CLINICAL CASE OF CRYPTOCOCCAL MENINGITIS IN A LIVER TRANSPLANT PATIENT

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ABCTRACT

The incidence of infections caused by Cryptococcus neoformans has increased significantly in recent years, especially in the settings of immune deficiency (HIV infection transplantation, etc.). Most often after inhalation of spores dissemination of yeast to the brain parenchyma occurs, leading to meningitis (meningo-encephalitis). Our clinical case, is a patient with cryptococcal meningitis after liver transplantation, who died despite the onset of antifungal therapy. This is further evidence of the severe prognosis of CNS cryptococcosis, especially in immunocompromised patients

Keywords: *meningitis, Cryptococcus neoformans, immunosuppression*

INTRODUCTION

The incidence of infections caused by Cryptococcus neoformans has increased significantly in recent years, especially in immunocompromised patients (14). Among these patients, the most common are HIV-positive and organ transplant recipients. The appearance of infections at a late stage after organ transplantation indicates a probable exogenous origin (inhalation of basidiospores or from untreated locus in sites with special blood circulation (1).

Scientific research has shown that in a large percentage of cases cryptococcal meningitis occurs more than 6 months post-transplantation as a result of immunosuppressive treatment against an acute graft rejection.

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Depending on the immunological status of the host acute dissemination with brain organotropism - infection of subarachnoidal space may occur prior to an invasion of brain parenchyma (meningo-encephalitis), (1). Clinically, it is most often described with headache, increased intracranial pressure, basal discharge symptoms, nausea, vomiting, a positive symptom of Kernig and Brudzinski.

Inadequate diagnosis, lack of microbiological examination, and consequently, r untimely antifungal therapy can lead to increased intracranial pressure and fatal result.

MATERIALS AND METHODS

Our clinical case is a 71 years old man of, diagnosed with hepatitis B in 2004, subsequently complicated by liver cirrhosis. On this occasion, in 2016 the patient was subjected to liver transplantation.

A broncho-alveolar lavage (BAL) sample was received at the National Reference Laboratory of Mycoses (National Center of Infectious and Parasitic Diseases) for detection of medically important fungi, with the observation of pulmonary mycosis. The microbiological culture from BAL was negative, but microscopy detected a lot of leukocytes.

A serum sample was tested alongside for the presence of antibodies to yeasts of the genus Candida and molds of the genus Aspergillus by indirect immunofluorescence (IIF), and for Cryptococcus antigen by latex – agglutination test (3).

The patient was transferred to the Clinic of Nervous Diseases with suspected meningitis, and on this occasion another clinical sample was received at the National Reference Laboratory - cerebrospinal fluid, again for fungal testing. A late - agglutination test was performed, which is a rapid test for detection of Candida and Cryptococcus antigens (2; 6) .

The cerebrospinal fluid was also cultured for bacteria and fungi detection. The strains were identified by biochemical tests and microscopy and the antifungal susceptibility was determined.

Serological testing with indirect immunofluorescence (IIF) did not show antibodies specific for fungi of the genus Candida and Aspergillus (Ig G 1:40 at a rate of up to 1: 160, IgA-negative, IgM-negative and Aspergillus-negative).

However, the latex–agglutination test for Cryptococcus antigens turned out positive (Fig.1). This is a qualitative

test for the detection of polysaccharide antigens (glucurono-xylomanan is the main component of Cryptococcus capsule) using latex particles loaded with monoclonall antibodies (7).

Fig.1 *Positive latex agglutination test*

The cultures were negative for bacrerial pathogen. On the universal culture medium for fungi Sabouraud dextrose agar a pure culture of white to cream-colored yeast and mucoid colonies were isolated in a significant amount, (Fig.2).

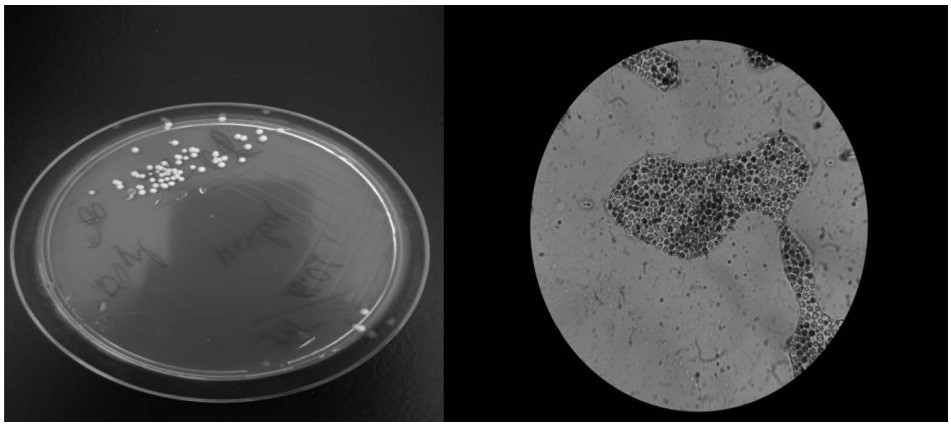

Fig.2 - *Macroscopic and microscopic view of cryptococci*

Single oval and budding yeast cells were visualized on a microscope slide (Fig. 2) .
The fungi from the pure culture were identified as Cryptococcus neoformans by Auxacolor biochemical identification test (Fig .3).

