New Microbiologica, 43, 2, 99-102, 2020, ISN 1121-7138

CASE REPORT

Cryptococcosis in an HIV-negative, HCV positive, immunosenescent patient: a case report

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SUMMARY

Cryptococcus species is still a very common opportunistic infection in AIDS patients. However, it is increasingly responsible for disease in otherwise immunocompromised individuals, such as transplant recipients and the heterogeneous group of patients with underlying immunologic diseases, hematologic disorders and organ failure syndromes.
Clinical presentation, prognosis, and outcomes are difficult to define given these varied host groups, and tailoring treatments to fit the necessities of each patient is likewise challenging.
Our patient was on treatment with steroids and direct-acting antiviral agents (DAAs) for a chronic HCV-related hepatitis, worsened by cryoglobulinemia, membranoproliferative glomerulonephritis and a low-grade B cells lymphoma.
We report a case of systemic cryptococcal infection in an immunosenescent, HIV-negative patient.

Received July 24, 2019 *Accepted* January 5, 2020

INTRODUCTION

Cryptococcus neoformans is a ubiquitous fungal pathogen that causes human disease ranging from asymptomatic colonization of the lungs to severe meningitis and cryptococcosis, which is a systemic fungal infection generally found in association with acquired immunodeficiency syndrome (AIDS) (Haddow *et al.*, 2010; Musubire *et al.*, 2018). This agent is also increasingly responsible for disease in HIV-negative populations (Pappas *et al.*, 2001). The annual incidence of cryptococcosis in HIV-negative patients is estimated at 0.2 to 0.9 per 100,000, depending on the geographical area studied (Hajjeh *et al.*, 1999). These non–HIV-infected individuals include patients who are receiving immunosuppressive agents, transplant recipients, and the heterogeneous group of patients with underlying disorders such as innate immunologic diseases, hematologic disorders, and organ failure syndromes. Moreover, in many centres, up to 20% of cases of cryptococcosis occur in phenotypically "normal" or otherwise clinically non-immunocompromised patients (Pappas *et al.*, 2001). Specifically, *Cryptococcus gattii* is more commonly identified in this type of immunocompetent population, whereas *Cryptococcus neoformans* is more involved in infections of immunocompromised patients (Harris *et al.*, 2011) .

Key words:
Cryptococcosis, immunosenescence, HCV, multifactorial immunosuppression.

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Clinical presentation, prognosis, and outcomes are difficult to define given these varied host groups, and no treatment regimen fitting all patients exists (Perfect *et al.*, 2010). A recent review sought relevant and recent research findings to develop treatment recommendations non-HIV immunocompromised patients. Clinical decision-making depends on the type of host, immunocompromised state, antifungal response and presence of neurological complications (Henao-martı and Chastain, 2018).
Because treatments are associated with toxicities and high cost, we need further studies and more affordable drugs for this selected population.
We report here a case of *Cryptococcus neoformans* as a cause of cryptococcosis in an HIV-negative, HCV positive and immunocompromised patient.

CASE REPORT

A 78-year-old female living in a rural area in Lombardy, Italy, was admitted to our Infectious disease ward from home because of fever, marked asthenia and a moderate headache. Drowsiness and disorientation had been present for about a week. She also had a purpuric rash involving the trunk, back and lower limbs bilaterally (*Figure 1*).
Her medical history revealed a hepatitis C (genotype 2, stiffness 10.3 kPa) virus infection. The patient was also suffering from cryoglobulinaemic type I (IgM/k) vasculitis (cryocrite 35%) with skin involvement and glomerulonephritis. She was undergoing treatment with prednisone 25 mg a day for these three conditions. She had also been diagnosed with a low-grade B-cell lymphoma (CD20+, CD79a+, CD5-, CD23-, Bcl-1-, CD10-) four years before. At

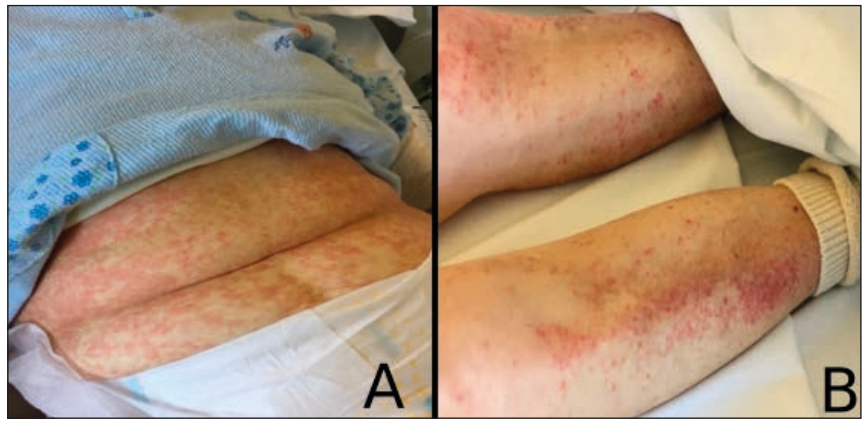

Figure 1 - *Purpuric rash involving the trunk (1-A) and lower limbs bilaterally (1-B) at the time of admission.*

the time of admission, she was in the sixth of an 8-week treatment with Glecaprevir/Pibrentasvir 300/120 mg and HCV-RNA had been undetectable after 4 weeks of treatment.

