BAKTERIJSKE INFEKCIJE KOD BOLESNIKA U PROGRAMU ALOGENE TRANSPLANTACIJE KOŠTANE SRŽI

ORIGINALNI RAD

ORIGINAL ARTICLE

BACTERIAL INFECTIONS IN PATIENTS IN ALLOGENIC STEM CELL TRANSPLANTATION PROGRAMS

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SAŽETAK

Uvod: Bakterijske infekcije su česte među bolesnicima u programu alogene transplantacije matičnih ćelija hematopoeze. Infekcije su najčešći uzrok obolјevanja i umiranja transplantiranih pacijenata. Najčešći patogeni su bakterije *Staphylococcus* i *Enterobacteriaceae*, koje izazivaju približno po 25% bakterijskih infekcija, a prate ih *Enterococcus* i *P. aeruginosa*.

Cilj: Cilj rada jeste određivanje učestalosti bakterijskih infekcija kod pacijenata u programu alogene transplantacije matičnih ćelija hematopoeze, na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije, kao i određivanje epidemiološke situacije, učestalosti različitih oblika kliničke slike bakterijskih infekcija i njihovog uticaja na preživljavanje.

Materijali i metode: U retrospektivnu kohortnu studiju uključeno je 58 bolesnika. Prisustvo bakterija određivano je standardnom bakteriološkom kultivacijom iz uzoraka. Dijagnoza lokalizovane bakterijske infekcije donosi se na osnovu postojanja karakterističnih kliničkih znakova, uz bakteriološku potvrdu. Klinička dijagnoza sepse postavlja se na osnovu pozitivne hemokulture i postojanja sistemskog inflamatornog odgovora. Lečenje bakterijske infekcije počinje se empirijski, a nastavlja antibioticima po antibiogramu. Preživljavanje bolesnika analizirano je Kaplan-Majerovom metodom i upoređivano uz pomoć log-rank testa.

Rezultati: Bakterijske infekcije su se javile kod 15 (25,9%) pacijenata. Najčešći izazivači su bile Gram-negativne bakterije (65,2%). Najčešća klinička slika je bila sepsa (59,0%). Letalitet od bakterijskih infekcija je bio 60,0%. Na osnovu Kaplan-Majerove krive, medijana preživljavanja bez bakterijskih infekcija je iznosila 106,0 meseci (95% CI 85,2 – 163,3), dok je za pacijente sa bakterijskim infekcijama iznosila 14,0 (95% CI 8,9 – 19,1). Log-rank testom uočena je značajna statistička razlika u preživljavanju ove dve grupe ($p < 0,001$). *Pseudomonas spp*. je bio osetljiv na kolistin i/ili meropenem. *Klebsiella pneumoniae* je bila osetljiva je na kolistin.

Zaključak: Bakterijske infekcije su česte na našoj klinici, izazvane su bolničkim i endogenim oportunističkim bakterijama. Prevenciju ovih infekcija treba usmeriti ka merama prevencije bolničkih infekcija.

Ključne reči: bakterijske infekcije, alogena transplantacija matičnih ćelija hematopoeze, sepsa

ABSTRACT

Introduction: Bacterial infections are common in patients enrolled in allogenic hematopoietic stem cell transplantation. Infections are the most common cause of illness and mortality in transplanted patients. The most common pathogens are *Staphylococcus* and *Enterobacteriaceae,* causing 25% of bacterial infections, each, followed by *Enterococcus* and *P. aeruginosa*.

Aim: The aim of the paper is calculating the frequency of bacterial infections in patients enrolled in allogenic hematopoietic stem cell transplantation, at the Clinic for Hematology of the University Clinical Center of Serbia, as well as analyzing the epidemiological situation, the frequency of different clinical presentations of bacterial infections, and their effect on overall survival.

Materials and methods: This retrospective cohort study enrolled 58 patients. The presence of bacteria was determined with standard microbial cultivation from samples. The diagnosis criteria for localized bacterial infection are a positive culture and characteristic clinical presentation. Sepsis is clinically diagnosed by a combination of a positive hemoculture and the existence of systemic inflammatory response. Management of bacterial infection starts empirically and is continued in keeping with antibiogram results. Patient survival was analyzed with the Kaplan-Meier method and compared with the log-rank test.

Results: Bacterial infections were registered in 15 (25.9%) patients. The most common pathogens were Gram-negative bacteria (65.2%). The most common clinical presentation was sepsis (59.0%). Bacterial infection lethality was 60%. The Kaplan-Meier curve showed the median value for estimated patient survival in patients without bacterial infection to be 106.0 months (95% CI 85.2 – 163.3), while, for patients with bacterial infection, it was 14.0 (95% CI 8.9 – 19.1). The log-rank test showed a significant difference in the length of survival between these two groups ($p < 0.001$). *Pseudomonas spp.* was sensitive to colistin and/or meropenem. *Klebsiella pneumoniae* was sensitive to colistin.

Conclusion: Bacterial infections are common at our clinic and are caused by endogenous opportunistic bacteria. Therefore, emphasis should be placed on the implementation of preventive measures aimed at preventing hospital-acquired infections.

Key words: bacterial infections, allogenic hematopoietic stem cell transplantation, sepsis

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UVOD

Bakterijske infekcije (BI) kod pacijenata sa imunosupresijom, a među njima su i bolesnici u programu alogene transplantacije matičnih ćelija hematopoeze (alo-TMĆH), česte su i potencijalno životno ugrožavajuće komplikacije lečenja. Infekcije su, uopšteno, najčešći uzrok oboljevanja i umiranja transplantiranih pacijenata [1,2]. One se mogu javiti *de novo*, mogu se preneti sa donora ili se aktivirati iz latentnih formi kod recipijenta [3].

