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CASE REPORT

Marrow talaromycosis as the initial presentation in a case of Burkitt lymphoma

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Abstract

Talaromyces marneffei is a thermally dimorphic fungus which causes opportunistic infections in immunocompromised individuals. The diagnosis of *T. marneffei* infection rests on the microscopic demonstration of the fungus in the tissues and/or isolation of the fungus from clinical specimens. In this report, we discuss a case involving a 23-year-old man who presented with a history of intermittent fever, cough and constitutional symptoms. Clinically, the patient exhibited pallor, jaundice, generalized seborrhoeic dermatitis, hepatomegaly, and small palpable cervical lymph nodes. A computed tomography (CT) scan of the abdomen showed homogenous hypodense lesions in both liver lobes. HIV screening result was reactive. Microscopic examination of the bone marrow aspirate smear and trephine biopsy identified fungal bodies, and culture of the marrow aspirate confirmed the presence of *T. marneffei*. Notably, the liver biopsy revealed Burkitt lymphoma alongside fungal bodies. He was treated with intravenous Amphotericin B but ultimately succumbed to the illness due to severe metabolic acidosis and multiorgan failure. This case underscores the importance of presumptive diagnosis through morphological or histological examination of bone marrow samples, as microbiologic culture methods can be time-consuming. Timely diagnosis and aggressive treatment are critical in managing patients with *T. marneffei* infection.

Keywords: Penicillium marneffei, *Talaromyces marneffei*, opportunistic infection, Bone marrow, Burkitt Lymphoma

INTRODUCTION

Talaromyces marneffei (*T. marneffei*), formerly known as *Penicillium marneffei*, was first discovered in 1956 from the hepatic lesions of a bamboo rat (*Rhizomys sinensis*) that succumbed to disseminated mycosis during captivity for experimental infections at the Pasteur Institute of South Vietnam.[1] *T. marneffei* is endemic in Southeast Asia and South China posing a significant opportunistic infection risk to immunocompromised individuals.[2] The incidence of systemic *T. marneffei* infection has escalated in recent years, correlating with the increasing incidence of human immunodeficiency virus (HIV) infections.[3] This infection typically manifests in the advance stages of HIV infection when the CD4 count falls below 50 cell/μL.[4] Clinically, the presentation can mimic disseminated histoplasmosis with patients exhibiting symptoms such as fever, weight loss, skin lesion, and malaise. Similar to acute disseminated histoplasmosis infection, involvement of the reticulo-endothelial system and consequent anaemia, leukopenia, lymphadenopathy, and hepatosplenomegaly are common.[4] Here, we report a case of disseminated talaromycosis involving the bone marrow and liver as an initial presentation of retroviral patient with Burkitt lymphoma.

CASE REPORT

A 23-year-old man presented with a two-month history of intermittent fever and cough, associated with loss of weight, loss of appetite, night sweats, lethargy, body weakness, skin lesions and shortness of breath. He had no significant past medical history and denied any high risk behaviour or intravenous drug usage.

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He reported taking traditional medication and protein supplements for several years. On examination, he was pale and jaundiced. Oral examination showed multiple pharyngeal ulcers. There were generalised seborrheic dermatitis over the face and nodular skin lesions all over the bodies. Hepatomegaly was present, measuring 15 cm below the right costal margin and a small palpable right cervical lymph node measuring 2×1 cm but no splenomegaly.

Laboratory investigations showed a haemoglobin (Hb) level of 9.5 g/dL, white cell count of 4.7 × 10^9/L predominantly neutrophils (82.2%), and platelet count of 329 × 10^9/L. Peripheral blood film showed moderate anaemia with leukoerythroblastic picture. The liver function test was abnormal: elevated bilirubin of 63.6 μmol/L, alanine transaminase, 233 U/L and alkaline phosphatase, 1318 U/L. The C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were high. Ultrasound abdomen demonstrated enlarged liver measuring 20.1 cm. Computed tomography (CT) scan revealed numerous homogeneous hypodense lesions over both liver lobes [Figure 3 (A)].

The bone marrow aspirate showed normocellular fragments with scattered macrophages and clusters of yeast-like fungal bodies' inclusions both intracellularly and extracellularly clusters of fungal bodies' inclusions, which stained positive for Periodic Acid Schiff (PAS) [Figure 1]. There was an increased erythrophagocytosis. Granulopoiesis and megakaryopoiesis were unremarkable. Trephine biopsy showed evidence of fungal infections highlighted by Grocott's methenamine silver (GMS) and periodic acid Schiff (PAS) stains [Figure 2]. There was no evidence of lymphomatous infiltration. *Talaromyces marneffei* was isolated from the blood and bone marrow aspirate culture.