Fig.3 - *Biochemical identification of yeast*

Cryptococci were also confirmed as urease-positive by an urease activity test (Fig .3).

The strain was further tested for sensitivity to several antifungals using the so-called. E - test and disco-diffusion test. The results are as follows: Fluconazole-S, Itraconazole-S, Voriconazole-S, Miconazole-S, Nystatin-S, Anidulafungin-R, Caspofungin R. Cryptococci are less sensitive to echinocandins due to the lower amount of target (β D-glucan) in their cell wall, but have been shown to be sensitive to azoles and polyenes.

On our recommendation, a serum sample was also tested for antibodies to Cryptococcus neoformans, and proved negative (3).

The recommended treatment scheme (SANFORD guide) for Cryptococcus neoformans includes three types of antifungals - Fluconazole, Amphotericin B and Flucytosine , followed by single use of Fluconazole (Amphotericin B and Flucytosine are not available on our market), (12; 13), (Table 1) .

Table 1. *Recommendations for therapy of SANFORD guide, USA*

Cryptococcosis (meningitis)	- **Liposomal Amphotericin B**(L-AmpB) 3-4 mg/kg iv q24h or - **Amphotericin B lipid complex**(ABLC) 5 mg/kg iv q 24h+Fluconazole 25 mg/kg po q6h -also in combination with :**Fluconazole** 400-800 mg po/ day/8 weeks	- **Liposomal Amphotericin B**(L-AmpB) 3-4 mg/kg iv q24h or **Amphotericin B lipid complex** 5 mg/kg iv q 24h or **Amphotericin B** 0.7-1 mg/kg iv q24h+**Fluconazole** 800-1200mg/day iv/po/2 weeks **- Liposomal Amphotericin B**3-4 mg/kg iv q24h or **Amphotericin B lipid complex** 5 mg/kg iv q 24h or **Amphotericin B** 0.7-1 mg/kg iv q24h/4-6 weeks -**Fluconazole** 800-1200/day iv/ po+**Flucytosine** 25 mg/kg po q6h/4-6 weeks -**Fluconazole** 1200-2000 mg po/ day/10-12 weeks

The patient was treated with Fluconazole i.v.-a loading dose of 800 mg, with supporting dose of 400 mg. According to the attending physician and relatives, his condition was improving as he became contact and conscious. However, a control puncture and cerebrospinal fluid examination were not performed, because a few days later the exitus letalis was reached.

DISSCUSSION

Infections caused by Cryptococcus spp are reported worldwide, including the United States and Europe.A patient with HIV-positive status and cryptococcal meningitis was also reported in Egypt (18). An association with eucalyptus trees has been demonstrated, but they can be isolated from various environmental locations, including birds.

In the recent years in Europe, infections caused by Cryptococcus neoformans Cryptococcus gatii and Cryptococcus deuterogatii have been on the rise (especially in HIV-positive patients),(17). In Spain, a case of cerebral cryptococcus has been described in an immunocompromised patient (19). Cryptococcal meningitis has also been reported in HIV-negative patients, but with other risk factors such as organ transplantation and chemotherapy. The patient in our clinical case is after an organ liver transplantion.

The culture study with isolation of yeast in pure culture once again proves that the previous latex-agglutination test is the best laboratory method, with great reliability in the diagnosis of cryptococcosis of the CNS. The test is highly sensitive and specific, and gives positive reaction even at very low microbial counts of cryptococci in the cerebrospinal fluid (1; 8).

According to EORTS (European Organization for Research and Treatment of Cancer) / MSGERC (Mycoses Study Group Education and Research Consortium), this serological test is accepted as criterion for proven invasive fungal disease (IFD), ie.

cryptococcal meningitis can be reliably diagnosed by antigen testing (Table 2), (16). Microscopic detection of encapsulated yeast should not be neglected, either.

Table 2.- *Criteria for "proven" IFD*

Type of fungus	Microscopic analysis	Cultural examination of sterile clinical material	Blood culture	Serological method	DNA
Yeast Molds	Histopathological, cyto pathological examination of biopsy material or direct microscopic examination in which yeast, pseudohyphae, true hyphae of biopsy material are visualized	Isolation of fungal strain from clinical material from the site of infection, with the exception of BAL,urine, paranasal sinus or mastoid sinus secretion	When positive for Candida, Cryptococcus Trichosporon, mold and others	Not applicable, except for detection of Cryptococcus antigen in cerebrospinal fluid, which confirms the diagnosis	Amplification of fungal DNA in combination with DNA sequencing

The symptoms of cryptococcal meningitis (headache, fever and fatigue) are not typical enough and are often confused with tuberculosis (20). Therefore, a fungal cause should be always considered. Cryptococcosis is one of the leading causes of illness and death in severely immunocompromised individuals. Timely application of antifungal therapy is vital in order to increase the chances for favorable outcome.

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