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# *Cryptococcus neoformans* meningoencephalitis in a patient with polyarteritis nodosa

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**Abstract** Case of 59-year-old male with chronic obstructive pulmonary disease and a number of comorbidities, who has developed meningoencephalitis caused by *Cryptococcus neoformans* var. *grubii* with polyarteritis nodosa diagnosed during hospitalization, was presented. Before evidence of meningoencephalitis, the patient was being treated with ketoconazole and low doses of fluconazole (200 mg/day) for alleged candidiasis. The dosage was increased (800 mg/day) following laboratory diagnosis of *C. neoformans* based on positive latex agglutination test and biochemical identification of encapsulated yeast isolated from the blood and CSF. Later, the yeast identification was confirmed by sequencing analysis. Owing to inadequate clinical response, fluconazole therapy was switched to voriconazole (400 mg/day) and later to intravenous amphotericin B (1.0 mg/kg per day). Despite of a temporary stabilization and improvement, which correlated with decline of cryptococcal antigen titers (from 1:1024 to 1:8), after 6 weeks, the patient's underlying condition deteriorated due to severe pancolitis and serious nosocomial bacterial infections. The patient died of multiorgan failure several days later. Our case demonstrates a possible connection between the development of life-threatening cryptococcosis and an autoimmune vasculitis disease and emphasizes that the outcome of the management of cryptococcal meningoencephalitis is highly dependent on early diagnosis, adequate treatment, including dosage, and last but not least control of underlying disease and risk factors.

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**Abbreviations**

| | |
|---|---|
| CSF | Cerebrospinal fluid |
| HIV | Human immunodeficiency virus |
| CSLI | Clinical and Laboratory Standards Institute |
| COPD | Chronic obstructive pulmonary disease |
| PCR | Polymerase chain reaction |
| CT | Computed tomography |
| MIC | Minimal inhibitory concentration |
| ESBL | Extended-spectrum beta-lactamase |
| CRP | C-reactive protein |

## Introduction

Cryptococcosis is an opportunistic fungal infection caused by encapsulated yeasts of *Cryptococcus* species with an increased affinity to the brain. There are five original serotypes of *C. neoformans* today associated with two species:

*C. neoformans* is serotype A, D, and AD; and *C. gattii* is serotype B and C (Ikeda et al. 1982). HIV-positive patients are mostly affected, and *C. neoformans*, especially subspecies *C. neoformans* var. *grubii* (serotype A), represents main etiology with over 600,000 casualties annually (Park et al. 2009). However, this mycosis also occurs in non-HIV persons with or without apparent predisposition and immunodeficiency such as organ transplant recipients or patients with metabolic disorders (cirrhosis, diabetes mellitus, renal insufficiency). In the case of immunocompetent patients, *C. gattii* is usually responsible for cryptococcosis (Pappas 2013). The cryptococcosis usually initiates in the lungs after inhalation of yeasts often with no pulmonary involvement and continues to spread hematogenously to the brain, where meningeal forms develop. The source of infection is the external environment, typically soil contaminated with bird droppings. But there is an additional internal reservoir—the prostate which may play role in some patients in relapse or reactivation of the mycosis as a result of immunosuppression (Larsen et al. 1989; Tortorano et al. 1997).

The diagnosis is based on microbiological examination of cerebrospinal fluid (CSF) and other biological materials because of low specificity of most clinical signs and symptoms. India ink staining of CSF sediment usually provides quick and relatively reliable information about a possible cryptococcal etiology based on visualization of capsule. The standard diagnostic method is detection of cryptococcal polysaccharide antigen by latex agglutination test or enzyme immunoassay. Amphotericin B and flucytosine in combination remain the drug of choice in the therapy of cryptococcal meningoencephalitis (Perfect et al. 2010; Day et al. 2013).

A case of cryptococcal meningoencephalitis is presented in a non-HIV patient whose treatment was complicated by a lack of optimal antifungal therapy and a number of underlying conditions, including polyarteritis nodosa as a probable primary predisposition to this infection.

