Blastomycosis of Bone
A Clinicopathologic Study

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ABSTRACT

Objectives: Blastomycosis osteomyelitis is a well-known but infrequently encountered complication of infection with the dimorphic mold, Blastomyces dermatitidis. Oftentimes, the diagnosis is unsuspected, resulting in a delay in making the diagnosis. The role of intraoperative consultation in making a rapid diagnosis has not been discussed previously.

Methods: Retrospective chart review of clinicopathologic information was conducted from all cases of blastomycosis osteomyelitis and arthritis diagnosed at Rush University Medical Center between 2000 and 2010.

Results: Fourteen cases of blastomycosis osteomyelitis and/or arthritis were identified, 12 of which clinically and radiologically presented as a bone tumor. The disease most commonly affected the lower extremities, particularly around the knee joint. Septic arthritis generally occurred secondary to osteomyelitis of the adjacent bone. Frozen section was performed in 10 cases, all of which were correctly diagnosed as granulomatous osteomyelitis. Two cases were culture negative, one of which showed many budding yeast forms typical of B dermatitidis on histology.

Conclusion: Blastomycosis osteomyelitis should be considered in the differential diagnosis of bone tumor, particularly when there is history of residence or travel in endemic areas. This disease can be correctly identified at frozen section, thus offering rapid diagnosis. There is an excellent correlation between morphologic and microbiologic studies.

Blastomyces dermatitidis is a dimorphic fungus that is endemic in the Mississippi and Ohio River valleys and around the Great Lakes in the United States and northwestern Ontario in Canada.1-4 The most common route of entry of the fungal spores is by inhalation, with the lung being the most frequent site of infection.3,5 Systemic infection with blastomycosis is well known, especially among immunocompromised patients. However, blastomycosis of the bones is relatively rare and often clinically unsuspected, leading to delay in diagnosis.2,4,6 Therefore, we undertook this retrospective study to characterize the clinicopathologic features of Blastomyces infection when it affects the bone and to assess the usefulness of intraoperative consultation for rapid diagnosis and management.

Materials and Methods

We searched the database of the Department of Pathology at Rush University Medical Center for all cases of blastomycosis
between 2000 and 2010 and identified those affecting the bone. Clinical, radiologic, and pathologic data were reviewed. The diagnosis of *B dermatitidis* was confirmed using histology and microbiology in the following manner.

All patients with a bone mass underwent biopsy. A portion of the biopsy specimens from some patients (at the discretion of the surgeon) was sent for intraoperative consultation. The frozen sections were cut 5 μm thick on a Leica CM1850 cryostat (Leica Biosystems, Buffalo Grove, IL) and stained by H&E as per standard protocol. The tissue was then fixed in 10% neutral buffered formalin. Decalcification of the tissue was performed in RDO rapid decalcifier solution (Apex Engineering, Aurora, IL), if required. The tissues were then paraffin embedded, cut into 5-μm sections, and stained with H&E. On histologic sections, the presence of characteristic granulomatous inflammation with admixed acute inflammatory infiltrate commanded a vigorous search for the fungus. A presumptive diagnosis of blastomycosis was made when yeast forms (15-20 μm) with a thick cell wall showing characteristic broad-based budding were seen. The diagnosis was supported using special stains for fungal forms such as Gomori methenamine silver (GMS) or periodic acid–Schiff (PAS), which were performed using an automatic stainer (Nexes special stainer; Ventana, Tucson, AZ). The cell wall of the yeast stained black on a green background with the GMS stain and magenta on a pink background with the PAS stain. All frozen and permanent tissue sections were reviewed by two pathologists (R.J. and V.B.R.).

