

# UTICAJ ANTIGLJIVIČNE PROFILAKSE NA POJAVU GLJIVIČNIH INFEKCIJA KOD BOLESNIKA U PROGRAMU ALOGENE TRANSPLANTACIJE

ORIGINALNI RAD

ORIGINAL ARTICLE

## INFLUENCE OF ANTIFUNGAL PROPHYLAXIS ON THE OCCURRENCE OF FUNGAL INFECTIONS IN PATIENTS UNDERGOING ALLOGENEIC TRANSPLANTATION

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### SAŽETAK

**Uvod:** Bolesnici sa hematološkim malignitetima, kao što su akutna mijeloidna leukemija i akutna limfoblastna leukemija (AML/ALL), mijelodisplastični sindrom (MDS) i oni koji su podvrgnuti alogenoj transplantaciji matičnih ćelija (aloTMČ) su pod najvećim rizikom od nastanka invazivnih gljivičnih infekcija (engl. *invasive fungal infections – IFI*). Najčešći među uzročnicima su *Candida spp.* i *Aspergillus spp.* Među strategijama za prevenciju nastanka invazivnih gljivičnih infekcija je blagovremena i adekvatna primena antigljivične profilakse koju preporučuje NCCN (*National Comprehensive Cancer Network*).

**Cilj:** Cilj istraživanja bila je analiza pojave *IFI* infekcija kod ovih pacijenata, kao i analiza uticaja i značaja pravovremene antigljivične profilakse za njihovo nastajanje.

**Materijal i metode:** U retrospektivnoj studiji, ispitivano je 42 bolesnika, prosečne starosti 35 godina, koji su bili u programu aloTMČ-a, u periodu od 2017. do 2019. godine, i kod kojih je primenjivana antigljivična profilaksa na Klinici za hematologiju, Univerzitetskog kliničkog centra Srbije (UKCS). Na osnovu informacija dobijenih iz istorija bolesti, formirane su baze podataka. Statistička analiza podataka obuhvatala je metode deskriptivne i analitičke statistike i urađena je u SPSS programu.

**Rezultati:** Klinički manifestnu infekciju u vidu oralne kandidijaze imalo je 19 (45,2%) bolesnika, dok se plućna aspergiloza razvila kod svega 3 (7,1%) bolesnika. Statistički značajna povezanost postojala je između klinički manifestne aspergiloze (7,1%) i prisustva antigena (galaktomanan) kod ovih bolesnika ( $p < 0,001$ ). Utvrđena je i statistički značajna povezanost između klinički manifestne aspergiloze i slabosti kalema: 2 (66,6%) naspram 1 (33,3%), ( $p = 0,016$ ).

**Zaključak:** Primena adekvatne antigljivične profilakse značajno smanjuje incidenciju pojave *IFI* kod bolesnika u programu aloTMČ-a, i na taj način doprinosi smanjenju morbiditeta i mortaliteta.

**Ključne reči:** antigljivična profilaksa, invazivne gljivične infekcije, aspergiloza, antigen

### ABSTRACT:

**Introduction:** Patients with hematologic malignancies, such as acute myeloid leukemia and acute lymphoblastic leukemia (AML/ALL), myelodysplastic syndrome (MDS), and those undergoing allogeneic stem cell transplantation (allo-SCT) are at the highest risk of invasive fungal infections (IFI). The most common causative agents are *Candida spp.* and *Aspergillus spp.* Among the strategies for preventing IFIs is the adequate implementation of antifungal prophylaxis recommended by the NCCN (*National Comprehensive Cancer Network*).

**Aim:** The aim of the study was to analyze the occurrence of IFIs in these patients, as well as to analyze the impact and importance of timely antifungal prophylaxis with regards to the development of these infections.

**Materials and methods:** The retrospective study included 42 patients, of the average age of 35 years, who underwent the allo-SCT program, between 2017 to 2019, and received antifungal prophylaxis at the Clinic for Hematology of the Clinical Center of Serbia (CCS). Based on information obtained from medical histories, databases were formed. Statistical analysis included descriptive statistical methods that were performed in the SPSS program.