At admission the patient was tachycardic (CF 110 bpm) with a blood pressure of 155/55 mmHg and a normal respiratory rate. On physical examination, she was disoriented and drowsy. Chest and abdominal examination were normal. The patient also presented with abdominal pain, vomiting and decreased faecal output.

A chest X-Ray did not show any parenchymal lesion. Blood tests showed a mild increase of C-reactive protein and procalcitonin without significant leucocytosis. In addition, she had an acute on chronic renal failure (creatinine 3,26 mg/dl) with considerable hyperuricemia (acid uric 22 mg/dl), electrolyte imbalances and metabolic acidosis (pH 7.28-HCO3 9.2 mmol/L). She was treated with volume expansion and high dose steroids plus administration of rasburicase for the reduction of hyperuricemia. The patient was seen by a nephrologist and haematologist who excluded the comorbidities as causes of the hyperuricemia.

Samples for blood cultures were drawn at admission and returned positive for *E. coli* after 77 hours and *Cryptococcus neoformans* with a growth time of 130 hours. Colonies grew on Sabouraud Dextrose Agar after 24h of incubation. Macroscopic and microscopic examinations of fungal isolation were performed. Colonies were cream-coloured smooth, mucoid and yeast-like; microscopic examination, using india ink preparation, showed wide gelatinous capsule. Identification of *Cryptococcus neoformans* was obtained using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) from Bruker Daltonics (Bremen, Germany), after direct transfer method according to the Bruker Daltonics protocol. Molecular characterization was performed on isolate. Molecular type and mating type of the isolate were identified by multiplex PCRs as previously described (Esposto *et al.*, 2004). Strains H99 (VNI, alphaA), JEC21 (VNIV, alphaD), and CBS132 (VNIII, alphaADa) were used as reference strains. *C. neoformans* antigen had been found positive in a serum sample with a titre of 1/400.

Since cryptococcosis was proven, a CD4 count was performed, which revealed immunodeficiency with 107/ul CD4 and a CD4/CD8 ratio of 1 (total lymphocytes 553 cells/ul). The patient was HIV negative, and HCV-RNA, HBV-DNA, HSV-1 and HSV-2 DNA were not detectable in peripheral blood. However, a mild positivity for EBV-DNA (3510 copies/ml; positive if >90) and CMV-DNA (2610 copies/ml; positive if > 90) was found.

CSF examination was performed and showed high levels of glucose and proteins but no cellularity, while cultural examination was negative; specifically, no evidence of *Cryptococcus neoformans* antigen was found in the CSF.

Fluconazole 300 mg *bid* and Cefotaxime 1 g every eight hours was started immediately. However, following a significant increase in transaminases (GOT 1172.0 mU/L), after a week of treatment, fluconazole was replaced with Amphotericin B, while Glecaprevir/Pibrentasvir was discontinued at week 7.

Since blood test for assessing the presence of cryoglobulinaemia was positive (cryocite 10% with no consumption of C3), we diagnosed the purpuric rash as a cryoglobulinaemic vasculitis.

An abdominal CT scan was performed and showed a partial obstruction of the large bowel, particularly of the splenic flexure and descending colon, and gastrectasy. These findings were suggestive of ischemic colitis, but, given the progressive worsening of her clinical condition, other investigations were deferred.

An MRI of the brain performed to evaluate a fluctuation of mental status showed no vasculitic lesions but a posterior reversible encephalopathy syndrome (PRES).

The patient was then transferred to the ICU, where she died.

We reported the case as a possible adverse event during antiviral therapy with Glecaprevir/Pibrentasvir, as Italian laws mandate that all major infections need to be reported as adverse events if DAAs are being administered to the patient.

DISCUSSION

Systemic cryptococcosis and cryptococcal meningitis are still regarded as infections typically striking HIV-positive patients with AIDS.

In our opinion, our patient suffered from multifactorial immunosuppression. This is a typical feature of cryptococcal infections in HIV-negative patients (Pandit *et al.*, 2006). This state is also suggested by the detection of CMV and EBV replication (Limaye *et al.*, 2008), as well as by her low CD4+ count. Reports of immunocompetent patients suffering from AIDS-associated infections have been published (Harris *et al.*, 2012, Zhu *et al.*, 2016).