Rizik od razvoja bakterijske infekcije kod ovih bolesnika zavisi od brojnih faktora, uključujući vrstu kalema, izvor matičnih ćelija, korišćenje mijeloablativnih režima, imunološku rekonstituciju, postojanje bolesti „kalem protiv domaćina" (engl. *graft versus host disease - GVHD*) [4], postojanje mukozitisa, kliconoštva, vaskularnih i drugih poremećaja. Pacijenti u programu alo-TMĆH su izrazito osetljivi na bakterijske infekcije, s obzirom da se, usled oštećenja mukoza hemioterapeuticima, gubi prirodna membrana i zaštita od prodora endogenih mikroorganizama. U toku kondicioniranja pacijenata za alo-TMĆH dolazi do ablacije koštane srži i neutropenije (čije se poboljšanje očekuje nakon engraftmenta), što dovodi do slabljenja imunološke komponente odbrane, te omogućava prodor bakterija i razvoj bakterijskih infekcija.

Ulazno mesto za bakterijske infekcije predstavlja mukoza creva, kada su u pitanju Gram-negativni izazivači. Kada je reč o Gram-pozitivnim bakterijama, najčešće ulazno mesto su vaskularni kateteri.

Studije pokazuju predominacije različitih izazivača u različitim transplantacionim centrima, tako da je određivanje epidemiološke situacije u ustanovi od ključnog značaja za profilaksu bakterijskih infekcija. Uopšteno gledano, najčešći izazivači bakterijskih infekcija kod bolesnika u programu alo-TMĆH su bolnički patogeni, te su infekcije nozokomijalne. Koagulaza negativan *Staphylococcus* i *Enterobacteriaceae* su najčešći patogeni i izazivaju približno po 25% bakterijskih infekcija. Prate ih *Enterococcus, P. aeruginosa* i *viridans streptococci*, kao i pseudomembranozni kolitis izazvan bakterijom *C. Difficile*, koja se javlja u 15% slučajeva, i to češće kod alogenih nego autolognih transplantacija [5].

Najčešće kliničke slike bakterijskih infekcija su febrilna neutropenija, koja se javlja u 80% bolesnika u programu alo-TMĆH [6], sepsa i septički šok, infekcije gastrointestinalnog trakta, pneumonije i infekcije mekih tkiva. Nešto ređe se javljaju infekcije urinarnog trakta i centralnog nervnog sistema [6].

U prethodnim decenijama, dobra antibakterijska profilaksa i promptno lečenje febrilne neutropenije dovele su do pada oboljevanja i smrtnosti od bakterijskih infekcija kod bolesnika u programu alo-TMĆH, ali se javlja novi izazov, a to je antimikrobna rezistencija. S vremenom se, naime, razvila grupa od šest

INTRODUCTION

Bacterial infections (BI) in immunosuppressed patients, which includes patients in the allogenic hematopoietic stem cell transplantation program (allo-HSCT), represent frequent and potentially life-threatening treatment complications. Infections are generally the most common cause of illness and death in transplanted patients [1,2]. They can occur as a *de novo* infection, or they may be transferred from the donor, or activated from latent forms in the recipient [3].

The risk of bacterial infection development in these patients depends on numerous factors, including the type of graft, the source of stem cells, the application of the myeloablative regimens, immune reconstitution, presence of graft versus host disease (GVHD) [4], presence of mucositis, being a germ carrier, presence of vascular and other disorders. Patients in the program of allo-HSCT are extremely sensitive to bacterial infections, since, due to the damage sustained by the mucosa and caused by chemotherapeutics, the natural membrane and protection against the invasion of endogenous microorganisms is lost. During patient conditioning for allo-HSCT, bone marrow ablation occurs as well as neutropenia (whose improvement is expected upon engraftment), thus leading to the weakening of the immunological component of defense and allowing for the invasion of bacteria and the development of bacterial infection.

The portal of entry for bacterial infections is the intestinal mucosa, when it comes to Gram-negative pathogens. When it comes to Gram-positive bacteria, the most common portal of entry are vascular catheters.

Studies have shown the predominance of different pathogens in different transplantation centers, which is why determining the epidemiological situation in a given institution is of crucial importance for the prophylaxis of bacterial infections. Generally speaking, the most common causative agents of bacterial infections in patients undergoing allo-HSCT are hospital pathogens, and the infections are therefore nosocomial. Coagulase-negative *Staphylococcus* and *Enterobacteriaceae* are the most common pathogens, and they case approximately 25% of bacterial infections, each. They are followed by *Enterococcus, P. aeruginosa* and *viridans streptococci,* as well as by pseudomembranous colitis caused by *C. Difficile*, which can be found in 15% of the cases, more frequently in allogenic than in autologous transplantations [5].

The most frequent clinical presentations of bacterial infections are the following: febrile neutropenia, which occurs in 80% of patients undergoing allo-HSCT [6], sepsis and septic shock, gastrointestinal tract infections, pneumonia, and soft tissue infections. Less frequently, infections of the urinary tract and the central nervous system are found [6].

multirezistentnih mikroorganizama, koja se naziva *ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* i *Enterobacter spp.*) [7]. Problem sa multirezistentnim bakterijama, posebno Gram-negativnim, izuzetno je značajan kod pacijenata sa produženom neutropenijom koji su na dugotrajnim hemioterapijskim režimima [8].

Cilj ovog rada je određivanje učestalosti bakterijskih infekcija kod pacijenata u programu alo-TMĆH na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije (UKCS), kao i određivanje epidemiološke situacije i rasprostranjenosti različitih patogena, određivanje učestalosti različitih kliničkih slika bakterijskih infekcija, kao i njihovog uticaja na preživljavanje bolesnika, sa posebnim osvrtom na septička stanja.

MATERIJALI I METODE

U retrospektivnu kohortnu studiju je uključeno 58 bolesnika sa Klinike za hematologiju UKCS-a, koji su bili u procesu alogene transplantacije koštane srži i praćeni u periodu od decembra 2017. godine zaključno sa decembrom 2020. godine. Bolesnici su imali dijagnoze akutne limfoblastne leukemije (ALL), akutne mijeloidne leukemije (AML), hronične limfocitne leukemije (HLL), Hočkinovog limfoma (HL), Nehočkinovog limfoma (NHL), mijelodisplastičnog sindroma (MDS), te mijelodisplastičnog/mijeloproliferativnog sindroma (MDS/MPS).