Initial HIV screening using electro-chemiluminescence immunoassay was negative. However, repeat testing was conducted due to a high level of suspicion, yielding a positive result. HIV confirmatory test with particle agglutination assay also showed reactive result. Further virology tests also revealed reactivity towards both cytomegalovirus and Epstein–Barr virus IgG. The patient was negative for both

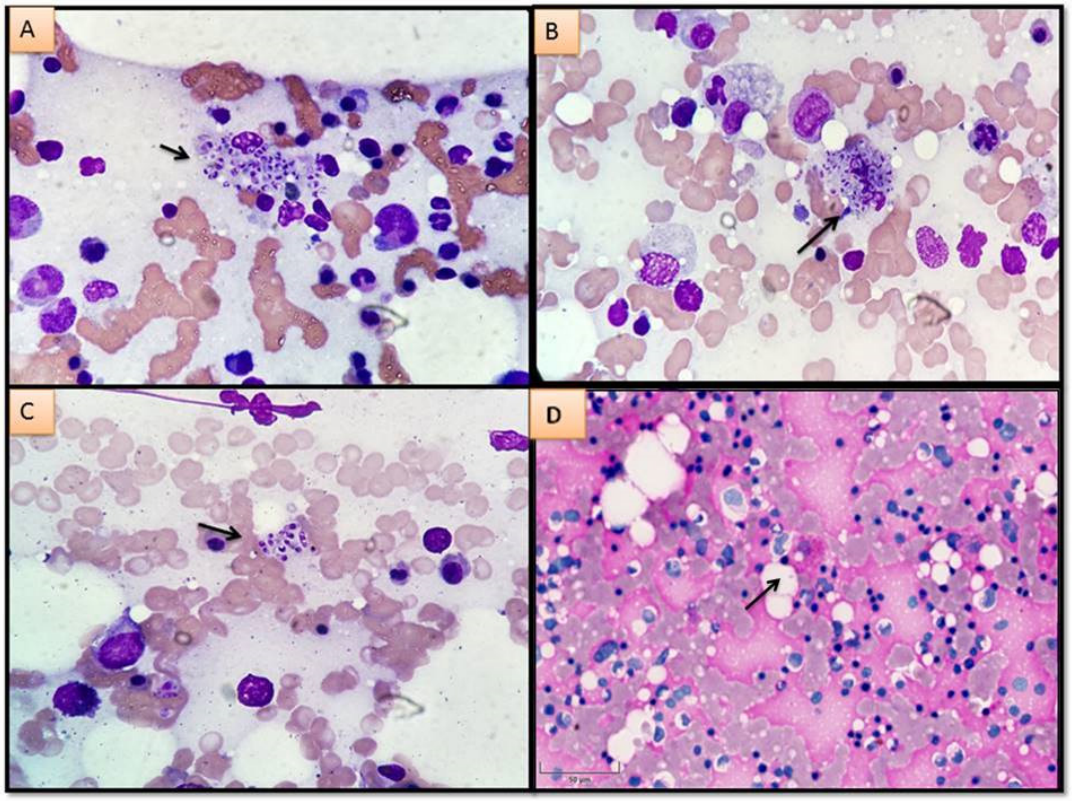

Figure 1. Bone marrow aspirate smear reveals intracellular and extracellular yeast-like fungal bodies (arrow) (MGG ×100) (A-C) and stained with magenta (arrow) (PAS stain ×100) (D)

