Case Report

Primary cutaneous cryptococcosis and a surprise finding in a chronically immunosuppressed patient

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Introduction: Primary cutaneous cryptococcosis is a rare form of cryptococcosis occurring after direct inoculation of *Cryptococcus* spp. yeast cells through skin injury and equally affects immunocompetent and immunocompromised individuals.

Case presentation: We report a case of a 61-year-old man who presented with an 8-month history of an ulcerative lesion on a finger, which was refractory to antimicrobials, following injury with a plant thorn. The patient had a medical history of myasthenia gravis and had been undergoing treatment with pyridostigmine and intermediate doses of prednisolone for more than 30 years. A skin-lesion culture was positive for *Cryptococcus neoformans* var. *neoformans*, which was molecularly confirmed and typed as serotype D, mating-type α , genotype AFLP2/VNIV. Histopathology revealed a diffuse type of inflammation, with many yeasts consistent with *Cryptococcus* spp. The serum cryptococcal antigen was positive at a titre of 1 : 32. Radiological investigation excluded disseminated disease but revealed the incidental presence of a clear cell renal cell carcinoma. The tumour was considered to be an additional factor of immunosuppression and could justify the histological findings. The patient was treated with 200 mg fluconazole twice daily, underwent surgical removal of the mass and received treatment with sunitinib, a receptor protein-tyrosine kinase inhibitor. At the 4 month follow-up, he had a remarkable clinical improvement. A year after, he remained symptom free and tolerated the anti-tumour therapy well.

Conclusion: The patient presented here had no evidence of a disseminated cryptococcal infection despite two concurrent causes of cellular immunity defect and a positive antigen titre.

Keywords: *Cryptococcus neoformans*; cutaneous ulcer; fluconazole; prednisolone; primary cutaneous cryptococcosis; renal carcinoma.

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Introduction

The basidiomycetous yeast genus *Cryptococcus* contains approximately 100 species but only two of them are well known for their ability to cause disease in humans and animals (Fonseca *et al.*, 2011). *Cryptococcus neoformans*,

with a worldwide occurrence, can be differentiated into two varieties named *grubii* (serotype A) and *neoformans* (serotype D). Its sibling, *Cryptococcus gattii* (serotypes B and C), is more restricted to tropical, subtropical and Mediterranean climate zones. *C. neoformans* can be isolated from decaying wood, fruits, vegetables, hay and dust, but a major way of spread is through bird droppings, especially those of pigeons. Entry of *Cryptococcus* into the

Abbreviations: AFLP, amplified fragment length polymorphism; CT, computed tomography; PCC, primary cutaneous cryptococcosis

human body occurs through the respiratory system, but also through skin abrasions and injuries (Chayakulkeeree & Perfect, 2006). In immunocompromised individuals, such as those with long-term corticosteroid use, transplant recipients, patients with haematological malignancies and particularly those with human immunodeficiency virus infection (Lu *et al.*, 2007; Jasch *et al.*, 2008; Singh *et al.*, 2008; Park *et al.*, 2009; Reisfeld-Zadok *et al.*, 2009), *Cryptococcus* can cause severe and life-threatening meningoencephalitis, pulmonary infection with cryptococcomas and skin lesions through haematogenous spread. The skin is the third most common organ involved in cryptococcosis; secondary cutaneous cryptococcosis affects about 15 % of patients with disseminated disease and 5 % of patients with meningitis (Chayakulkeeree & Perfect, 2006). Primary cutaneous cryptococcosis (PCC) is a rare form of cryptococcosis considered a distinct clinical entity (Christianson *et al.*, 2003; Neuville *et al.*, 2003) affecting both immunocompetent and immunocompromised individuals through direct inoculation of the yeasts, after injury with contaminated material. Here we present a patient with clinically, microbiologically and histologically diagnosed PCC, with a history of myasthenia gravis under corticosteroid treatment.