Mikrobiološke metode

Prisustvo bakterija u izolatu određivano je standardnom bakteriološkom kultivacijom iz dobijenih uzoraka.

Kao uzorci korišćeni su krv, urin, sputum, stolica, potom brisevi kože, sluznica i promena na mekim tkivima.

Klinički kriterijumi

Klinička dijagnoza lokalizovane bakterijske infekcije donošena je na osnovu postojanja karakterističnih kliničkih znakova, uz bakteriološku potvrdu.

Klinička dijagnoza sepse postavljana je na osnovu pozitivne hemokulture i postojanja sistemskog inflamatornog odgovora (Tabela 1).

In the previous decades, good antibacterial prophylaxis and prompt treatment of febrile neutropenia have led to a drop in the morbidity and mortality from bacterial infections, in patients undergoing allo-HSCT. However, a new challenge has emerged – antimicrobial resistance. Namely, with time, a group of six multiresistant microorganisms has emerged, and has been labeled ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) [7]. The problem of multiresistant bacteria, especially Gram-negative ones, is of extreme importance in patients with prolonged neutropenia undergoing long-term chemotherapy regimens [8].

The aim of this paper is to determine the frequency of bacterial infections in patients undergoing allo-HSCT at the Clinic for Hematology of the University Clinical Center of Serbia (UCCS); determine the epidemiological situation and the distribution of different pathogens; determine the frequency of different clinical presentations of bacterial infections, as well as their impact on patient survival, with a special insight into septic states.

MATERIALS AND METHODS

This retrospective cohort study involved 58 patients treated at the Clinic for Hematology of the UCCS, who were in the process of allogenic bone marrow transplantation and were followed up from December 2017 until December 2020. The diagnoses of the patients were as follows: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), myelodysplastic syndrome (MDS), myelodysplastic/myeloproliferative syndrome (MDS/MPS).

Microbiological methos

The presence of bacteria in the isolate was determined with standard microbial cultivation from the obtained samples.

The following were used: blood samples, urine specimens, sputum samples, stool samples, skin swabs, mucosal swabs, and swabs of soft tissue lesions.

Tabela 1. Kriterijumi za sindrom sistemskog inflamatornog odgovora (SIRS); (postojanje dva ili više simptoma od navedenih)

Table 1. Criteria for systemic inflammatory response syndrome (SIRS); (presence of two or more of the presented symptoms)

Telesna temperatura / *Body temperature*	< 36°C or > 38°C
Srčana frekvencija / *Heart rate*	> 90/min
Respiratorna frekvencija / *Respiratory rate*	> 20/min ili / *or* $RaSO_2$ < 32 mmHG
Leukociti / *WBC*	< 4 x 10^9/L (< 4,000/mm^3) ili / *or* >12 x 10^9/L (12,000/mm^3) ili / *or* 10% nezrelih formi / 10% *immature forms*

Klinička dijagnoza septičkog šoka postavljana je na osnovu postojanja hipotenzije i kardiovaskularne nestabilnosti kod pacijenata sa potvrđenom sepsom.

Profilaksa i lečenje

Svi pacijenti uključeni u program alo-TMĆH dobijali su antimikrobnu profilaksu. Hinolonski preparati (levofloksacin 500 mg p.o.) i cefelasporini (cefepim i.v.) korišćeni su za antibakterijsku profilaksu. Baktrim (2 x 400 mg p.o.) je korišćen za prevenciju reaktivacije *Toxoplasma gondii* i *Pneumocystis carinii*. Za antiviralnu profilaksu korišćen je aciklovir 3 x 400 mg p.o., a za profilaksu gljivičnih infekcija micafungin 50 mg i.v., do engraftmenta, potom posakonazol 3 x 200 mg p.o., do 90 dana nakon engraftmenta.

Lečenje bakterijskih infekcija najčešće se počinje empirijski, antibioticima širokog spektra, do dobijanja rezultata antibiograma. Nakon toga, antibiotici se primenjuju po antibiogramu, što je bio slučaj i sa našim pacijentima.

Za lečenje pojedinih pacijenata sa infekcijama kože i mekih tkiva korišćene su i hirurške metode, dok su pacijenti u septičkom šoku dobijali suportivnu terapiju.

Pre sprovođenja alo-TMĆH procedure, bolesnici su prolazili kroz kondicione režime, koji podrazumevaju primenu hemioterapijskih agenasa i/ili radioterapije, pre transplantacije. Koriste se dva režima – mijeloablativno kondicioniranje (engl. *myeloablative conditioning – MAC*) i kondicioniranje smanjenog intenziteta (engl. *reduced-intensity conditioning – RIC*). *MAC* je agresivniji i dovodi do potpunog opustošenja koštane srži (aplazije).

Statistička obrada

Inicijalno je formirana baza podataka grupisanjem i tabeliranjem rezultata po ispitivanim obeležjima bolesnika. Deskriptivni statistički parametri izraženi su kroz medijanu i raspodele relativnih frekvencija. Ukupno preživljavanje bolesnika obuhvatalo je period od momenta dijagnoze do smrtnog ishoda ili zaključno sa decembrom 2020. godine, kod živih bolesnika. Preživljavanje bolesnika analizirano je Kaplan-Majerovom metodom i upoređivano log-rank metodom. Za statističku obradu podataka korišćen je softver *SPSS 23.0 za Microsoft Windows*.

REZULTATI

Studijom je obuhvaćeno 58 pacijenata – 33 (56,9%) muškog pola i 25 (43,1%) ženskog pola, medijane starosti 38,40 godina, u opsegu od 19 do 58 godina. Učestalost dijagnoza bolesnika obuhvaćenih istraživanjem je bila: ALL (31,0%), AML (44,8%), HLL (1,7%), Hočkinov limfom (13,8%), MDS (1,7%), MDS/MPS (3,4%), NHL (3,4%) (Tabela 2).

