Cryptococcus neoformans associated death in a non-HIV patient

Simin Aisel Florescu1,2, Emanoil Ceausu1,2,*, Corneliu Petru Popescu1,2, Elena Nediu2, Maria Nica1,2, Petronela Ionescu2, Sebastian Smadu2, Daniel Codreanu2, Petre Iacob Calistru1,2

Abstract: Background: In the past 20 years, fungal infections have become more frequent in human pathology because of the rise in the number of patients with immune deficiencies (HIV infections, neoplasm, immuno-suppressive drugs, etc). The biggest influence in the epidemiology of C. neoformans infection in the past 40 years is closely tied to the emergence and evolution of HIV infections on a global scale.

Methods: In “Dr. Victor Babes” Clinical Hospital for Infectious and Tropical Diseases, Bucharest, in the last 10 years were recorded 27 cases of cryptococcal disease in HIV positive patients, and only 3 cases in HIV negative patients. In this article is present a case of C. neoformans meningitis in a HIV negative patient with severe cellular immunity deficiency of an unknown cause.

Results: Multiple laboratory tests were performed on a patient with meningoencephalitis diagnosis at the time of hospital admission. The most important was cerebrospinal fluid analysis, particularly microscopic examination after sediment staining with India ink, leading to visualization of transparent and colourless spots on a black background, suggestive for Cryptococcus spp. Antigen test for the detection of Cryptococcus neoformans in the cerebrospinal fluid was positive. HIV serology was negative and the bacterial etiology was also excluded. Cellular mediated immunity evaluation showed a severe immunosuppression but the cellular immunity deficit cause remained unidentified. Despite the etiological treatment performed according to the antifungigram for 36 days, the patient’s condition progressively altered, dying at day 50 of hospitalization.

Key Words: cryptococcal meningitis, mortality, HIV negative.

INTRODUCTION

In the past 20 years, fungal infections have become more frequent in human pathology because of the rise in the number of patients with immune deficiencies (HIV infections, neoplasm, immuno-suppressive drugs, etc).

The most common fungi associated with human pathologies are Candida spp, Aspergillus spp, Fusarium spp, Mucor spp and Cryptococcus spp. The asexual fungus Cryptococcus neoformans has been classified on the basis of its polyzaharide capsule – glucuronoxylomannan (GXM) into four serotypes A through D [1]. Cryptococcus spp is widely spread worldwide and it is found in the highest concentrations in pigeon and chicken excrements, bird guano. Even so, the infection can be found in humans without any prior contact with birds [2]. The biggest influence in the epidemiology of C. neoformans infection in the past 40 years is closely tied to the emergence and evolution of HIV infections on a global scale. Epidemiological data suggests that in Sub-Saharan Africa, C. neoformans is responsible for more than half a million deaths annually, exceeding tuberculosis related mortality [3].

In France, the Pasteur Institute published in 2015 the results of the Crypto A/D study on 230 HIV positive and negative patients [4]. The results showed that the number of cases of Cryptococcus neoformans infections in HIV positive patients has dropped after reaching a peak during the HIV epidemic between the years 1994 to
1996, while the number of cases in HIV negative patients has maintained constant, as shown in Figure 1.

![Figure 1](image_url)

**Figure 1.** Epidemiological evolution of cases of infection with C. neoformans [4].

In humans, Cryptococcus neoformans presence ranges from an asymptomatic colonization of the pulmonary tissue to a life-threatening disease (meningitis or Cryptococcosis, also known as cryptococcal disease). The disease rarely appear in immunocompetent individuals, as the most frequent cases are present in people with immune deficiencies, especially in HIV infected patients with severe immunodepression and a fall of CD4 count under 200/mm³ [5].

Before the HIV epidemic, cryptococcal disease was found in patient with sarcoidosis, oncological diseases, other cellular mediated immunity deficiencies or glucocorticoid treatments [6]. In HIV negative patients diagnosed with cryptococcal disease, from 10% to 40% do not present an apparent immunity deficiency. It is possible that subtle defects at a lymphocyte cell level could explain the appearance of the disease in apparently immunocompetent hosts [6].

