UČESTALOST INFEKCIJA TOKOM PRIMENE VISOKODOZNE HEMIOTERAPIJE SA AUTOLOGNOM TRANSPLANTACIJOM MATIČNIH ĆELIJA

ORIGINALNI RAD

ORIGINAL ARTICLE

INCIDENCE OF INFECTIONS DURING THE APPLICATION OF HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

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SAŽETAK

Uvod: Autologna transplantacija matičnih ćelija hematopoeze (ATMĆH) je standardni oblik lečenja hematoloških maligniteta. Promene u strategiji ATMĆH-a i unapređenje potporne nege značajno su smanjili incidenciju i obrazac ispoljavanja infekcija kod pacijenata.

Cilj rada: Ispitati učestalost infekcija u prvih 30 dana nakon ATMĆH-a, kao i eventualni uticaj broja $CD34^+$ matičnih ćelija u transplantatu i parametara engraftmenta: *ALC*500_20 (engl. *absolute lymphocyte count* $0{,}5x10^9$/L na dan +20), *ANC*500_11 (engl. *absolute neutrophil count* $0{,}5x10^9$/L na dan +11), i *PLT*20_13 (engl. *platelets* $20x10^9$/L na dan +13), na pojavu infekcija.

Materijali i metode: U retrospektivnoj kohortnoj studiji ispitivano je 80 bolesnika starosti iznad 20 godina sa dijagnozom multiplog mijeloma (MM), nehočkinskih limfoma (NHL) i Hočkinovog limfoma (HL), lečenih na Klinici za hematologiju Kliničkog centra Srbije, u periodu od jula 2006. do decembra 2017. godine. Zabeležene su sve epizode povišene telesne temperature i/ili dokumentovane infekcije u toku neutropenije.

Rezultati: Prosečno preživljavanje nakon ATMĆH-a iznosilo je 34,5 meseci, a kod 54 pacijenta (67,5%) je dokumentovan uzročnik infekcije. Gram pozitivne bakterijske infekcije su bile pet puta češće u odnosu na gram negativne. Kod gram pozitivnih izolata najčešći je bio koagulaza negativni *staphylococcus – CoNS* (engl. *coagulase-negative staphylococcus*), 37,0%, i *Streptococcus a haemoliticus,* 12,4%. Među gram negativnim izolatima najčešća je bila *Escherichia coli,* 62,5%, a *Klebsiella spp.* i *Ralstonia pickettii su bile zastupljene* sa podjednakom učestalošću od 12,5% . Gljivične infekcije su bile retke (10,0% *Candida spp*). Virusne infekcije su verifikovane kod 5 (6,3%) pacijenata (*Herpes zoster* virus, 3,8% i H1N1, 2,5%).

Zaključak: Broj $CD34^+$ matičnih ćelija u transplantatu, kao i brzina rekonstitucije hematopoeze, odnosno postizanje *ALC*500_20, *ANC*500_11 i *PLT*20_13, nisu bili statistički značajni za nastanak infekcija u ranoj fazi nakon ATMĆH-a.

Ključne reči: autologna transplantacija matičnih ćelija, limfomi, multipli mijelom, infekcija

ABSTRACT

Introduction: Autologous stem cell transplantation (AHSCT) is a well-established therapy for hematologic malignancies. Changes in transplantation strategies and improvement in supportive care have significantly altered the incidence and pattern of infections in these patients.

Aim: Evaluating the frequency of infections in the first 30 days after AHSCT, as well as the possible influence of the number of CD34 + stem cells in the graft and of the engraftment parameters: ALC500_20 (absolute lymphocyte count $0.5x10^9$ / L per day + 20), ANC500_11 (absolute neutrophil count $0.5x10^9$ / L per day + 11), and PLT20_13 (platelets $20x10^9$ / L per day + 13), on the occurrence of infections.

Materials and methods: The retrospective cohort study examined 80 patients above the age of 20 years, diagnosed with multiple myeloma (MM), non-Hodgkin's (NHL) or Hodgkin's lymphoma (HL), treated at the Clinic for Hematology of the Clinical Center of Serbia, in the period between July 2006 and December 2017. All episodes of fever and/or documented infection during neutropenia have been reported.

Results: The average survival after AHSCT was 34.5 months. A total of 54 patients (67.5%) had a documented infection. Gram-positive infections were five times more common than gram-negative. In gram-positive isolates, coagulase-negative staphylococcus – CoNS was the most common (37.0%) pathogen, followed by *Streptococcus a haemolyticus* (12.4%). Among gram-negative isolates, *Escherichia coli* was present in 62.5% of the cases, while *Klebsiella spp*. and *Ralstonia pickettii* were represented with an equal frequency of 12.5%. Fungal infections were rare (*Candida spp*., 10.0%). Viral infections were verified in 5 (6.3%) patients (*Herpes zoster* virus 3.8% and H1N1 2.5%).

Conclusion: The number of CD34+ stem cells in the graft, as well as the rate of hematopoietic reconstitution, i.e., the achievement of ALC500_20, ANC500_11, and PLT20_13, were not statistically significant for the development of infections in the early phase after AHSCT.

Key words: autologous stem cell transplantation, lymphoma, multiple myeloma, infection

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UVOD

Autologna transplantacija matičnih ćelija hematopoeze (ATMĆH) predstavlja standardni pristup u lečenju hematoloških maligniteta. Glavne indikacije za ATMĆH su multipli mijelom i limfomi. U novije vreme, ovaj pristup pokazuje i sve veći potencijal u lečenju solidnih tumora, kao i nekih neuroloških i autoimunih oboljenja [1-4].

