Tropical Biomedicine 28(2): 444–449 (2011)

Research Note

Cryptococcal osteomyelitis of the femur: A case report and review of literature

Zainal, A.I.[1*], Wong, S.L.[2], Pan, K.L.[3], Wong, O.L.[4] and Tzar, M.N.[5]
[1] Department of Pathology, [2] Radiology and [3] Orthopaedics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Malaysia
[4] Department of Pathology, Sarawak General Hospital, Sarawak, Malaysia
[5] Department of Medical Microbiology and Immunology, Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, Malaysia
[*] Correspondence author email: rzabidin@fmhs.unimas.my
Received 15 November 2010; received in revised form 10 April 2011; accepted 11 April 2011

Abstract. Fungal osteomyelitis is a rare opportunistic infection. It exhibits some clinical and radiological similarities to several other bone pathologies. A diagnostic delay may result in significant increase in morbidity. We report a case of a 37-year-old man with underlying hypogammaglobulinaemia presented with isolated cryptococcal osteomyelitis of the femur.

INTRODUCTION

Fungal infections are more often seen in debilitated patients, particularly among young immunocompromised hosts and the lung is the usual portal of entry (Bullogh, 1997). Very occasionally they cause osteomyelitis and avascular necrosis of bone. The infections may become fatal if not treated promptly; hence an early diagnosis is vital. Gurevitz *et al.* (1994) reported that fungal infections among immunocompetent patients are rare occasions. The commonly described infections include blastomycosis, coccidoidomycosis, cryptococcosis and rarely actinomycosis (Bullogh, 1997).

In this report we highlighted the clinicopathological features of this rare bone infection followed by a literature view on the diagnosis and treatment.

Case report

A 37-year-old man presented with a slowly enlarging right knee swelling for seven months duration associated with joint stiffness, recurrent fever and significant loss of weight and appetite. On physical examination the mass exhibited surface erythema with limited range of movement. The knee joint was warm and tender on palpation. No discharging sinus or other joint involvement was detected. The man had a previous history of pulmonary tuberculosis and had completed treatment four years prior to this presentation.

Radiograph of the knee showed non-expansile osteolytic lesions with a narrow zone of transition within the distal femur medial condyle (Figure 1). There was a mild marginal sclerosis noted around the lesions. The lesion did not seem to abut the articular surface. There was no regional periosteal reaction noted. Overall radiographic features were suggestive of Brodie's abscess.

Subsequently, surgical drainage and curettage of the lesion were performed. Intraoperatively, there was a 2 cm cavity at the medial cortex of distal right femur 2 cm from the joint line. Patchy whitish areas of

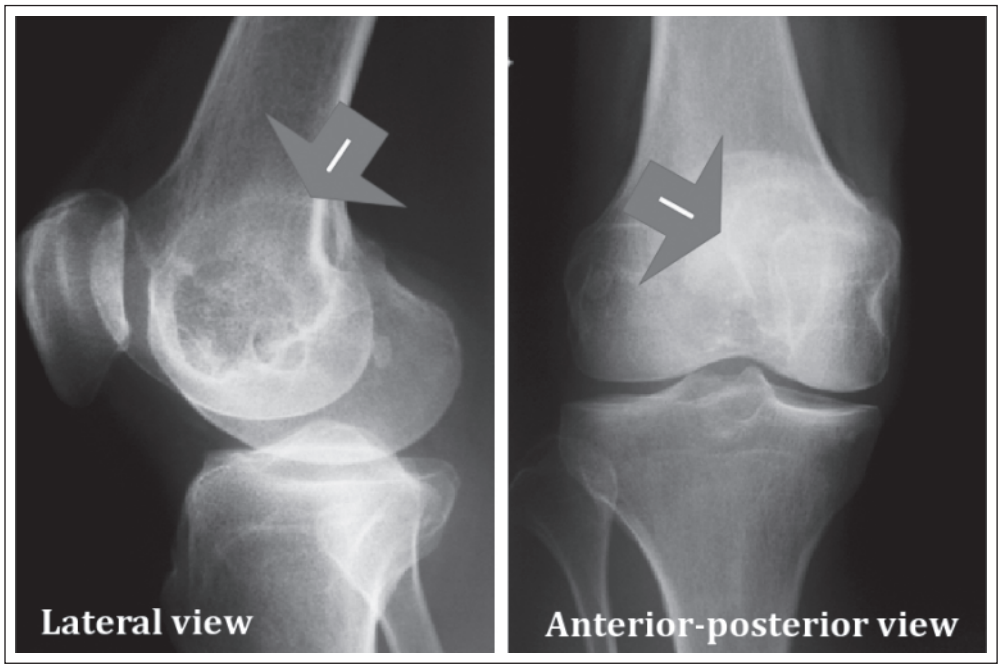

Figure 1. The radiograph of the distal femur osteolytic lesions
The arrows point at the distal femur lesion. No regional periosteal reaction noted

degenerated tissue were seen lining the cavity. Approximately 3 mls of purulent exudates were drained-out.

