Case presentation

Systemic infections due to fluconazole resistant Cryptococcus neoformans

Infecție sistemică cu Cryptococcus neoformans rezistent la fluconazol

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Abstract

A case of systemic infection with Fluconazole resistant Cryptococcus neoformans is presented in this article, with an emphasis on the case management difficulties and a short review of data from the literature regarding this issue.

Keywords : HIV/AIDS, Cryptococcus neoformans, heteroresistance

Rezumat

Prezentăm în acest articol un caz de infecție sistemică cu Cryptococcus neoformans rezistent la Fluconazol, cu accent pe dificultățile de management al cazului, dar și cu trecerea în revistă a unor date din literatura de specialitate privind această problematică.

Cuvinte cheie: HIV/SIDA, Cryptococcus neoformans, heterorezistență

The patient P.V., male, aged 24, residing in Țicleni/Gorj county, diagnosed with HIV infection in 2004, noncompliant to medical recommendations regarding HIV monitoring and treatment, having severe immunosuppression (last known CD_4 count = 8 cells/mm³), having discontinued antiretroviral therapy on his own initiative, was hospitalized between 2 - 15 March 2011 at the County Hospital Targu-Jiu for fever, vomiting, stiff neck, dry cough and bilateral purulent otorrhea. The patient refused the lumbar puncture and was empirically treated with Ceftriaxone 4 g/day under which he became afebrile after 72 hours. At the request of the patient, for further investigation, he was transferred to the Clinical Infectious Diseases Hospital from Craiova on March 15. Physical examination revealed the presence of an eruption on the face of the patient (papules with an

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Photo 1. A. Aspects of cutaneous cryptococcosis with bacterial superinfection (October 2011); B. Aspects after the treatment with Voriconazole (December 2011)

ulcerated center, suggestive of cutaneous cryptococcosis) and intense neck stiffness. The patient accepted the lumbar puncture, the exploration of the cerebrospinal fluid (CSF) revealing a nonpyogenic hypertensive cerebrospinal fluid, with 1225 nucleate cellular elements/mm³ (including yeasts, blood elements being entirely mononuclear cells), normal proteinorrachia, hypoglycorrachia and frequent fungal vegetative formations visible on China ink stained smear (microscopic suggestive for *Cryptococcus neoformans*).

The germ was microbiologically identified based on the following criteria: round unicellular encapsulated germ of 15-20 microns in diameter, with no sporulation or filamentation, China ink stained, which grows in 72 hours on Sabouraud media (white to cream coloured opaque colony having several millimeters in diameter); a urease positive test has been performed outside the hospital. Melanin formation and serotype were not determined.

Full development of CSF changes is presented in *Appendix 1*.

Because the hospital could not provide the required antifungal treatment the patient was transferred to the "V. Babes" Hospital for Infectious and Tropical Diseases – Bucharest. Here the patient was hospitalized during March 16 to October 13. The CSF examination was emphasized once again the pathogenic fungus (both microscopy and culture were positive). The etiological treatment was initially performed with Fluconazole 800 mg/day (in accordance with the current guidelines (1- 3), but also with the susceptibility phenotype of the yeast) until July 6, 2011.

However the evolution was unfavorable, the facial papules (in wich the presence of *Cryptococcus neoformans* was demonstrated by skin biopsy) grew larger, became confluent, showed a necrotic character with secondary bacterial superinfection – see photo 1A -(methicillin-resistant *Staphylococcus aureus* and *Corynebacterium striatum* were isolated by culture); concurrently, the meningeal clinical syndrome did not improve while in the CSF the fungal vegetative elements persisted. *Staphylococcus aureus* from the skin infection spread and affected the right eye (*Photo 1*).

On July 5, 2011 two fungal strains (both belonging to *Cryptococcus neoformans* species, but having two different susceptibility patterns to antifungals) were found by culture. For the second strain an assessment of minimum inhibitory concentration (MIC) was made, which revealed the following: Amphotericin B - susceptible (MIC = 0.19 microgram/ml), Flucytosine - susceptible (MIC \leq 1 mg/ml), Flucytosine - highly resistant (MIC > 256 mg/ml), Voriconazole - susceptible (MIC = 0.047 mg/ml).