**Results:** Nineteen (45.2%) patients presented with the clinical manifestation of oral candidiasis. Invasive pulmonary aspergillosis developed in only 3 (7.1%) patients. There was a statistically significant association between clinically manifest aspergillosis (7.1%) and the presence of antigens (Galactomannan) in these patients ( $p < 0.001$ ). There was also a statistically significant association between clinically manifest aspergillosis and graft weakness: 2 (66.6%) vs. 1 (33.3%), ( $p = 0.016$ ).

**Conclusion:** The use of adequate antifungal prophylaxis significantly reduces the incidence of IFIs in patients undergoing the allo-SCT program, and this contributes to the reduction of morbidity and mortality.

**Key words:** antifungal prophylaxis, invasive fungal infections, aspergillosis, antigen

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Primljeno • Received: May 17, 2021;

Revidirano • Revised: May 28, 2021;

Prihvaćeno • Accepted: June 6, 2021;

Online first: June 25, 2021.

DOI: 10.5937/smcl2-32279

## UVOD

S pojavom poboljšanih hemioterapijskih režima, mogućnosti alogene transplantacije matičnih ćelija (alo-TMĆ), i novih bioloških ciljanih terapija, ishod mnogih ozbiljnih hematoloških oboljenja se stalno poboljšava [1]. Međutim, invazivne gljivične infekcije (engl. *invasive fungal infections – IFI*) su i dalje vodeći infektivni uzrok morbiditeta i mortaliteta kod bolesnika sa hematološkim malignitetima [8].

Bolesnici sa hematološkim malignitetima, kao što su akutna mijeloidna leukemija i akutna limfoblastna leukemija (AML/ALL), mijelodisplastični sindrom (MDS) i oni koji su podvrgnuti alogenoj transplantaciji matičnih ćelija hematopoeze (aloHTMĆ) su u najvećem riziku od nastanka *IFI* infekcija [9], pri čemu je incidencija najviša kod akutne mijeloidne leukemije (AML) [10]. Imajući ovo u vidu, gljivične infekcije i dalje ostaju izazov kod ovih, tzv. „rizičnih“ bolesnika. Pored toga, nastanku gljivičnih infekcija dodatno doprinosi neprimjenjena antigljivična profilaksa širokog spektra dejstva. [2,3,4]. Invazivne gljivične infekcije (*IFI*) su infekcije visoke incidencije, ugrožavajuće su po život pacijenta, i zahtevaju ulaganje značajnih finansijskih sredstava kod bolesnika na programu alogene transplantacije matičnih ćelija (aloTMĆ) [7]. Najčešći među patogenim uzročnicima infekcije su *Candida* spp. i *Aspergillus* spp. Infekcije izazvane ovim vrstama, posebno, *Aspergillus* spp., još uvek su u porastu u ovoj populaciji bolesnika, i značajan su uzrok morbiditeta i mortaliteta, posebno u kontekstu produžene neutropenije i imunosupresivnog lečenja [5,7].

Prevenција i lečenje invazivnih gljivičnih infekcija kod bolesnika u programu aloTMĆ-a predstavlja veliki izazov. Stoga su u protekle dve decenije učinjeni veliki naponi kako bi se pronašla adekvatna strategija za sprečavanje nastanka teških *IFI* infekcija u ovoj populaciji bolesnika. Među strategijama za poboljšanje ishoda je blagovremena i adekvatna primena antigljivične profilakse [6]. Trenutno postoji nekoliko antigljivičnih lekova koje preporučuje Nacionalna sveobuhvatna mreža protiv raka za profilaksu *IFI* (*National Comprehensive Cancer Network – NCCN*). Tu spadaju: flukonazol, itrakonazol, vorikonazol, posakonazol i mikafungin [6]. Dostupnost ovih novih triazola (vorikonazol, posakonazol), karakterističnih za širi spektar, u poslednje vreme, promenila je ulogu antigljivične profilakse. Primena posakonazola i mikafungina značajno je poboljšala efikasnost antigljivične profilakse u ovoj populaciji bolesnika [11,12,13].

Cilj ovog istraživanja bila je analiza pojave manifestnih gljivičnih infekcija kod bolesnika u programu alogene transplantacije matičnih ćelija hematopoeze, kao i analiza uticaja i značaja pravovremene antigljivične profilakse za njihovo nastajanje.

