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Pismo urednika

Od osnivanja do danas, vođen idejom da se stručni i naučni dokazi o zdravstvenim temama sa prostora Srbije i van njenih granica objavljuju, razmenjuju i procenjuju, Srpski medicinski časopis Lekarske komore kontinuirano doprinosi unapređenju medicine, kao nauke i prakse. Više od 60 eminentnih stručnjaka, među kojima su osnivač i izdavač, članovi uredništva, Izdavačkog saveta i Uređivačkog odbora, zajedno sa recenzentima, autorima i saradnicima, udružilo je svoje napore u razvoj Časopisa i usmerilo ih ka novim idejama i inicijativama za unapređenje struke, zdravstvene politike i zdravlja društva i pojedinca.

Za nepunih godinu dana, i tokom pandemije Kovid-19, zajedno sa ovom četvrtom sveskom, Srpski medicinski časopis Lekarske komore objavio je 38 rukopisa kategorisanih kao aktuelne teme, originalni i pregledni radovi, prikazi slučajeva, editorijali i drugi članci, ukupno 127 autora. Činjenica da su do sada objavljene uvodne reči upućene od strane istaknutih i uvaženih ličnosti, naših dekana, akademika, i međunarodno priznatih eksperata, kao i da su objavljeni radovi, dela stručnjaka iz različitih disciplina medicinskih nauka, čini nas ponosnim na postignut uspeh.

U ovoj svesci, pažnja je usmerena prema aktuelnim temama, kao što su kvalitet vazduha u Srbiji (autor: Stankov S.), pandemija Kovid-19 (autorke: Fišeković-Kremić i Stojanović-Ristić), karcinomi (autori: Stardelova i saradnici), nedovoljno definisani uzroci smrti (autorka: Rosić N.), nezadovoljene zdravstvene potrebe (autori: Todorović i saradnici), kao i ka originalnim radovima mladih naučnih istraživača u oblasti hematologije i transplantacije matičnih ćelija (urednička reč: prof. dr Milena Todorović Balint).

Nadamo se da ovi rezultati mogu da podstaknu vodeće stručnjake da kontinuirano doprinose ovom časopisu u vidu svog originalnog ostvarenja, preglednog rada, komentara, izveštaja, stručnog viđenja, kritički argumentovanog mišljenja, prikaza slučaja, opisa problema, inovacije, mogućeg pristupa rešavanju problema ili dokaza kako se može i treba raditi.

Naša naredna sveska biće posvećena "Iskustvima sa pandemijom Kovid-19 i efektima pandemije na zdravlje i životnu i radnu sredinu". Više o temi, možete pročitati u tekstu Poziva autorima.

Dragi autori, Srpski medicinski časopis Lekarske komore vizionarski prati razvoj medicinske misli, i kao vaš partner, podržava da se vaše profesionalno mišljenje čuje i ostavi trag u savremenim društvenim tokovima. Vaši rukopisi su dobrodošli!

S poštovanjem,
Prof. dr Milena Šantrić Milićević
Glavni i odgovorni urednik Srpskog medicinskog časopisa Lekarske komore

A Word from the Editor

Since its inception onwards, guided by the idea that professional and scientific evidence on health-related topics, pertaining to Serbia and other countries, should be published, exchanged, and evaluated, the Serbian Journal of the Medical Chamber has continuously contributed to the progress of medical scientific research and practice. More than 60 renowned experts, including the founder, publisher, members of the editorial staff, the Publisher's Advisory Board, and the Editorial Board, together with reviewers, authors and associates, have pooled their efforts into the development of the Journal, directing their energy towards new ideas and initiatives for improving the medical profession, health policy and the health of the society and the individual.

In under a year, throughout the Covid-19 pandemic, including this issue, the Serbian Journal of the Medical Chamber has published 38 manuscripts, categorized as current topics, original articles, review articles, case reports, editorials, and other articles, written by a total of 127 authors. The fact that the letters of introduction published so far have been written by prominent and eminent individuals, deans of our faculties, academicians, and internationally recognized experts, as well as the fact that the papers published thus far have been written by experts in various medical disciplines, make us proud of the success we have achieved.

In this issue, the focus of the Journal is directed towards current topics, such as air quality in Serbia (author: Stankov S.), the Covid-19 pandemic (authors: Fišeković-Kremić and Stojanović-Ristić), carcinomas (authors: Stardelova et al.), ill-defined causes of death (author: Rosić N.), unmet health needs (authors: Todorović et al.), as well as towards original articles written by young researchers, in the area of hematology and stem-cell transplantation (word from the editor: Professor Milena Todorović Balint, PhD).

We hope that these results may inspire leading experts to continuously contribute to the Journal with their original articles, review articles, comments, reports, expert opinions, critical comments, case reports, problem descriptions, innovations, possible approaches to solving problems, and proof of good and recommended practices.

Our next issue will be dedicated to "Experiences with the Covid-19 pandemic and its effects on health and the living and work environment". You are invited to read more on this topic in the Call for papers.

Dear authors, the Serbian Journal of the Medical Chamber follows the development of medical science and thought with a vision into the future, and, as your partner, supports you in publishing your professional opinion so that it can leave a trace in contemporary social development. Your manuscripts are welcome and appreciated!

Sincerely,
Professor Milena Šantrić Milićević, PhD
Editor in Chief, Serbian Journal of the Medical Chamber

ЛЕКАРСКА КОМОРА СРБИЈЕ – SERBIAN MEDICAL CHAMBER
РЕГИОНАЛНА ЛЕКАРСКА КОМОРА БЕОГРАДА – REGIONAL MEDICAL CHAMBER OF BELGRADE

Позив ауторима за достављање радова
Искуства са пандемијом Ковид-19 и ефекти пандемије на здравље и животну и радну средину

Српски медицински часопис Лекарске коморе позива ауторе да предају стручне и научне чланке у вези са искуствима са пандемијом Ковид-19 и ефектима пандемије на здравље и животну и радну средину.

Позив је отворен од 28. маја 2021. године. Молимо вас да се пријавите путем интернета. Прво издање Часописа посвећено овој теми саставићемо од рукописа пристиглих до 30. јуна, 2021. године. Бићемо спремни да објавимо и друго такво издање, ако буде више радова на ову тему.

Позив је отворен за пријављивање оригиналних чланака, прегледа литературе, методолошких истраживања, студија пресека и лонгитудиналних студија, студијаслучаја, коментара/мишљења, и рукописа других формата. Молимо вас да шаљете цео чланак са сажетком.

Линк за регитрацију и субмитовање радова је:
https://aseestant.ceon.rs/index.php/smclk/information/authors

О овом позиву за радове

Нема сумње да ће свет из ове пандемије изаћи искуснији и мудрији. Због пандемије Ковид-19, били смо принуђени да извршимо многе промене у свом непосредномздравственом, животном и радном окружењу. Научили смо да се брже реорганизујемо, живимо другачије и преиспитујемо своје границе. Међутим, немају сви иста искуства са пандемијом Ковид-19. Ефекти и трајање пандемије и даље су углавном неуједначени и недовољно идентификовани, како међу различитим земљама тако и унутар истих држава. Упркос различитим доказима, и даље постоји суштински недостатак података на основу којих би се могли донети ваљани закључци и дугорочна предвиђања, а посебно недостатак информација из праксе у различитим контекстима (нпр. живот у неодговарајућим условима, или рад уз недостатак ресурса и капацитета у здравственим организацијама, који је постојао и пре пандемије Ковид- 19).

Радујемо се читању ваших рукописа који извештавају о знању, ставовима, понашању и искуствима група становника директно и индиректно изложених СаРС-КоВ-2 и здравствених радника директно и индиректно укључених у борбу против Ковид-19. Такође поздрављамо описе промена направљених у социјалним, економским,технолошким, организационим, образовним, еколошким и културним окружењима због Ковид-19, који су разматрани са становишта: јавног здравља, промоције здравља, комуникације у кризним догађајима, пружања здравствених услуга, управљањаздравственом заштитом и службом, здравствене политике и/или здравственог система.

На крају, добродошли су и рукописи о интервенцијама усмереним на ублажавање негативних ефеката Ковид-19 на здравље и функционисање људи, здравствених радника и здравствених установа и радујемо се што ћемо добити публикације које се баве добром здравственом праксом током пандемије Ковид-19.

У писању радова, молимо вас да се руководите упутством за ауторе за врсту чланка, дужину и формат и правила за двоструко слепу рецензију.

С радошћу очекујемо ваше рукописе!

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РЕГИОНАЛНА ЛЕКАРСКА КОМОРА БЕОГРАДА – REGIONAL MEDICAL CHAMBER OF BELGRADE

Call for papers

Experiences with the Covid-19 pandemic and its effects on health and the living and work environment

The Serbian Journal of the Medical Chamber invites authors to submit professional and scientific articles related to their experience with the Covid-19 pandemic and the effects of the pandemic on health and the living and work environment.

The call is open as of May 28, 2021. Please apply online. We will compile the first edition of the Journal dedicated to this topic from the manuscripts received by June 30, 2021. We are prepared to publish another such edition if we receive more articles covering this topic.

The call is open for the submission of original articles, literature reviews, methodological research, cross-sectional and longitudinal studies, case studies, comments/opinions, and manuscripts of other formats. Kindly, submit a full article with an abstract.

Link for registration and submissions:
https://aseestant.ceon.rs/index.php/smclk/information/authors

About this call for papers:

There is no doubt that the world will emerge from this pandemic more experienced and wiser. Due to the Covid-19 pandemic, we were forced to make many changes in our immediate health, living and work environment. We have learned to reorganize faster, live differently and challenge our limits. However, not everyone has had the same experience with the Covid-19 pandemic. The effects and duration of the pandemic remain largely uneven and insufficiently identified among and within countries. Despite diverse evidence, there is still a critical lack of data that would be the basis for sound conclusions and long-term predictions, particularly a lack of information stemming from health practice in different contexts (e.g., residing in inadequate living conditions, or working with a lack of resources and capacities, a situation present in health organizations even before Covid- 19).

We look forward to reading your manuscripts, which report on the knowledge, attitudes, behavior, and experiences of population groups directly and indirectly exposed to SaRSCoV-2 and health workers directly and indirectly engaged in the fight against Covid-19.

We also welcome the descriptions of the changes made in the social, economic, technological, organizational, educational, environmental and cultural settings, due to Covid19, considered from the point of view of public health, health promotion, crisis communication, healthcare and health service management, health policy and /or the health care system. Finally, we welcome the manuscripts on interventions aimed mitigating the negative effects of Covid-19 on the health and functioning of people, health workers and health facilities, and look forward to receiving publications dealing with good health practices during the Covid-19 pandemic.

The author's guidelines for the type of article, length and format and rules for double-blind review apply.

We look forward to your submissions!

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LETTER TO THE EDITOR

ON THE IMPORTANCE OF HYGIENIC MEASURES IN THE CONTROL OF AIRBORNE INFECTIOUS DISEASES

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Poštovano uredništvo,

Infektivne bolesti su se tokom istorije čovečanstva najviše širile u obliku epidemija, a kao odgovorni za njihovu pojavu su već duže vreme označeni patogeni mikroorganizmi. Međutim, patogeni mikroorganizmi čine samo mali deo mikrobioma ljudi, životinja i biljaka [1].

Da bismo razumeli ulogu mikroorganizama u etiologiji bolesti, moramo imati na umu da uslovi okoline u kojima se nalaze mikroorganizmi igraju odlučujuću ulogu u njihovom funkcionisanju u odnosu na životnu sredinu, a time i u odnosu na domaćine, kao njihovo specifično okruženje.

U akutnim zaraznim bolestima patogeni mikroorganizam najčešće stimuliše razne litičke, pre svega proteolitičke reakcije, pomoću različitih proteaza [2-5], pa prema tome on ima ulogu katalizatora patološkog procesa. Uloga katalizatora je samo da ubrza reakciju, pri čemu je ne može pokrenuti ili promeniti ravnotežni položaj reakcije [6]. Stoga, bar kada je reč o zapaljenskom procesu, takozvani biološki uzroci bolesti nisu stvarni uzroci, već samo katalizatori već postojećeg patološkog procesa, dok su stvarni neposredni uzroci po svojoj prirodi samo fizički ili hemijski faktori. Otuda ekstremna varijabilnost lokacije i intenziteta patoloških procesa sa istim biološkim uzročnikom kod različitih jedinki iste vrste domaćina. Otuda i jasno određenje većine mikroorganizama koji mogu da „izazovu" bolest kao oportunističkih patogena, za razliku od takozvanih striktnih patogena [7]. Međutim, „striktni" patogeni

Dear Editors,

Infectious diseases have spread primarily in the form of epidemics throughout the history of mankind and pathogenic microorganisms have long been held responsible for their occurrence. However, pathogenic microorganisms make up only a small portion of the microbiome of humans, animals and plants [1].

In order to understand the role of microorganisms in disease etiology, we have to remember that the environmental conditions in which microorganisms are found play a decisive role in their functioning in relation to the environment, and thus in relation to their hosts, as their specific environment.

In acute infectious diseases, the pathogenic microorganism most commonly stimulates various lytic, primarily proteolytic reactions, through various proteases [2-5], thus it has a role of catalyst in the pathological process. The role of catalyst is only to accelerate the reaction, it cannot initiate the reaction, nor can it change the equilibrium position of the reaction [6]. Therefore, at least when it comes to the inflammatory process, the so-called biological causes of disease are not the actual causes, but only catalysts of an already existing pathological process, while the real immediate causes are, by their nature, only physical or chemical factors. Hence, the extreme variability of both the location and intensity of pathological processes with the same biological causative agent in different individuals of the same host species. Consequently, the clear designation of most microorganisms that can "cause" the disease as

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su u osnovi takođe oportunistički, uslovni patogeni, u smislu da su im potrebni odgovarajući uslovi okoline da bi se manifestovala njihova patogenost, ali se oni razlikuju od uslovnih patogena samo po tome što ne mogu da prežive u različitim uslovima, zbog loše prilagodljivosti promenama u svojem okruženju. Dakle, patogenost i virulentnost mikroorganizama nisu njihova inherentna i nepromenljiva svojstva, već su samo rezultat patogenosti neživih faktora okoline koji deluju na domaćina i koji često ostaju van fokusa medicinskih istraživanja.

„Učiteljica života", istorija, daje nam odgovor na pitanje od glavne praktične važnosti - kako sprečiti pojavu zaraznih bolesti? Čak i pre otkrića zaraznih agenasa i specifičnih antimikrobnih lekova, mnoge higijenske mere bile su dovoljne da suzbiju većinu zaraznih bolesti, poput kolere i kuge u ranijim vekovima, koje su se javljale u obliku epidemija koje su desetkovale ljudsku populaciju. One su svedene na zanemarljivu učestalost u sredinama u kojima su primenjene takve mere. Konkretno, uvođenje kanalizacionog sistema i stalno uklanjanje čvrstog, posebno organskog otpada iz naseljenih područja doprineli su da nekadašnje epidemije kolere i kuge padnu u zaborav. Tako je, na primer, kuga uspešno kontrolisana direktnim merama deratizacije i dezinsekcije [8], a redovno uklanjanje čvrstog otpada sigurno je doprinelo kontroli vektora i kuge i drugih opasnih zaraznih bolesti, jer je uklonjeno plodno tlo za održavanje i umnožavanje odgovarajućih vektora. Suzbijanje kolere ostvareno je uvođenjem higijenski bezbednog snabdevanja vodom za piće [9], a u Srbiji je, 1915. godine, eliminisana stravična epidemija pegavog tifusa razvojem i sistematskim korišćenjem takozvanog srpskog bureta, a bez značajnog doprinosa bilo kakvih specifičnih antimikrobnih ili imunostimulišućih lekova [10].