The patient was on long-term treatment with steroids for cryoglobulinemia and a related membranoproliferative glomerulonephritis causing chronic kidney disease. These

are well known extra-hepatic manifestations of HCV infection, due to the lymphotropism of HCV (Negro *et al.*, 2015). The patient also had an underlying haematological condition, low-grade B-cell lymphoma, which is also significantly associated with chronic HCV infection (Su *et al.*, Younossi *et al.*, 2016). While cryptococcosis is significantly associated with non-Hodgkin lymphomas, severe cryptococcal infection usually strikes patients undergoing chemotherapy or with aggressive forms of the disease (Chen *et al.*, 2018; Schmalzle *et al.*, 2016; Vigouroux *et al.*, 2000).

It is now known that HCV eradication by antiviral treatment is not only beneficial in preventing the development of extrahepatic manifestations, but also in improving many disorders caused by HCV infection. Antiviral therapy was limited, until recently, by the many side effects and contraindications of interferon-based treatment. Currently, the availability of Interferon-free regimens solves this issue, allowing for enhanced safety and efficacy to provide universal treatment of HCV-related extrahepatic manifestations (Degasperi *et al.*, 2017).

In a cohort study, Gragnani *et al.* showed that treatment induced HCV eradication led to a sustained disappearance of mixed cryoglobulinemia in nearly all patients (97%), with a complete resolution of its signs and symptoms in 56% (Gragnani *et al.*, 2015). It should however be noted that many patients in this cohort were not cirrhotic.

It is likely because of these effects on extrahepatic morbidity that patients with sustained virologic response were found to have a reduced liver-unrelated mortality (Innes *et al.*, 2015). However, despite the successful treatment and undetectable HCV-RNA and the fact that no sign of advanced cirrhosis was present, our patient developed a clear exacerbation of the extrahepatic manifestations.

The patient also developed a severe cryptococcal infection. Lee and colleagues reported the first case of cryptococcal meningitis in a non-cirrhotic patient with chronic HCV infection on pegylated (PEG)-interferon-alfa and ribavirin (Tae Hee *et al.*, 2014). In our case, the whole case course was suggestive of a double immune reconstitution syndrome due to the sudden clearance of both HCV and *Cryptococcus*. This is most typical of HIV-infected patients or patients having received a solid organ transplant (Haddow *et al.*, 2010).

Although various adverse events have been reported during combination therapy with pegylated (PEG)-interferon-α and ribavirin, opportunistic infections, especially cryptococcal meningitis, are rare (Okanoue *et al.*, 1996). We found no previous report of Cryptococcosis in patients treated with DAAs. However, a recent study found that cases of other opportunistic infections, including AIDS defining infections, have been reported in patients HIV/HCV co-infected patients treated with DAAs (Macías *et al.*, 2019). It should be noted that in this study all patients were undergoing ART for a period of time long enough to exclude paradoxical IRIS due to ART initiation. These patients had shown a complete suppression of HIV viremia (Macías *et al.*, 2019). With regard to the specific safety of Glecaprevir/pibrentasvir (Maviret), the most commonly reported adverse reactions (incidence ≥ 10%) are headache and fatigue. The European Commission granted a marketing authorization valid throughout the European Union for this drug on 26 July 2017.

Given the recent approval, long-term monitoring of patients undergoing this treatment is necessary, and reporting suspected adverse reactions is important to allow continued monitoring of its cost/benefit. We are aware that the topic is somewhat controversial, and we felt that the reporting of our case could contribute to the debate.

For these reasons and in observance of Italian law, we reported the case as a possible adverse reaction to Glecaprevir/pibrentasvir.

Most of the available information on the treatment of cryptococcal infections comes from studies conducted on HIV-positive patients. Combined antifungal therapy with amphotericin B plus flucytosine improves survival among patients with cryptococcal meningitis (Day *et al.*, 2013).

A recent open-label, randomized, and multicentre trial on HIV-infected adults with cryptococcal meningitis in a resource-limited setting concluded that one week of amphotericin B plus flucytosine and 2 weeks of fluconazole plus flucytosine were also effective as induction therapy for cryptococcal meningitis (Molloy *et al.*, 2018)

Although the 2010 IDSA guidelines recommend therapeutic strategies for HIV negative patients with cryptococcosis, few studies are available to support this recommendation (Garelnabi and May, 2018).

Multifactorial immunosuppression can be as severe as that of AIDS and justifies an index of suspicion for opportunistic infections such as cryptococcosis, but its best treatment in HIV-negative patients remains to be determined.

Acknowledgements

The authors are grateful to Dr Sam Goblirsch for reviewing the manuscript and for helpful criticism.

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