**Image 1A.** Many refractile, round, broad-based budding yeast cells are seen within the giant cells (H&E, ×400). **B.** Gomori methenamine silver stain highlights numerous organisms, many of which show the characteristic broad-based budding (×400). **C.** A frozen section showing acute inflammation with giant cells harboring the yeasts (H&E, ×400). **D.** A touch preparation showing the budding yeast cells (arrows) in an inflammatory background (Diff-Quick, ×400).
Representative tissues from all patients were also submitted for microbiologic studies. A portion of all specimens was stained with calcofluor white and examined under a fluorescent microscope to look for yeast forms. Then, the tissue was cut and planted on brain heart infusion (BHI) agar with 5% sheep blood, chloramphenicol, and gentamicin (Remel, Lenexa, KS); Sabouraud’s BHI agar (Remel); and inhibitory mold agar (Remel). For specimens collected from nonsterile sites such as bronchioalveolar lavage, Sabouraud’s agar with cycloheximide and chloramphenicol (Mycosel agar; Remel) was also used. The plates were incubated at 30°C for 4 weeks and were inspected on days 1, 2, 4, and 7 for the first 7 days followed by once a week. The growth was considered suggestive of Blastomyces if the colonies were white to cream to beige, were velvety in appearance, and the growth was restrictive. Then, the colonies were subcultured on BHI with 5% sheep blood and buffered charcoal yeast agar to allow conversion to yeast form at 37°C. The colony grown on the subculture was teased and mounted on a glass slide. The fresh mount was stained with lactophenol aniline blue dye and examined under the microscope to look for morphology of the fungus. Fine fungal hyphae and short conidiophores were considered suggestive of Blastomyces, and a presumptive diagnosis was made. After 2005, the presumptive colonies were sent for confirmation by DNA probe (Gen-Probe, San Diego, CA). In one patient, urine enzyme immunoassay for B dermatitidis antigen was also used to arrive at the diagnosis (Mira Vista Diagnostics, Indianapolis, IN).

A definite case of blastomycosis was defined by positive culture and DNA probe (after 2005) from any source/site. The presence of yeast forms consistent with blastomycosis seen on histology without culture positivity was defined as probable blastomycosis. History of culture-proven blastomycosis with recurring disease, including osteomyelitis but negative culture, was also regarded as probable blastomycosis when the histology showed suppurative and granulomatous inflammation.

Results

A summary of patient characteristics is presented in Table 1.

Demographics

A total of 54 patients with a diagnosis of blastomycosis were discovered by review of the pathology database, of whom 14 (10 males, 4 females) had bone involvement. The age ranged between 13 and 63 years (median, 42.5 years). Only one child was identified. Most patients were adults between the third and sixth decades of life. The mean age for a female was lower than that for a male (23.5 years for women vs 47.3 years for men). Almost all patients (13 were from northeastern Indiana, and one patient was from northwestern Illinois. The race was known for 10 patients, of whom six were white, two were African American, and two were Hispanic.

Sites of Involvement

The bone lesions were left-sided in seven, right-sided in five, and midline in four patients. Involvement of long bones was seen in nine, axial skeleton in four (three vertebrae and one sternum), and joints in four Figure 1. A predilection for long bones of the lower extremity, particularly around the knee joint, was observed. Spinal vertebrae were the second most commonly infected site. Joint involvement was noted in four patients, three of whom had extension of bone infections into the adjacent joint space (distal femur and sternum infection into the adjacent knee joint and sternoclavicular joint, respectively), whereas one patient had only septic arthritis of the knee joint.

Four patients had concurrent and two had histories of pulmonary blastomycosis. Three of those with concurrent lung infection were symptomatic with respiratory complaints (cough and shortness of breath). Another patient with systemic blastomycosis had lung and skin infection followed by septic arthritis.

Presenting Symptoms and Signs

Twelve patients had localized symptoms and signs of a bone mass. One had pain and tenderness of the knee joint, and the other patient, who had a history of systemic blastomycosis, had draining sinus from the calf. There was no history of injury or trauma in any of the patients, making inoculation through the skin less likely. However, one patient’s attention was drawn to the mass due to severe pain following an injury at work. In addition, respiratory complaints were present in three patients. None of the 14 patients had any history of immunosuppression.