Do sada je zajednica učinila puno na unapređenju higijenskih standarda ličnog i porodičnog stanovanja, procesa u proizvodnji hrane i stočarstvu, sigurnog odlaganja komunalnog čvrstog i tečnog otpada, kao i na higijeni poslovnih objekata i procesa. Međutim, jedan važan segment higijenske prakse ostao je, do danas, prilično nerazvijen i zanemaren, a to je briga o higijeni atmosfere, pre svega atmosfere ljudskih naselja. Danas se atmosfera neprekidno i sistematski zagađuje određenim gasovima, parama, kao i česticama, uključujući radioaktivne, toksične i zarazne čestice. Direktni rezultat ovog stanja razvoja higijenske prakse je da su danas jedina vrsta epidemije koja pogađa opštu populaciju, epidemije koje se šire putem atmosfere, naime respiratorne epidemije. I dok veći deo javnosti vidi specifičnu vakcinu kao jedinu nadu za spas od trenutne respiratorne pandemije COVID-19, malo ko razume da bi to mo-

opportunistic pathogens, as opposed to the so-called strict pathogens [7]. However, "strict" pathogens are basically also opportunistic, conditional pathogens, in the sense that they need appropriate environmental conditions in order to manifest their pathogenicity. However, they differ from conditional pathogens only in that they cannot survive in different conditions, due to their poor adaptability to changes in their environment. Thus, pathogenicity and virulence of microorganisms are not their inherent and invariable properties, they are merely the result of pathogenicity of inanimate environmental factors, which act on the host, and which usually remain out of the focus of medical research.

"Life's teacher" – history, offers the answer to the question of key practical significance: how do we prevent the occurrence of infectious diseases? Even before the discovery of infectious agents and specific antimicrobial drugs, many hygienic measures were sufficient to reduce most infectious diseases, such as cholera and plague in earlier centuries, which broke out in the form of epidemics that decimated the human population. These infections were reduced to a negligible incidence in the environments where such measures were applied. In particular, the introduction of the sewage system and the regular removal of solid, especially organic waste from populated areas have contributed to the past epidemics of cholera and plague falling into oblivion. Thus, for example, plague was successfully controlled by direct deratization and disinsection measures [8], and regular solid waste removal certainly contributed to the control of vectors of both plague and other dangerous infectious diseases, by removing the substrate necessary for the preservation and the multiplication of the appropriate vectors. The control over cholera was established by the introduction of a hygienically safe supply of drinking water [9] and, in Serbia, in 1915, a terrible epidemic of spotted fever was eliminated by developing and systematically using the so-called Serbian barrel, without significant contribution of any specific antimicrobial or immunostimulating drugs [10].

So far, the community has made significant strides in the improvement of hygienic standards of personal and family housing, processes in food production and animal husbandry, the safe disposal of municipal solid and liquid waste, as well as in the domain of the hygiene of business facilities and processes. However, one important segment of hygiene practice has remained quite undeveloped and neglected to this day, and that is the care for the hygiene of the atmosphere, primarily the atmosphere of human settlements. Today, the atmosphere is constantly and systematically polluted with specific gases, vapors as well as particles, including radioactive, toxic and infectious particles. The direct result of this state of development of hygiene practice is that, nowadays, the only type of ep-

glo biti samo privremeno rešenje problema. Na primer, nedavno je javno izneto mišljenje nekih medicinskih stručnjaka da bi, čak i nakon eventualne eliminacije COVID-19 samo vakcinacijom, mogao kasnije da sledi neki COVID-22, -23 ili -24, i tako u nedogled. Ovakvo predviđanje se može izvesti direktno iz lekcija istorije, uključujući i one prethodno spomenute. Šta bi se, na primer, dogodilo kada bismo se danas borili protiv kuge i kolere samo vakcinama i antibioticima, a s druge strane nastavili da živimo u naseljima u kojima se neprestano nagomilavaju ljudski i životinjski izmet i čvrsti organski otpad? Odgovor je da bismo u tom slučaju sigurno mogli očekivati stalnu pojavu novih, otpornijih sojeva patogenih bakterija ili drugih patogena i ponovnu pojavu bolesti u drugim oblicima, jer same vakcine i antibiotici ne bi bili dovoljni da poništе povoljne uslove za održavanje i razvoj bolesti.

Da bi se postigla trajna kontrola širenja respiratornih epidemija, potrebno je uvesti trajne i sistematske mere za održavanje higijene atmosfere ljudskih naselja. Za početak, bilo bi potrebno da se usredsredimo na uklanjanje zagađenja česticama, jer ne zaboravimo da su virusi i bakterije koje prouzrokuju respiratorne bolesti čestice u svom fizičkom obliku. Ali, važnije od fizičkog uklanjanja zaraznih mikroorganizama iz atmosfere bilo bi uklanjanje otrovnih čestica u atmosferi, koje same po sebi izazivaju patološki proces, koji mikroorganizmi onda samo ubrzavaju. Što se tiče štetnih sastojaka u atmosferi savremenih gradova, grupa stručnjaka američke Agencije za zaštitu životne sredine zaključila je da su trenutni standardi za sadržaj čestica PM2,5 u vazduhu nedovoljni za zaštitu javnog zdravlja, a ovaj zaključak je zasnovan na značajnim i sveobuhvatnim dokazima iz epidemioloških studija, toksikoloških studija na životinjama i studija kontrolisane izloženosti ljudi [11].

Iako se javno zdravstveni odgovor na toksičnost čestica, kao nejasan rizik koji uglavnom ima posledice u dužem vremenskom periodu, može odložiti na neko vreme, nedavna istraživanja pokazuju da toksične čestice u atmosferi igraju ključnu ulogu u širenju trenutne pandemije COVID-19 i da stoga njihovo sistematsko uklanjanje predstavlja cilj visokog prioriteta za javno zdravlje. Tako je, na primer, utvrđena nesporna i visoka korelacija između koncentracije čestica PM2,5 u vazduhu, s jedne strane, i incidencije i mortaliteta od COVID-19, s druge strane [12-15].

Najveće površine na kojima se talože čestice organskog i neorganskog porekla su otvoreni javni prostori, ulice, trgovi, parkovi itd. Nataložene čestice se prirodnim padavinama ispiraju i odvode na niži teren, tako da naselja na uzvišenjima imaju prednost u odnosu na naselja u ravnici. Ali, u sušnim periodima, bez obidemic affecting the general population are epidemics of diseases that spread through the atmosphere, i.e., epidemics of respiratory (airborne) diseases. While the majority of the public sees the specific vaccine as the only hope of salvation from the current respiratory COVID-19 pandemic, very few understand that the vaccine might be only a temporary solution to the problem. For example, some medical professionals have recently publicly expressed the opinion that, even after the possible elimination of COVID-19 solely by means of vaccination, subsequently, a COVID-22, -23 or -24, may follow, and so on, indefinitely. This prediction can be deduced directly from the lessons of history, including those mentioned above. For example, what would happen if, at this day and age, we fought plague and cholera only with vaccines and antibiotics, and on the other hand continued to live in settlements where human and animal excrements and solid organic waste accumulated continuously? The answer is that in that case we could certainly expect a constant emergence of new, more resistant strains of pathogenic bacteria or other pathogens and the reappearance of diseases in altered forms, because vaccines and antibiotics alone would not be sufficient to nullify the favorable conditions for disease persistence and development.

For the purpose of achieving permanent control over the epidemics of airborne diseases, there is a need to introduce permanent and systematic measures to maintain the hygiene of the atmosphere of human settlements. For a start, we should focus on removing particle pollution, as we mustn't forget that viruses and bacteria causing respiratory diseases are, in their physical form, particles. However, what would be more important than physically removing infectious agents from the atmosphere is the removal of toxic particles from the atmosphere, which themselves cause a pathological process, which is then merely accelerated by microorganisms. Regarding harmful ingredients in the atmosphere of modern cities, a group of experts of the US Environmental Protection Agency concluded that the current PM2.5 standards are insufficient to protect public health, and this conclusion was based on substantial and comprehensive evidence from epidemiologic studies, toxicologic studies on animals, and controlled human exposure studies [11].

While public response to particulate matter toxicity, as an obscure risk that mainly has consequences in the long term, may be delayed for some time, recent research indicates that toxic particles in the atmosphere play an essential role in spreading the current COVID-19 pandemic, and that therefore their systematic removal is a high public health priority. Thus, for example, an undeniable and high correlation was found between the concentration of PM2.5 particles in the air, on one hand, and both incidence and mortality from COVID-19, on the other [12-15].

zira na konfiguraciju tla, čestice ostaju na tlu i zatim se strujanjem vazduha neprestano podižu u atmosferu. U prošlosti su javna komunalna preduzeća povremeno prala ulice, prvenstveno kolovoze, što je očigledno retka praksa u današnje vreme. Danas je golim okom vidljivo da se u većim naseljima neprestano stvara prašina, kako u saobraćaju, tako i tokom aktivnosti građevinskih firmi, tokom procesa sagorevanja goriva u toplanama i preduzećima, kao i sagorevanjem čvrstih materija u kućama, na privatnim i javnim površinama. Zbog toga postoji potreba za redovnim (npr. svake noći posle suvog dana, a bez jakog vetra) pranjem javnih površina. Poželjno je da se to izvodi hlorisanom ili ozonovanom vodom da bi se izvršila kako dezinfekcija zarazne prašine, tako i detoksikacija toksičnih organskih čestica [16,17]. Ovo svakako podrazumeva stalni porast obima redovnog rada, kao i standardizaciju kvaliteta rada javnih komunalnih preduzeća, odnosno preduzeća čiji bi se zadatak zasnivao na javno-privatnom partnerstvu, uz trajnu javnu kontrolu kvaliteta rada ovih preduzeća.

Pored redovnog čišćenja javnih površina od čvrstih čestica, neophodno je obavezati najvažnije generatore čestica (građevinska preduzeća, toplane, vozila) na sve moguće mere zadržavanja čestica na izvoru, uz njihovo sigurno odlaganje. Takođe treba posvetiti dužnu pažnju redovnoj dezinfekciji zatvorenih javnih prostora (zgrade javne uprave, bolnice, škole, kulturne institucije, itd.). Trenutno, kao moguća praktična opcija, postoji postupak noćnog ozoniranja prostorija ozonizatorima na bazi električnog luka [18] ili UV lampama od 185 nm [19]. Ove mere svakako treba pažljivo planirati i pripremiti za sprovođenje, a po verifikaciji njihovog efekta u manjim oglednim zajednicama, ne bi trebalo da ih i dalje posmatramo kao privremene mere, već kao mere koje su trajne i održive isto kao i mere lične higijene, vodovoda i kanalizacije i odlaganja čvrstog otpada.

Zdravstveni radnici i saradnici bi svakako trebalo da se založe i pomognu u uvođenju ovih i drugih neophodnih higijenskih mera za zaštitu atmosfere i odbranu od respiratornih infekcija, i da tako na najbolji način doprinesu daljem unapređenju javnog zdravlja.

Sukob interesa: Nije prijavljen.

The largest areas where particles of organic and inorganic origin are deposited are open public spaces, streets, squares, parks, etc. The deposited particles are washed away by natural precipitation and drained to lower areas, so that the settlements on elevated terrain are at an advantage, in that respect, over the settlements located on lower terrain. However, in dry periods, regardless of the ground surface configuration, particles remain on the ground and are then constantly lifted into the atmosphere by air flow. In the past, public utility companies occasionally washed the streets, primarily roads, which is obviously a rare practice nowadays. Today, it is visible to the naked eye that dust is constantly being generated in larger settlements, both in traffic, as well as during the activities of construction companies, during processes of burning fuel in heating plants and companies, as well as by burning solid materials in houses, private and public areas. Therefore, there is a need for regular (e.g., every night after a dry day with no strong wind) washing of public areas. It should preferably be done with chlorinated or ozonated water so as to inactivate infectious dust, as well as to detoxify toxic organic particles [16,17]. This certainly implies a permanent increase in the extent of regular work as well as the standardization of performance quality of public utility companies, or companies whose task would be based on public-private partnership, with permanent public control of the performance quality of these companies.

In addition to the regular cleaning of public areas from particulate matter, it is necessary to compel major particle generators (construction companies, heating plants, vehicles) to apply all available measures for retaining particles at their source, and safely disposing of them. Due attention should also be paid to regular disinfection of closed public spaces (public administration buildings, hospitals, schools, cultural institutions, etc.). As a current practical option, there is a procedure of night ozonation of rooms with arc-based ozonizers [18] or UV lamps of 185 nm [19]. These measures should certainly be carefully planned and prepared for implementation, and, upon verification of their effect at sentinel sites, they should cease to be seen as temporary measures, but rather measures that are as permanent and sustainable as personal hygiene measures, water supply, and sewage system and solid waste disposal.

Health professionals should certainly take every possible step to advocate and help to introduce these and other necessary hygiene measures aimed at protecting the air we breathe and defending against airborne infections, and thus contribute in the best way to the further improvement of public health.

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SaRS-CoV-2: EPIDEMIOLOŠKE KARAKTERISTIKE, KLINIČKA SLIKA, DIJAGNOSTIKA I PREVENCIJA – PREGLED DOSADAŠNJIH SAZNANJA

AKTUELNA TEMA

CURRENT TOPIC

SaRS-CoV-2: EPIDEMIOLOGICAL CHARACTERISTICS, CLINICAL CHARACTERISTICS, DIAGNOSIS AND PREVENTION – A REVIEW OF CURRENT KNOWLEDGE

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SAŽETAK

Bolest *SARS-Cov-2* se pojavila decembra meseca 2019. godine, kada je potvrđeno da je novi korona virus uzročnik oboljenja. Cilj ovog rada je da rezimira dosadašnja istraživanja o epidemiološkim karakteristikama, uzrocima, kliničkoj slici, dijagnostici, prevenciji i kontroli nove bolesti uzrokovane koronavirusom. *SARS-Cov-2* virus pripada rodu beta-koronavirusa, jednolančanim RNK virusima. Omotač ima presudnu ulogu u patogenosti virusa. Virusna infekcija može izazvati prekomernu imunološku reakciju kod domaćina, koja je označena kao "citokinska oluja", čiji efekat je obimno oštećenje tkiva. Opisana su tri glavna puta prenosa virusa: kapljičnim putem, direktnim kontaktom i aerosolom. Smatra se da inkubacioni period iznosi 1-14 dana. Klinička slika može da varira od asimptomatske, preko blage, do teške forme, koja se može završiti i smrtnim ishodom. Glavne kliničke manifestacije uključuju povišenu telesnu temperaturu, kašalj i kratak dah. Nazalna kongestija, curenje iz nosa, gušobolja, glavobolja, bolovi u mišićima, prolivaste stolice, gubitak čula ukusa i/ili mirisa se takođe prijavljuju. Dijagnostički postupci su: klinička slika, radiografija pluća, biohemijske analize, epidemiološka anamneza. Za postavljanje etiološke dijagnoze služi pozitivan nalaz nazofaringealnog ili orofaringealnog brisa (brzi Ag test, i/ili reverzna transkriptaza-lančana reakcija polimeraze (*RT-PCR*)). *SARS-Cov-2* infekcija je pogodila veliki broj ljudi i zemalja širom sveta. Primena preventivnih mera, vakcinacija, rano prepoznavanje inficiranih osoba i njihova izolacija, za sada su najefikasniji način borbe sa ovim virusom. Po završetku pandemije, moći će da se proceni zdravstveni, socijalni i ekonomski uticaj infekcije ovim virusom.