Praćenjem pacijenata u inicijalnoj fazi nakon transplantacije, dobijeni su podaci koji pokazuju da su infekcije značajan uzrok mortaliteta u 2,0% slučaja. Infekcija se definiše kao izolacija ili detekcija patoloških mikroorganizma, asocirana sa simptomima bolesti [5]. Uočeno je da, kod odraslih pacijenata, gram-pozitivne bakterijske infekcije (GP) prevazilaze broj infekcija izazvanih gram-negativnim bakterijama (GN), kao i da se bakterijemija javlja kod petine ispitanika [6-9]. Invazivne gljivične infekcije, najčešće *Candida*-om i *Aspergillus*-om, su neuobičajene [10]. Ukoliko se ipak jave, predstavljaju predominantne uzročnike u pre-engraftment fazi [11]. Trajanje neutropenije ≥10 dana nakon ATMĆH-a, povezano je sa pojavom ranih infekcija nakon ATMĆH-a [12].

Osim podataka o infekcijama, u prvih nekoliko dana posle ATMĆH-a, treba uzeti u razmatranje i podatke o komplikacijama u toku prve godine nakon procedure, kao bitnu implikaciju dugogodišnjeg preživljavanja.

Najčešće virusne infekcije zahvataju respiratorni trakt i kožu, a posledice su reaktivacije *Herpes zoster* virusa. Prema Bartonu i Kolisu, tokom prve godine nakon ATMĆH-a, pacijenti imaju nizak rizik za smrtni ishod usled infekcija [5]. Tokom protekle dve decenije, učinjene su bitne izmene u strategiji lečenja, sa poboljšanjem antivirusne i antigljivične profilakse, što je dovelo do značajnih promena u incidenciji posttransplantacionih infekcija. Upotreba faktora rasta uz autolognu potporu matičnim ćelijama značajno je skratila trajanje neutropenije. Poznavanje same etiologije i učestalosti infekcija važno je za planiranje odgovarajuće strategije prevencije i njihovog empirijskog lečenja.

Cilj rada bilo je ispitivanje učestalosti infekcija u prvih 30 dana nakon ATMĆH-a, kao i eventualnog uticaja broja $CD34^+$ matičnih ćelija u transplantatu i parametara engraftmenta: *ALC*500_20 (engl. *absolute lymphocyte count* 0,5x10^9/L na dan +20), *ANC*500_11 (engl. *absolute neuthrophil count* 0,5x10^9/L na dan +11), i *PLT*20_13 (engl. *platelets* 20x10^9/L na dan +13), na pojavu infekcija.

MATERIJALI I METODE

U retrospektivnoj kohortnoj studiji ispitivano je 80 bolesnika starosti iznad 20 godina, sa dijagnozom multiplog mijeloma (MM), nehočkinskih limfoma (NHL) i Hočkinovog limfoma (HL), lečenih na Klinici za

INTRODUCTION

Autologous hematopoietic stem cell transplantation (AHSCT) represents the standard approach to the treatment of hematological malignancies. The main indications for AHSCT are multiple myeloma and lymphoma. As of late, this approach has shown a growing potential in the treatment of solid tumors, as well as in the treatment of some neurological and autoimmune diseases [1–4].

Patient follow-up, in the initial phase following transplantation, has provided data showing that infections are a significant cause of mortality in 2.0% of the cases. Infection is defined as the isolation and detection of pathological microorganisms, associated with symptoms of disease [5]. In adults, it has been detected that gram-positive bacterial infections (GP) outnumber the number of infections caused by gram-negative bacteria (GN), as well as that bacteriaemia occurs in one fifth of the subjects [6-9]. Invasive fungal infections, usually caused by *Candida* or *Aspergillus,* are unusual [10]. However, if they do occur, they are predominant causative agents in the pre-engraftment phase [11]. Duration of neutropenia ≥10 days following AHSCT has been linked to early infections following AHSCT [12].

Apart from data on infections in the first few days following AHSCT, data related to complications during the first year following the procedure should be taken into consideration, as an important implication of long-term survival.

The most common viral infections include the respiratory tract and the skin, and are caused by the reactivation of the *Herpes zoster* virus. According to Barton and Collis, during the first two years following AHSCT, patients have low risk of a lethal outcome caused by infection [5]. Over the past two decades, significant changes have been made in treatment strategy, with improvements in antiviral and antifungal prophylaxis, which has led to significant changes in the incidence of post-transplant infections. The use of the growth factor with autologous stem cell support has significantly shortened the duration of neutropenia. The understanding of infection etiology and of the frequency of infection is important for planning the appropriate prevention strategy and for empirical treatment of infection.

The aim of this paper was analyzing the frequency of infections in the first 30 days following AHSCT, as well as investigating the possible influence of the number of $CD34^+$ stem cells in the graft and of the engraftment parameters: ALC500_20 (absolute lymphocyte count 0.5x10^9/L per day +20), ANC500_11 (absolute neuthrophil count 0.5x10^9/L per day +11), and PLT20_13 (platelets 20x10^9/L per day +13), on the occurrence of infections.

Hematologiju Kliničkog centra Srbije (KCS), u periodu od jula 2006. do decembra 2017. godine.

Mikrobiološke metode

Nakon ATMĆH-a, infekcije su dijagnostikovane izolacijom uzročnika iz kulture tkiva (bakterije, gljivice), dokazivanjem gljivičnih antigena i antitela (gljivice), i tehnikom reakcije lančane polimeraze - PCR (virusi). Bakterijska infekcija podrazumeva bakterijemiju i/ili pozitivan bris kože i/ili sluznice. U analizu su uključene samo infekcije koje su se pojavile 0 - 30 dana nakon transplantacije, u odsustvu komplikacija. Zabeležene su sve epizode povišene telesne temperature i/ili dokumentovane infekcije u toku neutropenije. Trajanje neutropenije se definiše kao vremenski period, izražen u danima, u kojem je broj neutrofila <500/mm^3, odnosno < 0,5x10^9/L. Prema preporukama Američkog udruženja infektologa (engl. *Infectious Diseases Society of America - IDSA*), febrilnost se definise kao prva epizoda povišene temperature >38,3°C, ili temperatura >38,0°C, izmerena najmanje dva puta u toku 12 sati, i zahteva ponavljane kliničke preglede, radiografski snimak grudnog koša i laboratorijsku i mikrobiološku analizu periferne venske krvi [13]. Pozitivna mikrobiološka kultura smatra se značajnom, ukoliko nije naglašena slučajna kontaminacija uzorka. Dijagnoza koagulaza negativnog *staphylococcus*-a (*CoNS*) podrazumeva dve pozitivne mikrobiološke kulture. Za dijagnozu pneumonije, neophodno je prisustvo povišene temperature uz radiološki potvrđen nalaz na plućima. Uspešan odgovor na terapiju znači smanjenje telesne temperature <37,5°C, uz klinički vidljive znakove poboljšanja unutar prva 24 sata.