## INTRODUCTION

With the emergence of improved chemotherapeutic regimens, and the possibility of allogenic stem cell transplantation (allo-SCT), as well as with new biologic targeted therapies, the outcome for many serious hematological diseases is constantly improving [1]. However, invasive fungal infections (IFI) remain the leading infective cause of morbidity and mortality in patients with hematological malignancies [8].

Patients with hematological malignancies, such as acute myeloid leukemia and acute lymphoblastic leukemia (AML/ALL), patients with myelodysplastic syndrome (MDS), as well as patients who undergo allogenic hematopoietic stem-cell transplantation (allo-HSCT), are at the highest risk of developing *IFI* infections [9], with the incidence of these infections being the highest in acute myeloid leukemia (AML) [10]. Bearing this in mind, fungal infections remain a challenge in these, so called, “risky” patients. Additionally, the lack of the application of prophylactic antifungal broad-spectrum therapy contributes to the development of fungal infections [2,3,4]. Invasive fungal infections (IFI) are infections of high incidence, they endanger the patient’s life, and they require the investment of significant financial resources in patients who are in the program of allogenic stem-cell transplantation (allo-SCT) [7]. The most common pathogens causing infection are *Candida* spp. and *Aspergillus* spp. Infections caused by these species of pathogens, especially, *Aspergillus* spp., are still on the rise in this population of patients, and they are a significant cause of morbidity and mortality, especially in the context of prolonged neutropenia and immunosuppressive treatment [5,7].

The prevention and treatment of fungal infections in patients who are in the allo-SCT program is a great challenge. This is why, in the previous two decades, great efforts have been made to find the appropriate strategy for preventing the development of severe IFIs in this population of patients. Timely and adequate application of antifungal prophylaxis is among the strategies for outcome improvement [6]. Currently, there are several antifungal drugs recommended by the National Comprehensive Cancer Network (NCCN) for *IFI* prophylaxis, namely: fluconazole, itraconazole, voriconazole, posaconazole, and micafungin [6]. The accessibility of these new triazoles (voriconazole, posaconazole), characteristic of the broad spectrum, has lately changed the role of antifungal prophylaxis. The application of posaconazole and micafungin has significantly improved the efficacy of antifungal prophylaxis in this population of patients [11,12,13].

The aim of this study was to analyze the occurrence of manifest fungal infections in patients included in

## METODE

Istraživanje je sprovedeno u vidu retrospektivne opservacione kohortne studije na osnovu baze podataka Klinike za hematologiju Kliničkog centra Srbije, u toku oktobra i novembra meseca 2019. godine. Studijom je obuhvaćeno 42 bolesnika, koji su bili podvrgnuti programu alogene transplantacije matičnih ćelija, u periodu od 2017. do 2019. godine. Bolesnicima je postavljena dijagnoza Hočkinovog limfoma (HL), Nehočkinovog limfoma (NHL), akutne leukemije (AML/ALL), hronične limfocitne leukemije (HLL), odnosno mijelodisplastičnog sindroma (MDS), u periodu od 2003. do 2019. godine.

Podaci o demografskim karakteristikama (starost, pol), podaci o postavljenoj dijagnozi, do kojih se, za potrebe istraživanja, došlo na osnovu patohistoloških i imunohistohemijskih analiza bioptiranog uzorka, kao i podaci o urađenoj alogenoj transplantaciji, dobijeni su iz medicinske dokumentacije bolesnika (istorija bolesti). Takođe, odatle su preuzeti i podaci o jačini primenjenog kondicionog režima (*Reduced-Intensity Conditioning – RIC; Myeloablative Conditioning – MAC*), zatim podaci o prisustvu/odsustvu specifičnih antitela (IgM, IgG) na *Candida spp* i *Aspergillus spp.*, do kojih se došlo na osnovu seroloških ispitivanja (*ELISA*), potom podaci o prisustvu/odsustvu antigena specifičnih za *Candida spp.* i *Aspergillus spp.* (*Candida manan test* i galaktomanan test), kao i podaci o klinički manifestnoj infekciji kod bolesnika u vidu oralne kandidijaze ili aspergiloze. Dijagnoza oralne kandidijaze postavljena je inspekcijom bukalne sluzokože i zasejavanjem, a uzorak je uzet sterilnim brisom sa bukalne sluzokože i jezika, dok je dijagnoza plućne aspergiloze postavljena na osnovu seroloških (galaktomanan test) i radioloških (rendgenografija i kompjuterizovana tomografija) ispitivanja.