An explanation might be that C. neoformans serotypes B and C can cause infections in immunocompetent hosts, opposed to C. neoformans serotypes A and D which cause infections predominantly in immunocompromised patients [7].

The motives for which the infection appears in apparently immunocompetent hosts have not yet been found. Studies conducted in Australia have shown a higher frequency of cryptococcal disease in the aboriginal population [8] than in the white population, while in Los Angeles the incidence has been reported to be twice as frequent in the Hispanic population as in the white population [9].

In contrast to HIV-infected individuals, some studies identified a highly activated antigen-presenting dendritic cell population within cerebrospinal fluid (CSF), accompanied by a highly active T-lymphocyte population with potentially damaging inflammatory cytokine responses. Elevated levels of CSF neurofilament light chains (NFL), a marker of axonal damage in severe central nervous system infections suggest a dysfunctional role to this acute inflammatory state. CSF macrophage proportions were reduced in patients with severe disease and biopsy and autopsy samples identified alternatively activated tissue macrophage populations that failed to appropriately phagocytose fungal cells [10].

The clinical signs and symptoms of C. neoformans vary in accordance with the site of the infection, along with usual infection signs as fever, weight loss, night sweats. The most frequent sites of infection are meningeal, followed by pulmonary infections. Most patients present with neurological manifestations that can vary from headaches to the alteration of mental status. Pulmonary cryptococcal disease presents with cough and minimal expectorations, pleuritic typical pain, and in very rare cases hemoptyis [11].

The diagnosis of cryptococcal meningitis is done through the examination of cerebrospinal fluid (CSF). Glucose levels are usually low, associated with a rise in protein levels. Giemsa stain examination shows pleocitosis with predominance in lymphocytes. Cryptococcus antigen testing can be positive, but the diagnosis is set through the examination of sediment with India Ink for the visualization of the encapsulated fungal elements. Microscopic examinations show round elements, capsulated, isolated or in groups, transparent and colorless on a black background, suggestive for Cryptococcus spp. Cultures present like cream coloured colonies, opaque, some creamy and some mucus-like, with a characteristic aspect of fungal colonies in the Cryptococcus spp. genus. The identification of fungal colonies is done through cultures swabs with Gram and Giemsa stain which present with round capsulated elements, with a honeycomb-like pattern. Biochemical identification is done through the VITEK2C method, or through mass spectrometry technique (MALDI-TOF). Quantitative antifungigram, with CMI levels, can be done with automated VITEK2C system using AST-YSO7 cards or through the E-test technique on RPMI 1620 medium [12].

Until the 1970s, the standard therapy for Cryptococcal meningitis was amphotericin B, later associated with 5-flucitosine which was found to be superior to the amphotericin B monotherapy. A qualitative leap of treatment was recorded with the introduction into therapy of liposomal Amphotericin B, currently considered the gold standard for C neoformans infection [13-15].

Other therapeutic options are Voriconazole, Posaconazole and Fluconazole, but Voriconazole and Posaconazole appear to be more potent and susceptible than Fluconazole [16].

The experience of treatment and current therapy of cryptococcal disease is based on evaluated antifungal
activity in HIV positive patients. Imidazole derivatives suppressive therapy is also recommended for HIV positive patients. Even so, there is no consensus regarding the duration of treatment in HIV negative patients.

Associated pathologies, organ failure and age over 60 years old are associated with a rise in mortality rates in HIV negative patients.

In a study performed in Taiwan between 2001 and 2009 on 88 patients diagnosed with cryptococcal disease, the mortality rate in the HIV negative group composed of 51 (88%) patients was 33% [17].

In “Dr. Victor Babes” Clinical Hospital for Infectious and Tropical Diseases, Bucharest, in the last 10 years we registered 27 cases of cryptococcal disease in HIV positive patients, and only 3 cases in HIV negative patients.