## Methods and the patient

### The strains

*Susceptibility testing* The *C. neoformans* isolates were obtained during hospitalization from liquor, blood, tracheal aspirate, and urine and were identified according to morphological (slimy colonies, encapsulated spherical blastospores) and biochemical features (urease production, ID 32C bioMérieux). Antifungal susceptibility testing (amphotericin B, ketoconazole, fluconazole, voriconazole, flucytosine) was performed by disk diffusion test (ITEST discs, Czech Republic), microdilution broth method, and Etest (bioMérieux, Czech Republic) following CSLI standards (M44-A, M27-A) and instructions of manufacturer (Clinical and Laboratory Standards Institute 2002; 2004). Mueller-Hinton agar was used in both of the agar diffusion tests (disk test, Etest). Additionally, two isolates were tested by Sensititre YeastOne (Trek Diagnostics).

*Molecular analysis* DNA was extracted from the CSF yeast isolate using QIAamp® DNA Mini Kit (Qiagen) protocol, and rRNA intergenic spacer region was amplified using PCR (McTaggart et al. 2011). The PCR products were cleaned and used in sequencing PCR reaction utilizing the primers as above and BigDye Term kit 3.1. Sequence analysis was performed on capillary sequencer ABI3130. *Cryptococcus* species was then identified based on the highest sequence similarity obtained when using BLAST® search at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

This result was confirmed based on the size of a PCR product recovered after amplification of the putative sugar transporter gene (Feng et al. 2013). The resulting products differ in the length (bp) according to the species of *Cryptococcus*: *C. neoformans* var. *grubii* 274 bp, *C. neoformans* var. *neoformans* 224 bp, and *Cryptococcus gattii* 170 bp.

### The patient

Fifty-nine-year-old man who was treated for many comorbidities including chronic cholecystitis and chronic obstructive pulmonary disease (COPD). He was non-smoker, regularly drinking 0.5 to 1.0 L of beer per day. In childhood, he was diagnosed with epilepsy, permanently taking antiepileptics (phenytoin, primidone) and later omeprazole for ulcerative duodenal disease. The first complains were preceded by myotonia for esophageal achalasia and operations of varices.

## Case report and discussion

### Case report

In the summer of 2004, the patient was examined owing to nonspecific problems (febrile, weakness, shortness of breath), which spontaneously normalized. The same year shingles of right half chest and neck developed. In August 2005, skin form of vasculitis was diagnosed, which spontaneously faded away. In early 2006, he was hospitalized for relapsing dyspnea, general weakness, and constipation. COPD II according to GOLD stages was diagnosed. Despite the negativity of some rheumatologic parameters (ANCA antibodies, ANA, ENA screening), other laboratory data (eosinophilia, high β2-microglobulin—8.87 mg/L, positive antibodies against leukocytes and thrombocytes) and clinical findings (splenomegaly, chronic inflammation of the duodenum) suggested a nonspecific chronic condition of connective tissue. The

patient was treated with β2-mimetics and low-dose corticosteroid (methylprednisolone, 16–20 mg/day). Additionally, the PCR positivity for *Pneumocystis jiroveci* from bronchoalveolar lavage fluid was reported, and the patient was treated with trimethoprim/sulfamethoxazole with good effect.

