



EPIDEMIOLOGIC AND THERAPEUTIC ACTUALITIES IN INVASIVE FUNGAL INFECTIONS

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Abstract. The invasive fungal infections (IFI) represent an increasing interest of the scientific world, 1.7 milliards of people being affected by the infection with fungi, some of them becoming invasive, with a highly rate of mortality even with specific treatment. The success of IFI treatment is provided by the time of initiation of the therapy and the type of the anti-fungal drug, taking into account the local epidemiologic data. We have retrospectively analyzed proven invasive fungal infections from the patients hospitalized in the "Prof. Dr. Matei Bals" INBI between January, 2010- April, 2016 analyzing the age, the gender, the status of HIV/NON-HIV, risk factors, clinical parameters, bio-markers use, fungi species identified by the Vitek® 2 technology, the initial anti-fungal therapy, the switch of the anti-fungal therapy, the survival. There were identified 79 positive samples with fungi, whereby in blood cultures and samples from the catheter head, *Candida albicans* represented the highest part of IFI with (43.63%=34 isolates), followed by *C. glabrata* (12.62% =10 isolates), *C. parapsilosis* (10.90% = 9 isolates), having the same percentage as *C. tropicalis* (10.90 % = 9 isolates) and other species of unidentified *Candida* (5.45% = 4 isolates) and rare species as *C. guilliermondii*, *C. krusei*, *C. kefyr*, *C. lipolytica*, *S. ciferii* representing (9.09%). One case of *Fusarium oxisporum* was isolated. *C. neoformans* was present in the cultures of spinal fluid in the ratio of 7.27%=6 isolates. Anti-fungal therapy was mostly initiated with fluconazole in a percentage of 58.33%, followed by voriconazole (22.22 %), caspofungin (13.88%), anidulafungin (5.55%) and switched accordingly with antifungal susceptibility obtained by Vitek® results in ratio of in 29 cases – a percentage of 37.14%. It is essential to know the local epidemiologic data in initiating the antifungal therapy and also to rapidly identify the pathogen agent. *C. albicans* is the ethiologic agent mostly involved in our study. Fluconazole is the first option in initiation of therapy.

Key words: Romania, invasive fungal infections (IFI), *C. albicans*, *C. glabrata*

Introduction

In the world 1.7 milliards of people are suffering annually of a fungal infection [1,2] part of them being invasive fungal infections (IFI), difficult to identify and treat, so called "hidden killers". Among the existent fungi species over 500 are with pathogen potential and the most frequent infections are determined by the type of *Candida spp.*, *Aspergillus spp.*, *Cryptococcus neoformans* and *Pneumocystis jirovecii*. Taking into consideration that the death rate is at the level of over 40% for the cases of invasive candidiasis [3], and in the invasive aspergillosis, the survival at 12 months is of 75% for the transplant of hematopoietic bone marrow

and 41% for the solid organ transplantation [4] also in cryptococcal meningitis the death rate is of 32% in conditions of anti-fungal therapy in co-association plus support with IFN gamma [5], the followed subject presents a great interest in the scientific world in finding some therapies for increasing the survival but also for diminishing factors of individual risk. Candidemia is on 4th place within the nosocomial septicemia processes at global level and represents the price of technology progression, medical invasiveness and also hand hygiene low compliance. The analysis published by Guinea et al. in 2014 [6] noted the fact that 5 species of *Candida* are responsible for 92% of candidemias. *C. albicans* represents the highest percentage of the candidemias. However there is a decrease at global level of 43.63% in the United States in *C. albicans* involvement and an increase of the involvement of *C. non-albicans* species. *C. parapsilosis* represents the 2nd place in Europe colonizing the hands