Radiology

Imaging studies were available for review in 11 patients. In all cases, neoplasm was included in the differential diagnosis, and in two cases, a radiologic diagnosis of malignancy was rendered. One of these patients had a juxta-articular, lytic, proximal tibial lesion that was thought to be a giant cell tumor. Another patient had a 1.8-cm pulmonary nodule with a thoracic vertebral lesion and extension into the epidural space. In this case, a diagnosis of lung cancer with metastatic disease was made. For destructive bone lesions with soft tissue extension, a diagnosis of sarcoma was considered. There were destructive, lytic lesions in eight patients. Two of these patients showed perilesional osteoporosis, and three had soft tissue extension of the process. Osteomyelitis was favored in the differential diagnosis in two patients.
### Table 1
**Summary of Patient Characteristics**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Race</th>
<th>Site</th>
<th>Radiology</th>
<th>Frozen Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>M</td>
<td>AA</td>
<td>Cervical vertebral body</td>
<td>Not available</td>
<td>Not performed</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>W</td>
<td>Right tibia; lung involvement present</td>
<td>Not available</td>
<td>Granulomatous and acute inflammation with necrosis</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>F</td>
<td>H</td>
<td>Left lateral femoral condyle eroding into the knee joint; pneumonia</td>
<td>Lytic lesion of distal femur with extension into the knee joint</td>
<td>Granulomatous and acute inflammation with yeast forms suggestive of blastomycosis</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>W</td>
<td>Sternum and sternoclavicular joint</td>
<td>Destructive lesion of sternum with soft tissue mass and extension into the sternoclavicular joint</td>
<td>Granulomatous inflammation with neutrophilic abscesses</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>M</td>
<td>W</td>
<td>Left humerus</td>
<td>Large destructive lesion with soft tissue mass suggestive of sarcoma</td>
<td>Granulomatous and acute inflammation with yeast forms suggestive of blastomycosis</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>F</td>
<td>AA</td>
<td>Left ankle</td>
<td>Large destructive lesion with soft tissue mass; differential diagnosis includes tumor and osteomyelitis</td>
<td>Granulomatous and acute inflammation with yeast forms suggestive of blastomycosis</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>M</td>
<td>W</td>
<td>T4 vertebra; lung involvement present</td>
<td>Extensive tumor of thoracic spine, epidural space with a solitary long nodule suggestive of tumor with metastasis</td>
<td>Granulomatous and acute inflammation with possible yeast forms suggestive of blastomycosis</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>W</td>
<td>Left ankle</td>
<td>Not available</td>
<td>Granulomatous and acute inflammation with yeast forms suggestive of blastomycosis</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>M</td>
<td>UNK</td>
<td>L1 vertebrae; presented initially with cough and back pain of 4 weeks' duration</td>
<td>Lytic lesion</td>
<td>Not performed</td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>M</td>
<td>UNK</td>
<td>Left tibia</td>
<td>Lytic lesion</td>
<td>Granulomatous inflammation and neutrophilic microabscesses</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>F</td>
<td>H</td>
<td>Right knee synovium, right femur</td>
<td>Distal femur lesion with intercortical breakthrough and extension into knee joint</td>
<td>Granulomatous inflammation and neutrophilic microabscesses</td>
</tr>
<tr>
<td>12</td>
<td>63</td>
<td>M</td>
<td>W</td>
<td>Right distal femur and both lungs</td>
<td>Ill-defined lesion in the medullary cavity of the distal femur and bilateral miliary lung nodules suggestive of tumor with metastases or osteomyelitis</td>
<td>Granulomatous and acute inflammation</td>
</tr>
<tr>
<td>13</td>
<td>57</td>
<td>M</td>
<td>UNK</td>
<td>Right distal femur; lung involvement present</td>
<td>Lytic lesion</td>
<td>Not performed</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>M</td>
<td>UNK</td>
<td>Left knee; systemic blastomycosis (lungs and multiple skin lesions)</td>
<td>Periarticular profound osteoporosis around knee joint</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

AA: African American; EIA, enzyme immunoassay; FNA, fine-needle aspiration; H, Hispanic; OSH, outside hospital; UNK, unknown; W, white.

* Denotes cases included in previous study from our institution.

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**Pathology**

Tissue was submitted to pathology in all 14 cases. A cytology specimen was submitted for one patient (case 1) and a biopsy specimen for all others. Of the 13 biopsy specimens, frozen section from a bone lesion was requested for 10 cases. In five of these, a diagnosis of blastomycosis was rendered during intraoperative consultation since characteristic broad-based budding yeast cells were visualized on frozen section slides, and/or touch preparations. All 10 cases showed granulomatous inflammation, which was typically admixed with neutrophilic infiltrate often forming microabscesses.