Case report

A 61-year-old Caucasian male was referred in March 2013 to the outpatient clinic of our hospital for cutaneous lesions on the dorsum of his left middle finger. These lesions consisted of a large skin ulceration and two small satellite lesions. The large ulceration was deep, round and painless with a grimy crater and heaped-up red and irregular borders, overlying the first interphalangeal joint (Fig. 1a). One of the satellite lesions was a firm, erythematous and oedematous nodular eruption with an initiating ulceration area on the top, and was located on the distal phalanx of the same finger. The other was an erythematous and oedematous papule overlying the knuckle of the middle finger. These lesions had developed gradually over an 8-month course. They began as cellulitis, subsequently becoming oedematous with overlying redness simulating lymphocutaneous disease, before the main lesion ulcerated. Physical examination did not reveal any proximal epitrochlear or axillary lymphadenopathy or fever.

During the 8-month period, the patient had received various antimicrobial regimens including β -lactams and clindamycin without any clinical improvement. He had a known history of myasthenia gravis, which was diagnosed at the age of 28 years and was being treated with pyridostigmine and 25 mg prednisolone daily. He did not report any other co-morbidities that could predispose to his condition. He lived in a rural area in southern Greece and, the patient recalled that he had a plant thorn injury of his left hand while gardening at home, before the lesions appeared. He did not report any travel outside Greece or his area of residence.

Fig. 1. (a) Lesions before treatment. (b) Periodic acid–Schiff staining of the core lesion biopsy revealing yeasts surrounded by a clear ‘halo’ and a diffuse inflammatory infiltrate in the dermis. Magnification $\times 40$.

Investigations

A swab sample was taken and cultured on Sabouraud glucose agar (SGA). After 2 days of incubation at 30 °C, the culture became positive for a mucoid yeast morphologically suggestive of *Cryptococcus*. Blood samples were taken for laboratory investigation. All routine biochemical parameters (glucose, creatinine, bilirubin, aspartate transaminase, alanine aminotransferase, γ -glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase and C-reactive protein) were within normal range. A blood count showed white blood cells at $9.9 \times 10^9 \text{ l}^{-1}$ with 79 % neutrophils. A biopsy of the core lesion was performed for histological and microbiological examination. A direct KOH preparation was negative for fungi, but *Cryptococcus*-like colonies grew on SGA.

Diagnosis

The cultured yeast was molecularly characterized using amplified fragment length polymorphism (AFLP) fingerprinting according to Arsic Arsenijevic *et al.* (2014), which showed that the isolate fell within the genotype

AFLP2/VNIV, which represents *C. neoformans* var. *neoformans*. Subsequently, a real-time PCR assay was performed (Arsic Arsenijevic *et al.*, 2014) and confirmed the identification of the isolate as *C. neoformans* var. *neoformans* serotype D, mating-type α . The isolate has been deposited in the public culture collection of the CBS-KNAW Fungal Biodiversity Center under accession number CBS 13015.

Histology showed a diffuse inflammatory infiltrate in the dermis, consisting of lymphocytes, plasma cells and histiocytes lacking granulomas or multinuclear giant cells. Periodic acid–Schiff staining revealed yeasts surrounded by a clear ‘halo’, features consistent with *Cryptococcus* spp. (Fig. 1b). Blood cultures were negative. An X-ray of the hand showed no bone involvement. The cryptococcal antigen in serum performed by latex agglutination (Pastorex Crypto Plus; Bio-Rad) was positive at a 1:32 dilution. A computed tomography (CT) scan showed no pulmonary involvement; nevertheless, it simultaneously disclosed the presence of a right kidney lesion extending to the perirenal adipose tissue and posterior aspect of Gerota’s fascia. Subsequently, a right nephrectomy was performed; histological examination showed clear cell renal cell carcinoma Fuhrman nuclear grade 2, with accompanying perirenal fat expansion.