Ključne reči: *SARS-Cov-2*, virus, pandemija, prevencija

ABSTRACT

The SARS-Cov-2 disease appeared in December 2019, when the new coronavirus was confirmed to be the cause of the disease. The objective of this article is to summarize previous research on the epidemiological characteristics, etiology, clinical characteristics, diagnosis, prevention, and control of the new SARS-Cov-2 infection. The SARS-Cov-2 virus belongs to the group of betacoronaviruses, which are single-stranded RNA viruses. The envelope has a crucial role in the pathogenicity of the virus. A viral infection can cause an excessive immune response in the patient, which is labeled as a "cytokine storm," and whose effect is extensive tissue damage. Three main routes of the transmission of the virus are: droplets, direct contact, aerosol. The incubation period is considered to be 1-14 days. The clinical manifestation ranges from asymptomatic, mild, to severe, and some cases end in death. The main clinical manifestations include fever, cough, and shortness of breath. Nasal congestion, a runny nose, a sore throat, headache, myalgia, diarrhea, loss of the sense of taste and/or smell have also been reported. The diagnostic procedures are the following: clinical manifestation, chest X-rays, biochemical analyses, epidemiological anamnesis. A positive nasopharyngeal or oropharyngeal swab (Ag test, and/or reverse transcription polymerase chain reaction (RT-PCR)) is used for etiological diagnosis. The SARS-Cov-2 infection has affected a large number of people and countries around the world. The application of preventive measures, early identification of infected persons, their isolation, and vaccination are currently the most effective mode in the battle against this virus. After the conclusion of the pandemic, it will be possible to estimate the health, social and economic impact of the virus.

Key words: SARS-Cov-2, virus, pandemic, prevention

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UVOD

Bolest *SARS-Cov-2* se pojavila krajem decembra 2019. godine u Vuhanu, Kina, kada je zapažena serija slučajeva pneumonija nepoznatog prouzrokovača. Nekoliko nedelja kasnije potvrđeno je da je novi korona virus uzročnik oboljenja. U Srbiji je, polovinom marta 2020. godine, nakon registrovanja prvih slučajeva i proglašenja pandemije od strane Svetske zdravstvene organizacije, uvedeno vanredno stanje [1,2,3,4]. Na početku epidemije, lekari primarne zdravstvene zaštite su bili u komunikaciji sa pacijentima putem telefona i u obavezi da u skladu sa anamnestičkim i epidemiološkim podacima koje dobiju od pacijenta, pruže sve neophodne savete i odrede potrebnu simptomatsku i/ili suportivnu terapiju, odgovarajući higijensko-dijetetski režim, savet da se ostane kod kuće, pa i kućnu izolaciju, ako je neophodna. Kasnije, kako je epidemija odmicala, a broj zaraženih i obolelih osoba se povećavao, formirane su Kovid ambulante na primarnom nivou, u kojima je lekar bio u obavezi da pacijenta sa sumnjom na *SARS-Cov-2* infekciju pregleda, uradi mu laboratorijske analize, radiografiju pluća, kao i nazofaringealni bris, i nakon toga ga uputi u izolaciju, leči, ili, ako je neophodno, uputi u dalji trijažni centar, radi hospitalizacije. Kako bi se sprečilo prenošenje infekcije, sledile su smernice Ministarstva zdravlja i Instituta za javno zdravlje Srbije, u cilju ranog otkrivanja i kontrole izvora infekcije i primene standardnih mera predostrožnosti, u smislu higijene ruku i mera respiratorne higijene, fizičke distance, važnosti upotrebe lične zaštitne opreme, izolacije sumnjivih slučajeva, i njihovog lečenja i transporta u Kovid bolnice, ako je neophodno [5,6]. Širenje *SARS-Cov-2* infekcije može biti usporeno ranim otkrivanjem, izolacijom, praćenjem kontakata, i masovnom vakcinacijom.

Cilj ovog rada je da rezimira dosadašnja istraživanja o epidemiološkim karakteristikama, uzrocima, kliničkoj slici, dijagnostici, prevenciji i kontroli nove koronavirus bolesti, *SARS-Cov-2* infekcije.

ETIOLOŠKE KARAKTERISTIKE

Koronavirusi su prvi put identifikovani kao patogeni za ljude krajem šezdesetih godina prošlog veka [7]. Novi *SARS-Cov-2* virus pripada rodu beta-koronavirusa. Čestice su okrugle, ovalne, često i polimorfne, prečnika od 60 – 140 nm i imaju omotač. Među funkcijama strukturnih proteina, omotač ima presudnu ulogu u patogenosti virusa. Do sada dostupni podaci ukazuju na to da ova virusna infekcija može izazvati prekomernu imunološku reakciju kod domaćina, koja je u celini označena kao "citokinska oluja", a čiji efekat je obimno oštećenje tkiva [8,9]. Novi koronavirus pokazao je mogućnost prenošenja sa životinja na čoveka i sa čoveka

INTRODUCTION

The SARS-Cov-2 disease appeared towards the end of December 2019, in Wuhan, China, when a series of pneumonia cases of unknown cause was registered. Several weeks later it was confirmed that a new coronavirus was the cause of the disease. In mid-March 2020, after the first cases had been registered and the pandemic had been declared by the World Health Organization, a state of emergency was declared in Serbia [1,2,3,4]. At the beginning of the epidemic, doctors in primary health care were in communication with patients by phone, and were obliged, in accordance with the anamnestic and epidemiological data received from the patients, to offer all necessary advice, as well as to prescribe necessary symptomatic and/or supportive therapy, to recommend the appropriate hygiene and dietary regimen, to provide advice on staying at home, and, if need be, instruct the patient on necessary home isolation. Later, as the epidemic progressed, and the number of infected and sick people increased, Covid clinics were established, at the level of primary health care, where, for each patient with suspected SARS-Cov-2 infection, the doctor was required to examine them, and perform laboratory analyses, a lung X-ray, a nasopharyngeal swab test; and after that, to instruct the patient to go into self-isolation, to treat the patient, or, if necessary, to refer the patient to the next-level triage center for hospitalization. In order to prevent the spread of the infection, guidelines were established by the Ministry of Health and the Institute for Public Health, for the purpose of early detection and control of infection sources and the application of standard precautionary measures related to: hand hygiene and respiratory hygiene, maintaining physical distance, the importance of the use of personal protective equipment, isolation of suspected Covid cases, their treatment and their transport to Covid hospitals, if necessary [5,6]. The spreading of the *SARS-Cov-2* infection can be slowed down by early detection, isolation, contact tracing and monitoring, and mass vaccination.

The goal of this paper is to give an overview of the research conducted so far regarding the epidemiological characteristics, causes, clinical presentation, diagnostics, prevention and control of the new coronavirus disease, the SARS-Cov-2 infection.

ETIOLOGICAL CHARACTERISTICS

Coronaviruses were first identified as pathogens affecting humans in the late 1960s [7]. The new SARS-Cov-2 virus belongs to the betacoronavirus genus. The particles are spherical, oval, and often polymorphous, 60 – 140 nm in diameter, with an envelope. Amongst the functions of the structural proteins, the envelope has a crucial role in the pathogenicity of the virus. Data available so

na čoveka. Prema istraživanju koje su sproveli Nanšan i Zonga (*Nanshan and Zhonga*) na 1.099 pacijenata koji su imali potvrđenu *SARS-Cov-2* infekciju, više od dve trećine pacijenata je imalo kontakt sa inficiranim ljudima, a svega 1,18% pacijenata je imalo podatak o izloženosti divljim životinjama [10].

EPIDEMIOLOŠKE KARAKTERISTIKE

Prema poslednjim smernicama, opisana su tri glavna puta prenosa virusa *COVID-19*:

1) kapljicama,

2) direktnim kontaktom,

3) aerosolom.

Prenos kapljicama se dešava kada respiratorne kapljice udišu ili gutaju zdrave osobe koje se nađu u blizini zaraženih osoba, koje te kapljice izbacuju pri govoru, kašljanju ili kijanju [11]. Dodirivanjem površina ili predmeta kontaminiranih virusom, a potom usta, nosa ili očiju, takođe predstavljaju put prenošenja infekcije. Kako se virus *SARS-Cov-2* može izolovati iz mokraće i stolice inficirane osobe, treba obratiti pažnju na mogućnost fekalno-oralne infekcije [12]. Procenjeno je da bi očekivani broj slučajeva koje bi direktno proizvela jedna zaražena osoba u populaciji bio 2,2 [13]. Kineski naučnici otkrili su da je izmet pacijenta pozitivnih na *SARS-Cov-2* u 6,5% slučajeva bio pozitivan na virus [10,14,15]. Infektivne kapljice lako mogu kontaminirati i epitel konjunktive očiju [16]. Epidemiološke studije su potvrdile da je polovina obolelih imala neku hroničnu bolest (51%) [17]. Neke studije su objavile starosnu distribuciju pacijenata, koja se kretala između 25 i 89 godina. Većina odraslih pacijenata je bila između 35 i 55 godina, mada je bilo identifikovanih slučajeva među decom i novorođenčadima [18]. Prosečna starost pacijenata bila je 59 godina; većina (59%) su osobe muškog pola [19]. Studija koja je sprovedena na devet novorođenih beba inficiranih ili obolelih majki, nije otkrila pozitivnost na virus kod novorođenčadi [20,21]. Međutim, ima podataka i da su novorođene bebe bile inficirane [22]. Mogućnost vertikalnog prenosa zahteva dalja istraživanja.

KLINIČKE KARAKTERISTIKE

Inkubacioni period iznosi 1-14 dana, prosečno 5,2 dana [19]. Dug inkubacioni period je razlog za veliku prenosivost virusa sa inficirane osobe na njenu okolinu. Kompletna klinička manifestacija bolesti još nije u potpunosti jasna, budući da se simptomi kreću od blage do teške kliničke slike, koja se ponekad može završiti i letalno, dok sa druge strane postoje i asimptomatski slučajevi. Smernice za definiciju slučaja pominju sledeće: povišenu telesnu temperaturu, smanjenje leukocita

far indicate that this viral infection may cause an excessive immune response in the host, which is, as a whole, marked as a "cytokine storm", whose effect is massive tissue damage [8,9]. The new coronavirus has demonstrated the ability of being transmitted from animal to human and from human to human. According to the research conducted by Nanshan and Zhonga on 1,099 patients with confirmed SARS-Cov-2 infection, more than two thirds of the patients had been in contact with infected people, and as few as 1.18% of patients had the data on exposure to wild animals in their medical history [10].

EPIDEMIOLOGICAL CHARACTERISTICS

According to the latest guidelines, three main transmission routes of the COVID-19 virus have been described:
1) transmission through droplets,
2) transmission through direct contact,
3) transmission via aerosol.

Droplet transmission occurs when respiratory droplets are inhaled or swallowed by healthy individuals who find themselves in close proximity to infected persons, who expel these droplets during speech, coughing or sneezing [11]. Touching surfaces or objects contaminated with the virus, and then touching one's nose, mouth or eyes, is also a route of transmission of the disease. As the SARS-Cov-2 can be isolated from urine and feces of infected persons, attention should be paid to the possibility of orofecal transmission of the infection [12]. It has been estimated that the expected number of cases that one infected person could produce in the population would be 2.2 [13]. Chinese scientists have discovered that the feces of SARS-Cov-2 positive patients was positive for the virus in 6.5% of the cases [10,14,15]. Infectious droplets can also easily contaminate the epithelium of the conjunctiva of the eye [16]. Epidemiological studies have confirmed that half of the diseased patients also suffered from some chronic disease (51%) [17]. The age distribution of patients was published in some studies, ranging from 25 to 89 years. Most of the adult patients were in the 35 to 55 age group, although there were also cases identified amongst children and newborns [18]. The average age of the patients was 59 years; most of them (59%) were male [19]. A study including nine babies born to infected or sick mothers did not discover Covid-19 positivity in the newborns [20,21]. However, there are data on newborn babies being infected [22]. The possibility of vertical transmission requires further research.

CLINICAL CHARACTERISTICS

The incubation period is 1-14 days, with the average being 5.2 days [19]. A long incubation period is the reason why there is high transmission of the virus from the infected person onto his/her environment. The complete

i/ili limfocita, radiografski nalaz na plućima. Na osnovu studije koja je obuhvatila 44.500 slučajeva potvrđene infekcije, blagi oblik bolesti bio je u 81%, ozbiljni i teški oblici oboljenja u 14%, kritičan oblik oboljenja sa poremećajem razmene gasova u plućima, šokno stanje, popuštanje i drugih organa u 5%, a 2,3 % do 5% obolelih sa smrtnim ishodom [23]. Glavne kliničke manifestacije uključuju povišenu telesnu temperaturu, kašalj, kratak dah. Nazalna kongestija, curenje iz nosa, gušobolja, glavobolja, bolovi u mišićima, prolivaste stolice, gubitak čula ukusa i/ili mirisa su takođe prijavljeni. Kod bolesnika sa hipoksijom moguća je konfuznost. Prosečno trajanje simptoma procenjeno je na 8 dana [24]. U istraživanju ranih kliničkih manifestacija, 87% pacijenata je imalo groznicu, 60% suv kašalj i oko 39% malaksalost [25,26]. Kada se radi o asimptomatskim slučajevima, rentgen nalaz pluća je normalan, test na *SARS-Cov-2* je pozitivan. Blagu kliničku sliku karakterišu simptomi akutne infekcije gornjih disajnih puteva, uključujući povišenu temperaturu, umor, bolove u mišićima, bol u guši, curenje iz nosa, kijanje, kašalj. Neki od pacijenata su imali i simptome oboljenja digestivnog trakta: muku, povraćanje, bol u trbuhu, prolivaste stolice. Pregled ovih pacijenata je samo pokazivao hiperemiju ždrela, dok je auskultatorni nalaz na plućima bio normalan. Na radiografiji pluća nije bilo znakova pneumonije. Umerena klinička slika praćena je groznicom, subfebrilnom temperaturom, suvim nadražajnim kašljem i zasićenjem krvi kiseonikom višim od 94%. Produbljivanjem simptoma umerene kliničke slike nastaje teška klinička slika. Nastavlja se febrilnost, dispneja, centralna cijanoza, zasićenje kiseonikom koje je niže od 90%, i javljaju se specifične promene viđene na skeneru pluća (CT). Postoji potreba za kiseoničkom potporom. Kritična klinička slika praćena je znacima respiratornog distres sindroma (RDS), respiratornim zastojem, stanjem šoka, encefalopatijom, i postoji potreba za mehaničkom ventilacijom. Laboratorijski nalazi usled citokinske oluje pokazuju porast fibrinogena, C reaktivnog proteina, D-dimera, i IL-6.