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of the staff and forming biofilm at the surface and in the catheters lumen. *C.glabrata* was identified more at young patients. *C.tropicalis* was present in candidemias at patients with cancer. *C.krusei* is present in a percentage of 3% according to the data published by Richardson & Lass Florl in 2008 [7], and of 2.63% (Guinea, 2014) [6]. *Fusarium oxisporon* is from the hyaline moulds family having septate hyphae, identified in post-transplant IFI that selects itself after the excess use of the azoles in prophylaxis. The species of *Candida spp.* changed in the last 2 decades. In the past *C. albicans* was the dominant pathogen, now this species still represents almost half of the blood cultures of the patients with fungal infections. *C.glabrata* becomes an important pathogen in North Europe, USA and Canada whereas *C.parapsilosis* is more present in South Europe, Asia and South America. The changes are due to the recommendations of treatment given by the susceptibility of azoles and echinocandins. The pathogen capacity varies as follows: *C.parapsilosis* and *C. krusei* are less virulent than *C. albicans*, *C.tropicalis* and *C.glabrata*. Other species that appear are *C. dubliniensis*, *C. lusitaniae*, *C.kefyr*, *C. guilliermondii* and are associated to some hosts or to some particular fields (e.g. *C. dubliniensis* is present at the patients infected with AIDS) [3]. An analysis of 6 years in Switzerland collected data during the period of time of 2004-2009 obtaining 1090 of blood cultures from which 675 (61% were *C.albicans*), 191 (17.5%) *C.glabrata*, 64 (5.4%) *C.tropicalis*, 59 (5.4%) *C.parapsilosis*, 33 (3%) *C.dubliniensis*, 22 (2%) *C.krusei* and 46 (4.2%) other species of *Candida* [8]. From 5201 patients in the analysis of Azie et al. in 2012 [9], within the project Prospective Alliance for Fungal Therapy (P.A.T.H.), the majority of the invasive candidemias benefited of the treatment with fluconazole, 1838 patients (48.3%), followed by the one with echinocandins 1293, (34%), amphotericin B deoxycholate 26 (0.7%) and voriconazole 75 (2%) and the invasive aspergillosis received treatment with voriconazole.

The American Company of Infectious Diseases in America (IDSA), in 2009, at the European Conference for Infections in leukemia (ECIL) in 2011 and the European Company of Microbiology and Infectious diseases (ESCMID) in 2012 worked out guides for the treatment of candidemia. All three companies presented consensus in recommendation of the echinocandins as first intention. The fluconazole is not recommended at first intention with exception of proven IFI with *C.parapsilosis*. The resistance at fluconazole in vitro in different parts of the world varies between 7-33%, the resistance at anidulafungin is at a level of 6.2%, from the species of *Candida* because of a mutation of FKS, and 30-35% from the isolated species were resistant to itraconazole and posaconazole and 15% were resistant to voriconazole. [10]

Materials and method

We analyzed the positive isolates from blood cultures and spinal fluid with fungi during the period of 2010-2016 within the microbiology laboratory at „Prof. Dr. Matei Bals INBI” of Bucharest identified by the

Vitek® self system and the data from the medical record regarding the age, gender, risk factors, paraclinical data, biomarkers use, type of initiated treatment and survival. It has been desired the current epidemiologic circumstances taking into account that there is no assessment of this kind of infections in Romanian and the correlation of the therapy used with the guides and data from international literature.

Results and discussions

Our data showed *C.albicans* representing the highest part of the IFI with 43.63%, followed by *C.glabrata* (12.62%), *C.parapsilosis* (10.90%), having the same incidence with *C.tropicalis* identified in blood cultures and *C.neoformans* (7.27%) identified in cephalorahidian liquid. Other species of *Candida* represented the difference until 100 %. We mention the presence of a case of *Fusarium oxisporum* identified at a patient with hepatic transplant realized at the Fundeni Institute. (Table I)

Species	Patients Lot (%)
<i>C. Albicans</i>	43.63
<i>C. Glabrata</i>	12.72
<i>C. Parapsilosis</i>	10.90
<i>C. Tropicalis</i>	10.90
<i>C. Neoformans</i>	7.27
<i>C. Spp</i>	5.45
Other ¹	9.09

¹ *C. lipolytica*, *S. Ciferii*, *F. Oxisporum*, *C. Guillemondii*

Tabel I. Fungal species isolated.

Regarding the initial antifungal treatment, the fluconazole represents the first option of treatment with 58.33%, followed by voriconazole 22.22%, caspofungin 13.88%, anidulafungin 5.55 %. Most of the charts were empiric initiated, on the base of the risk factors and infection signs considering a probable fungal infection. Lately, the result of the blood cultures guided the therapy according to susceptibility to antifungals. (Table II, Figure 1)

Initial antifungal treatment	Patients Lot (%)
Fluconazole	58.33
Voriconazole	22.22
Caspofungin	13.88
Anidulafungin	5.55

Tabel II. Initial antifungal treatment.