Svi bolesnici su primali antigljivičnu profilaksu: mikafungin 50 mg intravenski ukupno 15 dana počevši od prvog dana primene kondicionog režima, a potom posakonazol u dozi od 5 ml suspenzije 3 puta na dan do D+100 (stoti dan od dana alogene transplantacije matičnih ćelija hematopoeze).

Statistička obrada je obuhvatila formiranje baze podataka, sa grupisanjem i tabeliranjem rezultata po ispitivanim karakteristikama bolesnika. Statistička analiza prikupljenih podataka podrazumevala je metode deskriptivne i analitičke statistike i urađena je u *SPSS* programu (*Statistical Package for the Social Sciences*), verzija 25. Za procenu značajnosti razlike u učestalosti različitih karakteristika korišćene su metode univarijantne analize, i to  $\chi^2$  kvadrat test i Fišerov *test* tačne verovatnoće za kategorijalne varijable. Nivo značajnosti bio je 0,05. Pored toga, ispitivali smo sveukupno preživljavanje bolesnika nakon alogene transplantacije (*overall survival – OS* nakon aloTMC-a).

the program of allogenic hemopoietic stem-cell transplantation, as well as to analyze the impact and importance of timely antifungal prophylaxis in relation to the development of these infections.

## METHODS

The research was carried out as a retrospective observational cohort study, based on the database of the Clinic for Hematology of the Clinical Center of Serbia, during October and November 2019. The study included 42 patients, who had undergone the program of allogenic stem-cell transplantation between 2017 to 2019. The patients were diagnosed with one of the following: Hodgkin lymphoma (HL), Non-Hodgkin lymphoma (NHL), acute leukemia (AML/ALL), chronic lymphocytic leukemia (CLL), and myelodysplastic syndrome (MDS), in the period between 2003 and 2019.

The data on the demographic characteristics (age, sex), data on the diagnosis, which were acquired for the purpose of the study from pathohistological and immunohistochemical analyses of biopsy samples, as well as data on the allogenic transplantation that had been carried out, were obtained from patient medical records (medical histories). The medical records were also the source of the following information: data on the intensity of the applied conditioning regimen (*Reduced-Intensity Conditioning – RIC; Myeloablative Conditioning – MAC*), data on the presence/absence of specific antibodies to *Candida spp* and *Aspergillus spp.* (IgM, IgG), which were obtained on the basis of serological testing (*ELISA*), data on the presence/absence of antigens specific to *Candida spp.* and *Aspergillus spp.* (*Candida mannan test* and galactomannan test), as well as data on clinically manifest infection in patients, in the form of candidiasis or aspergillosis. Oral candidiasis was diagnosed by the inspection of the buccal mucosa and cultivation of the sample that was taken with a sterile swab from the buccal mucosa and the tongue, while the diagnosis of pulmonary aspergillosis was established based on serological (galactomannan test) and radiological (X-ray, CT scan) analyses and tests.

All patients received antifungal prophylaxis: micafungin 50 mg, intravenously, for a total of 15 days, beginning with the first day of performing the conditioning regimen, upon which they received posaconazole in a dose of a 5 ml suspension, three times a day until D+100 (day 100 as of the day of allogeneic hemopoietic stem cell transplant).

Statistical processing included the forming of a database, with grouping and tabular presentation of the results by tested patient characteristics. Statistical analysis of the collected data included descriptive and analytical statistical methods and it was performed in the

## REZULTATI

Tokom navedenog dvogodišnjeg perioda, na Odeljenju transplantacije koštane srži Klinike za hematologiju Kliničkog centra Srbije, koje postoji od 2017. godine, u program alogene transplantacije bilo je uključeno ukupno 42 bolesnika.

U **Tabeli 1** prikazane su demografske i kliničke karakteristike bolesnika.