Profilaksa

Svi pacijenti su dobijali antivirusnu profilaksu - aciklovir, 2 x 400 mg p.o., 3 nedelje, u posttransplantacionom periodu. Kao antigljivična profilaksa primenjivan je flukonazol, 150 mg p.o. do +30 dana (d) nakon transplantacije.

Statistička obrada

Za statističku obradu podataka korišćen je softver *SPSS* 17.0. Statistička obrada je obuhvatila formiranje baze podataka, sa grupisanjem i tabeliranjem rezultata po ispitivanim obeležjima bolesnika. Deskriptivni statistički parametri su izraženi kroz aritmetičku sredinu sa merama disperzije (standardna devijacija - SD, standardna greška - *SE*), medijanu, mod, i raspodelu relativnih frekvencija. Za procenu značajnosti razlike učestalosti posmatranih obeležja korišćen je hi-kvadratni test. Ukupno preživljavanje bolesnika je obuhvatalo period od momenta dijagnoze do smrtnog ishoda ili zaključno sa podacima iz decembra 2017. godine, kod

MATERIALS AND METHODS

In this retrospective cohort study, 80 patients older than 20 years, diagnosed with multiple myeloma (MM), non-Hodgkin lymphoma (NHL), or Hodgkin's lymphoma (HL), treated at the Clinic for Hematology of the Clinical Center of Serbia (CCS), in the period between July 2006 and December 2017, were examined.

Microbiology methods

After AHSCT, infections were diagnosed by isolating the pathogens from tissue cultures (bacteria, fungi), by proving fungal antigens and antibodies (fungi), and with the technique of polymerase chain reaction (*PCR*) (viruses). Bacterial infection exists when there is bacteremia and/or a positive skin or mucosal swab result. Only infections occurring 0 to 30 days following transplantation, in the absence of complications, were included in the analysis. All episodes of elevated body temperature and/or documented infections during neutropenia were recorded. The duration of neutropenia is defined as the number of days during which the neutrophil count is <500/mm^3, i.e., < 0.5x10^9/L. According to the Infectious Diseases Society of America (IDSA) guidelines, fever is defined as a single episode of elevated body temperature of >38.3°C, or body temperature of >38.0°C, measured at least twice in the space of 12 hours, and it requires repeated clinical examinations, radiographic imaging of the thorax, and laboratory and microbiological analyses of peripheral venous blood [13]. A positive microbiological culture is considered significant, if accidental contamination of the sample is not noted. The diagnosis of the coagulase-negative staphylococcus (CoNS) entails two positive microbiological cultures. For diagnosing pneumonia, it is necessary to confirm elevated body temperature as well as to have a radiologically confirmed finding of the lungs. Successful response to therapy entails the lowering of body temperature of <37.5°C, with clinically visible signs of improvement within the first 24 hours.

Prophylaxis

All of the patients received antiviral prophylaxis – acyclovir, 2 x 400 mg p.o., 3 weeks, in the post-transplant period. As antifungal prophylaxis, fluconazole was administered, 150 mg p.o. until +30 days (d) following transplantation.

Statistical analysis

Data was processed with the use of the *SPSS* 17.0 software package. Statistical analysis included the formation of a database, with the grouping and presentation of results in tables, according to the analyzed characteristics of the patients. Descriptive statistical parameters are presented as the arithmetic mean with dispersion

živih bolesnika. Ishod lečenja se smatrao povoljnim ukoliko je postignuta kompletna remisija (KR) ili parcijalna remisija (PR). Preživljavanje bolesnika u odnosu na lečenje je računato Kaplan-Majerovom metodom. Kriterijum za statističku značajnost je bio $p < 0,050$.

REZULTATI

Studijom je obuhvaćeno 80 pacijenata, prosečne starosti 35,4±12,2 godina, u rasponu od 20 do 62 godine. Grupu pacijenata sa multiplim mijelomom činilo je 50 osoba, 28 (56,0%) muškaraca i 22 (44,0%) žene. Prosečna starost u vreme dijagnostikovanja MM-a je bila 53,6±7 godine, a prosečna starost u vreme ATMĆH-a je iznosila 55,8±6,8 godine. (Tabela 1). Od sveukupnog broja ispitanika, 30 je bilo iz grupe obolelih od limfoma, i to 20 (66,7%) žena i 10 (33,3%) muškaraca. Obolelih od Hočkinovog limfoma je bilo 18 (60,0%), a od nehočkinskih limfoma, 12 (40,0%). Prosečna starost u vreme dijagnostikovanja limfoma je bila 35,4±12,1 godina, a prosečna starost u vreme ATMĆH-a je bila 37,6±11,6 godina. U vreme postavljanja dijagnoze, 9 pacijenata (30,0%) je imalo I i II klinički stadijum (KS), dok je III-V KS imao 21 (70,0%) pacijent. Demografske i kliničke karakteristike pacijenata sa HL-om i NHL-om navedene su u tabelama (Tabela 2).

Na osnovu Kaplan-Majerove analize, očekivano vreme preživljavanja nakon ATMĆH-a je iznosilo 34,5 meseci (Grafikon 1, Kliničke karakteristike bolesnika sa multiplim mijelomom).