DIJAGNOZA

Za pacijente sa sumnjom na *SARS-Cov-2* infekciju, dijagnoza se postavlja na osnovu kliničke slike, radiografskog nalaza pluća, i biohemijskih analiza. Kliničke manifestacije su u vidu akutnih respiratornih simptoma, povišene telesne temperature, i nalaza radiografije pluća, koji pokazuje znake pneumonije. Od značaja su i pozitivni epidemiološki podaci o putovanjima ili kontaktima sa osobama obolelim od *SARS-Cov-2* infekcije. Etiološka dijagnoza se postavlja na osnovu pozitivnog nalaza nazofaringealnog ili orofaringealnog brisa, *RT-PCR* metodom i/ili Ag brzim testom. Od laboratorijskih

clinical manifestation of the disease is not as yet completely clear, since the symptoms range from those typical for the mild clinical presentation to those characteristic of severe clinical presentation, which may sometimes end in death, while, on the other hand, there are also asymptomatic cases. The guidelines for defining a case of Covid-19 state the following: elevated body temperature, decreased leukocyte and/or lymphocyte count, an abnormal finding on lung X-ray. Based on a study including 44,500 cases with confirmed infection, the mild form of the disease was present in 81% of the cases, serious and severe forms of the disease in 14% of the cases, the critical form with disturbed pulmonary gas exchange, state of shock, failure of other organs, was present in 5% of the cases, and in 2.3% – 5% of the cases, the disease ended in death [23]. The main clinical manifestations include elevated body temperature, coughing, shortness of breath. Nasal congestion, a runny nose, a sore throat, headache, myalgia, diarrhea, loss of the sense of taste and/or smell, have also been reported. In patients with hypoxia, confusion is possible. The average duration of the symptoms is estimated to be 8 days [24]. In a research of early clinical manifestations, 87% patients had a fever, 60% had a dry cough, and around 39% experienced fatigue and weakness [25,26]. In asymptomatic cases, the X-ray finding is normal, while the *SARS-Cov-2* test is positive. Mild clinical presentation is characterized by symptoms of acute upper respiratory tract infection, including elevated body temperature, fatigue, myalgia, a sore throat, a runny nose, sneezing, and coughing. Some of the patients experienced symptoms related to the digestive tract: nausea, vomiting, pain in the abdomen, diarrhea. On examination, these patients presented only with hyperemia of the pharynx, while the auscultatory finding was normal. Pulmonary X-ray showed know signs of pneumonia. Moderate clinical presentation is accompanied by fever, subfebrile body temperature, dry irritating cough, and blood oxygen levels above 94%. Further exacerbation of symptoms typical of the moderate clinical presentation leads to severe clinical presentation. Febrility continues, there is dyspnea, central cyanosis; the blood oxygen level drops below 90%, and specific changes visible on a CT scan occur. There is a need for supplemental oxygen. Critical clinical presentation is accompanied by signs of respiratory distress syndrome (RDS), respiratory arrest, state of shock, encephalopathy, and there is a need for mechanical ventilation. Laboratory findings related to the cytokine storm show elevated levels of fibrinogen levels, C-reactive protein, D-dimer, and IL-6.

DIAGNOSIS

For patients suspected to be infected with SARS-Cov-2, diagnosis is determined on the basis of clinical

analiza krvi, u ranim fazama bolesti, broj perifernih leukocita je smanjen ili normalan, broj limfocita je smanjen, dok je povišen nivo sedimentacije eritrocita, odnosno CRP-a.

Broj trombocita je niži kod obolelih sa težom kliničkom slikom, a trombocitopenija je nezavisni prediktor mortaliteta kod obolelih sa težom kliničkom slikom [27]. Kod težih slučajeva, broj limfocita se progresivno smanjuje, a povišeni su D-dimer, serumski kalcitonin, troponin i feritin, naročito kod onih pacijenata koji zahtevaju smeštaj u jedinice intenzivne nege [17,28]. Zbog promena na plućima, važno je uraditi i radiografiju pluća. Najčešće promene, vidljive na rentgenskom snimku pluća, su zadebljao intersticijum, dominantno periferno i u donjim plućnim poljima, retikularne promene i konsolidacije. Ukoliko su prisutni respiratorni simptomi, a rentgenski nalaz pluća je normalan, ukoliko je došlo do kliničkog pogoršanja, ili da bi se isključile potencijalne komplikacije, preporučen je CT pluća. Neprozirnost plućnog parenhima u vidu mlečnog stakla i konsolidacija, sa ili bez vaskularnog uvećanja, kao i zadebljanja interlobularnih septi, su uobičajeni CT nalazi *SARS-Cov-2* pozitivnih pacijenata. Pleuralni izliv je redak [29,30,31].

Analizirana je dinamika antitela kod inficiranih pacijenata. IgM antitelo se otkriva u krvi pacijenata od 3 do 6 dana od početka bolesti, dok se prisustvo IgG antitela beleži od osmog dana. Kako početak bolesti odmiče, titar IgM antitela se postepeno smanjuje, od druge nedelje bolesti. IgG antitela dostižu titar od najmanje četvorostrukog porasta tokom perioda rekovalescencije, što ukazuje da imaju zaštitnu ulogu [32]. Istraživanja su pokazala snažnu povezanost titra ukupnih antitela na *SARS-Cov-2* i težine kliničke slike. Bolesnici koji su imali težu kliničku sliku stvarali su više titrove antitela, posle preležane bolesti.

PREVENCIJA

Najbolja mera prevencije bolesti je vakcinacija i izbegavanje izlaganja virusu. Lekari opšte medicine, kao i drugi zdravstveni radnici, koji na primarnom nivou leče obolele ili sumnjive na *SARS-Cov-2* infekciju, trebalo bi da preduzmu mere predostrožnosti, pre svega da imaju naviku nošenja maske na licu i pranja ruku sapunom ili dezinfekcionim sredstvom, kao i da izbegavaju dodirivanje lica prljavim rukama, te da poštuju fizičku distancu.

Asimptomatski kontakti se upućuju u kućnu izolaciju, u trajanju od 14 dana. Pacijenti sa blagim simptomima se upućuju na kućno lečenje i pod zdravstvenim nadzorom su lekara primarne zdravstvene zaštite iz Kovid ambulanti. Pacijenti sa težim kliničkim formama bolesti upućuju se na konsultativno lečenje u Kovid bolnice.

presentation, pulmonary X-ray findings, and biochemical laboratory analyses. Clinical manifestations are in the form of acute respiratory symptoms, elevated body temperature, and a pulmonary X-ray finding indicating signs of pneumonia. Positive epidemiological data on travel or contact with patients afflicted with the *SARS-Cov-2* infection, are also relevant. Etiological diagnosis is established on the basis of a positive nasopharyngeal or oropharyngeal swab, tested with the RT-PCR method and/or the Ag rapid test. As far as laboratory blood analyses are concerned, in the early stages of the disease, the peripheral leukocyte count is either decreased or normal, the lymphocyte count is decreased, while the erythrocyte sedimentation rate and the level of CRP are elevated.

The thrombocyte count is lower in patients with severe clinical presentation, and thrombocytopenia is an independent predictor of mortality in patients with severe clinical presentation [27]. In the more severe cases, the lymphocyte count progressively decreases, while D-dimer, serum calcitonin, troponin, and ferritin are elevated, especially in patients requiring intensive care [17,28]. Due to changes in the lungs, it is important to perform a pulmonary X-ray. The changes most commonly found on pulmonary X-rays are the thickening of the interstitium, dominantly peripherally and in the inferior lung fields, as well as reticular changes and consolidations. If respiratory symptoms are present, but the pulmonary X-ray is normal, in case the patient's condition clinically worsens, or if potential complications need to be excluded, it is recommended to perform a CT scan of the lungs. Ground-glass opacity of the parenchyma of the lungs and consolidation, with or without vascular enlargement, as well as interlobular septal thickening, are common findings on pulmonary CT scans for SARS-Cov-2 positive patients. Pleural effusion is rare [29,30,31].

The dynamics of the antibodies in infected patients has been analyzed. The IgM antibody is registered in the patient's blood three to six days after the onset of disease, while the presence of the IgG antibody is registered as of the eighth day. As the disease develops, the IgM antibody titer gradually decreases, as of the second week of disease. The IgG antibody titer increases to at least its quadruple value during the period of convalescence, indicating the protective role of these antibodies [32]. Research has shown a strong connection between the SARS-Cov-2 total antibody titer and the severity of clinical presentation. Patients who had had a more severe clinical presentation had higher titers of antibodies, after the illness.

PREVENTION

The best preventive measure against the disease is vaccination and avoiding exposure to the virus. General

Trenutno je u razvoju više potencijalnih vakcina. Do sada je njih 8 administrativno prihvaćeno u svetu, a četiri vakcine su dobile upotrebnu dozvolu u Srbiji. U trenutku pisanja ovog rada, dostupne vakcine u Srbiji su *BioNTech/Pfizer, Sinopharm BBIBP-CorV, Sputnik V, Oxford/AstraZeneca*, sa trenutno 26,9% potpuno vakcinisanih građana i 34,1% koji su primili jednu dozu [5].

Masovna i sveobuhvatna vakcinacija bi mogla biti najuspešnije sredstvo u borbi protiv *SARS-Cov-2* infekcije.

ZAKLJUČAK

Iako ne može odražavati celokupno istraživanje o *SARS-Cov-2* infekciji širom sveta, ovaj rad može pružiti informacije za buduća proučavanja i kontrolu bolesti. Tek kada se pandemija završi, moći će da se proceni zdravstveni, ekonomski i socijalni uticaj ove globalne katastrofe. *COVID-19* je nova bolest izazvana koronavirusom koja je pogodila veliki broj ljudi i zemalja širom sveta. Većina pacijenata će imati blagu kliničku sliku ali moguće su i teške forme praćene respiratornim distres sindromom, višestrukim zastojem organa, pa čak i smrtnim ishodom. Primena preventivnih mera, rano prepoznavanje inficiranih osoba, njihova izolacija i vakcinacija, za sada su najefikasniji načini borbe sa ovim virusom. Brz napredak nauke i javnog zdravlja koji je postignut pri suočavanju sa pandemijom *COVID-19* neuporediv je, ali još uvek postoji potreba za ubrzanjem protokola koji vode ka brzoj dijagnostici, terapiji i lečenju. Korist od vakcinacije bila bi vrlo velika, ako bi obuhvat populacije bio veliki, i na taj način bi se sprečile ponovljene epidemije.

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practitioners, as well as other health care workers, who, at the primary health care level, treat SARS-Cov-2 patients and those suspected of being infected, should implement preventive measures, primarily, they should habitually wear face masks and wash their hands with soap or disinfectant, they should avoid touching their face with dirty hands and observe physical distance.

Asymptomatic contacts should be instructed to self-isolate at home for a period of 14 days. Patients with mild symptoms are to be directed to convalesce at home under the supervision of the doctors working at primary health care Covid facilities. Patients with more severe clinical forms of the disease are to be refereed for consultative treatment in Covid hospitals.

At the moment, a number of potential vaccines are being developed. So far, eight vaccines have administratively been accepted in the world, and four vaccines have been approved for use in Serbia. At the moment when this paper is being written, the vaccines available in Serbia are the following: *BioNTech/Pfizer, Sinopharm BBIBP-CorV, Sputnik V, Oxford/AstraZeneca*, with currently 26.9% of the population fully vaccinated and 34.1% of the population having received the first dose [5].

Mass and general vaccination could be the most effective means in the fight against the SARS-Cov-2 infection.

CONCLUSION

Although it cannot reflect the entire research on the SARS-Cov-2 infection worldwide, this paper can offer information for future research and disease control. It is only once the pandemic is over that the health, economic and social impact of this global catastrophe can be assessed. COVID-19 is a new disease caused by coronavirus, which has afflicted a great number of people and countries all over the world. Most of the patients will develop a mild clinical presentation, but severe forms of the disease are also possible, resulting in respiratory distress syndrome, multipole organ failure, and even death. Enforcing preventive measures, early detection of infected persons, the isolation of such persons, and vaccination, are, at the moment, the most efficient and effective ways to fight this virus. The rapid development of science and public health, which has been achieved in facing the COVID-19 pandemic, is unprecedented. However, there is still a need to accelerate the protocols leading to swift diagnosis, therapy and treatment. The benefits from vaccination could be great, provided that the scope of the population covered by the vaccine is large, thus preventing repeated outbreaks of the epidemic.

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NEPOZNATI I LOŠE DEFINISANI UZROCI SMRTI U MORTALITETU STANOVNIKA SRBIJE, HRVATSKE, SEVERNE MAKEDONIJE I SLOVENIJE, U PERIODU OD 2007. DO 2016. GODINE

ORIGINALNI RAD

ORIGINAL ARTICLE

UNKNOWN AND ILL-DEFINED CAUSES OF DEATH IN THE MORTALITY OF THE POPULATIONS OF SERBIA, CROATIA, NORTH MACEDONIA, AND SLOVENIA, IN THE PERIOD BETWEEN 2007 AND 2016

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SAŽETAK

Uvod: Podaci o uzroku smrti čine kamen temeljac za analizu zdravstvene situacije i bolesti u zemljama, i daju veliki doprinos izgradnji dokaza za zdravstvene politike.

Cilj: Cilj ovog istraživanja je bio da se utvrdi u kojoj meri su u Srbiji, Hrvatskoj, Severnoj Makedoniji i Sloveniji, u desetogodišnjem periodu, između 2007. i 2016. godine, kao osnovni uzrok smrti, korišćene dijagnoze iz grupe – Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu (R00-R99), Međunarodne klasifikacije bolesti (MKB - X revizija).

Materijal i metode: U ovom istraživanju korišćene su metode deskriptivne i analitičke statistike. Urađena je analiza podataka o uzrocima smrti (R00-R99 MKB - X revizija) prema polu, tokom desetogodišnjeg perioda (2007 – 2016). Takođe je kao analitički metod korišćena linearna regresija za analizu trenda.

