Acquisition of FKS2 mutation after echinocandin treatment of infective endocarditis by *Candida glabrata*

Silvia Corcione[1], Antonio D'Avolio[2], Daniela Pasero[3], Alice Trentalange[1], Nicole Pagani[1], Maurizio Sanguinetti[4], Francesco Giuseppe De Rosa[1]

[1]Department of Medical Sciences, Infectious Diseases, University of Turin, City of Health and Sciences, Turin, Italy;
[2]Department of Medical Sciences, University of Turin Laboratory of Clinical Pharmacology and Pharmacogenetics, Turin, Italy;
[3]Division of Anesthesia and Critical Care, Department of Surgical Sciences, University of Turin, City of Health and Science, Molinette Hospital, Turin, Italy;
[4]Institute of Microbiology, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario Agostino Gemelli, Rome, Italy

SUMMARY

Bloodstream infections caused by non-albicans Candida species are increasing and echinocandins have been extensively used especially in patients with hemodynamic instability, previous antifungal treatment, and hospital risk factors for intrinsic or acquired resistance to azoles. *Candida glabrata* resistance to echinocandins is reported and is generally associated with previous use of echinocandins; *FKS* gene mutations have been associated with a worse outcome. We report the case of a 65-year-old woman who developed candidemia and endocarditis by C. *glabrata* with a newly acquired *FKS* mutation 24 months after successful treatment of infective endocarditis by *C. glabrata* with a double dosage of anidulafungin (200 mg daily) followed by oral voriconazole. Driven by high echinocandin MICs the strain taken by intraoperative cultures was further analyzed in a referral microbiology laboratory, confirming the new onset of point mutation S633P of the FKS2 gene.

Keywords: FKS, echinocandins, fungal endocarditis, MIC, *C. glabrata.*

INTRODUCTION

The incidence of systemic candidiasis caused by non-*albicans* species, such as *C. glabrata*, has increased due to the growing population of immunosuppressed and elderly patients and the widespread use of azoles [1, 2]. *Candida glabrata* is more likely to be non-susceptible to azoles in comparison to other Candida species, thus echinocandins are increasingly being used as empiric and targeted antifungal therapy [3, 4]. Widespread echinocandin usage has been accompanied by reports of emerging drug resistance among clinical isolates mediated by point mutations in FKS1 (all Candida species) or FKS2 (*C. glabrata*) hot spots that result in attenuated echinocandin activity [5]. The incidence of *FKS* mutations varies among institutions, with reports ranging from 3% to 18% [5, 6].

CASE REPORT

We report a 65-year-old woman who developed candidemia and endocarditis by *C. glabrata* with *FKS* mutation 24 months after a successful treatment of infective endocarditis by *C. glabrata* with a double dosage of anidulafungin (200 mg daily) followed by oral voriconazole [7].
The patient was affected by several important

Corresponding author
Silvia Corcione
E-mail: silvia.corcione@unito.it

co-morbidities: hepatitis C virus (HCV)-related cirrhosis, pancytopenia due to spleen enlargement, previous episodes of autoimmune hemolytic anemia and thrombocytopenia. Moreover, she undergone to several different cardiac surgeries: in 1980, aortic and mitral valve substitution, in 2001, prosthetic mitral valve implanted for infective endocarditis (IE), and in 2005, bioprosthetic tricuspid valve implant and intravascular electrocatheters), last surgical tricuspid valve replacement on May 2011 for *Candida glabrata* endocarditis.

The patient remained stable until March 2013, when she was admitted at our hospital due to fever, weight loss and asthenia. Considering her previous history and the suspect of infective endocarditis, blood cultures were drawn and antibiotic and antifungal therapy was started (caspofungin 70 mg on the first day then 50 mg/day). Blood cultures resulted positive for *C. glabrata. In vitro* sensitivities determined with Sensitre YeastOne (Trek Diagnostic, Inc.) had the following results: fluconazole >256 mg/l, caspofungin 16 mg/l, anidulafungin 4 mg/l, micafungin 2 mg/l; voriconazole 16 mg/l, posaconazole >8 mg/l, itraconazole >16 mg/l, amphotericin B 1 mg/l. Transesophageal echocardiography (TEE) showed vegetations on prosthetic mitral valve and bioprosthetic tricuspid valve. Due to the high intraoperative risk, cardiosurgery was delayed and antifungal treatment was continued with caspofungin and voriconazole was added (200 mg bid) [8-10]. After two weeks of therapy, TEE showed a slight reduction of the vegetations and the patient underwent cardiosurgery. Intraoperatory cultures were positive for *C. glabrata* with the same *in vitro* sensitivities described above. This strain was further analyzed in a referral microbiology laboratory, reporting the point mutation in hot spots of *FKS* genes and it was compared to the strain isolated in the previous hospitalization in 2011. The *FKS* mutation in *C. glabrata* was detected in the *FKS*2 gene and was well characterized as S663P mutation.