Posmatrajući parametre engraftmenta kroz rekonstituciju limfocitne loze tj. *ALC*500_20 (apsolutni broj

measurements (standard deviation – SD, standard error – SE), the median, mode, and the distribution of relative frequencies. The Chi-square test was used for assessing the significance of the difference in the frequencies of the observed markers. Patient overall survival covered the period from the moment of diagnosis until the lethal outcome, or ending with data from December 2017, in living patients. The treatment outcome was considered favorable if complete remission (CR) or partial remission (PR) was achieved. Patient survival in relation to treatment was calculated with the Kaplan-Meier method. The criterion for statistical significance was $p < 0.05$.

RESULTS

The study included 80 patients, whose average age was 35.4±12.2 years, within the age range of 20 - 62 years. The group of patients with multiple myeloma included 50 people, 28 (56%) men and 22 (44.0%) women. The average age at the time of the diagnosis of MM was 53.6±7 years, while the average age at the time of AHSCT was 55.6±6.8 years. (Table 1). Of the total number of subjects, 30 belonged to the group of lymphoma patients, namely 20 (66.7%) women and 10 (33.3%) men. There were 18 (60,0%) patients suffering from Hodgkin lymphoma, and 12 (40.0%) patients suffering from non-Hodgkin lymphoma. The average age at the time of the diagnosis of lymphoma was 35.4±12.1 years, while the average age at the time of AHSCT was 37.6±11.6 years. At the time of diagnosis, 9 (30.0%) patients were in clinical stage (CS) I and II, while 21 (70.0%) were in CS III-V. The demographic and

Tabela 1. Kliničke karakteristike bolesnika sa multiplim mijelomom

Table 1. Clinical characteristics of patients with multiple myeloma

	Broj pacijenata (%) / *Number of patients (%)*
Medijana starosti, godine / *Median age, years*	54
Opseg / *Range*	39 - 63
Pol / *Gender*	
Muškarci / *Men*	28 (56.0%)
Žene / *Women*	22 (44.0%
Klinički stadijum I / *Clinical stage I*	7 (14.3%)
Klinički stadijum II / *Clinical stage II*	26 (53.1%)
Klinička stadijum III / *Clinical stage III*	16 (32.7%)
Tip M komponente / *Type of M component*	
IgG kappa / *IgG kappa*	29 (61.2%)
IgG lambda / *IgG lambda*	11 (22.5%)
IgA kappa / *IgA kappa*	3 (6.1%)
IgA lambda / *IgA lambda*	5 (11.9%)
Odgovor na terapiju: / *Response to therapy*	
KR / *CR*	(18.4%)
PR / *PR*	32 (65.3%)
VDPR / *VGPR*	4 (16.3%)

KR: kompletna remisija; PR: parcijalna remisija; VDPR: veoma dobra parcijalna remisija

CR: complete remission; PR: partial remission; VGPR: very good partial remission

Tabela 2. Kliničke karakteristike pacijenata sa Hočkinovim limfomom (HL) i nehočkinskim limfomima (NHL)

Table 2. Clinical features of patients with Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL)

	HI / *HL*	NHL / *NHL*
	Broj pacijenata (%) / *Number of patients (%)*	**Broj pacijenata (%) / *Number of patients (%)***
Medijana starosti, godine / *Median age, years*	27	41
Opseg / *Range*	20 - 55	20 - 62
Pol / *Gender*		
Muškarci / *Male*	13 (72.2%)	7 (58.3%)
Žene / *Female*	5 (27.8%)	5 (41.7%)
Klinički stadijum I-II / *Clinical stage I-II*	7 (38.9%)	2 (16.7%)
Klinički stadijum III-IV / Clinical stage III-IV	11 (61.1%)	10 (83.3%)
B simptomi / *B symptoms*		
Da / *Yes*	15 (83.3%)	5 (41.7%)
Ne / *No*	3 (16.7%)	7 (58.3%)
Bulky tumorska masa / *Bulky tumor mass*		
Da / *Yes*	9 (50,0%)	1 (8.3%)
Ne / *No*	9 (50,0%)	11 (91.7%)
Terapijski odgovor / *Therapeutic response*		
CR+PR / *CR+PR*	9 (50,0%)	8 (66.7%)
SD+PD / *SD+PD*	9 (50,0%)	4 (33.3%)

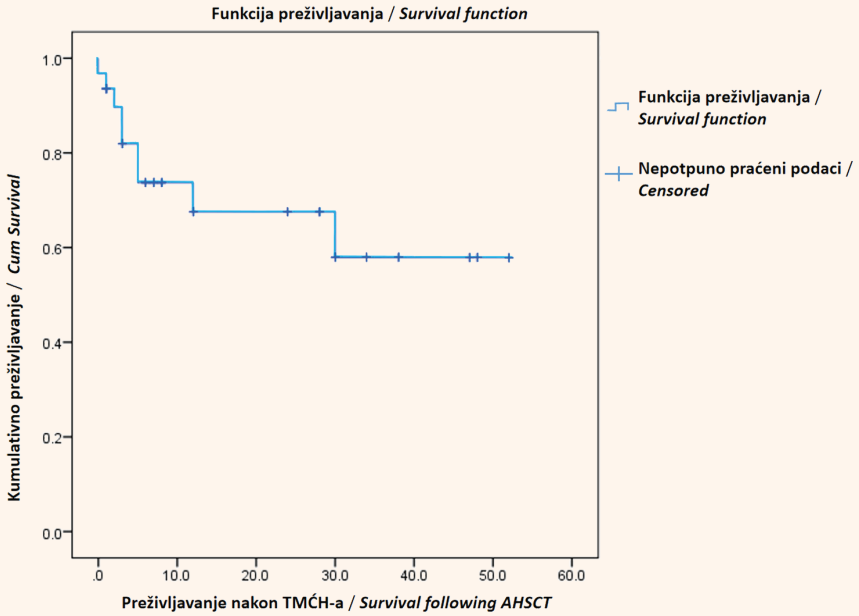
KR: kompletna remisija; PR: parcijalna remisija; SD: stabilna bolest; PD: progresivna bolest

CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease

limfocita 0,5x10^9/L na dan +20), došli smo do zaključka da nije bilo statistički značajne povezanosti između navedenog parametra i preživljavanja ($p = 0,547$). Očekivano preživljavanje nakon ATMĆH-a, kod pacijenata koji su dostigli ALC500_20 iznosilo je 34,7 meseci, a kod onih koji nisu 35,6 meseci. Uticaj dostizanja ANC500_11

clinical characteristics of patients with HL and NHL are presented in the tables (Table 2).