Date	CD ₄ count (cells/mm ³)	CD ₈ count (cells/mm ³)	CD ₄ / CD ₈ ratio			
March 22	10	105	0,04			
April 28	21	494	0,04			
June 28	122	938	0,04			
September 01	21	99	0,04			

Table 1. Evolution of CD₄ and CD₈ lymphocytes counts after antiretroviral treatment re-runs

From July 7, 2011 treatment with Voriconazole i.v. 200 mg bid was initiated under which the meningeal syndrome resolved and the number of CSF fungal vegetative formations began to decline. High cost of antifungal medication and financial difficulties led to cessation of Voriconazole administration on August 7 and the retaking of Fluconazole therapy (heteroresistance may be reversible, however it is not clear how much time is needed for the process to occur). Contextually, the meningeal clinical syndrome reappeared, the evolution of skin lesions worsened and elevated liver enzymes were detected (interpreted as Fluconazole drug-related liver toxicity, with further favorable evolution under conservative treatment). Voriconazole was restarted in September 9, but from October 13, although the patient's condition required continuation of this therapy, the hospital from Bucharest could not provide the necessary antifungal medication and the patient was transferred back to Craiova. It is to be noted that on March 30 antiretroviral therapy resumed with Abacavir + Lamivudine + boosted Lopinavir, the evolution of CD₄ and CD₈ lymphocytes counts being presented in Table 1.

In Craiova, due to administrative and financial efforts, the Voriconazole treatment continued. Considering the falling of the CD_4 count, an overlapping secondary opportunistic infection (masked by the systemic cryptococcosis) was suspected; chest X-ray showed a miliary aspect, while the computed tomography revealed pulmonary apical involvements and interlobular fissures thickenings suggestive for pulmonary tuberculosis. Sputum was negative for bK. Antimycobacterial regimen was started with Isoniazid + Pyrazinamide + Ethambutol + Ciprofloxacin, Rifampicin being excluded given its antagonism with the antifungal medication. A brain MRI examination ruled out intracranial expansive masses and the lumbar punctures performed on October 30 and November 25 showed a decrease in cellular elements (25, respectively 10/mm³) and did not reveal any fungal vegetative formations. Although a pulmonary superinfection with Pseudomonas aeruginosa had been diagnosed along with an episode of thoracic herpes zoster and the recurrence of hepatic cytolysis (probably due to the antimycobacterial regimen), the health condition of the patient markedly improved, allowing the discharge on December 5. Given the residence county possibilities for further antiretroviral therapy, Kivexa (combined Abacavir + Lamivudine) + Enfuvirtide regimen was chosen. Secondary prophylaxis of fungal infection with Voriconazole, antimycobacterial regimes for the next 6 months and specific monitoring will be continued.

Final diagnosis was as follows: 1. HIV infection clinical and immunological C3 category (AIDS); 2. Systemic infection with Fluconazole resistant *Cryptococcus neoformans;* 3. Miliary tuberculosis; 4. Pulmonary superinfection with *Pseudomonas aeruginosa;* 5. Cutaneous superinfection with methicillin-resistant *Staphylococcus aureus* and *Corynebacterium striatum;* 6. Right palpebral and ocular infection due to methicillin-resistant *Staphylococcus aureus;* 7. Thoracic herpes zoster; 8. Fluconazole and antimycobacterian drug-related liver toxicity.

The authors support the diagnosis of systemic infection due to *Cryptococcus neo-formans* based on detection of cutaneous and meningeal septic metastasis; several blood cultures performed both in Craiova and Bucharest were negative, however there is no doubt that blood spreading of the pathogen has indeed occurred during the illness.