We are presenting a case of C. neoformans meningitis in a HIV negative patient with severe cellular immunity deficiency of an unknown cause.

**Case History**

Forty-one years old male patient from Bucharest hospitalized at “Dr. Victor Babes” Clinical Hospital for Infectious and Tropical Diseases in the middle of October 2016 for nausea, vomiting and mental status alteration. The diagnosis of admission was assumed to be meningoencephalitis.

The disease onset was slow, insidious, 14 days prior to hospitalization, with intermittent headaches and progressive neurological deterioration, bradypsychia and bradylalia, tendency of aggressiveness. The patient had prior consultations in emergency rooms in Bucharest, where because of a prior diagnosis of mixed depressive-anxious syndrome in current treatment with Paroxetinum, the new symptoms were considered to be part of an exacerbation of the psychiatric disorder. The appearance of a fever syndrome in the middle of October 2016, documented at an Emergency Room department, as well as the increase in severity of the nausea and vomiting symptoms led to the patient’s transfer to our clinic. The patient had no history of cerebral trauma, convulsions, chronic cough, weight loss, intravenous drug use, blood transfusions or personal or professional behaviour associated with transmissible diseases. Family denied a history of tuberculosis, other chronic diseases or use of immunosuppressive drugs of any form.

Clinical examination at admission: patient was febrile (38.1 degrees Celsius), altered sensorium, subtle neck stiffness, positive Kerning and Brudzinski, symmetrical pupils, aphasia. Examination of the rest of the systems appeared to be normal.

Laboratory work indicated leukocytosis (18.950/ mm³) with neutrophilia (87.31%), normal serum electrolytes, renal function and liver function. Serological testing for HIV, HTLV, HBV, and HCV were negative.

Because of the suspected diagnosis of meningoencephalitis, the patient underwent a lumbar puncture and the cerebrospinal fluid was sent to the laboratory for analysis. The biochemical examination showed an albumin level of 1.254 g/L, chloride of 6.8 g/L, glucose level of 0.25 g/L.

Fuchs – Rosenthal chamber element count showed 200 elements/mm³.

Sediment coloration with India Ink stain highlighted fungal capsulated elements. Microscopic examination showed round elements, capsulated, isolated and in groups, transparent and colorless on a black background suggestive for Cryptococcus spp (Fig. 2).

Sediment examination with Giemsa stain highlighted 200 elements with the following formula: 70% mononuclear cells and 30% polymorph nuclear neutrophils and frequent round capsulated elements. (Fig. 3).

Gram stain did not show any Gram +/- cocci or bacilli, only capsulated round elements. Ziehl-Neelson stain examination did not highlight the presence of any bacilli. These examinations were performed with the

![Figure 2. Microscopic examination – China stain.](image1)

![Figure 3. Giemsa stained smear - LCR sediment.](image2)
specific reason of excluding any bacterial etiology.

Antigen test for the detection of Cryptococcus neoformans in the cerebrospinal fluid was positive.

Inoculation of CSF sediment was done on solid plates (BHI agar, Columbia Agar, Chocolate Agar, Sabouraud Agar). At 24 and 48 hour, all plates showed cultures of cream, opaque, some mucous colonies with the characteristic look of Cryptococcus spp genus colonies.

Bacterial cultures were negative at 5 days, suggesting an exclusive fungal etiology.

The identification of fungal colonies began with Gram and Giemsa coloured stain smears, which revealed round, encapsulated formations in the "honeycomb". Identification based on biochemical features was performed in VITEK2C automated system with YST cards. The mass spectrometry technique (MALDI-TOF) identified the same species of Cryptococcus neoformans as in the VITEK2C system. Quantitative antifungigram, with CMI values, was performed in VITEK2C automated system with AST-YSO7 cards and through the E-test technique on RPMI 1620 medium. The isolated Cryptococcus strain presented resistance to Fluconazole and susceptibility to Voriconazole.