Based on the Kaplan-Meier analysis, the expected length of survival following AHSCT was 34.5 months (Graph 1, Clinical characteristics of patients with multiple myeloma).



Grafikon 1. Ukupno preživljavanje nakon ATMĆH-a

Graph 1. Overall survival following AHSCT

Tabela 3. Uticaj broja *ALC500_20, ANC500_11, PLT20_13* na pojavu infekcije

Table 3. Influence of ALC500_20, ANC500_11, PLT20_13 on the occurrence of infection

Infekcija / *Infection*		**Infekcija / *Infection***	**Bez infekcije/ No *Infection***	**p**
ALC500_20	Dostignuta vrednost/ *Achieved value*	15 (39.5%)	23 (60.5%)	0.424
	Nedostignutavrednost / *Value not achieved*	27 (69.2%)	12 (30.8%)	
ANC500_11	Dostignuta vrednost/ *Achieved value*	18 (39.1%)	28 (60.9%)	0.356
	Nedostignutavrednost / *Value not achieved*	8 (28.6%)	20 (71.4%)	
PLT20_13	Dostignuta vrednost / *Achieved value*	22 (37.3%)	37 (62.7%)	0.550
	Nedostignutavrednost / *Value not achieved*	5 (29.4%)	12 (70.6%)	

(apsolutni broj neutrofila 0,5x10^9/L na dan +11) na preživljavanje nakon ATMĆH-a takođe nije pokazalo statističku značajnost (p = 0,550). Ni rekonstitucija megakariocitne loze, tj. dostizanje *PLT*20_13 (broj trombocita 20x10^9/L na dan +13) nije statistički značajno uticalo na post-transplantaciono preživljavanje (p = 0,070).

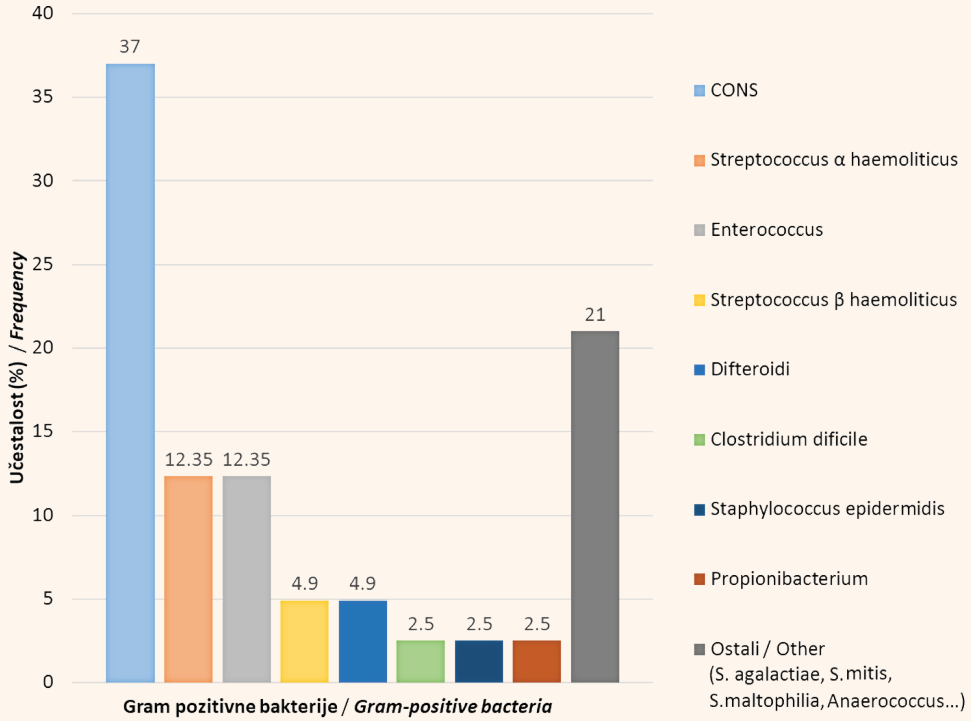
Medijana broja primenjenih CD34$^+$ matičnih ćelija u transplantatu iznosila je 6,7x10^6 kg telesne mase (tm), (opseg: 2,3 - 15,5x10^6/kg tm). Nije potvrđena razlika između broja prisutnih CD34$^+$ matičnih ćelija u transplantatu i rekonstitucije autologne hematopoeze tj. dostizanja vrednosti *ALC*500_20, *ANC*500_11 i *PLT*20_13. Povezanost rekonstitucije hematopoeze tj. dostizanja broja *ALC*500_20, *ANC*500_11 i *PLT*20_13 i pojave infekcije takođe nije imala statističku značajnost (Tabela 3).