Clinical criteria

Clinical diagnosis of localized bacterial infection was established on the basis of the presence of characteristic signs, with bacteriologic confirmation of infection.

Clinical diagnosis of sepsis was established on the basis of a positive blood culture and the presence of systemic inflammatory response (Table 1).

Clinical diagnosis of septic shock was established on the basis of the presence of hypotension and cardiovascular instability in a patient with confirmed sepsis.

Prophylaxis and treatment

All patients undergoing allo-HSCT received antimicrobial prophylaxis. Quinolone antibiotics (levofloxacin 500 mg p.o.) and cephalosporins (cefepime i.v.) were used as antibacterial prophylaxis. Bactrim (2 x 400 mg p.o.) was used for preventing the reactivation of *Toxoplasma gondii* and *Pneumocystis carinii*. Acyclovir 3 x 400 mg p.o. was used for antiviral prophylaxis, while for prophylaxis of fungal infections, micafungin 50 mg i.v. was used until engraftment, and then posaconazole 3 x 200 mg p.o., until 90 days following engraftment.

In most cases, bacterial infections are initially treated empirically, with broad-spectrum antibiotics, until the antibiogram results are received. After that, antibiotics are administered according to the antibiogram, which was the case with our patients as well.

Surgical methods were applied in treating patients with skin infections and infections of soft tissues, while patients in septic shock received supportive therapy.

Prior to allo-HSCT, patients underwent conditioning regimens, which involve the application of chemotherapeutical agents and/or radiotherapy, prior to transplantation. Two regimens are in use – myeloablative conditioning (MAC) and reduced-intensity conditioning (RIC). MAC is more aggressive and leads to complete destruction of the bone marrow (aplasia).

Statistical data processing

Initially, a database was formed by grouping results and categorizing them in tables, according to the studied patient characteristics. Descriptive statistical parameters were expressed as the median and the distributions of relative frequencies. The overall patient survival covered the period from the time of diagnosis until the lethal outcome or until December 2020, for surviving patients. Patient survival was analyzed with the Kaplan-Meier method and compared with the log-rank test. Statistical data processing was performed using the SPSS 23.0 software for Microsoft Windows.

Tabela 2. Učestalost dijagnoza hematoloških oboljenja

Table 2. Frequency of hematological diseases

Dijagnoza / *Diagnosis*	Broj (%) / *Number (%*
ALL	18 (31.0%)
AML	26 (44.8%)
HLL	1 (1.7%)
HL	8 (13.8%)
MDS	1 (1.7%)
MDS/MPS	2 (3.4%)
NHL	2 (3.4%)

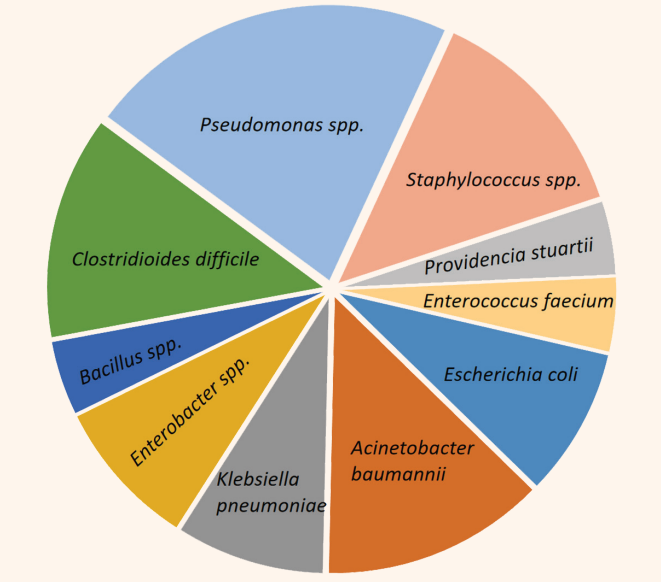
ALL – akutna limfoblastna leukemija; AML – akutna mijeloidna leukemija; HLL – hronična limfocitna leukemija; HL – Hočkinov limfom; MDS – mijelodisplastični sindrom; MDS/MPS – mijelodisplastični/mijeloproliferativni sindrom; NHL – Nehočkinov limfom

ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; CLL – chronic lymphocytic leukemia; HL – Hodgkin lymphoma; MDS – myelodysplastic syndrome; MDS/MPS – myelodysplastic/myeloproliferative syndrome; NHL – non-Hodgkin lymphoma

Tabela 3. Učestalost izazivača bakterijskih infekcija

Table 3. Frequency of bacterial pathogens

Izazivač / *Pathogen*	Broj (%) / *Number (%)*
Escherichia coli	2 (9.0%)
Acinetobacter baumannii	3 (13.0%)
Klebsiella pneumoniae	2 (9.0%)
Bacillus spp.	1 (4.0%)
Clostridioides difficile	3 (13.0%)
Pseudomonas spp.	5 (22.0%)
Staphylococcus spp.	3 (13.0%)
Providencia stuartii	1 (4.0%)
Enterobacter spp.	2 (9.0%)
Enterococcus faecium	1 (4.0%)



Grafikon 1. Učestalost izazivača bakterijskih infekcija

Figure 1. Frequency of bacterial pathogens

RESULTS

The study involved 58 patients – 33 (56.9%) male patients and 25 (43.1%) female patients. Their median age was 38.40 years (range: 19 – 58). The frequency of the diagnoses for patients involved in the study were as follows: ALL (31.0%), AML (44.8%), CLL (1.7%), Hodgkin lymphoma (13.8%), MDS (1.7%), MDS/MPS (3.4%), NHL (3.4%) (Table 2).

More of the analyzed patients, 43 (74.1%) of them, were on the MAC regimen, while a lesser number was conditioned with the RIC regimen, i.e., 15 (25.9%) patients.