The final pathology (including cytology) also showed granulomatous inflammation with admixed neutrophilic abscesses in all patients. Varying numbers of broad-based budding yeast forms consistent with blastomycosis were seen in all but one patient. This patient had culture-proven systemic blastomycosis that was partially treated due to noncompliance with drug therapy.

Of the other five cases in which a diagnosis of blastomycosis was not rendered at the time of frozen section, sampling error was identified as the cause of discrepancy between intraoperative and final diagnosis in two cases. Upon
review of frozen section slides, two cases showed rare yeast forms that were initially missed. In one case, the original frozen section slide was not available for review. However, the permanent sections of frozen tissue showed several organisms. In all these cases, the discrepancy was minor and did not cause any direct harm to the patient.

Microbiology

Tissues were sent for microbiologic studies in all 14 cases. The specimens for histology and microbiologic studies were procured as follows: wound debridement (n = 8), bone biopsy (n = 3), laminectomy (n = 1), corpectomy (n = 1), and fine-needle aspiration of the bone lesion (n = 1). In two patients, bronchioalveolar lavage fluid and bronchial biopsy specimens also were sent. Of the three culture plates were positive in 12 patients. Confirmation using DNA probes was done in five patients (after 2005). In addition, urine enzyme immunoassay for Blastomyces was positive in one patient whose transbronchial biopsy specimen grew *B. dermatitidis*. The two culture-negative patients had a history of blastomycosis (3 months and 1 year) with intermittent...
Figure 1  Site distribution of blastomycosis osteomyelitis. Four patients had Blastomyces arthritis, one of whom had isolated septic arthritis, whereas others had extension of the infection into the joint space from the adjacent bone (one from the sternum and two from the long bones).

Discussion

Blastomycosis osteomyelitis is a relatively uncommon and often clinically unsuspected disease. In addition to a small series of five patients reported from our institution previously,1 there has been only one other case series of 45 patients with blastomycosis of bones and joints published in the past decade.2 To our knowledge, the utility of frozen section in the diagnosis of bone blastomycosis has not been explored before.

Lower extremities, particularly around the knee joint, were the most common site of involvement, noted in 50% of our patients. The second most common site of involvement was the vertebrae. A similar distribution of sites was also observed in previous case series.1-3 Blastomyces septic arthritis is much less common than osteomyelitis and occurs secondary to accompanying osteomyelitis.11,12 We encountered four cases of septic arthritis, three of which had adjacent osteomyelitis. One patient with isolated septic arthritis of the knee had a history of partially treated disseminated blastomycosis.

Blastomycosis is typically acquired by inhalation of conidiospores present in the soil in endemic areas and begins as a pulmonary infection followed by hematogenous spread to other body sites. Skin inoculation secondary to trauma is another mode of entry for the organism. In the series of 45 patients with osseous blastomycosis reported by Oppenheimer et al,2 cutaneous disease was present in 33 (73%) patients and lung involvement in 29 (64%) patients. However, typical systemic signs and symptoms of Blastomyces infection were generally absent in our patients, and most had only a localized bone mass as the chief symptom. This may be at least in part due to the active orthopedic oncology service at our institution that attracts referrals for potential bone tumors from the area. The radiographic appearance is neither typical nor specific for osteomyelitis. The age (second to sixth decades) and site (around knee joint) predilection also overlap with bone tumors. Therefore, a working diagnosis of tumor is often entertained clinically.2,4,13 In our case series, a previous or concurrent history of pulmonary symptoms was present in 50%. Only two patients had clinical features of bone/ joint infection, whereas all the others had a painless mass. Tumors, including sarcoma, giant cell tumor, and metastases, were a strong diagnostic consideration in approximately 45% of cases.

When infection masquerades as tumor, oftentimes there is a significant delay in diagnosis.4,6,13 In a previous case series of five patients reported from our institution (includes cases 2, 3, 4, and 13 in this study), the average delay in diagnosis was 4.7 months (143 days).4 The Illinois Department of Public Health reported a twofold increase in risk of mortality with a delay in diagnosis of more than 128 days.14 In the case series published by Oppenheimer et al,2 a delay in diagnosis of 14 to 50 days was observed. Despite the fact that there were no mortalities in our case series, there was significant morbidity, with three patients requiring 2 or more weeks of hospital stay and two also requiring rehabilitation. One patient underwent irrigation and debridement multiple times before being referred to our institution, where diagnosis was established and definitive treatment initiated. This increases cost of care, disability, and inconvenience caused to the patients and their families.