Rezultati: U Srbiji su, u toku desetogodišnjeg perioda, dijagnoze R00-R99 bile među pet najčešćih grupa dijagnoza smrti, odnosno u svakoj godini su zauzimale treće mesto, sa procentualnom zastupljenošću od 4,7%. U posmatranom periodu, u zemljama iz okruženja, zabeležen je porast stope umrlih lica u Sloveniji, sa najvećom stopom u 2016. godini (19,9), dok se u Hrvatskoj uočava pad stope umrlih lica sa dijagnozama iz grupe XVIII MKB-X (R00-R99). U Makedoniji je stopa imala linearan trend, sa blagim padom u 2012. (52,3) i 2013. godini (58,7). U posmatranom periodu, zapažen je porast stope broja umrlih stanovnika Srbije sa nepoznatim uzrokom smrti, sa naročito visokim stopama u 2009. i 2016. godini. Uporednom analizom utvrđeno je da su R00-R99 dijagnoze više zastupljene u mortalitetnoj statistici Srbije, u odnosu na Sloveniju i Hrvatsku, a manje u odnosu na Severnu Makedoniju.

Zaključak: Potrebne su hitne intervencije u cilju poboljšanja kvaliteta podataka o osnovnom uzroku smrti u potvrdama o smrti. Potrebno je poboljšati podatke o mortalitetu i podatke o uzrocima smrti u posmatranim zemljama.

Ključne reči: mortalitet, potvrda o smrti, uzroci smrti, MKB-X

ABSTRACT

Introduction: Data on the cause of death form the cornerstone for analyzing the health situation and disease in countries, and they make a major contribution to building evidence for health policies.

Aim: The aim of this study was to determine the extent to which diagnoses from the group – Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (R00-R99), International Classification of Diseases (ICD - Revision X) were used as the main cause of death in Serbia, Croatia, North Macedonia, and Slovenia in the ten-year period, 2007 – 2016.

Materials and methods: Methods of descriptive and analytical statistics were used in this research. An analysis of data on the causes of death (R00-R99 ICD - Revision X), by gender, during the ten-year period (2007 – 2016) was performed. Linear regression was also used as an analytical method to analyze the trend.

Results: During the ten-year period, in Serbia, the R00-R99 diagnoses were among the five most common groups of diagnoses of death, i.e., in each year they took third place, with a percentage of 4.7%. In the observed period, in the surrounding countries, there was an increase in the death rate in Slovenia, with the highest rate in 2016 (19.9), while in Croatia there was a decrease in the death rate related to the diagnoses from group XVIII ICD - X (R00-R99). In Macedonia, the rate had a linear trend, with a slight decline in 2012 (52.3) and 2013 (58.7). In the observed period, an increase in the death rate of the population of Serbia with an unknown cause of death was observed, with particularly high rates in 2009 and 2016. Comparative analysis has shown that R00-R99 diagnoses are represented more in the mortality statistics of Serbia than in Slovenia and Croatia, and less than in Northern Macedonia.

Conclusion: Urgent interventions are needed to improve the quality of mortality statistics and data on the causes of death in the described countries.

Key words: mortality, death certificate, causes of death, ICD-X

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UVOD

Politike i programi za borbu protiv bolesti i povreda zasnivaju se na pravovremenim informacijama o prirodi i obimu zdravstvenih problema. Najčešće korišćeni podaci za potrebe zdravstvenih politika su statistički podaci o broju ljudi koji umiru, i to prema starosti i polu, kao i prema uzroku smrti. Podaci o uzroku smrti čine kamen temeljac za analizu zdravstvene situacije i bolesti u zemljama, i daju veliki doprinos izgradnji baza podataka za zdravstvene politike [1]. Svetska zdravstvena organizacija (SZO) preporučuje izbegavanje korišćenja neutvrđenih i nepoznatih uzroka smrti u potvrdi o smrti, jer se smatra da ta terminologija ne daje informacije o uslovima koji su doveli do smrti [2]. Takođe, postoje sugestije da stopa smrtnosti za smrt koja se pripisuje simptomima, znacima i loše definisanim uzrocima smrti može biti potencijalni pokazatelj pristupa i korišćenja zdravstvenih usluga [3]. Procenat smrti koje su loše definisane ili kod kojih je uzrok smrti nepoznat, jedan je od pokazatelja i samog kvaliteta podataka o uzroku smrti. Pouzdanost ovih podataka je neophodna, ne samo za procenu trendova i varijacija u zdravlju stanovništva, već i za procenu nejednakosti u zdravlju između grupa stanovništva [4].

Mortalitetni podaci obezbeđuju najvažnije zdravstvene pokazatelje za ocenu i poređenje zdravstvenog stanja na lokalnom, državnom i međunarodnom nivou, jer se u svakoj razvijenoj zemlji, kao i u većini zemalja u razvoju, redovno i sveobuhvatno prikupljaju [5]. Takođe, pouzdani i validni podaci o mortalitetu su važni za epidemiološka istraživanja kao i za javno-zdravstvenu politiku i prioritetne ciljeve [6]. Statistika mortaliteta je jedan od osnovnih izvora zdravstvenih informacija i u mnogim zemljama je najpouzdaniji izvor zdravstvenih podataka [7].

Međunarodna klasifikacija bolesti, njena deseta revizija (MKB-X) spada u referentnu klasifikaciju SZO. Široko je prihvaćena i preporučuje se kao vodič za međunarodno izveštavanje o zdravlju. Svrha MKB je da omogući sistematsko evidentiranje, analizu, tumačenje i poređenje prikupljenih podataka o smrtnosti i obolevanju, u različitim zemljama ili regionima, i za različite vremenske periode [8]. Međunarodna klasifikacija - MKB je podeljena po grupama i ukupno ima 21 grupu. Grupe od I do XVII se odnose na bolesti i druga bolesna stanja, a Grupa XIX na povrede, trovanje i posledice spoljnjih uzroka. Preostale grupe upotpunjuju okvir mogućih stanja obuhvaćenih dijagnostičkim podacima. Grupa XVIII obuhvata simptome, znake i patološke kliničke i laboratorijske nalaze, koji nisu klasifikovani na nekom drugom mestu [8]. Sa tačke gledišta prevencije smrti, važno je prekinuti lanac događaja koji vodi neželjenom ishodu, bolesti, nesposobnosti.

INTRODUCTION

Policies and programs for fighting diseases and injuries are based on timely information on the nature and scope of health problems. The most commonly used data for the purpose of health policies are statistical data on the number of deceased people, given by age and sex, as well as by cause of death. The data on cause of death represent the cornerstone of the analysis of the health situation and disease in countries, and they greatly contribute to the development of databases for health policies [1]. The World Health Organization (WHO) recommends avoiding the use of undetermined and unknown causes of death in the death certificate, as the belief is that this terminology does not offer information on the conditions that had led to death [2]. Also, there are suggestions that the death rate for deaths attributed to symptoms, signs and ill-defined causes of death may be a potential indicator of access to and use of health services [3]. The percentage of ill-defined deaths or deaths with unknown cause, is one of the indicators of the very quality of the data on the cause of death. The reliability of these data is necessary, not only for the assessment of trends and variations in the population's health, but also for the assessment of inequalities in health among the population groups [4].

Mortality data provide the most important health indicators for the assessment and comparison of the health status, at the local, state, and international levels, since in each developed country, as well as in most developing countries, these data are regularly and comprehensively collected [5]. Also, reliable and valid data on mortality are important for epidemiological research, as well as for public health policy and priority goals [6]. Mortality statistics is one of the basic sources of health-related information, and, in many countries, it is the most reliable source of health-related data [7].

The International Classification of Diseases, Tenth Revision (ICD-X), is reference classification of the WHO. It is widely accepted and recommended as a guidebook for international reporting on health. The purpose of ICD is to enable systematic recording, analysis, interpreting, and comparison of collected data on mortality and morbidity, in different countries or regions, and for different time periods [8]. The ICD is divided into groups and has a total of 21 groups. Groups I to XVII relate to diseases and other states of illness; Group XIX relates to injuries, poisoning, and consequences of external factors. The remaining groups complete the framework of possible conditions covered by diagnostic data. Group XVIII comprises symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified [8]. From the point of view of death prevention, it is important to break the chain of events which leads to an adverse outcome, illness or disability.

Najefektivniji javno-zdravstveni cilj je da spreči dejstvo uzročnika. Zbog toga, osnovni uzrok smrti je definisan kao *„(a) bolest ili povreda koja je pokrenula niz bolesnih stanja koja su direktno dovela do smrti ili b) okolnosti nesrećnog slučaja ili nasilja koje su uzrokovale smrtonosnu povredu"* [9, str. 35, MKB-X, knjiga 2]. Ova pravila obezbeđuju međunarodnu uporedivost podataka o mortalitetu i pomažu u standardizaciji upravljanja nejasnom medicinskom dokumentacijom. Pravilo je da, kada se u potvrdu o smrti unese više od jednog stanja, treba izabrati samo jedno osnovno stanje koje je dovelo do svih drugih. Iako postoje pravilnici koji se primenjuju, kada su u pitanju podaci o mortalitetu, međunarodna uporedivost je ipak ograničena, zbog razlika u zdravstvenim sistemima i nacionalno modifikovanim pravilima [9,10].

Sledeća stanja se smatraju nepoznatim i loše definisanim stanjima: MKB-X, R00-R94 ili R95-R99, Grupa XVIII – Simptomi, znaci i patološki klinički nalazi neklasifikovani na drugom mestu (Prilog 1). Kategorije iz ove grupe ne treba koristiti kao šifre za „glavno stanje", osim ako simptom, znak ili nenormalni nalaz nije bio očigledno glavno stanje za lečenje ili ispitivanje u toku epizode lečenja i nije bio povezan sa drugim stanjem koje je lekar označio [11].

Cilj ovog istraživanja je bio da se utvrdi u kojoj su meri, kao osnovni uzrok smrti, korišćene dijagnoze iz grupe – Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu (R00-R99), MKB – X revizija. Ova analiza će omogućiti da se uporedi kvalitet podataka o uzroku smrti u našoj zemlji u odnosu na zemlje regiona i to: Hrvatsku, Severnu Makedoniju i Sloveniju, u desetogodišnjem periodu (2007 – 2016).

METODE

Jedinice posmatranja i ispitivane varijable

Jedinice posmatranja su: ukupan broj stanovnika, udeo u ukupnom mortalitetu (%), i stopa umrlih zbog nepoznatih i loše definisanih uzroka smrti (R00-R99 MKB - X revizija) u Srbiji, Hrvatskoj, Severnoj Makedoniji i Sloveniji, u periodu od 2007. do 2016. godine. Prikazani su i rezultati analize trenda podataka o udelu umrlih zbog R00-R99 u ukupnom mortalitetu (%).

Izvori podataka

U istraživanju su korišćeni objavljeni podaci Republičkog zavoda za statistiku o mortalitetu stanovnika Srbije [12-15]. Za međunarodno poređenje korišćeni su zvanični podaci mortalitetne statistike Hrvatske [16-17], Makedonije [18,19] i Slovenije [20,21].

Statistička analiza

U ovom istraživanju, korišćene su metode deskriptivne i analitičke statistike. Urađena je analiza podataka

The most effective public health goal is to prevent the impact of the causative agent. This is why the underlying cause of death is defined as "*the disease or injury that initiated the train of events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury*" [9, p. 35, ICD-X, Volume 2]. These rules provide for international comparability of data on mortality and help in the standardization of managing unclear medical documentation. The rule is that, when more than one condition is entered into the death certificate, only one underlying condition should be selected as the one leading to all the others. Although there are rulebooks in use, when it comes to mortality data, international comparability is still limited, due to the differences amongst health systems, as well as nationally modified rules [9,10].

The following states are considered unknown and ill-defined: ICD-X, R00-R94 or R95-R99, Group XVIII – Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (Appendix 1). The categories from this group should not be used as codes for the "underlying cause", unless the symptom, sign, or abnormal finding was the obvious underlying condition that was treated and investigated during the treatment episode and was not connected with any other condition marked by the doctor [11].

The aim of this study was to determine the extent of the use of the diagnoses from the group - Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (R00-R99), ICD, Tenth Revision, as the underlying cause of death. This analysis will enable the comparison of the quality of the data on cause of death in our country with that of the other countries in the region: Croatia, North Macedonia, and Slovenia, over a ten-year period (2007 – 2016).

METHODS

Units of observation and tested variables

The units of observation are the following: the overall population, the participation in total mortality (%), and the rate of persons deceased due to unknown and ill-defined causes of death (R00-R99 ICD – Revision X) in Serbia, Croatia, North Macedonia, and Slovenia, in the period 2007 – 2016. The results of the analysis of the data trend regarding the participation of persons deceased due to R00-R99 in overall mortality (%) is also presented.

Data sources

Data published by the Statistical Office of the Republic of Serbia on the mortality of the citizens of Serbia were used in the study [12-15]. Official mortality statistics data of Croatia [16-17], North Macedonia [18,19], and Slovenia [20,21] were used for the purpose of international comparison.

o uzrocima smrti (R00-R99 MKB - X revizija) prema polu, starosti i regionima Srbije, kao i linija trenda i bazični i lančani indeks promene tokom desetogodišnjeg perioda (2007 – 2016). Za analizu trenda korišćena je linearna regresija uz pomoć programa američkog Nacionalnog instituta za karcinome (engl. *Joinpoint Regression Trend Analysis Software - Version 4.9.0.0)*. Najvažniji rezultati istraživanja prikazani su grafički i tabelarno, zatim kroz procente i stope učešća dijagnoza iz grupe R00-R99 MKB-X u mortalitetnoj statistici stanovnika Srbije i zemalja iz regiona, i prodiskutovani su u svetlu relevantne literature.

REZULTATI

U posmatranom periodu, zapaža se porast stope broja umrlih na 100.000 stanovnika Srbije sa dijagnozom iz grupe MKB XVIII – Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu (u nastavku teksta, R00-R99). Najviša stopa se beleži 2009. godine (72,0/100.000) kao i 2016. godine (70,6/100.000) (Grafikon 1).

Tokom posmatranog perioda, u Republici Sloveniji, uočen je porast stope umrlih lica od R00-R99, a najveća stopa se uočava u 2016. godini – 19,9/100.000 (Grafikon 2).

U Republici Hrvatskoj, u posmatranom periodu od 2008. do 2015. godine (2015. godina je bila poslednja godina sa dostupnim podacima u vreme izvođenja studije), uočava se pad stope umrlih lica sa dijagnozama iz grupe R00-R99, tako da su vrednosti u 2006. i u 2015. godini bile iste (Grafikon 3).

Od 2007. do 2016. godine, u Republici Severnoj Makedoniji, stopa umrlih lica sa dijagnozom uzroka smrti iz grupe R00-R99 imala je linearan trend, sa blagim padom u 2012. i 2013. godini (Grafikon 4).