Bacterial infections occurred in 15 (25.9%) patients, of whom three patients had polymicrobial infection. This is why the total number of isolated bacteria was 23. The most frequent pathogens were Gram-negative opportunistic bacteria (15; 65.2%), while Gram-positive pathogens caused infections in 34.8 % of the cases. The most common pathogens were *Pseudomonas spp.* and *Acinetobacter baumannii*, while the least common were *Enterococcus faecium*, *Providencia stuartii*, and *Bacillus* spp. The frequencies of the isolated pathogens have been presented in detail in Table 3 and Graph 1 (Table 3 and Figure 1).

The following samples were taken for bacteriological testing: blood samples, urine specimens, stool samples, sputum samples, and swabs taken from the skin, mucosa and soft tissue lesions. The total number of samples taken for laboratory testing was 17. Sampling was most frequently done from blood (47.1%), followed by swabs taken from the skin, mucosa, and soft tissue lesions (23.5%), urine (11.8%), stool samples (11.8%), and finally sputum (5.9%).

The total number of clinical presentations of the infections was 22. Of that number, the most common was sepsis, with or without septic shock (13 patients; 59.0% of infections), followed by infections of the skin, mucosa, and soft tissues (18.0%), pseudomembranous colitis

Tabela 4. Učestalost kliničkih slika bakterijskih infekcija

Table 4. Frequency of bacterial infection clinical presentations

Klinička slika bakterijske infekcije / *Clinical presentation of bacterial infection*	**Broj (%) / *Number (%)***
Sepsa i septički šok / *Sepsis or septic shock*	13 (59.0%)
Infekcije kože, sluzokože i mekih tkiva / *Skin, mucosal and soft tissue infections*	4 (18.0%)
Pneumonija / *Pneumonia*	1 (4.5%)
Enterokolitis / *Enterocolitis*	1 (4.5%)
Pseudomembranozni kolitis / *Pseudomembranous colitis*	3 (14.0%)

Veći broj ispitivanih bolesnika, njih 43 (74,1%), bilo je na MAC kondicionom režimu, dok je 15 (25,9%) kondicionirano RIC režimom.

Bakterijske infekcije su se javile kod 15 (25,9%) pacijenata, od toga je polimikrobnu infekciju imalo tri pacijenta. Stoga je broj izolovanih bakterija bio 23. Najčešći izazivači su bile Gram-negativne oportunističke bakterije (15; 65,2%), dok su Gram-pozitivni izazivači dovodili do infekcija u 34,8 % slučajeva. Kao najčešći izazivači pojavili su se *Pseudomonas spp.* i *Acinetobacter baumannii*, dok su najređi bili Enterococcus faecium, *Providencia stuartii* i *Bacillus spp*. Detaljni prikaz učestalosti izolovanih izazivača dat je u Tabeli 3 i Grafikonu 1 (Tabela 3 i Grafikon 1).

Uzorci za bakteriološka ispitivanja uzeti su iz krvi, urina, stolice, sputuma i briseva kože, sluznica i mekotkivnih promena. Ukupni broj uzoraka za laboratorijsku dijagnostiku bio je 17. Najčešće je uzorkovana krv (47,1%), zatim brisevi kože, sluznica i mekotkivnih promena (23,5%), potom urin (11,8%), stolica (11,8%) i sputum (5,9%).

Ukupni broj kliničkih slika infekcija je bio 22. Od toga je najčešća bila sepsa sa ili bez septičkog šoka (13 (14.0%), and finally, infections occurring individually – pneumonia and enterocolitis (4.5%, each) (Table 4).

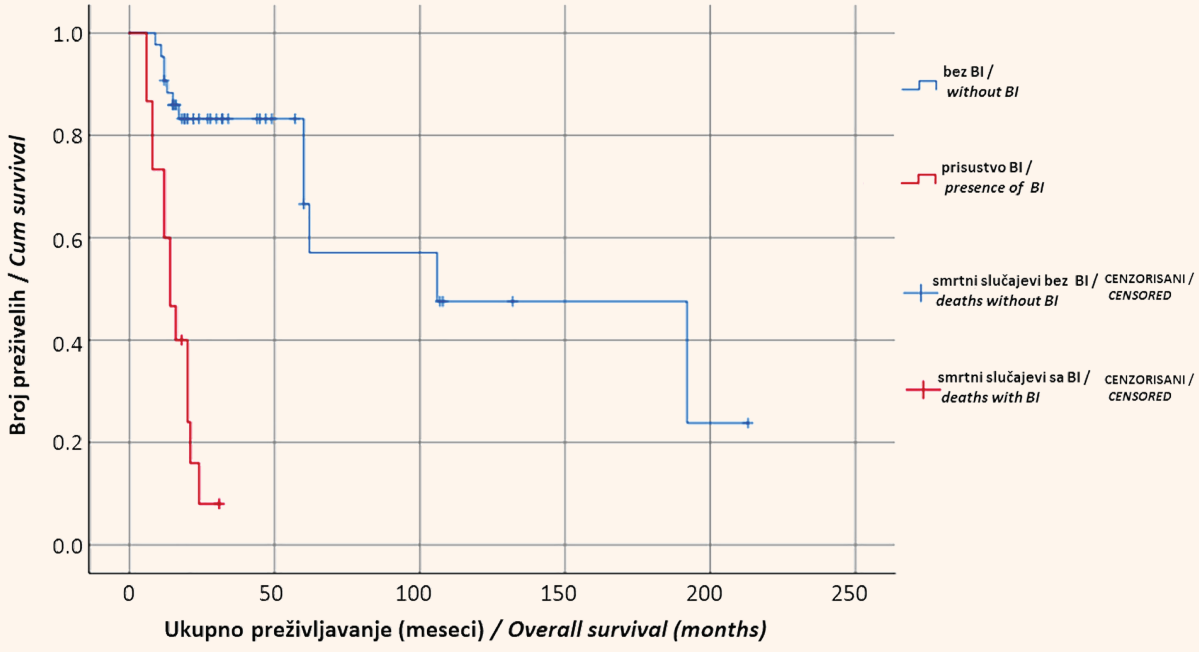
The number of patients who died due to bacterial infections was 9, of whom 8 clinically presented with sepsis. Therefore, lethality of bacterial infections was 60.0%, while the mortality rate related to bacterial infections amongst patients involved in the allo-HSCT program was 15.5%.