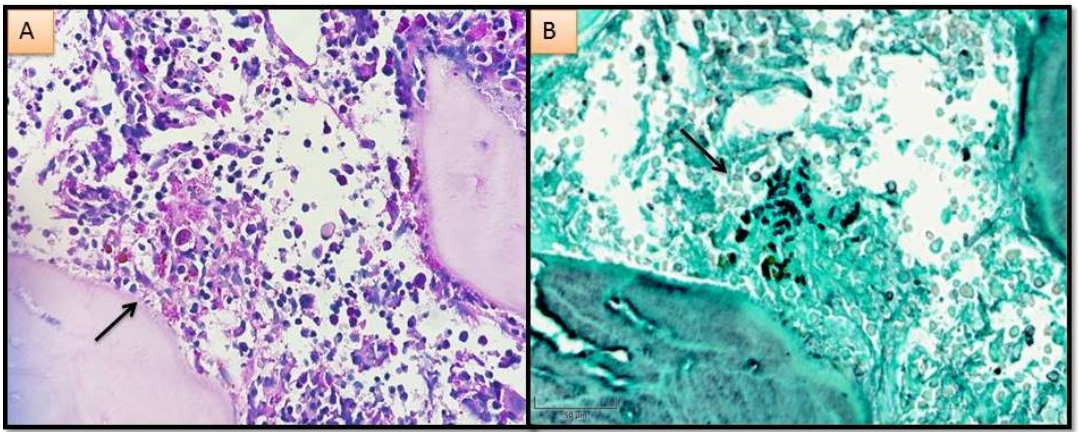

Figure 2. Trephine biopsy showed few colonies of yeast-form cells which are small, round to sausage-shaped (arrows) highlighted by PAS stain (×40) in (A) and GMS stain (×40) in (B)

Aspergillus and Candida antigens by enzyme-linked immunosorbent assay (ELISA) method. Acid fast bacilli (AFB) screening was also negative.

Liver and skin tissue biopsies showed fungal bodies staining positively with GMS. There was also malignant cell infiltration forming sheets and clusters within the liver sinusoids seen in haematoxylin and eosin (H&E) staining of the liver biopsy. The cells were medium in size with round to oval hyperchromatic nuclei with inconspicuous nucleoli and the background showed numerous apoptotic bodies with starry-sky appearance [Figure 3(B)]. The malignant cells were positive towards CD20, CD10, BCL6 and BCL2 (weak) with high Ki-67 proliferation index (~90%) in favour of high-grade B-cell lymphoma likely Burkitt lymphoma with concomitant fungal infection. FISH analysis confirmed the presence of MYC gene rearrangement supporting the diagnosis of Burkitt lymphoma. He was treated with intravenous Amphotericin B but unfortunately succumbed to severe disseminated talaromycosis with multiorgan failure after two weeks.

DISCUSSION

This case illustrates the importance of prompt detection of severe disseminated talaromycosis in an immunocompromised patient with concomitant Burkitt lymphoma. Systemic fungal infections are more commonly seen in immunodeficient patients, for example in patients with acquired immune deficiency syndrome (AIDS). Besides HIV-positive patients,

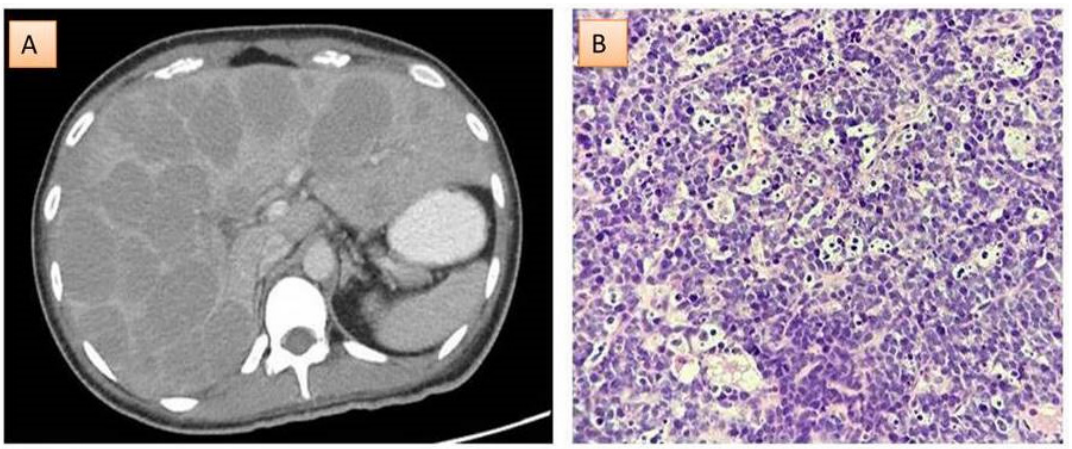

Figure 3. (A) Computed tomography (CT) scan revealed numerous homogeneous hypodense lesions over both liver lobes (B) Liver biopsy showed infiltration of small to moderate malignant cells and numerous background apoptotic bodies showing starry-sky appearance (H&E ×40)

T. marneffei infections have been reported in other immunocompromised non-HIV patients, such as renal transplant recipients, patients with autoimmune disease such as systemic lupus erythematosus and patients who are receiving corticosteroid therapy, anti-cancer targeted therapy such as anti CD20 monoclonal antibodies and kinase inhibitors.[5]