U posmatranom periodu, udeo R00-R99 u ukupnom mortalitetu je bio statistički značajno viši u Srbiji

Statistical analysis

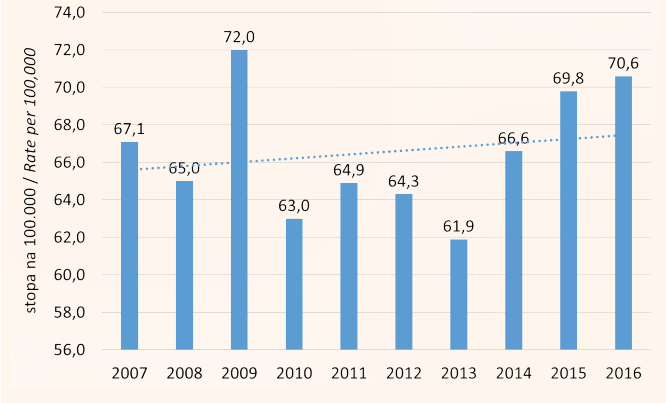
Methods of descriptive and analytical statistics have been used in this study. Analysis of data on the causes of death (R00-R99 ICD - Revision X), by sex, age and regions in Serbia, as well as the trend line, the basic index and the chain index of change, over a ten-year period (2007 – 2016) have been carried out. Linear regression with the support of the program of the American National Cancer Institute (Joinpoint Regression Trend Analysis Software - Version 4.9.0.0) was used for trend analysis. The most significant results of the study are represented in graphs and tables, through percentages and the rate of participation of the diagnoses from the R00-R99 ICD-X group in the mortality statistics of the citizens of Serbia and the countries form the region, and have been discussed in the context of relevant reference literature.

RESULTS

In the observed period, a rise in the rate of deceased persons per 100,000 citizens of Serbia with a diagnosis from the group ICD XVIII – Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (hereinafter: R00-R99) was recorded. The highest rate was recorded in 2009 (72.0/100,000) as well as in 2016 (70.6/100,000) (Figure 1).

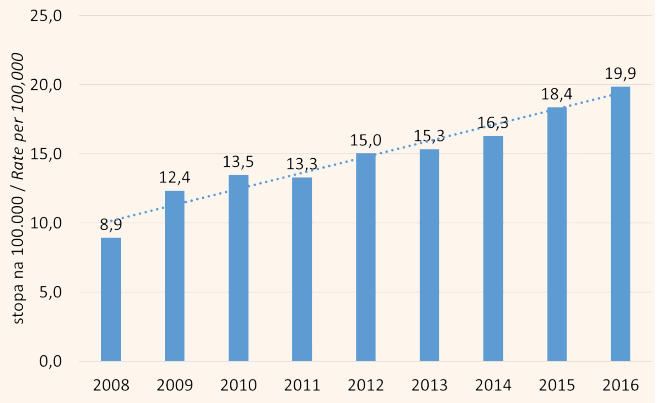
During the observed period, in the Republic of Slovenia, a rise in the rate of persons deceased from R00-R99 was recorded, with the highest rate noted in 2016 – 19.9/100,000 (Figure 2).

In the Republic of Croatia, during the observed period, between 2008 and 2015 (at the time of the study being carried out, year 2015 was the last year with available data), a drop in the rate of deceased persons with diagnoses from the R00-R99 group was recorded, thereby the values in 2006 and 2015 were the same (Figure 3).



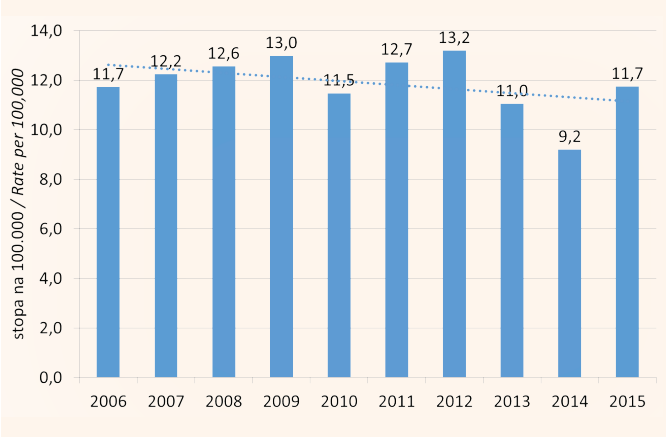
Grafikon 1. Stopa umrlih lica na 100.000 stanovnika sa dijagnozama iz grupe XVIII MKB-X (R00-R99) u Republici Srbiji, od 2007. do 2016. godine

Figure 1. The rate of deceased persons per 100,000 citizens with the diagnosis from the XVIII ICD-X (R00-R99) group, in the Republic of Serbia, between 2007 and 2016



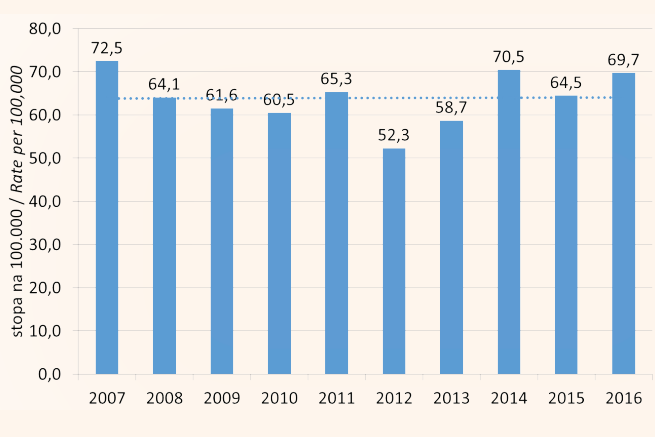
Grafikon 2. Stopa umrlih lica na 100.000 stanovnika sa dijagnozama iz grupe XVIII MKB-X (R00-R99) u Republici Sloveniji, od 2008. do 2016. godine

Figure 2. The rate of deceased persons per 100,000 citizens with the diagnosis from the XVIII ICD-X (R00-R99) group, in the Republic of Slovenia, between 2008 and 2016



Grafikon 3. Stopa umrlih lica na 100.000 stanovnika sa dijagnozama iz grupe XVIII MKB-X (R00-R99) u Republici Hrvatskoj, od 2008. do 2015. godine

Figure 3. The rate of deceased persons per 100,000 citizens with the diagnosis from the XVIII ICD-X (R00-R99) group, in the Republic of Croatia, between 2008 and 2015



Grafikon 4. Stopa umrlih lica sa dijagnozama iz grupe XVIII MKB-X (R00-R99) u Republici Severnoj Makedoniji, od 2007. do 2016. godine

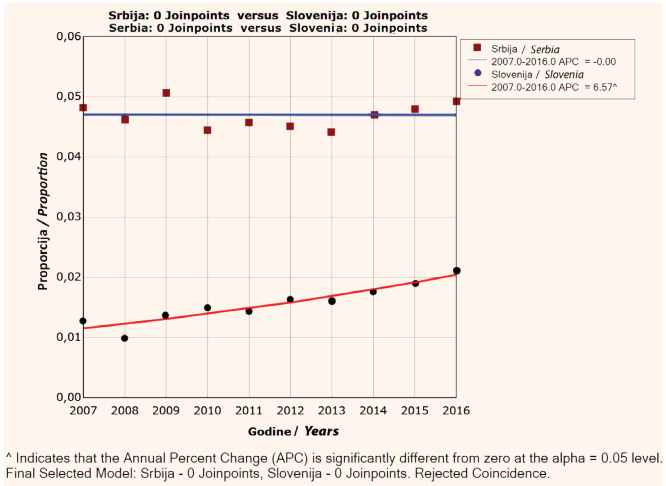
Figure 4. The rate of deceased persons with the diagnosis from the XVIII ICD-X (R00-R99) group, in the Republic of North Macedonia, between 2007 and 2016

u odnosu na Sloveniju ($p < 0,001$) (Grafikon 5). U posmatranom periodu, u Srbiji ne postoji statistički značajan trend promena proporcija R00-R99 (APC = 0,0; $p = 0,995$), već linearni stagnantni trend. U Sloveniji postoji statistički značajan linearni trend porasta proporcija R00-R99 u posmatranom periodu sa prosečnom stopom godišnje promene od 6,6%. (APC = 6,6; $p < 0,001$).

U posmatranom periodu, udeo R00-99 u ukupnom mortalitetu je bio statistički značajno niži u Srbiji u odnosu na Severnu Makedoniju ($p < 0,001$) (Grafikon 6), iako ni u Srbiji ni u Severnoj Makedoniji ne postoji statistički značajan trend promena proporcija R00-R99 u

Between 2007 and 2016, in the Republic of North Macedonia, the rate of deceased persons with cause of death diagnoses from the R00-R99 group had a linear trend, with a mild decrease in 2012 and 2103 (Figure 4).

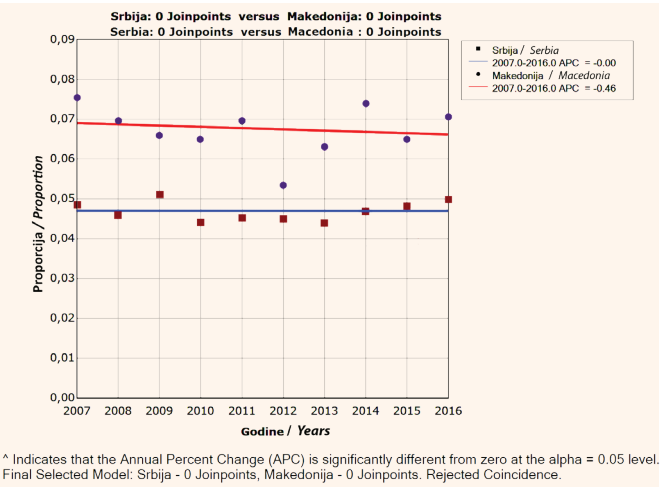
In the observed period, the participation of R00-R99 in the overall mortality was statistically significantly higher in Serbia than in Slovenia ($p < 0.001$) (Figure 5). In the observed period, in Serbia, there is no statistically significant trend of change in the proportion of R00-R99 (APC = 0.0; $p = 0.995$), rather, there is a linear trend of stagnation. In Slovenia, within the observed period, there is a statistically significant linear trend of



^ Indicates that the Annual Percent Change (APC) is significantly different from zero at the alpha = 0.05 level.
Final Selected Model: Srbija - 0 Joinpoints, Slovenija - 0 Joinpoints. Rejected Coincidence.

Grafikon 5. Analiza trenda za dijagnoze R00-R99 (Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu), poređenje između Republike Slovenije i Republike Srbije, od 2007. do 2016. godine

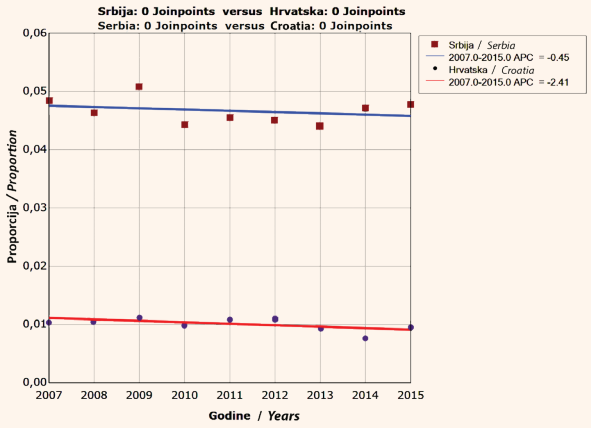
Figure 5. Trend Analysis for the diagnoses R00-R99 (Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified), comparison between the Republic of Slovenia and the republic of Serbia, between 2007 and 2016



^ Indicates that the Annual Percent Change (APC) is significantly different from zero at the alpha = 0.05 level.
Final Selected Model: Srbija - 0 Joinpoints, Makedonija - 0 Joinpoints. Rejected Coincidence.

Grafikon 6. Analiza trenda za dijagnoze R00-R99 (Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu), poređenje između Republike Srbije i Republike Severne Makedonije, od 2007. do 2016. godine

Figure 6. Analysis of the trend for the diagnoses R00-R99 (Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified), comparison between the Republic of Serbia and the Republic of North Macedonia, between 2007 and 2016



^ Indicates that the Annual Percent Change (APC) is significantly different from zero at the alpha = 0.05 level.
Final Selected Model: Srbija - 0 Joinpoints, Hrvatska - 0 Joinpoints. Rejected Coincidence.

Grafikon 7. Analiza trenda za dijagnoze R00-R99 (Simptomi, znaci i patološki klinički i laboratorijski nalazi neklasifikovani na drugom mestu), poređenje između Republike Srbije i Republike Hrvatske od 2007. do 2015. godine

Figure 7. Analysis of the trend for the diagnoses R00-R99 (Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified), comparison between the Republic of Serbia and the Republic of Croatia, between 2007 and 2015

posmatranom periodu (APC = 0,0; $p = 0,995$ i APC = -0,5; $p = 0,669$, respektivno). U obe zemlje, zastupljen je stabilan linearni trend koji ukazuje na stagniranje i minimalne promene.

Za Hrvatsku su, u vreme istraživanja, bili dostupni samo podaci od 2008. do 2015. godine.

U tom periodu, vrednosti proporcija R00-R99 su statistički značajno više u Srbiji u odnosu na Hrvatsku ($p < 0,001$) (Grafikon 7). I u Srbiji i u Hrvatskoj postoji linerani trend promena proporcija R00-R99 (APC = -0,4; $p = 0,464$, i APC = -2,4; $p = 0,068$, respektivno), odnosno, nema statistički značajnih promena.

DISKUSIJA

Uprkos preporukama SZO da se izbegava korišćenje neutvrđenih i nepoznatih uzroka smrti u potvrdi o smrti, jer se smatra da ta terminologija ne daje informacije o uslovima koji su doveli do smrti [3], kao i postojanju zakonske regulative o načinu popunjavanja potvrde o smrti u Srbiji i zemljama u okruženju, u posmatranim zemljama, veliki broj ovih dijagnoza je zastupljen kao osnovni uzrok smrti u potvrdama o smrti. U periodu od 2007. do 2016. godine, u Srbiji, dijagnoze iz grupe XVIII MKB-X (R00-R99) su bile među pet najčešćih grupa dijagnoza navedenih kao osnovni uzrok smrti, odnosno u svakoj godini su prema rangu zauzimale treće mesto, a zabeležen je i njihov porast u 2009. i 2016. godini. Takođe, u posmatranom periodu, postoji porast stope umrlih lica u Sloveniji, gde se najveća stopa uočava u 2016. godini, dok je u Hrvatskoj i Severnoj Makedoniji uočen blagi pad stopa umrlih lica sa dijagnozama R00-R99.

increase in the proportion of R00-R99, with an average rate of annual change of 6.6%. (APC = 6.6; $p < 0.001$).

In the observed period, the participation of R00-99 in the overall mortality was statistically significantly smaller in Serbia than in North Macedonia ($p < 0.001$) (Figure 6), although there isn't a statistically significant trend of change in the proportion of R00-R99, in the observed period, either in Serbia or in North Macedonia (APC = 0.0; $p = 0.995$ and APC = -0.5; $p = 0.669$, respectively). In both countries, a stable linear trend is present, indicating stagnation and minimal change.

