungal including voriconazole.

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