Based on the Kaplan-Meier survival curve, the median of survival in patients without proven bacterial infection was 106.00 months (95% CI 85.2 – 163.3), while, for patients with bacterial infections, it was 14.0 months (95% CI 8.9 – 19.1). Based on the log-rank test, a statistically significant difference was registered in the survival of these two groups of patients ($p < 0.001$) (Figure 2).

Fisher's exact test was used to compare the frequency of bacterial infections in relation to the conditioning regimen (MAC or RIC). The test did not show a statistically significant difference in frequency ($p = 0.308$).

Descriptive data

Of a total of 13 patients with sepsis, two patients had septic shock.



Grafikon 2. Učestalost izazivača bakterijskih infekcija

Figure 2. Frequency of bacterial pathogens

pacijenata; 59,0% infekcija), slede je infekcije kože, sluznica i mekih tkiva sa 18,0%, pseudomembranozni kolitis sa 14,0%, i najzad infekcije koje su se pojedinačno javljale – pneumonija i enterokolitis sa po 4,5% (Tabela 4).

Broj pacijenata koji su umrli usled bakterijskih infekcija bio je 9, od toga je 8 prezentovalo kliničkom slikom sepse. Stoga je letalitet bakterijskih infekcija bio 60,0%, dok je 15,5% bolesnika u programu alo-TMĆH umrlo usled posledica bakterijskih infekcija.

Na osnovu Kaplan-Majerove krive preživljavanja, medijana preživljavanja pacijenata bez dokazane bakterijske infekcije je iznosila 106,00 meseci (95% CI 85,2 – 163,3), dok je za pacijente sa bakterijskom infekcijom iznosila 14,0 meseci (95% CI 8,9 – 19,1). Na osnovu log-rank testa, uočena je značajna statistička razlika u preživljavanju ove dve grupe pacijenata ($p < 0,001$) (Grafikon 2).

Fišerovim testom tačne verovatnoće poređena je učestalost bakterijskih infekcija u odnosu na kondicioni režim (MAC ili RIC). Test nije pokazao statistički značajnu razliku u učestalostima ($p = 0,308$).

Deskriptivni podaci

Od ukupno 13 pacijenata sa sepsom, dva bolesnika su imala septički šok.

Četiri infekcije kože, sluznica i mekih tkiva bile su sledeće: mukozitis, flegmona ušne školjke, flegmona vulve i flegmona perianalne regije.

Polimikrobna infekcija javila se u tri slučaja, sa sledećim izazivačima:

- *Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter spp.*
- *Pseudomonas spp., Enterobacter spp.*
- *Klebsiella pneumoniae,* koagulaza-negativni *Staphylococcus, Providencia stuartii*.

Imali smo antibiogram za 11 izazivača, a rezultati antibiograma su bili sledeći: *Pseudomonas spp.* je u svim dobijenim analizama bio osetljiv na kolistin i/ili meropenem; *Staphylococcus aureus* je bio osetljiv na amikacin, vankomicin i rifampicin; *Enterococcus faecium* je bio osetljiv na vankomicin i meropenem; u svim slučajevima, *Klebsiella pneumoniae* je bila multirezistentna, ali osetljiva na kolistin.

DISKUSIJA

Učestalost bakterijskih infekcija kod bolesnika u programu alo-TMĆH, na Klinici za hemotologiju UKCS-a, nalazi se u opsegu učestalosti, u odnosu na dosadašnju literaturu. U našoj ustanovi, učestalost bakterijskih infekcija se pokazala nešto manjom u odnosu na izveštaj koji je podnela *Polish Pediatric Group for Hematopoietic Stem Cell Transplantation*, kod pedijatrijskih pacijenata u programu alo-TMĆH, gde je učestalost iznosila 40% [9], dok je u našoj studiji bila 25,9%.

The four infections of skin, mucosa, and soft tissues were as follows: mucositis, phlegmon of the earlobe, phlegmon of the vulva, and phlegmon of the perianal region.

Polymicrobial infection occurred in three cases, with the following pathogens:

- *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter spp.*
- *Pseudomonas spp.*, *Enterobacter spp.*
- *Klebsiella pneumoniae*, coagulase-negative *Staphylococcus*, *Providencia stuartii*.

We had an antibiogram for 11 pathogens, and the antibiogram results were as follows: in all the laboratory results, *Pseudomonas spp.* was sensitive to colistin and/or meropenem; *Staphylococcus aureus* was sensitive to amikacin, vancomycin, and rifampicin; *Enterococcus faecium* was sensitive to vancomycin and meropenem; in all cases, *Klebsiella pneumoniae* was multiresistant, but sensitive to colistin.

DISCUSSION

The frequency of bacterial infections in patients involved in the program of allo-HSCT, at the Clinic for Hematology of the UCCS, falls within the range reported in other publications. At our hospital, the frequency of bacterial infections has proven to be somewhat smaller than reported by the Polish Pediatric Group for Hematopoietic Stem Cell Transplantation, in pediatric patients involved in the allo-HSCT program, where the frequency was 40% [9], while it was 25.9% in our study.

A study by Balletto and Mikulska [5] showed that Gram-positive pathogens were more frequent in patients undergoing allo-HSCT (60%), as compared to Gram-negative bacteria (40%). However, the frequencies in different hospitals are different. These frequencies range from 85%/15% to the advantage of Gram-positive bacteria to 74%/26% to the advantage of Gram-negative bacteria, which is in keeping with our result, which was 65.22%/34.78% to the advantage of Gram-negative pathogens.