Discussions

Comparing the analysis realized by Guinea et al. in 2014 [6] where it is noted that 5 species of *Candida* are responsible for 92% of the candidemias, we can observe that 5 species are now present in the analysis

from INBI. *C.albicans* is decreasing at 43.63%, similar as in the United States and Spain, as well as is at global level. From the species of *C. non-albicans* there were identified *C.glabrata*, *C.parapsilosis*, *C.tropicalis*, *C.krusei* that together with *C.albicans* represented a percent of 89% being closed by the percent presented in the studies [6]. *C.glabrata*, that in the analysis presented by Richardson & Lass Florl [7] in 2008 was identified of 20% in America and 15% in Europe presented a smaller percentage, being however on the second place, in our analysis. This specie is resistant at fluconazole and is selected after the use of fluconazole in prophylaxis and therapy in community.

The studies show the presence of *C.parapsilosis* on the 2nd place in Europe but in our analysis it is on 3rd place closer to *C.glabrata*. *C. parapsilosis* colonizes the hands of the staff and forms biofilm on the surface and in the catheters lumen explaining this result, corroborated with the results obtained using the ATP bioluminescence technique (tri-phosphate adenosine) where we noted the deficiencies in the hands hygiene of the medical staff that by horizontal transmission contaminated the medical devices. It was observed by genotyping, noticing that the genotypes of *Candida* species from the hands of the medical staff are similar to those identified at patients. So, it is needed to take measures of control and hygiene of the hands of the medical staff as well as resources for hygiene products in hospitals. *C.tropicalis* was presented in candidemias at patients with cancer and, is situated on the 4th place. *C.krusei* was identified in one blood culture, in an inferior percent with the data published by Richardson&Lass Florl [7] in 2008, and Guinea in 2014 [6]. One case had concomitantly a triple implication of *C.kefyr* + *C.lusitaniae* + *C.parapsilosis*. Five cases had concomitantly double fungal infection *C.albicans* / *C. glabrata*, *Candida glabrata* / *Candida lipolytica*, *C.albicans* / *C.parapsilosis*. The study of the biofilm realized by the species of *Candida* showed that some species penetrates the endothelial wall, creating a space for the other specie [11]. *Fusarium oxisporum* was identified in an postransplant subject. [7].

Regarding the treatment, the fluconazole represents the most used anti-fungal in our study, followed by voriconazole and echinocandin. The results are closer with the analysis of P.A.T.H., having fluconazole the first option, followed by echinocandins. Amphotericin B was not used on our analysis because there is no longer registered in Romania.

ESCMID recommends the initiation with echinocandins in case of suspicion of candidemia and voriconazole in case of invasive aspergillosis until the receiving of blood culture results that became positive in 3-4 days.

From an analysis of a tertiary hospital on 162 cases, it is noted the fact that the identification of the fungi is realized in an interval of 2.2 +1.3 days with a variability between species (from 0.6–7.9 days) and time until the therapy initiation was of 3.5 +2.1 days. By using modern techniques (T2Candida Panel, MALDI-TOF, PNA-FISH) we can identify the 5th species of *Candida* in a shorter period of time: T2CandidaPanel in 0.6+-0.2 days, 2.6 ± 1.3 days for PNA-FISH (fluorescence in situ hybridization using peptide nucleic acid probes) 2.5 ± 1.4 days MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) [12].

To these, other technologies of identification can

be added including PCR (Polymerase Chain Reaction) used now in our institute together with the MALDI – TOF system.

Conclusions

Knowing the epidemiological data in every country and especially in every hospital it is vital in the orientation of the therapeutically strategies. In the current epidemiologic background, the fluconazole does not represent anymore an anti-fungal of first intention fully justified in the empiric therapy, excepting the case of candidemia with *C. parapsilosis*. That is why the guidelines recommend the echinocandins as first intended treatment.

Taking into consideration that in our epidemiological analysis *C. glabrata*, a species considered a priori potentially resistant to fluconazole is on the 2nd place as presence, there is a risk in administrating fluconazole as first intention.

The investments in techniques of fast identification of fungi will be the guarantor of the success of the anti-fungal therapies and the prevention of resistance in the next future.

The hygiene in hospitals, the diminishing of the invasiveness and the immunosuppressors, the care of using any antibiotic and anti-fungal are part of preventive methods. The hand hygiene of determines the decrease of the candidemias, especially of those with *C.parapsilosis* but also of the infections associated to the health care.

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