According to the previous reported results antifungal therapy was switched to liposomal amphotericin B (3 mg/kg/die) that was interrupted after two days, because to infusion intolerance (chest pain, dyspnea and a slow resolution itchy rash extended to all body) that had already occurred in 2011. Micafungin at the dosage of 150 mg/die was started because of its favorable tolerability profile, scarce drug interactions and no need of any adjustment for renal or hepatic insufficiency [11]. Moreover, micafungin seems to be superior *in vitro* to the other echinocandins in limiting the emergence of resistance among *C. glabrata* [6]. Postoperative recovery was complicated by renal failure, dyspnea and anasarcatic status, which lead to fatal outcome in June 2013.

DISCUSSION

According to IDSA and ESCMID guidelines, echinocandins are preferred as the initial therapy for candidemia but resistance to this class are emerging [8, 12, 13].

Guidelines also recommend to treat candida endocarditis of prosthetic valve with liposomal amphotericin B or caspofungin followed by fluconazole for life-long suppressive when surgery is not possible [8, 12]. In our patient caspofungin was combined to an azole to enhance the patient's chances to get to surgery as there is some evidence suggesting that combinations of voriconazole and caspofungin may be effective [9, 10, 14].

Echinocandins work by inhibiting the β-1,3-glucan synthase enzyme (encoded by *FKS*1, *FKS*2, and *FKS*3 genes in *C. glabrata*), which synthesizes a major component of the fungal cell wall. Mutations in FKS1 or FKS2 have been associated with elevated echinocandin minimal inhibitory concentrations (MICs), reduced glucan synthase enzyme sensitivity and outcomes in animal models, with statistically significant differences observed between specific mutations [5, 15].

In a recent study it has been reported that any prior echinocandin therapy is a significant predictor of FKS (OR, 19.65; 95% CI, 7.19-58.1) [16]. In contrast with these data Beyda et al. described isolates with FKS1 I634V mutation without any prior echinocandin exposure, suggesting that, although the low number of cases reported, continuous surveillance of FKS mutations is needed to understand their epidemiologic and clinical significance [17].

In addition, an increasing number of reports of *Candida spp.* isolates with elevated echinocandin MICs are being reported [18]. Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established clinical

MIC breakpoints (CBPs) and epidemiological cutoff values (ECVs) for anidulafungin and micafungin tests against *Candida spp* [19-22]. These interpretive criteria are both predictive of *in vivo* outcome and help distinguishing wild-type (WT) strains (no intrinsic or acquired resistance mutations) from non-WT strains which contain acquired resistance mutations in the *FKS* genes. Caspofungin MIC showed a great intra and interlaboratory variation as much as anidulafungin or micafungin have been considered to be used as a surrogate for clinical *in vitro* testing of echinocandins [23].

Echinocandin resistance in *C. glabrata* species is still a sporadic event, notwithstanding it jeopardizes the treatment and cure of patients who often already have many other comorbidities. To surpass the intrinsic trouble connected to *in vitro* susceptibility-based assays new methods based on DNA analysis have been developed to rapidly identify *C. glabrata* echinocandin-resistance and to better direct target therapy [24, 25]. Understanding both clinical and molecular factors leading to new mutations can help in developing better diagnostic tools and therapeutic strategies to overcome resistance.

Conflicts of Interest

None to declare.

REFERENCES

[1] Yapar N. Epidemiology and risk factors for invasive candidiasis. *Ther. Clin. Risk Manag*. 10, 95-105, 2014.

[2] De Rosa F., Corcione S., Raviolo S., et al. Candidemia, and infections *Clostridium difficile* and carbapenemase-producing Enterobacteriaceae: new enteropathogenetic opportunistic syndromes? *Infez. Med.* 23, 2, 105-116, 2015.

[3] Whaley S.G., Berkow E.L., Rybak J.M., Nishimoto A.T., Barker K.S., Rogers P.D. Azole antifungal resistance in *Candida albicans* and emerging non-albicans Candida species. *Front Microbiol*. 7, 2017. doi:10.3389/fmicb.2016.02173

[4] Grandesso S., Sapino B., Mazzucato S, et al. Study on *in vitro* susceptibility of *Candida* spp. isolated from blood culture. *Infez. Med.* 20, 1, 25-30, 2012.

[5] Hori Y., Shibuya K. Role of FKS gene in the susceptibility of pathogenic fungi to echinocandins. *Med. Mycol. J*. 59, 2, E31-E40. 2018.