Studijom je obuhvaćeno 42 bolesnika kod kojih je postavljeno sledećih šest dijagnoza: 19% HL, 4,8% NHL, 33,3% AML, 35,7% ALL, 2,4% HLL, i 4,8% MDS. U ispitivanoj grupi, muškaraca je bilo 21 (50%), a isto toliko i žena. Prosečna starost iznosila je 35 ± 12,02 godina. Najmlađi bolesnik je imao 14, a najstariji 56 godina, u vreme postavljanja dijagnoze. Kod 12 bolesnika (28,6%) primenjen je RIC kondicioni režim aloTMC-a dok je kod 30 (71,4%) primenjen MAC kondicioni režim.

U **Tabeli 2** prikazani su podaci o prisustvu specifičnih IgG i IgM antitela i antigena za *Candida spp.* i *Aspergillus spp.*

**Tabela 1.** Demografske i kliničke karakteristike bolesnika

**Table 1.** Patient demographic and clinical characteristics

Varijabla / Variable	Broj / Number	Procenat / Percentage
Pol / Sex		
Muškarci / Men	21	50%
Žene / Women	21	50%
Starost / Age		
	35 ± 12.02	
Dijagnoza / Diagnosis		
HL	8	19%
NHL	2	4.8%
AML	14	33.3%
ALL	15	35.7%
HLL	1	2.4%
MDS	2	4.8%
Klinički manifestna infekcija / Clinically manifest infection		
Oralna kandidijaza / Oral candidiasis	19	45.2%
Plućna aspergiloza / Pulmonary aspergillosis	3	7.1%
Jačina kondicionog režima / Conditioning regimen intensity		
MAC	30	71.4%
RIC	12	28.6%

Legenda: HL (Hoćkinov limfom); NHL (Nehoćkinov limfom); AML (akutna mijeloidna leukemija); ALL (akutna limfoblastna leukemija); HLL (hronična limfocitna leukemija); MDS (mijelodisplastični sindrom); MAC (mijeloablativni kondicioni režim, engl. *myeloablative conditioning*); RIC (kondicioni režim redukovano intenziteta, engl. *reduced-intensity conditioning*)

Legend: HL (Hodgkin lymphoma); NHL (Non-Hodgkin lymphoma); AML (acute myeloid leukemia); ALL (acute lymphoblastic leukemia); CLL (chronic lymphocytic leukemia); MDS (myelodysplastic syndrome); MAC (myeloablative conditioning); RIC (reduced-intensity conditioning).

SPSS program (Statistical Package for the Social Sciences), version 25. The significance of the difference in the frequency of the different characteristics was assessed by means of univariate analysis methods, namely the  $\chi^2$  squared test and Fisher's exact test of independence for categorical variables. The significance level was 0.05. Additionally, we tested the overall survival of patients upon allogenic transplantation (OS after allo-SCT).

## RESULTS

During the abovementioned two-year period, at the Department for Bone Marrow Transplant of the CCS Clinic for Hematology, which was established in 2017, a total of 42 patients were included in the program of allogenic transplantation.

**Table 1** shows the demographic and clinical characteristics of the patients.

The study included 42 patients in whom the following six diagnoses were established: 19% HL, 4.8% NHL, 33.3% AML, 35.7% ALL, 2.4% CLL, and 4.8% MDS. In the analyzed group, there were 21 men (50%), and the same number of women. The average age was 35 ± 12.02 years. The youngest patient was 14, while the oldest one was 56 years old, at the time of diagnosis. The RIC conditioning regimen of allo-SCT was carried out in 12 patients (28.6%), while the MAC conditioning regimen was applied in 30 patients (71.4%).

**Table 2** shows the data on the presence of specific IgG and IgM antibodies and antigens to *Candida spp.* and *Aspergillus spp.*

In 29 patients (69%) the presence of antibodies to *Candida spp.* was registered, while in 13 patients (31%) the antibody test was negative. The positive antibody test for *Aspergillus spp.* was registered in 50% of the patients. The *Candida spp.* mannan test was positive in one patient (2.4%), while the positive galactomannan test was registered in 4 patients (9.5%).