Data from medical literature and discussion

Cryptococcus neoformans was isolated for the first time in 1950; before 1955 only 300 cases of infection with this fungus were documented (4), while currently over 950.000 infections/year are worldwide estimated, mostly associated with immunosuppression secondary to HIV infection (5). Ubiquitous, but most common in decaying vegetation or wood, this organism is spread on the ground by birds droppings (birds seem to have just the role of carriers, being resistant to infection due to higher body temperature than mammals) (5). Although there were no reports of human-to-human spread (6, 7), clusters of familial cases were found, probably through contact with a common source of infection; contextually, the brother-in-law of P.V. (also HIV positive) was diagnosed in the same period of time with meningitis due to Fluconazole resistant Cryptococcus neoformans; he, unfortunately, died in Bucharest in Octomber 2011. The germ is usually susceptible to Amphotericin B, Flucytosine, and triazoles; Cryptococcus gatii strains are significantly more resistant to Fluconazole compared with Cryptococcus neoformans strains (86% vs 46%) (8), the triazole antifungal activity being insignificant against other non-neoformans / non-gattii strains (9).

The mechanisms of Fluconazole resistance are not fully understood. The germ shows heteroresistance to this antifungal (defined as the presence of subsets of the population that grows up in the presence of Fluconazole), this feature being associated with germ virulence (10). Heteroresistance was also demonstrated on strains isolated before the emergence of triazole (1979), but, comparatively, the MIC was twice as high for species studied from 1990 to 2000 (10). Another mechanism by which the fungus adapts to increased concentrations of Fluconazole is chromosome duplication, particularly of chromosome 1 that contains two resistance genes: ERG11 - the target of triazoles, respectively AFR1 - encoding for the Fluconazole transporter (11). Note that regarding P.V. (but also his brother-in-law) no data on previous exposure to Fluconazole exists. That raises the question whether there are "more heteroresistant" strains compared to most of the isolates. In vitro heteroresistance can be reversed, either by growing the fungi on Fluconazole-free medium (thus excluding the selective pressure of the drug), or by increasing incubation temperature (heteroresistance is decreased at 35 °C, respectively abolished at 40 °C) (10, 12, 13); practical consequences would consist of limiting the use of Fluconazole (in order to limit the emergence and multiplication of heteroresistant subsets) and of raising of the body temperature as adjuvant treatment; also note that P.V was afebrile throughout the hospitalization in infectious disease clinics, which might have favored the emergence of heteroresistance. The Fluconazole antifungal activity against resistant strains is restored by combining it with Polymyxin B (14), but this drug is not available in our country. With the increasing number of cases treated with this triazole, resistant cases have been reported, their number, presently small, being on the rise (15-17). The case described is one of them and the treatment of the patient is based on Voriconazole considering the literature data and taking into account the fact that Amphotericin B is not available in Romania. A susceptibility study which involved the testing of over 27,000 fungal strains (out of which 10% were Cryptococcus neoformans) established that only 1% are in vitro resistant to Voriconazole (MIC > 4 mg/ml) and, more importantly, that 71% of Fluconazole-resistant strains are susceptible to