At the time of the conducting of the research, there were only data from the period between 2008 and 2015 available for Croatia. In that period the values of the proportion of R00-R99 were statistically significantly greater in Serbia, as compared to Croatia ($p < 0.001$) (Figure 7). There is a linear trend of change in the proportion of R00-R99 (APC = -0.4; $p = 0.464$, and APC = -2.4; $p = 0.068$, respectively) both in Serbia and Croatia, i.e., there are no statistically significant changes.

DISCUSSION

Despite WHO recommendations to avoid the use of undetermined and unknown causes of death in the death certificate, due to the belief that this terminology does not offer information on the conditions that had led to death [3], as well as the existence of legislation regulating the way that the death certificate should be filled out both in Serbia, as well as in the countries from the region, in the observed countries, a large number of these diagnoses is prevalent as the underlying cause of death in the death certificate. In the period between 2007 and 2016, in Serbia, the diagnoses from the group XVIII ICD-X (R00-R99) were amongst the five most common groups of diagnoses stated as the underlying cause of death, i.e., in each year, they were ranked third; also, an increase in their numbers was registered in years 2009 and 2016. Additionally, in the observed period, in Slovenia, there was an increase in the death rate, with the highest rate observed in 2016, while in Croatia and North Macedonia, a mild decrease of death rates of persons diagnosed with R00-R99 as the cause of death was noted.

An increase or continuation of the level of ill-defined causes of death in the mortality statistics represents a great challenge for researchers all over Europe. Experience from other countries teaches us the way to improve data on mortality and cause of death, and, as an example, we can use the fact that the Government of Turkey has, as of 2009, conducted several reforms, at the Turkish Statistical Institute, to improve the system of reporting on the cause of death [22]. After analysis, it has been noted that there is a significant

Porast ili održavanje nedovoljno definisanih uzroka smrti u mortalitetnoj statistici predstavlja veliki izazov za istraživače širom Evrope. Iskustva iz drugih zemalja nas uče kako bismo mogli da poboljšamo podatke o mortalitetu i uzrocima smrti, a kao primer imamo da je Vlada Turske, od 2009. godine, na Turskom institutu za statistiku, sprovela nekoliko reformi za poboljšanje sistema izveštavanja o uzroku smrti [22]. Nakon analize, uočeno je da u značajnoj meri nedostaju informacije za demografske i epidemiološke varijable, naročito kada su u pitanju smrti odojčadi i detaljno evidentiranje uzroka smrti [22].

Pojava nedovoljno definisanih i loše šifriranih osnovnih uzroka smrti u potvrdi o smrti dovodi do smanjenja korisnosti ovih podataka, naročito kada pristup šifriranju nije standardizovan [23]. Takav je primer iz istraživanja koje ukazuje da u sistemu postoji visok stepen nedoslednosti u šifriranju mentalnih poremećaja i poremećaja ponašanja, bolesti nervnog sistema, endokrinih poremećaja, određenih kardiovaskularnih bolesti i loše definisanih uzroka smrti, kao osnovnog uzroka smrti [23]. U cilju poboljšanja podataka mortalitetne statistike, uvođenje softverskog sistema za šifriranje podataka o uzroku smrti u našoj zemlji bi doprinelo boljem i kvalitetnijem izveštavanju. Od januara 2017. godine, u zemljama EU su u upotrebi softverske verzije MKB-X. Po primeni novog softvera za šifriranje uzroka smrti, Nacionalni registar Škotske registrovao je prvo povećanje, a zatim i smanjenje broja smrti sa određenom dijagnozom kao osnovnim uzrokom smrti [24]. Nacionalni centar za zdravstvenu statistiku američkog Centra za kontrolu i prevenciju bolesti je razvio automatsko šifriranje uzroka smrti, a razvijeni softveri imaju široku primenu, te je smanjen rizik od sistemskih grešaka usled neujednačene interpretacije i primene pravila šifriranja. Softver je na raspolaganju za automatizaciju šifriranja medicinskih informacija na potvrdi o smrti prema uputstvima SZO. Ovo je jedan od retkih izvora podataka vezanih za zdravlje koji su uporedivi za mala geografska područja i dostupni u dugom vremenskom periodu [25].

Ograničenja istraživanja

Za neke uzroke smrti, statistika mortaliteta je odraz prakse šifriranja, a ne stvarne epidemiološke situacije, te u tom smislu treba posmatrati pouzdanost ovih rezultata istraživanja. Pokrivenost i kvalitet podataka o uzroku smrti variraju između zemalja, i čak validne, pouzdane i uporedive procene trendova uzroka smrti, u najboljim sistemima, ograničene su problemima kao što su promene u MKB-X, upotreba tabelarnih listi u kojima se gube značajni detalji o uzroku smrti, kao i mnogi smrtni slučajevi kojima je kao uzrok smrti dodeljena dijagnoza koja se ne može smatrati osnovnim uzrokom smrti. Naime, podregistracija je naročito ozbiljna

lack of data related to demographic and epidemiological variables, especially when it comes to the death of infants and to the detailed recording of the cause of death [22].

The occurrence of ill-defined and poorly coded underlying causes of death in the death certificate undermines the usefulness of this data, especially when the access to coding is not standardized [23]. Such is the example from research indicating that there is a high degree of inconsistency in the system related to the coding of mental disorders and behavioral disorders, diseases of the nervous system, endocrine disorders, certain cardiovascular diseases and ill-defined causes of death, as the underlying cause of death [23]. For the purpose of improving mortality statistics data, the introduction of a software system for the coding of data on the cause of death in Serbia would contribute to better reporting. As of January 2017, software versions of ICD-X have been in use in the EU countries. Upon the implementation of the new software for coding the cause of death, the National Records of Scotland registered the first increase, and then decrease in the number of deaths with a certain diagnosis as the underlying cause of death [24]. The National Center for Health Statistics of the American National Center for Disease Control and Prevention developed an automatic coding system of causes of death, and the developed computer programs are widely in use, which has reduced the risk of systemic errors caused by inconsistent interpretation and application of coding rules. The software is available for the automatization of the coding of medical data in the death certificate, in keeping with WHO guidelines. This is one of the rare sources of health-related data comparable for small geographic areas and available for a longer period of time [25].

Study limitations

For some causes of death, mortality statistics reflects the coding practice rather than the actual epidemiological situation, and, therefore, the reliability of these research results should be observed in that context. The coverage and quality of data on cause of death vary amongst countries, and even valid, reliable, and comparable assessments of the trends of the cause of death, in the best of systems, are limited by problems such as the changes in the ICD-X, the use of tabular lists where significant details on cause of death are lost, as well as by many deaths which have been assigned a diagnosis that cannot be considered an underlying cause of death. Namely, underregistration is particularly serious in rural areas with poor transport and a poor accessibility to health care centers [26].

It is necessary to research a longer period and a greater number of variables (e.g., by sex, by age,

u ruralnim područjima, u oblastima sa lošim transportom i slabom dostupnošću zdravstvenih centara [26].

Potrebno je istraživanjem obuhvatiti veći period i veći broj varijabli (na primer, po polu, starosti i pojedinačnim dijagnozama, i po izvoru podataka u potvrdama o smrti), a zatim uraditi uporednu analizu sa dijagnozama obducenata, kako bi se bolje razumeo problem i definisale ciljane intervencije. Zdravstvene ustanove, posebno bolnice, predstavljaju česte izvore podataka o smrtnosti stanovništva prema starosti, polu i uzroku. Ove statistike su važni markeri kvaliteta bolničke nege i obezbeđuju neophodna ulaganja, kako za nacionalne tako i za zdravstvene politike na lokalnom nivou. Neophodna je kontinuirana edukacija, kako lekara u zdravstvenim ustanovama tako i mrtvozornika, koji se bave popunjavanjem potvrde o smrti, o izgledu, sadržaju i pravilnom načinu šifriranja podataka o uzroku smrti. Takođe je veoma važno da donosioci odluka u zdravstvenom sistemu prepoznaju značaj kvalitetnih i tačnih podataka o mortalitetu, te je neophodno ulaganje u jačanje vitalnog sistema registracije, kako bi došlo do poboljšanja na svim nivoima, u smislu ispravno šifriranih osnovnih uzroka smrti i njihovog generisanja u konačne izveštaje o mortalitetnoj statistici.

ZAKLJUČAK

U posmatranom periodu zapažen je porast stope broja umrlih stanovnika Srbije sa nepoznatim uzrokom smrti, uz naročito visoke stope u 2009. i 2016. godini. Uporednom analizom, utvrđeno je da su R00-R99 dijagnoze više zastupljene u mortalitetnoj statistici Srbije, u odnosu na Sloveniju i Hrvatsku, a manje u odnosu na Severnu Makedoniju. Potrebne su hitne intervencije u cilju poboljšanja kvaliteta podataka o osnovnom uzroku smrti u potvrdama o smrti, kako bi se povećalo poverenje u njihovu tačnost i kako bi ti podaci bili primarni dokazi na kojima se zasnivaju epidemiološka istraživanja i zdravstvene politike. Ovo istraživanje je omogućilo da se predstavi šira slika kvaliteta šifriranja podataka o osnovnom uzroku smrti u potvrdi o smrti, na regionalnom nivou.

Izjave zahvalnosti

Istraživanje je deo master teze iz oblasti javnog zdravlja dr Nataše Rosić, pod naslovom „Zastupljenost nepoznatih i loše definisanih uzroka smrti u mortalitetu stanovnika Srbije: implikacije na unapređenje kvaliteta mortalitetne statistike" odbranjene na Medicinskom fakultetu Univerziteta u Beogradu (mentor: prof. dr Milena Šantrić Milićević; Beograd, septembar 2018.)

Sukob interesa: Nije prijavljen.

and individual diagnoses, as well as by the source of data in the death certificates), and then to perform a comparative analysis against the diagnoses of the medical examiners, in order to better understand the problem and define the target interventions. Health care institutions, especially hospitals, are a common source of population mortality data, by age, sex, and cause. These statistical data are important markers of the quality of hospital care and provide for necessary investments, for both national and local health care policies. Continued training is necessary, with respect to the appearance, content and proper coding of data on the cause of death, both for doctors in health care institutions and for medical examiners, who are involved in filling out death certificates. It is also very important that decision makers in the health care system should recognize the significance of precise and quality data on mortality, which is why investing into the strengthening of the vital registration system is necessary, in order to achieve improvement at all levels, in the sense of properly coded underlying causes of death and the incorporation of these data into final reports on mortality statistics.

CONCLUSION

In the observed period, an increase in the death rate of Serbian citizens with unknown cause of death has been noted, with exceptionally high rates in 2009 and 2016. Comparative analysis has shown that R00-R99 diagnoses are more present in the mortality statistics of Serbia, as compared to Slovenia and Croatia, and less, as compared to North Macedonia. Urgent interventions are necessary in order to improve the quality of data on the underlying cause of death in death certificates, with the aim of improving the trust in their accuracy and providing for these data to be the primary proof which epidemiological research and health policies are based on. This research has provided a broader view of the quality of coding data on the underlying cause of death in death certificates, at the regional level.

Acknowledgements

The study is a part of the master thesis in the area of public health by Dr. Nataša Rosić, titled: *The participation of unknown and ill-defined causes of death in the mortality of the population of Serbia: implications for the improvement of mortality statistics quality*, defended at the Faculty of Medicine of the University of Belgrade (mentor: Professor Milena Šantrić Milićević, PhD; Belgrade, September 2018.)

Conflict of interest: None declared.

PRILOG 1

Grupa XVIII u MKB-X, *Simptomi, znaci i patološki klinički nalazi neklasifikovani na drugom mestu* sadrži trinaest podgrupa, a to su:

R00-R09 Simptomi i znaci sistema za krvotok i sistema za disanje

R10-R19 Simptomi i znaci sistema za varenje i trbuha

R20-R23 Simptomi i znaci kože i potkožnog tkiva

R25-R29 Simptomi i znaci nervnog sistema i mišićno-koštanog sistema

R30-R39 Simptomi i znaci mokraćnog sistema

R40-R46 Simptomi i znaci poimanja, čulnog opažanja, emocija i ponašanja

R47-R49 Simptomi i znaci poremećaja govora i poremećaja glasa

R50-R69 Opšti simptomi i znaci

R70-R79 Patološki nalazi krvi, bez dijagnoze

R80-R82 Patološki nalazi mokraće, bez dijagnoze

R83-R89 Patološki nalazi drugih telesnih tečnosti, supstanci i tkiva, bez dijagnoze

R90-R94 Patološki nalazi kod dijagnostike i funkcionalnih ispitivanja, bez dijagnoze

R95-R99 Neoznačeni i nepoznati uzroci smrti

APPENDIX 1

Group XVIII in the ICD-X, *Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified* comprises the following 13 subgroups:

R00-R09 Symptoms and signs involving the circulatory and respiratory systems

R10-R19 Symptoms and signs involving the digestive system and abdomen

R20-R23 Symptoms and signs involving the skin and subcutaneous tissue

R25-R29 Symptoms and signs involving the nervous and musculoskeletal systems

R30-R39 Symptoms and signs involving the urinary system

R40-R46 Symptoms and signs involving cognition, perception, emotional state and behavior

R47-R49 Symptoms and signs involving speech and voice

R50-R69 General symptoms and signs

R70-R79 Abnormal findings on examination of blood, without diagnosis

R80-R82 Abnormal findings on examination of urine, without diagnosis

R83-R89 Abnormal findings on examination of other body fluids, substances and tissues, without diagnosis

R90-R94 Abnormal findings on diagnostic imaging and in function studies, without diagnosis

R95-R99 Ill-defined and unknown causes of mortality

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EZOFAGEALNI, GASTRIČNI, KOLOREKTALNI, PANKREATIČNI, HEPATOCELULARNI KARCINOMI I HOLANGIOKARCINOMI U SEVERNOJ MAKEDONIJI: SERIJA PACIJENATA LEČENIH NA UNIVERZITETSKOJ KLINICI, IZMEĐU 2015. I 2019. GODINE

ORIGINALNI RAD

ORIGINAL ARTICLE

ESOPHAGEAL, GASTRIC, COLORECTAL, PANCREATIC, HEPATOCELLULAR CARCINOMAS AND CHOLANGIOCARCINOMAS IN NORTHERN MACEDONIA: A SERIES OF PATIENTS TREATED AT THE UNIVERSITY CLINIC, BETWEEN 2015 AND 2019

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SAŽETAK

Uvod: Globalno opterećenje društva gastrointestinalnim kancerom (GIK) raste. Tumori želuca, debelog creva i jetre su među pet najčešćih gastrointestinalnih karcinoma kod muškaraca i žena širom sveta. Incidencija GIK-a pokazuje značajne varijacije u Evropi i Severnoj Americi. Ovaj rad razmatra znake konvergencije bolničkog morbiditeta u Severnoj Makedoniji ka procenjenim vrednostima globalnog morbiditeta.

Cilj: Cilj rada je da opiše bolnički morbiditet od gastrointestinalnih kancera na Univerzitetskoj klinici u Severnoj Makedoniji.