Posmatranjem učestalosti infekcija, dobili smo podatke da su dominantni uzročnici infekcija bile bakterije, dok su gljivične i virusne infekcije bile retke. Nakon ATMĆH-a, 29 (36,3%) pacijenata nije imalo bakterijsku infekciju, kod 25 (31,3%) pacijenata je izolovan jedan uzročnik, a kod 26 (32,5%) pacijenta je izolovano ≥2 bakterijskih uzročnika. Od ukupnog broja pozitivnih bakterioloških kultura, GP bakterije su bili izazivači u 83,5% slučaja infekcije, što je čak pet puta veća učestalost u odnosu na GN bakterije. Najčešći GP uzročnici su bili koagulaza negativni *staphylococcus* (*CoNS*), pronađen u 37,0% izolata, i *Streptococcus a haemoliticus,* pronađen u 12,4% izolata. Ostali važniji GP uzročnici su dati u Grafikonu 2 (Grafikon 2). Među GN bakterijama najčešći izazivači su bili *Escherichia coli,* 62,5%, *Klebsiella spp.,* i *Ralstonia pickettii,* sa podjednakom učestalošću od 12,5% (Grafikon 3). Gljivične infekcije su se javile kod 8 (10,0%) pacijenata, i jedini izolovani uzročnik je bila *Candida spp.* Virusne infekcije su se ređe javljale, kod svega 5 (6,3%) pacijenata, a izolovani su *Herpes zoster* virus (HZV) kod 3 (3,8%) i H1N1 virus kod 2 (2,5%) pacijenta. Nijedna parazitarna infekcija nije zabeležena.

Observing the engraftment parameters through the reconstitution of the lymphocyte lineage, i.e., ALC500_20 (absolute lymphocyte count 0,5x10^9/L per day +20), we have come to the conclusion that there was no statistically significant link between the said parameter and survival (p = 0.547). Expected survival following AHSCT, in patients who had reached ALC500_20 was 34.7 months, and in those patients who had not reached this value, it was 35.6 months. The influence of reaching ANC500_11 (absolute neutrophil count 0.5x10^9/L per day +11) on survival following AHSCT also failed to demonstrate statistical significance (p = 0.550). The reconstitution of the megakaryocyte lineage, i.e., reaching PLT20_13 (platelet count 20x10^9/L per day +13) demonstrated no statistically significant impact on post-transplant survival (p = 0.070).

The median value of the number of CD34$^+$ stem cells in the graft was 6.7x10^6 kg body mass (bm), (range: 2.3 – 15.5x10^6/kg bm). No difference was confirmed between the number of CD34$^+$ stem cells present in the graft and the reconstitution of autologous hematopoiesis, i.e., achieving the values of ALC500_20, ANC500_11, and PLT20_13. The link between hematopoiesis reconstitution, i.e., reaching the values ALC500_20, ANC500_11, and PLT20_13 and the development of infection also showed no statistical significance (Table 3).

Regarding the frequency of infections, we obtained data confirming that the dominant pathogens in the infections were bacteria, while fungal and viral infections were rare. Following AHSCT, 29 (36.3%) patients did not have a bacterial infection, in 25 (31.6%) patients, one pathogen was isolated, while in 26 (32.5%) patients ≥2 bacterial pathogens were isolated. Of the total number of positive bacterial cultures, GP bacteria were found in 83.5% cases of infection, which was as much as five time the frequency of GN bacteria. The most common GP pathogens were the coagulase-negative

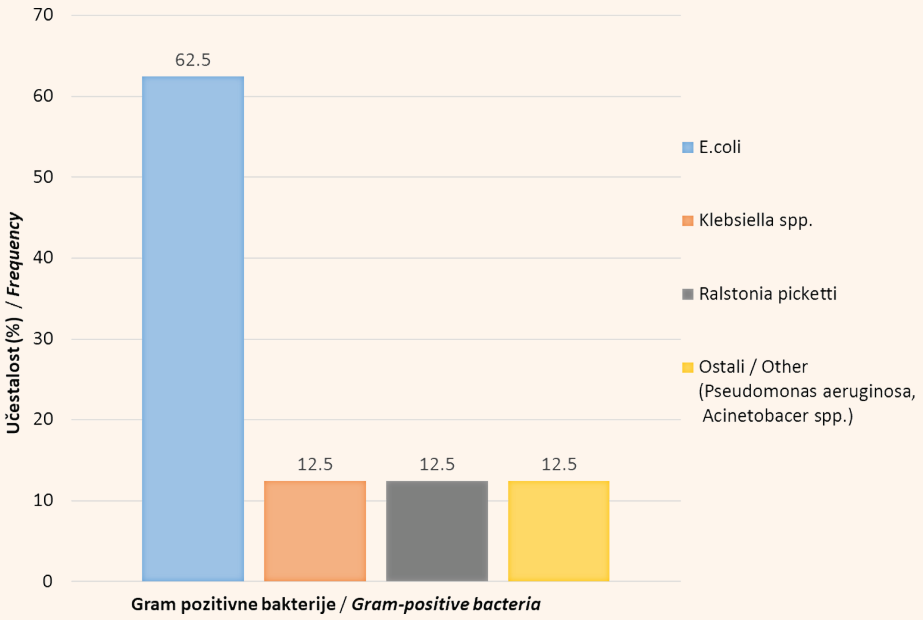


Grafikon 2. Učestalost gram pozitivnih bakterija kao uzročnika infekcija kod bolesnika lečenih autolognom transplantacijom do d +30

Graph 2. Frequency of gram-positive bacteria as the cause of infection in autologous transplant patients up to d + 30

Smrtni ishod je zabeležen kod 11 pacijenata, od čega je 9 pacijenata preminulo zbog razvoja infekcije. Šest pacijenata je preminulo zbog bakterijskih infekcija, 2 pacijenta obolela od limfoma su preminula kao posledica H1N1 virusne infekcije, usled razvoja respiratorne insuficijencije. *Candida spp.* sepsa je uzrokovala smrtni ishod kod jedne pacijentkinje obolele od

staphylococcus, found in 37.0% of isolates, and *Streptococcus α haemoliticus,* found in 12.4% of isolates. The remaining important GP pathogens are presented in Graph 2 (Graph 2). Amongst GN bacteria, the most common agents of infection were *Escherichia coli,* 62.5%, *Klebsiella spp.,* and *Ralstonia pickettii,* with the same frequency of occurrence of 12.5% (Graph 3). Fungal



Grafikon 3. Učestalost gram negativnih bakterija kao uzročnika infekcija kod bolesnika lečenih autolognom transplantacijom do d +30

Graph 3. Frequency of gram-negative bacteria as the cause of infection in autologous transplant patients, up to d + 30

limfoma. Poređenjem vitalnog statusa kod pacijenata sa infekcijom dobili smo podatak o smrtnom ishodu kod 6 (21,4%) osoba, dok je u grupi onih bez infekcije smrtni ishod nastao kod 5 (10,0%) pacijenata. Hi-kvadrat test pokazao je da ne postoji statistički značajna razlika u odnosu na preživljavanje kod pacijenata sa i bez infekcije ($p = 0,164$).

