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Prevention of fungal infection in the early period after liver transplantation

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The article discusses the risk and incidence of fungal infections in the early period after orthotopic liver transplantation, their diagnosis, treatment and prevention.

The patients after liver transplantation are at high risk for developing invasive fungal infection. The presence of high-risk factors is an absolute indication for prophylactic antifungal medications.

Keywords: liver transplantation, fungal infections, antifungal therapy.

Fungal infection is a clinically significant problem in transplantation. The development of fungal infection in patients undergoing liver transplantation is still associated with a poor outcome, despite a number of commercially available anti-fungal drugs. Thus, some authors have reported mortality rates from 30 to 77% among the cases of the invasive candidiasis development, and 65-90% among aspergillosis cases. The delay in administering an antifungal therapy has a negative impact on the outcomes [1-6].

The optimal approach to prevention of the antifungal infection has not been standardized yet. A number of studies demonstrated the isolation of resistant Candida strains in patients receiving a prevention therapy with fluconazole [7].

A meta-analysis of published studies on the prevention of fungal infection has shown a decrease in the rates of undiagnosed fungal infection, but the overall mortality rates and the need to administer an empirical therapy for suspected fungal infection have not reduced [8]. Given the lack of accurate data on a clinically beneficial effect of the prevention, its high costs, potential toxicity, and the risk of pathogen resistance development, many transplant centers refrain from administering a universal antifungal prevention therapy nowadays [9].

A so-called targeted approach is the most commonly used, i.e. when prevention therapy is administered in the patients of a high-risk group only.

We should note that the term "high risk patients" has not been defined anyway yet, and the transplant specialists are often guided by various sets of criteria for stratifying the patients with increased risks of fungal infection.

There are a great number of factors making the patients vulnerable to invasive mycoses. The most important of these factors include liver re-transplantation, a fulminant hepatic failure as an indication to transplantation, an acute kidney injury in the postoperative period requiring the use of dialysis techniques, relaparotomy in the early postoperative period, a massive blood loss (requiring more than 20 units of blood components to be transfused for correction during surgery).

Other factors include the surgery duration for more than 12 hours, a disseminated ($\geq$ 2 loci) superficial colonization with Candida spp., and

making a hepatico-jejunal anastomosis versus a choledocho-choledochal anastomosis [10-13].

The choice of antifungal drugs for targeted prophylaxis, their dosages, and the duration of prevention therapy course also remain controversial.

Fluconazole and different forms of amphotericin B have been widely used in clinical practice for targeted prophylaxis. Meanwhile, the fluconazole-resistant strains of *Candida krusei* and *Candida glabrata* are identified more and more frequently. One should remember that azoles influence the plasma levels of the most commonly used immunosuppressants: cyclosporine, tacrolimus, and everolimus.

The duration of prevention therapy has not been standardized either, ranging from 5-7 days to 4 weeks or longer post-transplantation [14].

The aim of the study was to assess the risk and the incidence of fungal infection in patients early after liver transplantation.

Material and Methods

The study included 80 patients who underwent liver transplantation in N.V.Sklifosovsky Research Institute for Emergency Medicine in the period from January 2014 to November 2015. There were 62 men (77.5%), and 18 women (22.5%) aged 48.1±11 years old. The patient disease severity before liver transplantation was scored 17.25±7.6 as per the Model for End-Stage Liver Disease (MELD) score.

Various kinds of biological material (throat swab, blood, urine, feces), as well as the removed peripheral drains and central venous catheters were sent to laboratory for study purposes. Tests for fungal infection were made twice weekly post-surgery till patient's discharge from hospital.

Initially, the biological material was cultured in the microbiology laboratory in accordance with standardized methodology. Blood was cultured using Bactec 9050 (BD, USA) analyzer for blood cultures in vials: Bactec™ Plus Aerobic/F Culture Vials for aerobic bacteria; Bactec™ Plus Anaerobic/F Culture Vials for anaerobic microorganisms; BactecTM Mycosis IC/F Culture Vials for fungi. Microorganism identification and antibiotics-susceptibility testing were performed by using an automated microbiological WalkAway 40 Analyzer (USA) or traditional microbiological techniques.

Polymerase chain reaction (PCR) was additionally made to study DNA for *Candida albicans*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Candida famata*, *Candida tropicalis*, *Candida guilliermondii* using kits manufactured by ZAO "Vector-Best".

The following data were recorded: risk factors, prevention therapy, complications.

Statistical data processing

Statistical processing of the data obtained in the study was performed using STATISTICA 8.0 Software package (StatSoft Inc., USA). The medians, upper and lower quartiles (ME [25-75%]) were calculated for variables with a distribution different from normal.