It can be noted that the bacteria isolated in our study are often opportunistic and that they are in the group of pathogens causing hospital-acquired infections, which is why the state of immunosuppression is not a key factor in their pathogenesis. Also, in the prevention of these infections, measures for preventing nosocomial infections are of the utmost importance.

There are data in literature on the occurrence of opportunistic mycobacterial infections and infection with *Nocardia asteroides* in transplanted patients; however, in our experience so far, this has not been the case, [3,11]. A significant factor in their development is immunosuppression, and the infections are chronic.

Studija koju su sproveli Baleto i Mikulska [5], pokazala je da su Gram-pozitivni izazivači bili češći kod pacijenata u programu alo-TMĆH (60%), u odnosu na Gram-negativne (40%). Međutim, učestalosti na različitim klinikama su različite. Učestalosti se kreću u spektru od 85%/15% u koristi Gram-pozitivnih do 74%/26% u korist Gram-negativnih, što se poklapa sa našim rezultatom od 65,22%/34,78% u korist Gram-negativnih izazivača.

Može se primetiti da su bakterije izolovane u našoj studiji često oportunističke i da spadaju u grupu izazivača bolničkih infekcija, tako da stanje imunosupresije nije ključni faktor u patogenezi. Takođe, u prevenciji ovih infekcija su najznačajnije mere prevencije nozokomijalnih infekcija.

U literaturi postoje podaci o pojavi oportunističkih mikobakterijskih infekcija i infekcija bakterijom *Nocardia asteroides* kod transplantiranih pacijenata, međutim, u našoj dosadašnjoj praksi to nije slučaj [3,11]. Za njihov razvoj, značajni faktor je imunosupresija, a infekcije su hronične.

U literaturi se, kao značajan faktor rizika za razvoj bakterijskih infekcija, navodi vrsta kondicionog režima [5]. *MAC* režim se dovodi u vezu sa češćim infekcijama, međutim, naše istraživanje nije pronašlo ovu vezu (p vrednost Fišerovog testa tačnih verovatnoća bila je veća od 0,05). Ovaj rezultat može se pripisati malom uzorku, ali i nejednakosti grupa pacijenata koji su kondicionirani različitim režimima. Zanimljivo je da su pacijenti na *MAC* protokolu imali 6,5 puta veću učestalost bakterijskih infekcija (*MAC/RIC* = 13/2).

Profil antimikrobne rezistencije bakterija iz naših izolata govori u prilog postojanja značajne rezistencije, na šta ukazuje i činjenica da je *Pseudomonas spp*. bio osetljiv samo na kolistin i/ili meropenem, koji spadaju u grupu rezervnih antibiotika. Interesantno je da nije zabeleženo postojanje vankomicin-rezistentnog *Enterococcus*-a (VRE). Ove podatke treba uzeti sa rezervom, zbog ograničenog uzorka, što je pre svega posledica nedostatka podataka u zdravstvenom informacionom sistemu i elektronskoj medicinskoj dokumentaciji. Antibiogram za *C. difficile* nije rađen zbog očekivanog rezultata i dobre empirijske terapije (metronidazol ili vankomicin p.o.).

Raspodela kliničkih slika bakterijskih infekcija u našem istraživanju odgovara literaturi; najčešća klinička slika kod ovih bolesnika je sepsa. U našoj populaciji se javila u 59% bakterijskih infekcija, dok se u studiji, koja je obuhvatila 313 ispitanika do 100. dana nakon transplantacije matičnih ćelija hematopoeze, pozitivna hemokultura javila u 64,1% obolelih [11].

U dugoročnom praćenju, *Pseudomonas spp.* se pokazao kao najčešći patogen koji dovodi do bakterijske infekcije kod pacijenata u programu alo-TMĆH [12], što se i u našoj studiji pokazalo tačnim.

The type of conditioning regimen is mentioned in literature as a significant risk factor for the development of bacterial infection [5]. The MAC regimen is believed to be linked with more frequent infections; however, our study did not establish this link (the p value of Fisher's exact test was greater than 0.05). This result could be attributed to the small size of the sample, but also to the inequality amongst the groups of patients conditioned with different regimens. It is interesting that the patients which were on the MAC regimen had a 6.5 times higher frequency of bacterial infections (MAC/RIC = 13/2).

The profile of the antimicrobial resistance of the bacteria found in our isolates speaks in favor of the existence of significant resistance, which is also indicated by the fact that *Pseudomonas spp.* was sensitive only to colistin and/or meropenem, which are in the reserved group of antibiotics. It is interesting to note that the presence of vancomycin-resistant *Enterococcus* (VRE) was not registered. These data should be taken with certain reservations, due to the limited size of the sample, which is primarily the result of the lack of data in the health information system and the electronic medical records. The antibiogram for *C. difficile* was not performed, due to the predictable result and good empirical therapy (metronidazole or vancomycin p.o.).

The distribution of clinical presentations of bacterial infections in our study is in keeping with other publications; the most common clinical presentation in these patients is sepsis. In our population, it presented in 59% of bacterial infections, while in a study involving 313 subjects who were followed up until day 100 after hematopoietic stem cell transplantation, a positive hemoculture occurred in 64.1% of the patients [11].

In long-term follow-up, *Pseudomonas spp.* has shown to be the most common pathogen that leads to bacterial infection in patients undergoing allo-HSCT [12], which has proven to be true in our study as well.

A study from 2019, involving a group of patients with multiple myeloma following bone marrow transplantation, determined that sepsis caused by enterococcus significantly affected overall patient survival [13]. Our study can provisionally be compared with this study, but it also shows, based on the Kaplan-Meir curve, a significant drop in survival in patients with bacterial infection, with the predominant BI being sepsis.

In our study, lethality due to bacterial infections was 60%, which is significantly higher than the range of expected lethality of 6% to 40%, reported in two studies which had the most extreme results [14,15].