In the first half of 2007, the patient was repeatedly hospitalized owing to anorexia, weight loss, and low-grade fever. In May 2007, leucopenia ($2.5\times10^9$/L) and anemia (hemoglobin 95 g/L) were diagnosed. Based on clinical picture and microbiological findings (*Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Branhamella catharalis* in the sputum; *Candida albicans* from the oral cavity), antibiotic therapy was started (ciprofloxacin, and clarithromycin, later changed to cefuroxime axetil), including ketoconazole (400 mg p.o. per day). Lumbar puncture revealed pleocytosis with prevailing lymphocytes. Suspicion of aseptic meningitis syndrome was aroused. Repeated blood cultures were negative except a yeast (non-*albicans Candida*) not identified to species-level. The patient was transferred to the university hospital (May 30, 2007) and antiinfective therapy was switched to G-crystalline penicillin, acyclovir, and fluconazole (200 mg/day). The patient's condition improved temporarily, but several days later, the short-term loss of consciousness developed, which was considered an attack of grand mal seizure. Control CSF examination showed persistent pleocytosis with 75 % of polymorphonuclear leukocytes. Meantime, yeast from CSF was identified as *C. neoformans*. The patient status aggravated. He had meningeal syndromes with a slowing psychomotor activity and a marked loss of weight (11 kg; 15 % drop in last 6 months) but was cardiopulmonary stable and afebrile with no relevant bronchoscopy finding. Laboratory showed elevated CRP (37), mineral imbalance, and leucopenia ($2.13\times10^9$/L) with anemia (hemoglobin 95 g/L). *C. neoformans* was repeatedly isolated from the blood and CSF. Fluconazole was increased to 800 mg i.v. per day, and antibiotic treatment reflected bacterial findings and their clinical relevance. The patient was serologically negative for HIV 1 and 2, and PCR testing did not detect any relevant pathogen (*Mycobacterium*, *Pneumocystis*, and *Legionella*). Abdominal pain with diarrhea led to a CT examination and coloscopy which revealed pancolitis with the colon and cecum involvement. *Clostridium difficile* toxin was borderline positive. Oral antibiotics were discontinued, metronidazole was introduced, and patient was switched to total parenteral nutrition. Laboratory findings suggested the progressive development of renal insufficiency, coagulopathy, and anemia. On June 6, the patient improved hematologically according to CSF examination but microscopy as well as culture remained *Cryptococcus* positive. In addition, biopsy revealed invasion of the large intestine with cryptococci (Fig. 1). On the other hand, antigenemia declined considerably during the first weeks when antigen titer dropped from 1:1,024 to 1:128. Due to suboptimal MIC values of fluconazole (8 mg/L) and inadequate clinical response, antifungal therapy was switched to intravenous voriconazole (200 mg b.i.d.) on June 12.

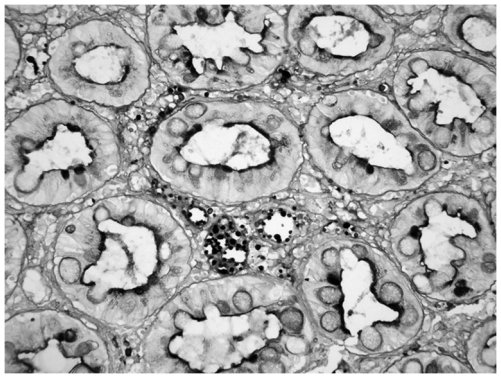

**Fig. 1** Cryptococcosis of the large bowel. Colonic mucosa with normal architecture and mild chronic inflammatory infiltrate. Small clusters of yeast cells are seen in interstitial tissue among crypts. Grocott methenamine-silver stain, magnification ×400

Despite intensive care, the patient's condition gradually deteriorated, especially due to acute respiratory distress syndrome, pneumothorax, and abdominal parameters. Coagulopathy and anemia required repeated administrations of blood transfer, plasma, and later plateletpheresis and the treatment with G-CSF (filgrastim). On June 13, he underwent colectomy with terminal ileostomia owing to a severe pancolitis and purulent peritonitis. Histological examination of bioptic samples revealed no cryptococci, but polyarteritis nodosa was diagnosed.

The patient obtained ventilatory and catecholamine support. Opportunistic bacteria (*P. aeruginosa*, *Klebsiella pneumoniae* ESBL positive) and herpes viruses (CMV) caused severe infections. On June 19, lumbar puncture showed a stationary hematological finding even when cryptococcal capsular antigen titer continued to decline to 1:8. On June 29, pulmonary bleeding had to be solved by selective embolization. Later, *Cryptococcus* antigen titer in CSF started to increase (to 1:128) and voriconazole was switched to conventional amphotericin B (1.0 mg/kg per day). Patients' status was further complicated by repeated nosocomial infections and, eventually, septic shock with multiorgan failure led to death.