Study results

Among 80 patients included in the study, 32 (40%) had the risk factors of invasive fungal infection (Table. 1). As several patients had a combination of events assessed as separate risk factors, the total number of the cases was 40. Table 1 demonstrates that the most common risk factors

included the use of broad spectrum antibiotics accounting for 37.5%, the use of renal replacement therapy making 22.5%, and neutropenia that occurred in 12.5% of cases.

Table 1. Risk factors of invasive fungal infection (n = 32)

	Number	%
Renal replacement therapy	9*	22.5
Liver retransplantation	1	2.5
Treatment of bacterial complications with broad-spectrum antibiotics	15*	37.5
Relaparotomy in the early postoperative period	3 *	7.5
Hepatico-enteric anastomosis	2	5
Using blood components >20 doses	5	12.5
Neutropenia $<2 \times 10^9$ /L	5	12.5
Total	40	100%

* Multiple risk factors in a patient.

Antifungal prevention therapy was administered in 79 patients (98.75%). Its duration made 9 (4; 12) days in all the patients. The majority of patients without high risk received the preventive therapy in short duration; antifungals were discontinued together with the termination of antimicrobial therapy.

Prevention was performed using either a lipid complex of Amphotericin B at a dose of 50 mg/day in 49 patients (61.25%), or echinocandins in a standard dose in 25 (31.25%), or fluconazole at a dose of 400 mg/day in 6 (7.5 %). There was the need to switch from fluconazole to

echinocandins in 3 cases: because of *Candida spp*. identified in blood in 1 case, and PCR-detected *Candida glabrata* in other 2 cases. The case report illustrating the clinical conversion from fluconazole to anidulafungin is presented below.

Patient D., a female of 54 years old, underwent liver retransplantation from cadaveric organ donor. Indications for surgery included recurrent primary biliary cirrhosis (at 6 years after primary liver transplantation), hepatocellular failure, hepatorenal syndrome with concomitant type 2 diabetes mellitus. Pre-operative disease severity was assessed as 10 by Child-Pugh score, and 18 by MELD.

Intraoperatively, the patient was stable, anesthesia was maintained with the use of Sevoran at a low flow rate of fresh gas. Of particular note was a decreased urine output under 50 mL/h at hepatectomy and at anhepatic stage, as well as the use of dopamine at a dose of 8-10 mcg/kg/min and norepinephrine at a dose of 200-300 ng/kg/min to stabilize the mean arterial pressure above 70 mmHg at anhepatic stage and in the first minutes after the venous reperfusion.

The calculated amount of blood loss was 800 mL, with 200 mL of washed autologous red blood cells (RBCs) being reinfused using the cell salvage autotransfusion device. Hepatico-jejunal anastomosis was made to ensure the bile outflow. The patient was extubated in the intensive care unit (ICU) at 8 hours after surgery. Immunosuppression therapy included daclizumab (20 mg intraoperatively after achieving hemostasis before suturing the laparotomy wound, and 20 mg on the 4^{th} postoperative day), cyclosporin, methylprednisolone, and mycophenolic acid. A preventive antibacterial and antifungal therapy was administered with cefotaxime at a dose of 2 g/day (with initial dosing at 30 minutes before the skin incision),

and fluconazole, 200 mg/day. The maximum increased cytolysis enzyme activities to 569, and 699 U/L for ALT, and AST, respectively, were documented on the 1st postoperative day. Right-side lower lobe pneumonia and a postoperative wound suppuration were diagnosed on the 5th postoperative day. Blood hematology demonstrated anemia (with hemoglobin of 78 g/L, RBC $3.83x10^{12}$ /L), leukocytosis of $12.87x10^{9}$ /L with leftward shift in differentials to myelocytes (3%); thrombocytopenia (with platelets being $78x10^{9}$ /L). Hypocoagulation was noted in hemostatic system with INR being 1.86. Blood biochemistry showed hyperbilirubinemia (total bilirubin being 35 mcmol/L), hyperazotemia (with creatinine 184 mmol/L, and urea 29 mmol/L), hypoalbuminaemia (with albumin of 29 g/L). Microbiology cultures of blood and abdominal contents revealed a multidrug-resistant pathogen *Acinetobacter spp.* susceptible to carbapenems. Blood cultures for fungi isolated *Candida spp*. PCR test results demonstrated DNA of *Candida parapsilosis*, *Candida albicans*, *Candida glabrata* in the samples from the throat, urine, and intestinal content. Considering all the above data, the patient was given meropenem at a dose of 6 g daily, linezolid 1200 mg/day, and anidulafungin at an initial dose of 200 mg/day, and further at a dose of 100 mg daily. After a 14-day anidulafungin therapy, the control PCR tests and the cultures of blood, sputum, urine, and intestinal content demonstrated no fungi.