DISKUSIJA

Naša studija je retrospektivno evaluirala razvoj infekcija nakon ATMĆH-a kod 80 odraslih pacijenata, od čega je ispitivano 50 (62,5%) pacijenata sa MM-om i 30 (37,5%) pacijenata sa limfomom.

Prevalencija GP infekcija je veća u odnosu na GN infekcije kod pacijenata sa solidnim tumorima, limfomima i MM-om [1,6-9] Bakterijemija se javila kod 63,8% naših pacijenata. Ova vrednost je za ~10,0% manja u odnosu na incidenciju bakterijemije kod pacijenata sa limfomima i MM-om u istraživanju koje su sproveli Kolin i saradnici [12]. U njihovom uzorku, kod 63,6% pacijenata nije izolovana nijedna patogena bakterijska kultura, kod 26,6% pacijenata je izolovan samo jedan bakterijski uzročnik, a preostalih 9,8% pacijenata je imalo ≥2 bakterijskih izolata [12]. Niska učestalost bakterijskih infekcija (21,3%) opisana je kod pacijenata sa MM-om, limfomima i solidnim tumorima [14].

Koagulaza negativni *staphylococcus* (*CoNS*) i *Streptococcus α haemoliticus* su bili najčešći GP uzročnici infekcije, dok su *E. coli* i *Klebsiella spp.* bili najčešći GN uzročnici bakterijemije u našoj grupi pacijenata, što korelira sa rezultatima drugih studija [15]. *Clostridium difficile* je uzročnik 10,0% infekcija, što je pet puta češće nego u našem istraživanju [5]. Nakon visokodozne hemioterapije i ATMĆH-a, invazivne gljivične infekcije su izuzetno retke kod pacijenata i retko se mogu dokazati. Svi pacijenti u našoj grupi su dobili antigljivičnu profilaksu, međutim kod 8 (10,0%) pacijenata je dokazana *Candida spp.* u brisu grla, ali uz pozitivan *Candida mannan* antigenski test, kod samo jednog pacijenta. U svojoj studiji, Gilbert i saradnici navode da se gljivice u krvi izoluju kod 4,0% pacijenata koji nisu koristili antigljivičnu profilaksu [16]. Rajh i saradnici su opisali infekciju *Candida glabrata*-om, otpornom na azolne antimikotike [17]. *Aspergillus flavus* je izolovan kog jednog pacijenta u plućima [15]. Što se virusnih infekcija tiče, HZV infekcija se javila kod 28,0% ispitanika, što je u odnosu na naše rezultate sedam puta češće. Međutim, u ovom istraživanju, prosečno vreme reaktivacije virusa je bilo 5 meseci, odnosno, u 91,0% slučajeva do reaktivacije je došlo u prvoj godini nakon transplantacije [18]. Antivirusna profilaksa aciklovirom je redukovala pojavu reaktivacije HSV-a.

infections occurred in 8 (10.0%) patients, and the only infectious agent isolated was *Candida spp.* Viral infections occurred less frequently, in only 5 (6.3%) patients, and the *Herpes zoster* virus (HZV), in 3 (3.8%) cases, and the H1N1 virus, in 2 (2.5%) were isolated. No parasitic infections were registered.

The lethal outcome was registered in 11 patients, of whom 9 patients died due to the development of infection. Six patients died due to bacterial infection, 2 patients suffering from lymphoma died as the result of H1N1 viral infection, due to the development of respiratory insufficiency. Sepsis caused by *Candida spp.* was the cause of death in one female patient suffering from lymphoma. By comparing the vital status in patients with infection we obtained data on the lethal outcome in 6 (21.4%) persons, while, in the group without infection, the lethal outcome was registered in 5 (10.0%) patients. The Chi-square test showed no statistically significant difference, related to survival, between patients with and without infection ($p = 0.164$).

DISCUSSION

Our study retrospectively evaluated the development of infections following AHSCT, in 80 adult patients, of whom 50 (62.5%) analyzed patients were suffering from MM while 30 (37.5%) patients had lymphoma.

The prevalence of GP infections is higher than the prevalence of GN infections, in patients with solid tumors, lymphoma, and MM [1,6-9]. Bacteriemia occurred in 63.8% of our patients. This value is by ~10,0% lower as compared to the bacteriemia incidence in patients with lymphoma and MM registered in a study by Collin et al. [12]. In their sample, in 63.6% of the patients no pathogen bacterial culture was isolated, in 26.6% of the patients, only one bacterial pathogen was isolated, while the remaining 9.8% of patients had ≥2 bacterial isolates [12]. A low frequency of bacterial infections (21.3%) was described in patients with MM, lymphoma and solid tumors [14].