## Causative agent

*C. neoformans* var. *grubii* was identified by DNA sequence analysis (identification probability of 100 %). This result was confirmed by analysis of PCR product size (274 bp) of tested strain by a singleplex PCR assay.

Susceptibility testing to five antifungal drugs provided a good agreement among used methods: all strains were susceptible to amphotericin B, voriconazole, ketoconazole, but intermediate to fluconazole. The results of flucytosine testing were dependent on the method used especially on the

composition of media (Rex et al. 1993). According to agar diffusion tests (disk test, Etest) and microbroth dilution ones, the strains were resistant and susceptible, respectively (Table 1). The results of Sensititre YeastOne showed general resistance to all echinocandins and, in accordance with the results of microdilution broth test, good susceptibility to other antifungal drugs, including itraconazole, posaconazole, and voriconazole (Table 1).

## Discussion

The cryptococcosis caused by *C. neoformans* in non-HIV patients is relatively rare and it is usually associated with *C. gattii*. These patients consist of two main groups at risk—organ transplant recipients and non-HIV, non-transplant patients (Pappas 2013). The latter are largely associated with Hodgkin lymphoma under immunosuppressive therapy (corticosteroids), patients with metabolic disorders (liver cirrhosis, diabetes mellitus, renal insufficiency) and autoimmune diseases (Pagano et al. 2004; Walker and Warnatz 2006; Kiertiburanakul et al. 2006; Chuang et al. 2008; Pappas 2013). There are several reports on cryptococcosis in systemic lupus erythematosus (Liou et al. 2003; Chen et al. 2007), rheumatoid arthritis (Hage et al. 2003; Moosbrugger et al. 2008), and Sjögren's syndrome (Schattner et al. 2004), but only few cases of cryptococcosis are mentioned as related to polyarteritis nodosa (Hiss et al. 1988; Bordin et al. 1996; Chávez-López et al. 2006; Riera-Mestre et al. 2006; Benešová et al. 2007). Usually, the elderly patients treated with corticosteroids for underlying vasculitis disease were predominantly affected. Unfortunately, in our patient, polyarteritis was diagnosed at the advanced stage and repeated severe opportunistic infections hampered effective immunosuppressive therapy. Patient's history suggested that the first problems of a systemic disease may have started in the period of 2004–2005, when he developed shingles and suspected cutaneous vasculitis. Approximately the same time, COPD developed which in association with early administration of corticosteroids could facilitate acquisition of cryptococci as suggested by some previous reports (Dupreval et al. 1977). As regards vasculitis, that was not confirmed histologically, and thus an impetigo-like form of herpes zoster cannot be excluded. Herpes infections are often related to a decreased cell-mediated immunity and could also explain the elevated levels of β2-microglobulin. This was suggested as a marker of herpes virus infection in differential diagnosis of aseptic viral meningitis (Peterslund et al. 1989; Takahashi et al. 1999). High values of β2-microglobulin were also reported in patients with autoimmune disease (Sjögren's syndrome) and idiopathic lymphocytopenia, who had cryptococcosis (Salit et al. 2007; Schattner et al. 2004). In particular, patients with idiopathic lymphocytopenia are often affected by both meningeal cryptococcosis and herpes virus infections (Zonios et al. 2007). Some authors pointed out a possible relationship between autoimmunity (polyarteritis) and this form of lymphocytopenia (Bordin et al 1996). This corresponds with the course of disease in our patient even if there was a lack of a detailed laboratory data on the numbers of CD4+lymphocytes