**Tabela 2.** Prisustvo specifičnih IgG i IgM antitela i antigena za *Candida spp.* i *Aspergillus spp.*

**Table 2.** Presence of specific IgG and IgM antibodies and antigens to *Candida spp.* and *Aspergillus*

Varijabla / Variable	Broj / Number	Procenat / Percentage
Pozitivna antitela na Candida-u / Positive antibodies to Candida	29	69%
Pozitivna antitela na Aspergillus / Positive antibodies to Aspergillus	21	50%
Pozitivan Candida manan / Positive Candida mannan	1	2.4%
Pozitivan galaktomanan / Positive galactomannan	4	9.5%

Kod 29 bolesnika (69%) zabeleženo je prisustvo antitela na *Candida spp.* dok su kod 13 pacijenata (31%) antitela bila negativna. Pozitivna antitela na *Aspergillus spp.* su zabeležena kod 50% bolesnika. Pozitivan *Candida spp.* manan test je zabeležen kod 1 bolesnika (2,4%) dok je pozitivan galaktomanan test zabeležen kod 4 bolesnika (9,5%). Klinički manifestnu infekciju, u vidu oralne kandidijaze, imalo je 19 bolesnika (45,2%), dok se plućna aspergiloza razvila kod svega 3 bolesnika (7,1%).

Ispitivano je da li postoji statistički značajna povezanost između klinički manifestne infekcije (oralna kandidijaza/plućna aspergiloza) i jačine primenjenog kondicionog režima, međutim statistički značajna povezanost nije nađena ( $p = 0,327$  MAC,  $p = 0,256$  RIC), odnosno nije bilo statistički značajne razlike u ispoljavanju manifestne gljivične infekcije između bolesnika koji su primali MAC i onih koji su primali RIC kondicioni režim. Takođe, nije bilo statistički značajne povezanosti između klinički manifestne infekcije i dijagnoze bolesnika ( $p = 0,580$ ).

Od troje bolesnika (7,1%) koji su imali klinički manifestnu plućnu aspergilozu, svo troje je imalo i pozitivan galaktomanan test (100%), što se pokazalo kao statistički značajno ( $p < 0,001$ ). Takođe, utvrđena je statistički značajna povezanost između klinički manifestne plućne aspergiloze i slabosti kalema: 2 (66,6%) naspram 1 (33,3%), ( $p = 0,016$ ).

Procenjena medijana preživljavanja bolesnika nakon aloTMČ-a, a bila je 56 meseci.

## DISKUSIJA

Invazivne gljivične infekcije (IFI) jesu značajan uzrok morbiditeta i mortaliteta kod bolesnika u programu alogene transplantacije matičnih ćelija (aloTMČ). U literaturi postoje brojni podaci o učestalosti IFI infekcija, značaju primarne antigljivične profilakse i njenom uticaju na smanjenje pojave gljivičnih infekcija, kod bolesnika koji su u programu aloTMČ-a.

Prospektivna studija, u koju je bilo uključeno 23 centra za transplantaciju u Sjedinjenim Američkim Državama, analizirala je epidemiologiju i faktore rizika kod IFI infekcija i dala podatke da je invazivna aspergiloza bila najčešća infekcija, dok su invazivna kandidijaza i ne-*Aspergillus* plesnima izazvane infekcije bile ređe [14]. Takođe, multicentrična prospektivna studija iz Brazila dala je rezultate da su IFI infekcije otkrivene kod 9,2% slučajeva, nakon aloTMČ-a [15]. U ovom istraživanju, učestalosti invazivne aspergiloze i kandidijaze su bile slične, dok je u prospektivnom istraživanju iz Italije, aspergiloza bila prva po učestalosti (81,1%), dok je kandidijaza bila znatno manje zastupljena (11%).

Na osnovu rezultata brojnih studija, primena mifungina, posakonazola i ostalih antigljivičnih lekova, kao antigljivične profilakse, jeste sveopšte prihvaćena

Clinically manifest infection, in the form of oral candidiasis, was present in 19 patients (45.2%), while pulmonary aspergillosis developed in only 3 patients (7.1%).