For the exclusion of tuberculosis etiology and for a quick result, a Real-time Polymerase Chain Reaction (PCR) test was done on cerebrospinal fluid for the detection of M. tuberculosis deoxyribonucleic acid (DNA), with negative result.

Cellular mediated immunity evaluation showed a severe immunosuppression with a T lymphocyte level of 374/mm³, without a humoral immunity deficiency (B lymphocyte 285/mm³). CD4 T Lymphocyte count was 118/mm³ with a CD4/CD8 ratio of 0.59. To exclude an HIV infection in a serological window, a RT-PCR HIV was performed, with negative results.

The cellular immunity deficit cause remained unidentified.

Magnetic Resonance Imaging (MRI) brain studies showed inflammatory and vasculitis lesions without any other pathological findings.

From the moment of hospitalization, and after the positive Indian Ink stain, the patient receives treatment with Fluconazole as well as corticoids and supportive medication. After culture results and antifungigram results that showed resistance to Fluconazole, the treatment was switched to Voriconazole.

Despite the etiological treatment performed according to the antifungigram for 36 days, the patient’s condition progressively altered, dying at day 50 of hospitalization.

At the post mortem examination, the leptomeninges was infiltrated with white-yellow deposits as well as the cerebral hemispheres, and hematoxylin eosin (HE) stain microscopic evaluation showed round elements with a double contouring, suggestive for C. neoformans (Figs 4 to 6).

Thoracic and abdominal cavity examination showed multiple lymph nodes with fungal micro-abscesses, as well as splenic micro-abscesses. Pulmonary tissue presented with fungal abscesses that appeared pseudo-tumoral with dimensions ranging from 5 to 8 centimeters, yellow in colour (Figs 7 and 8).
Pulmonary microscopic examination showed fungal fragments in the alveolar and pulmonary tissue (Figs 9 and 10).

The cause of death was disseminated cryptococcal disease in an immunosuppressed patient of an unknown cause.

**DISCUSSIONS**

The rise in incidence of fungal infections in the past years makes this specific pathology an important diagnosis and treatment problem. In the last years we have faced a rise in the frequency of fungal diseases, predominantly because of the rise in the number of patients with associated immunosuppression – HIV patients, patients with oncological diseases, patients on immunosuppressive medications, etc.

Most common immunodeficiencies are of known causes, such as HIV infections, oncological diseases or suppressive drug treatments, but also of unknown causes. These unknown causes could be explained through the lack of diagnosis due to the limits of medical laboratories which usually use routine tests, or through the emergence of new immunity related diseases yet to be discovered which could have an infectious diseases etiology.

The restoring of the immune function is very important for the positive evolution of the disease. If in the case of patient with a known HIV infection, the antiretroviral treatment restores in time the immune system which along with the etiological treatment of the disease can help the clinical evolution in a positive way, in the case of an immune deficiency of unknown cause the clinical evolution can be unfavourable due to the persistence of the main cause of the diseases.

Although building an infrastructure for the report of fungal infections frequency, diagnosis methods, comorbidities and treatment methods would be difficult to implement, it would involve global interest in the elaboration of clear recommendations for the diagnosis and treatment.

Literature data for the treatment of C. neoformans are based on reviews of the disease in HIV infected individuals, and there is lack of therapeutically guidelines for the treatment of non HIV infected patient.
Studies performed until now show that the immune response differs in HIV negative patients than HIV positive patients, and this should be closely investigated for the development of future treatment guidelines.

CONCLUSIONS

Most cases of Cryptococcus meningitis and cryptococcal disease appear in HIV positive patients, but they can also develop in cases of non-HIV related severe immunosuppression.

Even with etiological treatment, the infection is difficult to control.

The antifungal agents recommended for the treatment of C. neoformans have different actions on the pathogen itself. In cases of a severe immunosuppression the disease can disseminate with unfavourable outcomes.

Conflict of interest. The authors declare that there is no conflict of interest.

References