**Table 1** Susceptibility of *Cryptococcus neoformans* isolates to antifungal drugs by different methods

| | | Amphotericin B | | Flucytosine | | Fluconazole | | Voriconazole | | Ketoconazole | | Itraconazole | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h |
| Disk test[b,c] (*n*=8) | GM | 16.2 | 15.8 | 6.0 | 6.0 | 22.8 | 15.3 | 32.8 | 24.3 | 36.3 | 35.7 | | |
| | SD | 0.83 | 1.12 | 0.0 | 0.0 | 4.18 | 4.17 | 1.45 | 2.18 | 2.45 | 3.33 | | |
| Etest[b,c] (*n*=8) | GM | 0.38 | 0.42 | 32 | 32 | 7.7 | 13.7 | 0.10 | 0.15 | 0.21 | 0.27 | | |
| | SD | 0.0 | 0.058 | 0.0 | 0.0 | 0.66 | 2.83 | 0.052 | 0.095 | 0.071 | 0.095 | | |
| Microdilution broth[a,b,d] (*n*=9) | GM | 0.125 | 0.25 | 3.4 | 3.4 | 7.4 | 8.0 | 0.125 | 0.25 | 0.5 | 0.5 | | |
| | SD | 0 | 0 | 0.83 | 0.83 | 1.26 | 2.95 | 0.0 | 0.0 | 0.0 | 0.0 | | |
| Microdilution broth[e] | | | | | | | | | | | | | |
| CRN-csf (*n*=1) | MIC | 0.25 | 0.25 | 4 | 8 | 4 | 8 | 0.06 | 0.06 | | | 0.03 | 0.06 |
| CRN-blood (*n*=1) | MIC | 0.25 | 0.25 | 4 | 8 | 8 | 8 | 0.06 | 0.12 | | | 0.06 | 0.12 |

*GM* geometric mean, *SD* standard deviation, *CRN Cryptococcus neoformans*, *CSF* cerebrospinal fluid

[a] MIC of amphotericin B and other drugs was read as 95 and 80 % decrease of optical density in contrast to control well, respectively

[b] *C. neoformans* isolates from CSF (*n*=5), blood (*n*=2), tracheal aspirate (*n*=1); in microdilution broth test plus urine isolate (*n*=1)

[c] Diameter (mm) of inhibition zone and MIC (mg/L) in disk test and Etest, respectively

[d] Microdilution broth test according to M27-A CSLI standard, MIC (mg/L)

[e] Sensititre YeastOne (Trek Diagnostics), not shown results of echinocandins (anidulafungin, micafungin, caspofungin) with all MICs >8 mg/L and posaconazole MIC=0.06 and 0.12 for CRN-csf and CRN-blood, respectively

in the blood. However, the absolute blood number of lymphocytes repeatedly showed a significant leucopenia from late 2006, accompanied by transient periods of neutropenia.