Treatment

The patient was started with 200 mg fluconazole twice daily, prednisolone was reduced to 2.5 mg daily, pyridostigmine remained unchanged, and he was referred to the urology department. He underwent surgical removal of the mass 5 weeks thereafter, at which point he stopped the antifungal therapy on his own. Five months later, he was started on anti-tumour treatment with sunitinib, a receptor protein-tyrosine kinase inhibitor.

Outcome and follow-up

The patient was followed up at frequent intervals for 8 weeks from the initiation of the antifungal treatment, with his lesion exhibiting obvious signs of granulation tissue and general improvement (Fig. 2). The cryptococcal serum antigen titre was reduced to 1:16. A year later, the lesions were completely healed and there were no signs of systemic infection, despite the early cessation of antifungal treatment. He tolerated the anti-tumour therapy well and further follow-up examinations were recommended.

Discussion

PCC was first described in the 1950s; but its existence as a distinct clinical entity has long been disputed. Instead, it was considered by some to be a manifestation of disseminated disease in immunocompromised individuals (Sarosi *et al.*, 1971; Noble & Fajardo, 1972; Hay, 1985;

Fig. 2. Lesions at 8 weeks after treatment.

Perfect & Seaworth, 1985; Kwon-Chung *et al.*, 2000). New evidence, however, has changed this concept (Ng & Loo, 1993; Christianson *et al.*, 2003; Neuville *et al.*, 2003; Baumgarten *et al.*, 2004; Lafleur *et al.*, 2004; Pietras *et al.*, 2010). In fact, Neuville and co-workers (2003) defined criteria that characterize PCC and can be used for the clinical diagnosis. The clinical and laboratory findings of the case presented here are consistent with this clinical entity, namely lesions confined to a limited body area, history of prior injury (in this case, a plant thorn), exposure to a contaminated source (probably the plant thorn), a rural area of living, no systemic signs, no extracutaneous sites positive for *Cryptococcus* and a favourable outcome. Moreover, the isolate involved belonged to serotype D, mostly associated with PCC (Neuville *et al.*, 2003). A skin ulcer developing chronically may be the result of a cutaneous infection or primary cutaneous cancer (Ingletton *et al.*, 1998). The differential diagnosis includes infection with common bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, atypical mycobacteria such as *M. marinum*, *M. avium* and *M. intracellulare*, *Nocardia* spp., parasites of the genus *Leishmania* and fungi. Coexistence of an infectious agent with Kaposi’s sarcoma has also been described (Pietras *et al.*, 2010).

Fungal genera usually associated with the described clinical presentation are *Blastomyces*, *Coccidioidomyces*, *Cryptococcus*, *Histoplasma*, *Sporothrix* and *Paracoccidioidomyces*. Except for *Cryptococcus*, these fungi presuppose residence or visit in endemic areas, such as certain areas in North and Latin America.

The history of multiple antimicrobial regimens without any result along with the history of cellular immunosuppression of the patient was more suggestive of a fungal or mycobacterial infection (Christianson *et al.* 2003; Lafleur *et al.* 2004). In this case report, we present a rare case of a composite lesion (papule, ulcer and lymphocutaneous pattern) of PCC. This is suggestive of the wide spectrum of lesions *Cryptococcus* can cause (papules, cellulitis, whitlow,

lymphocutaneous morphology, ulcer, pyoderma gangrenosum, chancre, discoid lupus erythematosus-like), even in the same patient (Miura *et al.*, 1971; Ng & Loo, 1993; Christianson *et al.*, 2003; Neuville *et al.*, 2003; Baumgarten *et al.*, 2004; Lafleur *et al.*, 2004; Jasch *et al.*, 2008; Posada *et al.*, 2009). The combination of a confined lesion in immunosuppressed patients, along with cellulitis, often leads to a delayed diagnosis of PCC and of recurrent trials of unsuccessful therapeutic regimens (Christianson *et al.*, 2003; Fallah *et al.*, 2011). In such cases, early culture, before empirical administration of antimicrobials, is of great importance.