Coagulase-negative *staphylococcus* (*CoNS*) and *Streptococcus α haemoliticus* were the most common GP pathogens, while *E. coli* and *Klebsiella spp.* were the most common causes of bacteriemia in our group of patients, which correlates with the results of other studies [15]. *Clostridium difficile* is the cause of 10.0% of infections, which is five times more frequent than was the case in our study [5]. Following high-dose chemotherapy and AHSCT, invasive fungal infections are extremely rare in patients and can rarely be proven. All of the patients in our group received antifungal prophylaxis. However, in 8 (10.0%) patients *Candida spp.* was confirmed with a throat swab, though a positive *Candida mannan* antigen test was noted in just one

Ne postoji statistički značajna razlika u učestalosti infekcija kod MM-a u odnosu na limfome [17]. Ovi podaci su važni jer većina pacijenata pre ATMĆH-a prolazi kroz višestruke cikluse hemioterapije, koji su praćeni teškom neutropenijom u trajanju >10 dana. Prema kliničkim rezultatima koje navode Boudi i saradnici, trajanje neutropenije je glavni faktor rizika za nastanak infekcije, nezavisno od osnovne bolesti [19]. U istraživanju koje su sproveli Mosad i saradnici, nakon ATMĆH-a je postojala niska incidencija febrilnih komplikacija [20]. Sa druge strane, neka istraživanja potvrđuju pojavu febrilne neutropenije sa incidencijom od 100% u posttransplantacionom periodu [9].

Viver i saradnici su zabeležili stopu smrtnosti od 1,5%, u grupi od 1.000 pacijenata podvrgnutim visokodoznoj hemioterapiji i ATMĆH-u, koja se direktno mogla pripisati infekcijama [21]. Kod pacijenata sa MM-om, limfomima i solidnim tumorima, snižen broj $CD34^+$ matičnih ćelija u transplantatu nije povezan sa povećanim rizikom od infekcija nakon transplantacije [22]. Zbog malog broja $CD34^+$ ćelija, pacijenti nakon autologne transplantacije mogu biti pod rizikom da razviju infekcije poput fatalne HSV pneumonije ili CMV retinitisa, kao i pacijenti nakon alogene transplantacije matičnih ćelija hematopoeze, a zbog odložene rekonstitucije hematopoeze [23].

Naša studija ima nekoliko ograničenja. Populacija pacijenata koju smo posmatrali bila je heterogena, a analiza je vršena retrospektivno. Samo mikrobiološki potvrđene infekcije su uključene u ispitivanje. Analizirane su rane infekcije od 0 - 30 dana posle transplantacije.

ZAKLJUČAK

U našem istraživanju, pacijenti su, nakon ATMĆH-a, imali nizak rizik od smrtnog ishoda usled infekcije. Pojava infekcije nije korelirala sa brojem infundovanih $CD34^+$ ćelija u transplantatu. Rekonstitucija autologne hematopoeze, tj. broj *ALC*500_20, *ANC*500_11 i *PLT*20_13 nije značajno uticao na pojavu infekcija. Bakterije su bile dominantni uzročnici infekcija, pri čemu su GP bakterije bile oko pet puta češće nego GN bakterije. Najčešći uzročnici iz grupe GP bakterija su bili *CoNS* i *Streptococcus a haemoliticus*. Najčešći GN uzročnici su bili *Escherichia coli* , *Klebsiella spp.* i *Ralstonia*. Incidencija infektivnih komplikacija nakon transplantacije se nije značajno razlikovala kod pacijenata sa limfomima i MM-om.

Primena profilaktičke terapije dovela je do smanjenja pojave virusnih i gljivičnih infekcija.

Sukob interesa: Nije prijavljen.

patient. In their study, Gilbert et al. state that fungi was registered in the blood in 4.0% of patients who had not used antifungal prophylaxis [16]. Reich et al. described infection with *Candida glabrata,* resistant to azole antimycotics [17]. *Aspergillus flavus* was isolated in the lungs of one patient [15]. As to viral infections, HZV infection occurred in 28.0% of the subjects, which is seven times more frequent than was the case with our patients. However, in this study, the average time of reactivation of the virus was 5 months, i.e., in 91.0% of the cases, reactivation occurred in the first year following transplantation [18]. Antiviral prophylaxis with acyclovir has reduced the occurrence of the HSV virus.

There is no statistically significant difference in the occurrence of infection between MM and lymphoma [17]. This information is important as most patients, prior to AHSCT, go through multiple cycles of chemotherapy, which are followed by severe neutropenia lasting >10 days. According to the clinical results reported by Bodey et al., the duration of neutropenia is the main risk factor for the development of infection, independently of the underlying disease [19]. In a study by Mossad et al., a low incidence of febrile complications was registered [20]. On the other hand, some studies have confirmed the occurrence of febrile neutropenia with an incidence of 100% in the post-transplant period [9].

Weaver et al. registered a 1.5% mortality rate, in a group of 1,000 patients who had been subjected to high-dose chemotherapy and AHSCT, which could directly be attributed to infection [21]. In patients with MM, lymphoma, and solid tumors, a decreased number of $CD34^+$ stem cells in the graft has not been connected to an increased risk of infection following transplantation [22]. Due to a small number of $CD34^+$ cells, after autologous transplantation, patients may be at risk of developing similar infections, such as the fatal HSV pneumonia or CMV retinitis, to those occurring in patients after allogenic HSCT, as the result of delayed hematopoietic reconstitution [23].

Our study has several limitations. The population of patients analyzed was heterogenous, and the analysis was performed retrospectively. Only microbiologically confirmed infections were included in the study. Early infections, occurring 0 – 30 days following transplantation were analyzed.

CONCLUSION

In our study, after AHSCT, patients had a low risk of lethal outcome due to infection. The occurrence of infection did not correlate with the number of $CD34^+$ cells infused in the graft. The reconstitution of autologous hematopoiesis, i.e., the number of ALC500_20, ANC500_11, and PLT20_13, did not significantly affect

the occurrence of infection. Bacteria were the predominant cause of infection, with GP bacteria being around five times more common than GN bacteria. The most common pathogens from the GP group of bacteria were CoNS and *Streptococcus a haemoliticus*. The most common GN pathogens were *Escherichia coli*, *Klebsiella spp., and Ralstonia*. The incidence of infectious complications following transplantation did not significantly differ between patients with lymphoma and MM patients.

The application of prophylactic therapy has led to a decrease in the occurrence of viral and fungal infections.

Conflict of interest: None declared.

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