In general, CD4+ T lymphocytes play a key role in the management of intracellular infections, including those caused by fungal agents such as dimorphic fungi and cryptococci (Shoham and Levitz 2005; Walker and Warnatz 2006). The patients with cryptococcosis can also have a changed cytokine profile (Netea et al. 2004; Zonios et al. 2007). The example is TNF-α factor, the levels of this proinflammatory cytokine are often lower in patients with cryptococcal infection either as result of pathological process or a specific therapy of autoimmune disease with monoclonal antibodies (e.g., infliximab, adalimumab) (Murphy et al. 1997; Hage et al. 2003; Jarvis et al. 2008). On the other hand, cryptococci developed a number of defense mechanisms to resist or to escape host immunity, most of them connected with capsular polysaccharides (glucuronoxylomannan) and melanization of cell wall (Liu et al. 2012). Due to propensity of cryptococci to CNS, therapeutic regimens are influenced by a limited ability of most of antifungal drug to cross the blood-brain barrier. Despite of a relatively poor penetration, amphotericin B (0.7–1.0 mg/kg per day) in combination with flucytosine (100 mg/day orally in four divided doses) remains a drug of choice in the induction treatment for cryptococcal meningitis (Day et al. 2013). If flucytosine is unavailable, amphotericin B alone or combined with fluconazole (400–800 mg/day) are considered the second line therapy. The use of high-dose fluconazole is an alternative to these combination regimens, especially in non-HIV patients (Saag et al. 2000; Pappas et al. 2001; Yao et al. 2005). Recent clinical studies suggested the need to increase dosage to 1,200–1,600 mg of fluconazole per day and, if possible, to add flucytosine (Day et al. 2013; Loyse et al. 2013). The study results are supported with latest experimental pharmacokinetics and pharmacodynamics data of fluconazole (Sudan et al. 2013). Echinocandins have no relevant effect on cryptococci due to absence of glucans in their cell wall; itraconazole is reserved for the maintenance and consolidation therapy (Saag et al. 2000; Shao et al. 2007). Therapeutic effect of the new triazoles, including voriconazole, remains to be confirmed, but the results of individual case studies are promising (Bandettini et al. 2009; Shao et al. 2007). However, it is important to stress that most of treatment recommendations result from the studies performed in HIV-positive patients. There is lack of relevant clinical data about the therapy of non-HIV patients. Especially the subgroup of non-HIV/non-transplant recipients is very heterogeneous one, and only case reports are typically available. Underlying conditions (immunosuppression, metabolic disorders) together with problematic yeast clearance from the CNS make therapy of cryptococcosis difficult (Day et al 2013). Frequency of secondary antifungal resistance is relatively low and is mainly associated with AIDS patients (Cheong and McCormack 2013). Cross-resistance to voriconazole and fluconazole is rare in contrast to fluconazole and itraconazole (Pfaller et al. 2005). Our isolates showed consistent susceptibility to all antifungals tested; the values of minimal inhibitory concentration (MIC) were in a narrow range except for fluconazole MICs that varied around the value interpreted as just susceptible for *Candida* species (MIC=8 mg/L) (Rex et al. 2001). However, this criterion is a bit vague because current standard methods do not specify interpretive breakpoints for cryptococci (Rex and Pfaller 2002) and, in addition, current breakpoints for susceptibility to fluconazole and voriconazole of individual *Candida* species are lower compared with previous ones (CSLI 2012). The use of in vitro test results for prediction of clinical outcome remains controversial, even if some studies suggested trend toward a better clinical outcome in patients infected with cryptococcal strains showing lower MICs (≤4–8 mg/L) (Menichetti et al. 1996; Witt et al. 1996; Jessup et al. 1998; Aller et al. 2000; Dannaoui et al. 2006).

The management of our patient was primarily influenced by late diagnosis of cryptococcosis, delayed, and inadequate antifungal therapy complicated with predisposing factors such as COPD, vasculitis, and previous long-term use of corticosteroids (16–20 mg/day). Early ketoconazole and low-dosage fluconazole therapy was inadequate for the treatment of cryptococcosis, especially if we consider that our patient was taking omeprazole which can affect bioavailability of ketoconazole through increasing stomach pH (Chin et al. 1995). As flucytosine is not registered in the Czech Republic, high dose fluconazole alone was used as an initial therapy (Pappas et al. 2001). Even if the patient's status gradually improved in response to increased fluconazole dose (800 mg/day), fluconazole was switched to voriconazole after 3 weeks because of persisting meningeal signs and aggravation of underlying conditions. Despite of a significant decrease of antigenemia (1:1,024 to 1:8), cryptococci were not eradicated completely and cryptococcal antigen titers started to increase again. It was evident that control of underlying disease failed, and the patient eventually died of serious complications associated with multiorgan failures.

Cryptococcosis in the Czech Republic is a rare fungal infection because of low prevalence of HIV infection especially in the regions outside of Prague (Benešová et al. 2007). On the other hand, owing to high mortality associated with cryptococcal meningoencephalitis, attention should be paid to non-HIV patients at risk of the mycosis (transplant recipients, lymphoproliferative and metabolic disorders, autoimmune diseases, immunosuppressive regimens). As soon as patient begins to show signs and symptoms of meningeal involvement, cryptococcal etiology must be considered and laboratory examination of CSF is necessary (antigen detection, culture, microscopy, PCR). If positive for cryptococci, immediate initiation of aggressive antifungal therapy (preferentially combination of amphotericin B and flucytosine) with adequate

dosing under monitoring of susceptibility and control of underlying conditions has to be applied.

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**Conflicts of interests** All authors declare that they have no conflict of interest.

**Ethical standards** All authors declare that the experiments comply with the current laws of the country in which they were performed.

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