As in the majority of the cases reported, patients with either PCC or systemic cryptococcosis have a cellular immunity defect. Corticosteroid use and post-transplant immune status have been identified as the most common causes, but other causes such as human immunodeficiency virus infection, malignancies or even a transient decrease in lymphocytes have also been identified (Ng & Loo, 1993; Christianson *et al.*, 2003; Neuville *et al.*, 2003; Baumgarten *et al.*, 2004; Lafleur *et al.*, 2004). However, there are reports of PCC in patients with intact immunity, a fact supporting the concept of a distinct clinical entity (Bellosta *et al.*, 1999; Lacaz Cda *et al.*, 2002; Revenga *et al.*, 2002). The significance of a positive blood antigen test in the setting of an apparent PCC is uncertain. It may indicate a serious skin infection, cryptococcal meningoencephalitis or an early manifestation of disseminated disease (Neuville *et al.*, 2003; Baumgarten *et al.*, 2004; Lafleur *et al.*, 2004). A positive antigen, however, should not exclude PCC (Hafner *et al.*, 2005). In this patient, the positive antigen titre, although low, posed a serious question whether the lesion was confined to the finger or was an early indicator of a systemic cryptococcal infection. Cutaneous lesions can be the only symptom and an early marker of disseminated disease. On the other hand, it is not unusual for PCC patients to have secondary antigenaemia due to severe local infection (Neuville *et al.*, 2003). Whether the natural history and risk of dissemination of PCC is the same in patients with positive or negative serum latex agglutination remains a question (Christianson *et al.*, 2003). Therefore, it was extremely important for this patient to have regular follow-up examinations during a 10-month period to exclude the possibility of developing systemic or disseminated disease (Neuville *et al.*, 2003; Ng & Loo, 1993; Sarosi *et al.*, 1971). In the case of persistent antigen titre, an unapparent colonization site should be excluded (Hafner *et al.*, 2005).

Considering the fact that our patient did not exhibit any systemic involvement such as lymphadenopathy or fever, as well as the fact that the chest CT scan was negative for pulmonary disease, a lumbar puncture and a CT scan of the head were not considered necessary. The incidental finding of a mass on the right kidney, in conjunction with the positive blood antigen, was highly suggestive of renal cryptococcoma. Surprisingly, the histological investigation revealed clear cell renal cell carcinoma. There have been

laboratory data on the kidney carcinoma ability to depress cellular immunity function secondary to its by-products (Das *et al.*, 2008a, b; Biswas *et al.*, 2009). As a result, two factors of cellular immunity dysfunction were identified in this patient; an iatrogenic one and a neoplastic one. In most reports of either PCC or skin manifestations of systemic cryptococcosis where only one immunosuppressive factor was identified, histological findings were consistent with granulomatous inflammation (Ng & Loo, 1993; Baumgarten *et al.*, 2004; Neuville *et al.*, 2003; Hafner *et al.*, 2005). This case is remarkable in that no evidence of systemic or disseminated disease was detected, despite the coexistence of two factors of cellular immunity dysfunction.

The Infectious Diseases Society of America guidelines for cryptococcal disease do not refer to confined skin disease in immunocompromised patients (Perfect *et al.*, 2010). Because of his medical history of chronic iatrogenic immunosuppression, the patient was prescribed 200 mg fluconazole twice daily, indefinitely. However, he stopped this treatment on his own after only 5 weeks, fortunately without consequences. There are reports stating discontinuation of corticosteroid treatment during cryptococcal infections (Anderson *et al.*, 1992). We preferred not to do so, considering a possible flare of myasthenia gravis (Lafleur *et al.*, 2004). Follow-up at 2, 5 and 8 weeks showed obvious granulation tissue in the base of the ulcer and epitheliopoiesis of the lesion, a sign of clinical improvement.

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