The existence of a statistically significant connection between clinically manifest infection (oral candidiasis/pulmonary aspergillosis) and the intensity of the applied conditioning regimen was analyzed, however, a statistically significant connection was not found ( $p = 0.327$  MAC,  $p = 0.256$  RIC), i.e., there was no statistically significant difference in the expression of manifest fungal infection between patients on the MAC and the ones on the RIC regimen. Also, there was no statistically significant connection between clinically manifest infection and the diagnosis of the patients ( $p = 0.580$ ).

Of the three patients (7.1%) who developed clinically manifest pulmonary aspergillosis, all three tested positive on the galactomannan test (100%), which proved statistically significant ( $p < 0.001$ ). Also, a statistically significant connection between clinically manifest pulmonary aspergillosis and graft weakness was established: 2 (66.6%) vs. 1 (33.3%), ( $p = 0.016$ ).

The approximated median length of patient survival after allo-SCT was 56 months.

## DISCUSSION

Invasive fungal infections (IFI) are a significant cause of morbidity and mortality in patients submitted to the program of allogenic stem-cell transplantation (allo-SCT). There are numerous data in literature on the frequency of IFI infections, the significance of primary antifungal prophylaxis, and its influence on the reduction of the occurrence of fungal infections, in patients submitted to the program of allo-SCT.

A prospective study, which included 23 transplantation centers in the United States of America, analyzed the epidemiology and risk factors in IFIs and provided data confirming invasive aspergillosis to be the most frequent infection, while invasive candidiasis and non-*Aspergillus spp.* related invasive fungal infections were less frequent [14]. Also, results of a multicentric prospective study from Brazil concluded that IFIs were found in 9.2% of cases, after allo-SCT [15]. In this study, the frequency of the occurrence of aspergillosis and the frequency of occurrence of candidiasis were similar, while, in a prospective study from Italy, aspergillosis was the most common infection (81.1%), while candidiasis was far less frequent (11%).

Based on the results of numerous studies, the application of micafungin, posaconazole, and other antifungal drugs as antifungal prophylaxis, is generally accepted for the prevention of IFIs in patients submitted to the allo-SCT program. This is why analyzing the effect of antifungal prophylaxis remains a challenge and the subject of many studies [17,18,19].

za prevenciju nastanka *IFI* infekcija kod bolesnika u programu aloTMČ-a. Zbog toga je ispitivanje uticaja antigljivične profilakse i dalje izazov i predmet mnogih istraživanja [17,18,19].

Istraživanje sprovedeno u Nemačkoj potvrđuje razliku između primene mono i kombinovane profilakse [19]. U ovoj studiji, upoređivana su dva profilaktička režima primenjena nakon aloTMČ-a, u vidu primene posakonazola *per os* (POS), i njegove kombinacije tj. premoščavanja (engl. *bridging*) sa intravenskim mikafunginom (POS-MIC). Bolesnici koji su primili POS-MIC, prema ovom istraživanju, imali su manju verovatnoću da će razviti invazivnu aspergilozu (RR 0,71, 95% CI 0,51 – 1,00) ili moguću *IFI* infekciju (RR 0,36, 95% 0,15 – 0,87). Ovi rezultati ukazuju na to da kombinacija posakonazola sa intravenskim mikafunginom (u vidu *bridging*-a) može poboljšati antigljivičnu profilaksu kao i smanjiti učestalost aspergiloze, što se pokazalo i u našem istraživanju.

U toku našeg istraživanja, ispitali smo da li postoji korelacija između klinički manifestne infekcije i jačine primenjenog kondicionog režima (MAC/RIC), međutim statistički značajna povezanost nije nađena, iako je bilo očekivano da u grupi bolesnika koji su primali MAC kondicioni režim bude veća učestalost *IFI* infekcija. Izvesno je da je mali broj pacijenata u našem uzorku uticao na dobijene rezultate.