[6] Shields R.K., Kline E.G., Healey K.R., et al. Spontaneous mutational frequency and FKS mutation rates vary by echinocandin agent against *Candida glabrata*. *Antimicrob. Agents Chemother*. 63 (1), 2019.

[7] De Rosa F.G., D'Avolio A., Corcione S., et al. Anidulafungin for *Candida glabrata* infective endocarditis. *Antimicrob. Agents Chemother*. 56, 8, 4552-4553, 2012.

[8] Pappas P.G., Kauffman C.A., Andes D.R., et al. Clinical practice guideline for the management of candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin. Infect. Dis*. 62, 4, e1-e50, 2016.

[9] Lopez-Ciudad V., Castro-Orjales M.J., Leon C., et al. Successful treatment of *Candida parapsilosis* mural endocarditis with combined caspofungin and voriconazole. *BMC Infect. Dis.* 6, 73, 2006.

[10] Falcone M., Barzaghi N., Carosi G., et al. Candida infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine (Baltimore).* 88, 160-168, 2009.

[11] De la Torre P., Reboli A.C. Micafungin: an evidence-based review of its place in therapy. *Core Evid*. 9, 27-39, 2014.

[12] Cornely O.A., Bassetti M., Calandra T., et al. Guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. *Clin. Microbiol. Infect*. 18,19-37, 2012.

[13] Pfaller M.A., Castanheira M., Lockhart S.R., Ahlquist A.M., Messer S.A., Jones R.N. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *J. Clin. Microbiol*. 50, 4, 1199-1203, 2012.

[14] Baltch A.L., Bopp L.H., Smith R.P., Ritz W.J., Michelsen P.B. Anticandidal effects of voriconazole and caspofungin, singly and in combination, against *Candida glabrata*, extracellularly and intracellularly in granulocyte-macrophage colony stimulating factor (GM-CSF)-activated human monocytes. *J. Antimicrob. Chemother.* 62, 6, 1285-1290, 2008.

[15] Healey K.R., Perlin D.S. Fungal resistance to echinocandins and the MDR phenomenon in *Candida glabrata*. *J. Fungi Basel Switz*. 4, 3, 2018.

[16] Alexander B.D., Johnson M.D., Pfeiffer C.D., et al. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin. Infect. Dis.* 56, 12, 1724-1732, 2013.

[17] Beyda N.D., John J., Kilic A., Alam M.J., Lasco T.M., Garey K.W. FKS mutant *Candida glabrata*: risk factors and outcomes in patients with candidemia. *Clin. Infect. Dis.* 59, 6, 819-825, 2014.

[18] Beyda N.D., Lewis R.E., Garey K.W. Echinocandin resistance in Candida species: mechanisms of reduced susceptibility and therapeutic approaches. *Ann. Pharmacother*. 46, 7-8, 1086-1096, 2012.

[19] Pfaller M.A., Messer S.A., Diekema D.J., Jones R.N., Castanheira M. Use of micafungin as a surrogate marker to predict susceptibility and resistance to caspofungin among 3,764 clinical isolates of Candida by use of CLSI methods and interpretive criteria. *J. Clin. Microbiol*. 52, 1, 108-114, 2014.

[20] Arendrup M.C., Cuenca-Estrella M., Lass-Flörl C., Hope W., EUCAST-AFST. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). *Clin. Microbiol. Infect.* 18, E246-E247, 2012.
[21] Arendrup M.C., Cuenca-Estrella M., Lass-Flörl C., Hope W.W, European Committee on Antimicrobial Susceptibility Testing - Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST). EUCAST technical note on Candida and micafungin, anidulafungin and fluconazole. *Mycoses* 57, 6, 377-379, 2014.
[22] Arendrup M.C., Pfaller M.A., Group the DFS. Caspofungin E-test susceptibility testing of Candida species: risk of misclassification of susceptible isolates of *C. glabrata* and *C. krusei* when adopting the revised CLSI caspofungin breakpoints. *Antimicrob. Agents Chemother*. 56, 7, 3965-3968, 2012.
[23] Espinel-Ingroff A., Arendrup M.C., Pfaller M.A., et al. Interlaboratory variability of caspofungin MICs for Candida spp Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? *Antimicrob. Agents Chemother*. 57, 12, 5836-5842, 2013.
[24] Zhao Y., Nagasaki Y., Kordalewska M., et al. Rapid detection of FKS-associated echinocandin resistance in *Candida glabrata*. *Antimicrob. Agents Chemother*. 60, 11, 6573-6577, 2016.
[25] Sariguzel F., Berk E., Koc N.A, et al. Evaluation of CHROMagar Candida, VITEK2 YST and VITEK® MS for identification of Candida strains isolated from blood cultures. *Infez. Med.* 23, 4, 318-322, 2016.