Histological examination of the cavity wall showed fibrocollagenous tissue with scattered clusters of rounded fungal organisms in yeast form. These yeasts were surrounded by aggregates of macrophages (Figure 2). The yeast displayed refractile appearance under polarized light. No hyphae form detected. The organism stained positive with Gomori methanamine silver (GMS) stain. The morphology of the fungus in histology was consistent with *Cryptococcus neoformans*. Post-operative serum cryptococcal antigen was noted to be positive (1:4096 titres). However, cultures from the tissue fragments and the aspirated pus were negative. No caseating granuloma was seen to suggest tuberculosis.

The patient was treated with two weeks course of intravenous amphotericin B 35 mg daily and followed by oral fluconazole 400 mg daily for the following six weeks. Lumbar puncture was performed post-operatively. No fungal element was detected in the cerebrospinal fluid while the culture study was negative. On further investigation, the patient was noted to have low immunoglobulins G, A and M (below the normal levels of an adult). The HIV, hepatitis B and C screenings were non-reactive. The patient was neither neutropaenic nor diabetic. He works as a farmer and denied any direct contact with birds. On follow up, his condition improved with no residual active lesion. He is currently treated for hypogammaglobulinaemia.

DISCUSSION

Pyogenic bacteria and mycobacteria are among the commonest cause of osteomyelitis. With the emergence of opportunistic infections in immunocompromised patients, the diagnosis and treatment of osteomyelitis have become more challenging (Rosenberg, 2004). *Cryptococcus neoformans* is an uncommon but treatable causal factor of osteomyelitis which may affect both normal and immunocompromised patients (Behrman *et al*., 1990; McClelland *et al*., 2007).

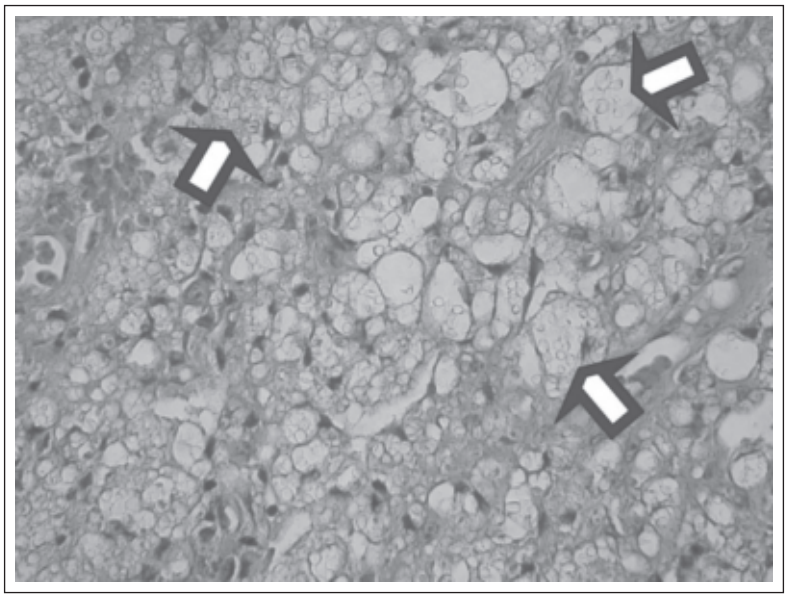

Figure 2. Tissue biopsy from the lesion (H&E 20X)
There were numerous scattered *Cryptococcus neoformans* organisms (blue arrows) surrounded and engulfed by aggregates of macrophages

Several predisposing factors of cryptococcosis have been identified including HIV infection, haematological disorders and several chronic diseases (Chayakulkeeree & Perfect, 2008). Cryptococcosis occurs primarily by inhalation of the infectious propagules, however, direct inoculation into tissue due to trauma or through gastrointestinal tract can be possible portals of entry occasionally (Chayakulkeeree & Perfect, 2008). In healthy individual the infection is commonly cleared or may remain in latent form for a prolong period of time (McClelland *et al*., 2007).

Most fungi are highly immunogenic and induce strong antibody response and T cell-mediated immunity. *Cryptococcus neoformans* possesses a polysaccharide capsule which inhibits phagocytosis. This protective mechanism can be overcome by host's complement and antibody opsonisation (Male *et al*., 2006). The capsule is one of the main pathogenic factors as acapsular strains are avirulent (McClelland *et al*., 2007). Reduced level of immunoglobulin in this case predisposed this patient towards this infection.