U Nemačkoj je, u periodu od 2013. do 2017. godine, rađeno retrospektivno istraživanje na 156 primalaca aloTMČ-a, na odeljenju intenzivne nege [21]. Standardni dijagnostički testovi izvedeni na bronhoalveolarnom lavatu (BAL) obuhvatali su konvencionalnu kulturu za bakterije i gljivice, direktnu mikroskopiju, galaktomanan i PCR testiranje za otkrivanje gljivičnih, bakterijskih i virusnih patogena. Prema dobijenim rezultatima, gljivične infekcije su bile najistaknutija grupa patogena i identifikovane su kod 28 pacijenata (42%). Najviše patogena je pripadalo vrstama plesni. Uzimajući u obzir rezultate kulture i testove iz BAL-a (galaktomanan i PCR) i krvi (galaktomanan), dijagnostička obrada pružila je mikološke dokaze o *Aspergillus spp.* kod 20 pacijenata (30%). Šesnaest (80%) ovih pacijenata imalo je dijagnozu invazivne aspergiloze.

Naša studija je takođe pokazala statistički značajnu povezanost između ispoljavanja slabosti kalema kod bolesnika nakon aloTMČ-a i razvoja klinički manifestne aspergiloze. Ovaj nalaz je očekivan jer slabost kalema prolongira imunokompromitovanost bolesnika nakon aloTMČ-a, pa samim tim povećava verovatnoću razvoja oportunističkih infekcija kao što je aspergiloza.

## ZAKLJUČAK

Ovo istraživanje je pokazalo da se invazivne gljivične infekcije javljaju i kod pacijenata u program

A study carried out in Germany confirms the difference between the application of mono and combination prophylaxis [19]. In this study, two prophylactic regimens applied after allo-SCT, were compared: the administering of posaconazole *per os* (POS), and its combination, i.e., bridging with intravenous micafungin (POS-MIC). According to this study, patients who received POS-MIC had a lesser probability of developing invasive aspergillosis (RR 0.71, 95% CI 0.51 – 1.00) or possible IFIs (RR 0.36, 95% 0.15 – 0.87). These results indicate that the combination of posaconazole with intravenous micafungin (in the form of bridging) may improve antifungal prophylaxis, as well as decrease the frequency of the occurrence of aspergillosis, which was also the result of our study.

In our study, we analyzed whether there was a correlation between the clinically manifest infection and the intensity of the applied conditioning regimen (MAC/RIC), however, a statistically significant connection was not found, although it was expected that, in the group submitted to the MAC conditioning regimen, the frequency of IFIs would be higher. Evidently, the small number of patients in our sample affected the results of the study.

In Germany, in the period between 2013 and 2017, a retrospective study was performed on 156 intensive care patients who were recipients of allo-SCT [21]. Standard diagnostic tests performed on bronchoalveolar lavage (BAL) specimens included conventional bacteria and fungi culture testing, direct microscopy, galactomannan and PCR testing for detecting fungal, bacterial and viral pathogens. According to the results of the tests, fungal infections were the leading group of pathogens, and were discovered in 28 patients (42%). Most of the pathogens belonged to species of molds. Based on the results from the cultures and the BAL tests (galactomannan and PCR), as well as the blood tests (galactomannan), diagnostic processing offered proof on *Aspergillus spp.* in 20 patients (30%). Sixteen (80%) of these patients were diagnosed with invasive aspergillosis.

Our study also demonstrated a statistically significant connection between the manifestation of graft weakness in patients after allo-SCT and the development of clinically manifest aspergillosis. This finding was expected, as graft weakness renders the patients to be immunocompromised longer, after allo-SCT, whereby it increases the probability of the development of opportunistic infections, such as aspergillosis.

## CONCLUSION

This study has shown that, while invasive fungal infections occur also in patients in the program of allo-SCT, who are treated with antifungal prophylaxis,

aloTMC-a, koji su na tretmanu antigljivične profilakse, ali se ove infekcije javljaju kod značajno manjeg broja ovakvih pacijenata. Rezultati ove studije pokazali su da je uvođenje dvojne kombinovane antigljivične profilakse (mikafungin i posakonazol) imalo za posledicu da incidencija IFI infekcija kod bolesnika u programu aloTMC-a bude značajno manja.

**Sukob interesa:** Nije prijavljen.

these infections affect a significantly lesser number of such patients. The results of this study have shown that the introduction of dual combination antifungal prophylaxis (micafungin and posaconazole) results in a significant decrease in the incidence of IFIs, in patients submitted to the allo-SCT program.

**Conflict of interest:** None declared.

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