The diagnosis using PCR test revealed DNA of least one of the tested *Candida* species in 20 patients (25%). Table 2 shows positive PCR results of testing the biological material.

Table 2. PCR test results

Pathogens	Material investigated					Total number of samples
	blood	throat swab	urine	feces	sputum	
Candida albicans	-	14	1	8	1	24
Candida glabrata	-	5	3	4	-	12
Candida parapsilosis	1	4	3	3	1	12
Candida tropicalis	-	4	2	3	-	9
Candida krusei	-	1	-	-	-	1
Candida famata	-	2	-	-	-	2
Candida guilliermondii	-	1	-	1	-	2
Candida spp	-	4	-	3	-	7

The most common type of Candida spp. in our study was *Candida albicans* (34.8%).

In general, the presence of isolated *Candida albicans* was seen in 2 patients (10%) only (See Figure); in all other cases, fungal colonization was associated with other types of *Candida* spp., specifically, with *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*.

Discussion of the results

The development of bacterial and invasive fungal infection is a clinically significant problem in patients after liver transplantation. Our study has shown the presence of high-risk factors for invasive fungal infections in 40% of liver transplant recipients. Fungi of *Candida* species have been isolated from a variety of biological fluids in a quarter of patients. However, only 34.8% of isolated pathogens belonged to *Candida albicans*.

Other pathogens belonged to those Candida spp. that were generally characterized as having stronger antifungal drug resistance.

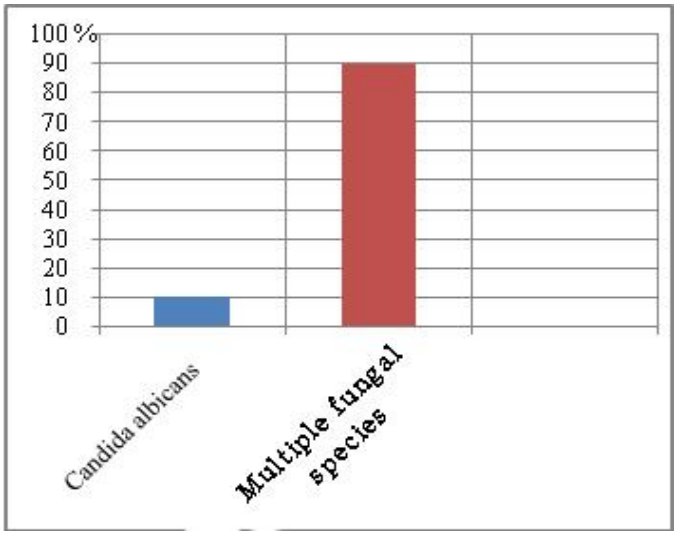

Figure. Incidence of single and multiple fugal species colonization

Most of the patients received the prophylactic therapy in short duration; the antifungal prophylaxis was completed together with the termination of prophylactic antibiotic therapy.

Based on the obtained results, we could identify a group of patients free from high-risk factors who did not need a preventive antifungal therapy in the early postoperative period. Further studies are necessary to define clear criteria for identification of such patients.

Aimed at preventing fungal infection, we used either an amphotericin B lipid complex, or echinocandins, or fluconazole. Three patients on fluconazole with confirmed fungal infection were switched to prevention therapy with echinocandins.

Fluconazole is a potent selective inhibitor of fungal enzymes required for the synthesis of ergosterol. Fluconazole is an efficient agent against most

strains of *Candida albicans* and *Cryptococcus neoformans*. However, *Candida krusei* and many *Candida glabrata* strains possess a natural resistance to fluconazole [15-19]. The data obtained in this study suggest that Candida glabrata ranks second by the incidence among pathogens causing fungal infection in patients after liver transplantation. In this regard, we consider the echinocandins (caspofungin, anidulafungin, mikamin) to be acceptable drugs for the prevention of invasive fungal infections in high-risk patients. Echinocandins selectively inhibit 1,3-b-D-glycan-synthetase, an important component of the fungal cell wall, and have proven their efficacy, demonstrating a favorable safety profile and advantages in the treatment of patients undergoing liver transplantation. These drugs are efficient against many fungi of Candida spp., including *Candida albicans, Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida dubliniensis*, and *Candida lusitaniae*, and *Candida guilliermondii*, and also *Aspergillus spp*. [20]. There were no cases of "breakthrough" fungal infection in our study when using echinocandins.

Conclusion

The patients after liver transplantation are at a high risk of invasive fungal infections. The presence of high-risk factors is an absolute indication for prophylactic antifungal therapy. Median duration of preventive therapy course makes 9 days. Fungal infection caused by Candida spp. is associated with the species other than *Candida albicans* in 65.8% of cases. In this regard, fluconazole is not the drug of choice for the prevention of fungal infection.

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