Item / Date	15 mar.	16 mar.	18 mar.	29 mar.	27 june	05 july	15 july	04 aug	12 aug.	17 aug.	30 aug.	08 sept.	03 oct.	30 oct.	25 nov.
Cells/mm ³	1225 (+yeasts)	1625 (+yeasts)	840 (+yeasts)	7	1350 (+yeasts)	1114	132	146 (+yeasts)	217	88	15 (+ yeasts)	135 (+ yeasts)	145 (+ yeasts)	25	10
Giemsa stained smear	100% Mo	Rare Mo	Rare Mo	-	100% Mo	85% Mo 15% PMN	95% Mo 5% PMN	Мо	60% PMN 40% Mo	Мо	-	Rare Mo	35% Mo 65% PMN	-	-
Pandy	-	+/-	+	++	++++	++++	++++	+++	++++	++++	++	+	++	-	-
Albumin(g/l)	0.2	0,2	0,3	0,99	0,5	1,7	3,1	0,6	2,9	1,8	0,7	0,6	0,8	0,2	0,2
Glucose (g/l)	0,15	0,12	0,23	0,73	0,25	0,25	0,25	0,25	0,11	0,34	0,25	0,25	0,25	0,25	0,35
Chlorate (g/l)	-		7,4	7,4	7,6	7,5	7,5	7,6	7,2	7,6	7,5	7,4	7,5	-	-
Methylen blue stained smear	++++													-	-
China ink stained smear		++++	++++	+	++++	++++	+	++	-	+	++	+++	++		
Culture	C.n.	<i>C.n.</i>	<i>C.n.</i>	<i>C.n.</i>	-	-	<i>C.n.</i>	<i>C.n.</i>	-	Neg	<i>C.n.</i>	C.n.	C.n.	Neg	Neg
Susceptibility	-	Flucyt-S, Fluco-S, AmfB-S	-	Flucyt-S, Fluco-S, AmfB-S	AmfB-S, Vorico- S, Itra-S, Posa-S, Fluco-S,	2 strains Strain 1 Flucyt-S, Fluco-S, AmfB-S Strain 2 Flucyt-S, Fluco-R, AmfB-S	-	AmfB-S, Fluco-S, Itra-S, Posa-S, Vorico-S	-	-	Vitek method Flucyt-S, AmfB-S, Fluco-I <u>ATB-</u> <u>Fungus</u> <u>method</u> Flucyt-S, Fluco-I, Vorico-S, Posa-S	2 strains Strain 1 Flucyt-S, Fluco-R, AmfB-S Strain 2 Flucyt-S, Fluco-S, AmfB-S	<u>Vitek</u> <u>method</u> Flucyt-S <u>E-test</u> <u>method</u> AmfB-S, Fluco-R, Vorico-S, Posa-S		
Ziehl Nielsen stained smear	Neg	Neg	Neg	Neg	Neg	Neg	-	-	Neg	Neg	Neg	-	Neg	Neg	Neg
Culture for bK	Neg	Neg	Neg	Neg	Neg	Neg	-	-	Neg	Neg	Neg	-	Neg	Neg	Neg
PCR-bK CSF	-	Neg	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 1 – The CSF evolution for the patient P.V.

Mo = mononuclears, PMN = poymorphonuclears, C.n. = Cryptococcus neoformans, AmfB = Amphotericin B, Flucyt = Flucytozine, Fluco = Fluconazole, Vorico = Voriconazole, Itra = Itraconazole, Posa = Posaconazole, S = susceptible, I = dose-dependent susceptible, R = resistent, bK = Koch bacillus, neg = negative

the second-generation triazole (18). Another study (over 3000 isolates tested) confirmed that, excepting Amphotericin B and Flucytosine, both Voriconazole and Posaconazole (secondgeneration triazoles) are active against Fluconazole-resistant strains (19). The ARTEMIS study (more than 11,000 strains tested between 1997-2007) showed high susceptibility to Voriconazole; it is also revealed that the percentage of Voriconazole-resistant strains remained relatively constant (less than 2%) (16).

The authors consider that a single type of *Cryptococcus neoformans* was involved and that we have observed the development of heteroresistance. Dual infections with different strains have been described (20), however it is highly improbable to be our case at least from two points of view: 1. while being hospitalized it was nearly impossible for the patient to get infected with another strain; 2. considering the possibility of simultaneous acquiring of two different strains before admission we have no explanation for the extremely long incubation of the second (Fluconazole resistant) strain. Still, the genotypic characterization of the strains was not available.

Concerning the patient P.V. CSF normalization as well as the healing of the skin lesions were finally obtained (see Photo 1B). For maintenance therapy, oral Voriconazole, 200 mg bid, was recommended, on the basis of a sole article which addressed this matter (21). No references were found regarding the duration of maintenance therapy, however the authors consider that it should be maintained until the disappearance of cryptococcal antigen in the CSF, combined with an increase of the CD₄ count above 200 cells/mm³.

At the end of February 2012 a CSF specimen were analzyed by "Victor Babes" Hospital – Bucharest using PCR technique, showing negative *Cryptococcus neoformans* DNA and the presence of 3 copies/ml of *Mycobacterium tuberculosis* DNA, thus confirming our supposition regarding the presence of tuberculosis (note that the patient already followed several months of antibacillary regimen). An ELISA test demonstrates that the cryptococcal antigen was still present in the CSF.

Conclusion

This case highlights the difficulties of treating Fluconazole resistant infections due to *Cryptococcus neoformans*. Particularity of the case resides in treating it with Voriconazole.

Abbreviations

CSF – cerebrospinal fluid MIC – minimum inhibitory concentration

Conflict of interest

The authors have nothing to declare.

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