Deep mycosis typically shows different level of aggressiveness. It most likely depends on the individual and the respective organism involved (Witte *et al*., 2000). Cyrptococcal osteomyelitis occurs commonly as a complication to chronic meningoencephalitis (Bullogh 1997). An isolated cryptococcal osteomyelitis is an uncommon condition (Witte *et al*., 2000). The spectrum of clinical presentation extends from asymptomatic with an osteolytic lesion to an infection with signs and symptoms of systemic involvement (Behrman *et al*., 1990). Sarcoidosis is the most common reported underlying disease in association with this mycosis, followed by tuberculosis and previous steroid treatment (Witte *et al*., 2000). The vertebra is the commonest reported osteomyelitis site while the other frequent sites include the pelvis, femur, spine and tibia (Bullogh, 1997; Liu, 1998).

Radiographically, the osteolytic femoral condyle lesion in especially in this age group displayed non-specific features which are commonly shared by several conditions including giant cell tumor, chondroblastoma, subchondral cyst or infective processes.

Cryptococcal osteomyelitis commonly exhibits osteolysis with or without local periosteal reaction (Rabia, 2006). An extensive periosteal reaction is an uncommon finding (Witte *et al.*, 2000). With the given history of slow progressive nature of the clinical presentation, the radiographic findings of this patient were more suggestive of tuberculous osteomyelitis or subacute pyogenic osteomyelitis. Clinical and radiological similarity to several other bone pathologies may cause delay in antifungal treatment initiation.

The early recognition of the organism by morphology, culture study and serological analysis may potentially improve the prognosis and treatment of this infection (Witte *et al.*, 2000). Histological evaluation is a quick and easy method to identify fungal organism. It is an important adjunct to culture study for diagnosis of fungal infection. Fungi organism can be identified based on their size and distinctive morphology (Haque, 2010). *Cryptococcus* is a basidiomycete that grows as haploid budding yeast (McClelland *et al.*, 2007). In tissue histology, the yeast cells of *Cryptococcus* are typically spherical and irregular in size ranging from 5 to 20 µm in diameter. The cells typically exhibit thick polysaccharide capsule (Anselmo *et al.*, 2003). The budding forms appear to be attached by a narrow constriction. Most fungi including *Cryptococcus* are readily demonstrated with common special stains for fungus including GMS and periodic acid Schiff (Haque, 2010). Fine needle aspiration with Diff-Quick stained slide has been reported to be useful for prompt diagnosis and exclusion of malignant neoplasm (Witte *et al.*, 2000).

Hence when a chronic granulomatous inflammation pattern was detected, it is important to perform a direct smear and culture study for acid fast bacilli and also for fungi (Bullogh, 1997). Pre-operative serological cryptococcal antigen may prove to be helpful (Gurevitz *et al.*, 1994).

In a properly collected and processed specimen, there are several reasons that might lead to a negative culture study. This includes low sensitivity of culture, host immunocompetence recovery and institution of antifungal agents prior to culture. In this case, visualization of yeast cells during histopathological examination had virtually confirmed the presence of yeasts in the tissue. The only question left was the confirmation of species.

Conventional methods of fungal culture have been known to yield unsatisfactory rate of isolation. To our knowledge, there is no published study that looked specifically into the sensitivity of conventional culture methods in isolating *Cryptococcus* species from clinical specimens. However, Telenti & Roberts (1989) reported that the best blood culture system (the Isolator system) could only detect 73% in a study on autopsy-proven disseminated candidiasis. Therefore, a negative culture does not exclude an active infection.

Another possible reason for the negative cultures is recovery of host immuno-competency. In previously treated cases of cryptococcal infection among HIV patients, antiretroviral therapy institution had led to subsequent improvement in CD4+ cell count and immune function. This condition, called the immune reconstitution inflammatory syndrome (IRIS), may sometimes cause clinical deterioration as a result of rapid and dysregulated immune response recovery. These cases usually presented with negative cryptococcal cultures (Bicanic & Harrison, 2010). Another study by Manfredi *et al.* (2003) showed that after the introduction of highly active anti-retroviral therapy (HAART), episodes of HIV-related cryptococcosis characterized by persistently negative cultures rose from 10.3% of years 1992-1996 to 87.5% after 1997. They concluded that the apparent absence of viable organisms might be explained by the increased immune response against a latent infection due to HAART use, the co-administration of suppressive antifungal therapy and the pro-inflammatory role played by cryptococcal antigen, even in the absence of active fungal replication. However in this case, the patient was neither diagnosed as HIV-positive nor did he receive any antifungal agents prior to sample collection for the culture studies.

The possibility of the cryptococcal antigen test being false positive is remote. The false positive rate of cryptococcal

capsular polysaccharide antigen testing is only 0% to 0.4% (Kauffman *et al*., 1981). In addition, a titre of 4096 in this case was too high to be ignored. Most of the false positive results of latex agglutination testing for cryptococcal polysaccharide antigen have initial reciprocal titers of 8 or less (Tanner *et al*., 1994).