T. marneffei infections occur late in the course of immunodeficiency, typically when the CD4 cell counts have plummeted. However, CD4 count was not done due to rapid deterioration of the disease. Interestingly, our patient had disseminated talaromycosis as an initial presentation of his underlying illness. This patient initially presented with classical clinical features of *T. marneffei* infection such as fever and hepatomegaly associated with clinical manifestation of HIV infection such as anaemia, weight loss and cachexia. The skin lesions which have been described in this patient was also found in more than 70% of cases of *T. marneffei* infection and it was one of the important clues towards the diagnosis.[4]

While there are no specific haematological markers for disseminated fungal infections, early detection relies on clinical suspicion and evidence of fungal presence in bone marrow and trephine biopsy. Cytopenia as detected in this patient is common in infections. Occasionally fungus may be seen in the peripheral blood film of immunocompromised hosts with systemic fungal infections, appearing free in circulation or within the neutrophils or monocytes.[6]

Diagnosing *T. marneffei* involves microscopic demonstration of the fungus in the tissues and/or isolation of the fungus from clinical specimens. Bone marrow culture is the most sensitive method, followed by skin biopsy and blood culture.[4] A study on 92 patients infected with *T. marneffei* in Chiang Mai Hospital, Thailand showed that the most sensitive clinical specimens for isolation of *T. marneffei* were bone-marrow and lymph-node biopsy samples showing 100% sensitivity while the sensitivity of blood culture was approximately 76%. However, bone-marrow aspiration and lymph node or skin biopsy are more invasive than blood cultures. In addition, in the absence of gram-negative bacteraemia, the sensitivity of blood culture may be higher.[7] Despite microbiologic culture being the gold standard for diagnosis of fungal infection by its high accuracy, the long duration for culture due to the slow growth rate of the fungus remains as a main draw-back for early diagnosis. Therefore, in cases with high suspicion of disseminated fungal infection, detection of the fungus by aspirated sample as illustrated in this case may aid the clinician towards early diagnosis, definitive treatment and improvement in patient outcome.

Another study involving 72 patients with culture proven *T. marneffei* infection showed 16 patients were found to have bone marrow involvement and has clearly illustrated the morphology of the fungus in bone marrow aspirate and trephine.[8] The aspirates showed histiocytic proliferation in which some appeared in clusters. The histiocytes were engorged with numerous yeast-form cells and some extracellular aggregates were found. Round pathogens that have been phagocytosed by histiocytes are found in a variety of other infections. These include protozoan infections (leishmaniasis, toxoplasmosis, pneumocytis), deep mycosis (histoplasmosis, blastomycosis, and cryptococcosis), and bacterial infections (rhinoscleroma and granuloma inguinale). All these need to be considered in the differential diagnosis. The yeast form observed in this case appeared as small, round to oval containing central or eccentric dot-like structure. Some of these cells have clear septation which is a characteristic feature of *T. marneffei*. Despite similar appearances, Histoplasma capsulatum typically shows narrow-based budding compared to the septate yeast form in *T. marneffei*.[9]

Tissue biopsy can provide presumptive diagnosis while awaiting fungal culture results, especially when culture growth is absent or not requested. However, morphological detection of fungus in bone marrow is not definitive; the diagnosis of fungal infection is not difficult when the organism is detectable in the bone marrow/tissue. Organism detection can be challenging in severely hypocellular or necrotic marrows following cytotoxic chemotherapy.[6]

In this case, fungal cell walls and cytoplasm did not stain well with haematoxylin and eosin (H&E), preventing obvious identification. High clinical suspicion of fungal infection should prompt searches for fungus and requests for special immunohistochemical stains in biopsy tissue. Grocott (methenamine) silver (GMS) stain or Periodic acid-Schiff (PAS) are the standard histological stains for fungi. The GMS stain is more sensitive than the PAS stain, but has a signal to noise issue in which it also stains inflammatory cells (lysosomes) and tissue reticulin, in addition to fungi. PAS staining has the slight advantage because the morphology of

the tissue adjacent to the fungi can still be better visualized, but this can be addressed using a GMS stain and H&E counterstain.[10] The organisms appear as unicellular round to oval cells which divide by cross-wall formation in macrophages or histiocytes. Extracellularly, it may appear as elongated or sausage-shaped cells with or without one or two septa.[4]

In conclusion, a high index of suspicion is essential for identifying *T. marneffei* in bone marrow aspirate and trephine biopsy specimens from immunocompromised individuals, particularly those who are retroviral-positive. Presumptive histological diagnosis of talaromycosis may facilitate early institution of appropriate antifungal therapy which is critical in high risk immunocompromised patients.

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