Our study has several limitations. The group of patients was heterogenous; the study is retrospective; the number of subjects was limited due to the fact that

Studija rađena 2019. godine, na grupi pacijenata sa multiplim mijelomom nakon transplantacije koštane srži, utvrdila je da je sepsa izazvana enterokokom značajno uticala na ukupno preživljavanje pacijenata [13]. Naša studija se uslovno može porediti sa ovom, ali takođe pokazuje, na osnovu Kaplan-Majerove krive, značajno smanjenje preživljavanja kod pacijenata sa bakterijskim infekcijama, a predominanta BI je bila sepsa.

U našoj studiji, letalitet usled bakterijskih infekcija je bio 60%, što je značajno veće od opsega očekivanog letaliteta od 6% do 40%, prikazanog u dve studije sa najekstremnijim rezultatima [14,15].

Naša studija ima nekoliko ograničenja. Grupa pacijenata je bila heterogena; studija je retrospektivna; broj ispitanika je bio ograničen zbog toga što se radi o mladom transplantacionom centru. Kao što je već navedeno, rezultati antibiograma nedostaju za određene pacijente, usled tehničkih problema koji su bili van naše kontrole. Zbog malog broja uzoraka, mali je bio i broj bakterijskih infekcija, pa su određeni parametri prikazani deskriptivno.

ZAKLJUČAK

Na našoj klinici, kod bolesnika u programu alo-TMĆH, bakterijske infekcije su česte, ali nisu samo posledica imunosupresije, već nastaju u kombinaciji imunosupresije i dejstva bolničkih i endogenih oportunističkih bakterija. Stoga je potrebno prevenciju ovih infekcija usmeriti ka opštim merama prevencije bolničkih infekcija i antimikrobnoj profilaksi endogenih, pre svega Gram-negativnih izazivača. Ovom studijom pokazali smo, u određenom stepenu, epidemiološku situaciju u našem Centru, što može značajno uticati na planiranje profilakse u daljem radu. Postoji ohrabrujući podatak, a to je da na našem odeljenju još uvek nema bakterija sa ekstremnim spektrom rezistencije, kao što su Pseudomonas rezistentan na karbapeneme, meticilin-rezistentni *Staphylococcus* (MRSA) i vankomicin-rezistentni *Enterococcus* (VRE).

Najčešće bakterijske infekcije predstavljene su sepsom, sa ili bez septičkog šoka. Njihova terapija je kompleksna, često multidiscplinarna, a mogu se komplikovati i mogu značajno pogoršati prognozu bolesnika. Poznavanje profila antimikrobne rezistencije omogućava početak nešto specifičnije empirijske terapije pre dobijenog antibiograma, što može značajno poboljšati ishode. Sasvim je sigurno da pri sumnji na Gram-negativne bakterije (*Pseudomonas spp.*, *Klebsiella pneumoniae*) standardna empirijska antibiotska terapija nailazi na ograničenja, usled rezistencije.

this is a new transplantation center. As already stated, the antibiogram results were lacking for some of the patients, due to technical difficulties, which were outside of our control. Due to a small number of samples, the number of identified bacterial infections was also small, which is why certain parameters were reported descriptively.

CONCLUSION

At our clinic, in patients undergoing allo-HSCT, bacterial infections are common, but are not only the result of immunosuppression, rather the combination of immunosuppression and the effect of hospital and endogenous opportunistic bacteria. This is why its is necessary to direct the prevention of these diseases towards comprehensive measures of preventing hospital-acquired infections and towards antimicrobial prophylaxis of endogenous, primarily Gram-negative pathogens. With this study we have shown, to a certain degree, the epidemiological situation at our Center, which, going forward, may significantly influence the planning of prophylaxis. The fact that bacteria with an extreme spectrum of resistance, such as carbapenem-resistant *Pseudomonas,* methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), have as yet not been registered on our ward, is encouraging.

The most frequent bacterial infections, described in this study, presented in the form of sepsis, with or without septic shock. Their treatment is complex, often necessitating a multidisciplinary approach, and they can cause complications and significantly worsen the patient's prognosis. Knowing the profile of antimicrobial resistance enables the introduction of more specific empirical therapy before the antibiogram is obtained, which may significantly improve outcomes. It is quite evident, however, that when Gram-negative bacteria (*Pseudomonas spp., Klebsiella pneumoniae*) are suspected, standard empirical antibiotic treatment has its limitations, due to resistance.

LIST OF ABBREVIATIONS AND ACRONYMS

BI – bacterial infections
allo-HSCT – allogenic hematopoietic stem cell transplantation (bone marrow transplantation)
GVHD – graft versus host disease
ALL – acute lymphoblastic leukemia
AML – acute myeloid leukemia
CLL – chronic lymphocytic leukemia
HL – Hodgkin lymphoma
NHL – non-Hodgkin lymphoma
MDS – myelodysplastic syndrome
MDS/MPS – myelodysplastic/myeloproliferative syndrome

SPISAK SKRAĆENICA

BI – bakterijske infekcije
alo-TMĆH – alogena transplantacija matičnih ćelija hematopoeze (transplantacija koštane srži)
GVHD – bolest „kalem protiv domaćina" (engl. graft versus host disease - GVHD)
ALL – akutna limfoblastna leukemija
AML – akutna mijeloidna leukemija
HLL – hronična limfocitna leukemija
HL – Hočkinov limfom
NHL – Nehočkinov limfom
MDS – mijelodisplastični sindrom
MDS/MPS – mijelodisplastični sindrom/mijeloproliferativni sindrom
SIRS – sindrom sistemskog inflamatornog odgovora (engl. systemic inflammatory response syndrome)
p.o. – per os
i.v. – intravenski
MAC – mijeloablativno kondicioniranje
RIC – kondicioniranje smanjenog intenziteta

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SIRS – systemic inflammatory response syndrome
p.o. – *per os* (administered orally)
i.v. – intravenous
MAC – myeloablative conditioning
RIC – reduced-intensity conditioning

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