Antifungal treatment and surgery are the two common therapeutic choices (Raftopoulos *et al*., 1998). Most cases of cryptococcal osteomyelitis were successfully treated with medical treatment alone or in combination with surgical debridement (Liu, 1998). The reported effective antifungal agents include amphotericin B and fluconazole as proven in this case (Behrman *et al*., 1990).

Cryptococcal osteomyelitis may lead to significant morbidity and mortality. It should be considered as one of the differential diagnoses of osteolytic osseous lesions particularly in immunocompromised or even in immunocompetent hosts.

Acknowledgements. The authors wish to thank Dr. Mohd Zulkarnaen A Narihan and Angela Chia Yin Yin for her assistance in writing this case.

REFERENCES

Anselmo, C., Lee, K., Lia, J.Y., Bergunio, R., Lum, A., Viduya, P., Jang, J., Goodman, C.D., Warner, S.L., Roemer, M. & Haller, B. (2003). A resident's fungal morphology [Online]. Available from: http://labmed.ucsf.edu/education (Accessed 7 November 2010)

Behrman, R.E., Masci, J.R. & Nicholas, P. (1990). Cryptococcal skeletal infections: case report and review. *Reviews of Infectious Diseases* **12**(2): 181-190.

Bicanic, T. & Harrison, T. (2010). Cryptococcus. In: *Spectrum of Disease and Treatment in Pathogenic Yeasts*, *the Yeast Handbook*. Ashbee HR and Bignell EM (editors) Springer-Verlag Berlin Heidelberg: 145-165.

Bullogh, P.G. (1997). *Bullogh and Vigoritta's Orthopaedic Pathology* 3rd edition. Mosby-Wolfe: 126-129.

Chayakulkeeree, M. & Perfect, J.R. (2008). Cryptococcosis. In: *Infectious Disease: Diagnosis and Treatment of Human Mycoses*. Hospenthal DR and Rinaldi MG Humana Press Inc., Totowa, NJ: 255-276.

Gurevitz, O., Goldschmied-Reuven, A., Block, C., Kopolovic, J., Farfel, Z. & Hassin, D. (1994). Cryptococcus neoformans vertebral osteomyelitis. *Journal of Medical and Veterinary Mycology*. **32**(4): 315-318.

Haque, A. (2010). Special stains use in fungal infections. *Connection*: 187-194.

Kauffman, C.A., Bergman, A.G., Severance, P.J. & McClatchey, K.D. (1981). Detection of cryptococcal antigen. Comparison of two latex agglutination tests. *American Journal of Clinical Pathology* **75**: 106-109.

Liu, Y.F. (1998). Cryptococcal osteomyelitis: Case report and review. *Diagnostic Microbiology and Infectious Diseases* **30**(1): 33-35.

Male, D., Brostoff, J., Roth, D.B. & Roitt, I. (2006). *Immunology* 7th edition. Mosby: 274-275.

Manfredi, R., Calza, L. & Chiodo, F. (2003). AIDS-associated cryptococcus infection before and after the highly active antiretroviral therapy era: Emerging management problems. *International Journal of Antimicrobial Agents* **22**: 449-452.

McClelland, E.E., Casadevall, A. & Eisenman, H.C. (2007). *New Insight in Medical Mycology*. Springer Netherlands: 130-151.

Rabia, A. (2006). Cryptococcal osteomyelitis of the humeral head initially diagnosed as avascular necrosis. *Southern Medical Journal* [Online]. Available from: http://www.thefreelibrary.com (Accessed 7 November 2011)

Raftopoulos, I., Mellerab, J.L., Harris, V. & Reyesab, H.M. (1998). Cryptococcal rib osteomyelitis in a pediatric patient. *Journal of Pediatric Surgery* **33**(5): 771-773.

Rosenberg, A.E. (2004). Bone, joints and soft tissue tumours. In: *Robbins and Contran Pathologic Basis of Disease* 7th edition. Elsevier Saunders Philadelphia: 1290-1292.

Tanner, D.C., Weinstein, M.P., Fedorciw, B., Joho, K.L., Thorpe, J.J. & Reller, L. (1994). Comparison of commercial kits for detection of cryptococcal antigen. *Journal of Clinical Microbiology* **32**: 1680-1684.

Telenti, A. & Roberts, G.D. (1989). Fungal blood cultures. *European Journal of Clinical Microbiology and Infectious Diseases* **8**: 825-831.

Witte, D.A., Chen, I., Brady, J., Ramzy, I., Truong, L.D. & Ostrowski, M.L. (2000). Cryptococcal osteomyelitis. Report of a case with aspiration biopsy of a humeral lesion with radiologic features of malignancy. *Acta Cytologica* **44**(5): 815-818.