Clinically, even if blastomycosis is considered in the differential diagnosis, this is a slow-growing organism, and...
culture and confirmation can take several weeks. Intraoperative consultation is known to be a particularly efficient tool for rapid preliminary diagnosis and management. The turnaround time for intraoperative consultation at most College of American Pathologists–accredited laboratories, including ours, is 20 minutes, and there is high concordance between intraoperative and final diagnoses.\(^{15,16}\)

All cases that underwent frozen section were correctly diagnosed as granulomatous osteomyelitis, with half showing budding yeast forms with thick walls consistent with *Blastomyces* and two additional cases showing identifiable yeast forms upon review. The organisms sometimes may be present but difficult to unequivocally identify and diagnose if too few in number.\(^{17}\) A failure to recognize the fungus at the time of frozen section can also be due to sampling error. Granulomas with microabscesses were considered highly suggestive of this infection, given the endemcity. *Blastomyces* and other deep mycoses have been shown to elicit a characteristic host response comprising granulomatous inflammation admixed with acute inflammatory infiltrate sometimes forming microabscesses.\(^9\) It is important to be aware that the yeast forms of *Blastomyces* can closely resemble endospores of *Coccidioides*, *Cryptococcus*, and even *Histoplasma*.\(^{17}\) In addition to staining with GMS and PAS, the absence of staining on Diff-Quik (particularly on frozen section touch preparation) and mucicarmine is also helpful.\(^{18}\) Multinucleation of the yeast has been reported to aid distinction from other fungi,\(^{19}\) although this was not observed in our cases. A definitive diagnosis of fungal osteomyelitis can only be possible if a diagnosis is suspected clinically and tissue procured in an aseptic and antisepctic manner is submitted for microbiologic studies. However, the diagnosis is often unsuspected, and the specimen is sent for frozen section to rule out a neoplastic process. A frozen section can not only effectively rule out tumor but also either suggest or confirm an infectious process, facilitating immediate additional sampling for microbiologic studies. This will also relieve the patient and family of stress while results of final pathology are awaited.

The final pathology results are usually available within 2 to 3 days. Therefore, frozen section followed by final pathology offers a quick alternative mode to establish the diagnosis and start treatment while the results of culture are pending. Furthermore, there is good concordance between surgical pathology and microbiology studies, as shown by our results and a previous study.\(^9\) Patel et al\(^9\) had shown concordance between pathology and microbiology in approximately 80% of cases. In partially treated cases, cultures may be negative due to prior drug exposure.

Although surgical pathology offers a definite advantage over microbiology in terms of turnaround time, both modalities have good concordance and complement each other. The same study by Patel et al\(^9\) showed that 10.25% of culture-positive cases were false negative on morphology. Conversely, 8.7% of cases that showed a characteristic appearance of *Blastomyces* on morphology showed other fungal organisms on culture, most notably *Candida albicans*, *Aspergillus*, and *Coccidioides immitis*.\(^9\)

The major limitation of our study is that, due to its retrospective nature, the details of treatment and imaging studies were not available for many patients. A review of available records showed the disease to respond well to prolonged antimicrobial therapy. As with any other prolonged therapies, patient compliance might be difficult to achieve, thereby limiting clearance of infection as seen in one of our patients.

**Conclusion**

For patients who have residence in or a history of travel to endemic areas, blastomycosis should be considered in the differential diagnosis of a bone mass with or without accompanying or prior history of pulmonary symptoms. The lower extremities around the knee joints followed by vertebrae are the favored sites. There are no clinical signs, symptoms, or radiologic features that are specifically suggestive of blastomycosis. Therefore, clinical suspicion and tissue procurement are mandatory for definitive diagnosis. Tissue should be submitted for both histologic and microbiologic studies in all such cases. Intraoperative consultation is a fast and efficient modality to triage cases, and its use should be encouraged.

**References**