Materijal i metode: Retrospektivna longitudinalna analiza obuhvatila je seriju slučajeva sa GIK-om, na najvećoj, Univerzitetskoj klinici za gastroenterohepatologiju (UKG) u Skoplju, tokom perioda 2015 – 2019. Deskriptivnim metodama statistike opisan je bolnički morbiditet od GIK-a i distribucija prema starosti, polu i lokalizaciji kancera.

Rezultati: U petogodišnjem periodu, na Univerzitetskoj klinici za gastroenterohepatologiju je lečen ukupno 2.831 pacijent sa GIK-om, od čega je 1.484 pacijenta imalo kolorektalni kancer, 763 pacijenta rak želuca i 88 pacijenata rak jednjaka. Iako su kanceri jetre bili manje zastupljeni, čak osmina takvih pacijenata (355 ili 13%) imala je nespecifični malignitet jetre. Većina pacijenata je bila u starosnoj grupi 60 – 69 godina, sa izuzetkom raka jednjaka. Primećen je porast incidencije raka pankreasa, skoro podjednak kada se razmatra distribucija po polu, i uglavnom u starosnim grupama 60 – 69 i 70 – 79 godina.

Zaključak: Bolnički morbiditet od GIK-a u severnoj Makedoniji pokazuje trend porasta, stoga je važno utvrditi koliko je skrining doprineo ranom otkrivanju ovih kancera, i osigurati pristup i dostupnost terapije za B i C hepatitis.

Ključne reči: gastrointestinalni karcinom, rak želuca, rak pankreasa, hepatocelularni kancer, rak jednjaka, kolorektalni kancer

ABSTRACT

Introduction: The global burden of gastrointestinal cancer (GIC) is growing. Stomach, colon and liver are among the five most common sites for GIC in men and women worldwide. The incidence of GIC shows significant variation in Europe and North America.

Aim: The aim of this paper is to describe hospital morbidity from GI cancer at the University Clinic in Northern Macedonia.

Materials and methods: A retrospective longitudinal analysis included a series of cases with GIC, at the University Clinic of Gastroenterohepatology (UCG) in Skopje, in the period 2015-2019. Descriptive statistical methods were used to describe hospital morbidity from GIC, and its distribution by age, sex, and cancer site.

Results: In a five-year period, a total of 2,831 patients with GIC were treated at the UCG, of which 1,484 patients had colorectal cancer, 763 patients had gastric cancer and 88 patients had esophageal cancer. Although liver cancers were less common, as many as one eighth of such patients (355 or 13%) had nonspecific liver malignancy. Most patients were in the 60 – 69 age group, with the exception of esophageal cancer. An increase in the incidence of pancreatic cancer was observed, almost equal, when considering the distribution by sex, and mainly in the age groups 60 –69 and 70–79 years.

Conclusion: Hospital morbidity due to GIC in North Macedonia shows an increasing trend, so it is important to determine how much screening has contributed to the early detection of these cancers and to ensure access to and availability of therapy for hepatitis B and C.

Key words: gastrointestinal carcinoma, gastric cancer, pancreatic cancer, hepatocellular cancer, esophageal cancer, colorectal cancer

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UVOD

Globalno opterećenje društva gastrointestinalnim kancerima (GIK) raste. Želudac, debelo crevo i jetra su među pet najčešćih lokalizacija gastrointestinalnih karcinoma kod muškaraca i žena širom sveta [1,2,3]. Incidencija GIK-a pokazuje značajne geografske varijacije, pri čemu je incidencija kolorektalnog kancera viša u Zapadnoj Evropi i Severnoj Americi, a incidencija kancera želuca i jetre je viša u Aziji i Africi. Prema Globalnoj opservatoriji za rak (*GLOBOCAN*) [4], stope incidencije i mortaliteta u Severnoj Makedoniji slične su onim koje su zabeležene u zemljama Zapadne Evrope. U 2020. godini, incidencija kolorektalnog kancera u Zapadnoj Makedoniji bila je 10,9 i 14,4 na 100.000 stanovnika, za muškarce i za žene, naspram 9,9% i 10%, respektivno, u Zapadnoj Evropi [4].

Prema podacima Direkcije za e-zdravlje (interni podaci), u 2018. godini, najviša incidencija u Makedoniji je zabeležena za GIK. Ovi podaci ukazuju na to da su više od jedne trećine svih kolorektalnih i gastričnih karcinoma novi slučajevi, naspram slučajeva hepatičnih i karcinoma pankerasa, gde su dve trećine svih slučajeva novi slučajevi. Na primer, 74% (304 od ukupno 402 slučaja) hepatičnih i karcinoma žučnih puteva (C22 u Međunarodnoj klasifikaciji bolesti X (MKB-X)), i 70% (338 od ukupno 486 slučajeva) karcinoma pankreasa (MKB-X: C25), naspram 35% (747 od ukupno 2.121 slučaja) karcinoma debelog creva (MKB-X: C18), 33% (486 od ukupno 1.486 slučajeva) rektalnih karcinoma (MKB-X: C20), i 36% (403 od ukupno 1.129 slučajeva) karcinoma rektosigmoidnog spoja (MKB-X: C19), i 49% (516 od ukupno 1.045 slučajeva) gastričnih karcinoma (MKB-X: C16). Slično ovim podacima, incidencija raka jetre i pankreasa ima agresivan trend, uprkos tome što su, prema podacima o prevalenciji, ovi karcinomi rangirani ispod karcinoma debelog creva, rektuma i želuca.

Otkriće gastričnog patogena iz prve grupe karcinogena [5], *Helicobacter pylori* (*H. pylori*), značajno je izmenilo lečenje i shvatanje ove bolesti, u smislu infektivne bolesti, koja je predvidiva i koja se može sprečiti [6-8].

Na osnovu procena *GLOBOCAN*-a iz 2018. godine, rak pankreasa je rangiran kao jedanaesti kancer po učestalosti u svetu, uzrokujući 4,5% svih smrti nastalih usled kancera u 2018. godini, dok je u Sjedinjenim Američkim Državama to treći najčešći kancer [9]. Do sada je poznato da, u svetu, incidencija i mortalitet od raka pankreasa koreliraju sa većom starošću i muškim polom [10,11].

Među različitim vrstama primarnih kancera jetre, peti kancer po učestalosti na svetskom nivou [12,13], hepatocelularni karcinom (HCK), kao i intrahepatični holangiokarcinom (IHK), su najčešći, čineći oko 70% i 15% slučajeva, respektivno [14-16].

Imajući u vidu značajne varijacije incidencije GIK-a u Evropi i Severnoj Americi, ovaj rad se bavi znacima

INTRODUCTION

The global burden of gastrointestinal cancers (GIC) is growing. The stomach, colon, and liver are among the five most common sites for GIC, in men and women worldwide [1,2,3]. The incidence of GIC shows significant geographic variation, with colorectal cancer incidence being higher in Western Europe and North America, and gastric and liver cancer incidence being higher in Asia and Africa. Based on the Global Cancer Observatory (GLOBOCAN) [4], the incidence and mortality rates in North Macedonia are similar to those of Western European countries. In 2020, the incidence of colorectal cancer in North Macedonia was 10.9 and 14.4 per 100,000 population, for men and women, as compared to 9.9% and 10%, respectively, in Western Europe [4].

According to the E-health Directorate (internal data), in 2018, the highest incidence in North Macedonia was observed for GIC. These data suggest that more than one third of all colorectal and gastric carcinoma cases are new cases, as opposed to liver and pancreatic cases where two thirds of all cases are new cases. For example, 74% (304 of a total of 402 cases) of liver and bile duct carcinomas (C22 of the International Classification of Diseases version X (ICD-X)), and 70% (338 of a total of 486 cases) of pancreatic carcinoma (ICD-X: C25), as opposed to 35% (747 of a total of 2,121 cases) of colon carcinoma (ICD-X: C18), 33% (486 of a total of 1,486 cases) of rectal carcinoma (ICD-X: C20), and 36% (403 of a total of 1,129 cases) of carcinoma at the recto-sigmoid junction (ICD-X: C19), and 49% (516 of a total of 1,045 cases) of gastric carcinoma (ICD-X: C16). Similarly to these data, the worldwide incidence of pancreatic and liver cancer has an aggressive trend, despite being ranked, according to prevalence data, below colon, rectal and gastric carcinoma.

The discovery of the group 1 carcinogen gastric pathogen [5], *Helicobacter pylori* (*H. pylori*), has considerably altered the treatment and concept of the disease towards an infectious disease, which is predictable and preventable [6-8].

Based on GLOBOCAN 2018 estimates, pancreatic cancer has ranked the 11th most common cancer in the world, making up 4.5% of all deaths caused by cancer in 2018, while it is the third most common cancer in the United States [9]. To date, it is known that incidence and mortality of pancreatic cancer correlate with increasing age and the male sex, worldwide [10,11].

Among the various types of primary liver cancers, the fifth most common cancer globally [12,13], hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the most common, accounting for roughly 70% and 15% of cases, respectively [14-16].

Given the significant variation of incidence of GIC in Europe and North America, this paper discusses the

konvergencije bolničkog morbiditeta u Severnoj Makedoniji prema morbiditetu na globalnom nivou. Cilj ovog rada jeste da opiše bolnički morbiditet od GIK-a na Univerzitetskoj klinici u Severnoj Makedoniji.

MATERIJALI I METODE

Retrospektivna longitudinalna analiza uključivala je seriju slučajeva GIK-a na Univerzitetskoj klinici za gastroenterohepatologiju (UKG) u Skoplju, u periodu između 2015. i 2019. godine.

Primenili smo deskriptivne statističke metode prilikom prikazivanja bolničkog morbiditeta, u odnosu na distribuciju GIK-a, u odnosu na starost, na pol, kao i u odnosu na lokalizaciju kancera, u petogodišnjem periodu, od 1. januara 2015. godine do 31. decembra 2019. godine.

Izvorni skup podataka je očišćen od duplikata i filtriran po jedinstvenim pacijentima. Podaci su analizirani u programu *MS Excel* za *macOS* (*Microsoft Corporation, Redmond, Washington, US*).

REZULTATI

Najnoviji podaci *GLOBOCAN*-a [4] pokazuju da su ukupna stopa incidencije standardizovane prema godinama

signs of convergence of hospital morbidity in North Macedonia towards the global morbidity. The aim of this paper is to describe hospital morbidity from GI cancer at the University clinic in Northern Macedonia.

MATERIALS AND METHODS

A retrospective longitudinal analysis included a series of GIC cases at the University Clinic of Gastroenterohepatology (UCG) in Skopje, during the period between 2015 and 2019.

We applied descriptive statistical methods to present hospital morbidity, by GIC distribution, by age, by sex, and by cancer site, for a five-year period, from January 1, 2015 to December 31, 2019.

The original dataset was cleaned from duplicates and filtered by unique patients. Data was analyzed in MS Excel software for macOS (Microsoft Corporation, Redmond, Washington, US).

RESULTS

The latest GLOBOCAN data [4] shows that the overall age-standardized incidence rate and the risk of developing cancer have decreased between 2018 and

Tabela 1a. Indikatori kancera, Severna Makedonija, 2018. i 2020. godina

Table 1a. Cancer indicators, North Macedonia, 2018 and 2020

	2018. godina / *2018. year*			2020. godina / *2020. year*		
	Muškarci *Males*	Žene *Females*	Oba pola *Both sexes*	Muškarci *Males*	Žene *Females*	Oba pola *Both sexes*
Populacija, br. *Population, n*	1,042,282	1,042,774	2,085,056	1,042,282	1,042,774	2,085,056
Novi slučajevi raka, br. *New cancer cases, n*	4,258	3,549	7,807	4,247	3,385	7,632
Stopa incidencije standardizovana prema godinama starosti (svet) / *Age-standardized incidence rate (world)*	260.1	208.2	230.8	253.8	193.8	220.4
Rizik za dobijanje raka pre 75. godine života (%) *Risk of developing cancer before the age of 75 years (%)*	27.1	21.2	24.0	26.2	19.7	22.7
Smrti uzrokovane rakom, br. *Cancer deaths, n*	2,532	1,584	4,116	2,584	1,640	4,224
Stopa smrtnosti standardizovana prema godinama starosti (svet) *Age-standardized mortality rate (world)*	149.4	83.4	113.9	148.1	84.1	113.6
Rizik za umiranja od raka pre 75. godine života (%) *Risk of dying from cancer before the age of 75 years (%)*	16.3	9.3	12.7	16.1	9.4	12.6
Slučajevi sa petogodišnjom prevalencije, br. *5-year prevalent cases, n*	8,252	9,154	17,406	9,581	9,265	18,846
Prvih 5 najčešćih kancera, sa izuzetkom kancera kože koji nisu melanomi (rangirano po slučajevima) *Top 5 most frequent cancers excluding non-melanoma skin cancer (ranked by cases)*	Pl / *L* P / *P* KR / *CR* B/ *B1* Ž/ *S*	D / *Br* KR / *CR* *CoU / CoU* Pl / *L* *CeU / CeU*	Pl / *L* D / *Br* KR / *CR* P / *P* *CoU / CoU*	Pl / *L* P / *P* KR / *CR* B/ *B1* Ž/ *S*	D / *Br* KR / *CR* *CoU / CoU* Pl / *L* *CeU / CeU*	Pl / *L* D / *Br* KR / *CR* P / *P* *CoU / CoU*

Izvor: Globalna opservatorija za rak [4]; Pl – pluća; P – prostata; KR – kolon-rektum; B – bešika; Ž – želudac; *CeU - cervix uteri; CoU- corpus uteri;* D – dojka; M – melanom

Source: Global Cancer Observatory [4]; L – lung; P – prostate; CR – colorectum; Bl – bladder; S – stomach; CeU - *cervix uteri*; CoU - *corpus uteri*; Br – breast

Tabela 1b. Indikatori kancera Evropske regije SZO u 2018. i Zapadne Evrope u 2020. godini

Table 1b. Cancer key indicators, WHO European Region in 2018 and Western Europe in 2020

	2018, godina / *2018, year*			2020, godina / *2020, year*		
	Muškarci *Males*	Žene *Females*	Oba pola *Both sexes*	Muškarci *Males*	Žene *Females*	Oba pola *Both sexes*
Populacija, br, *Population, n*	447,845,844	447,986,637	2,085,056	96,374,578	99,771,743	196,146,321
Stopa incidencije standardizovana prema godinama starosti (svet) / *Age-standardized incidence rate (world)*	311.3	239.8	268.6	365.3	294.3	325.0
Rizik za dobijanje raka pre 75, godine života (%) *Risk of developing cancer before the age of 75 years (%)*	31.1	23.6	27.0	34.9	27.9	31.2
Stopa smrtnosti standardizovana prema godinama starosti (svet) *Age-standardized mortality rate (world)*	144.4	85.9	111.0	127.1	83.9	103.3
Rizik za umiranja od raka pre 75, godine života (%) *Risk of dying from cancer before the age of 75 years (%)*	15.1	9.1	11.9	13.0	8.8	10.8
Prvih 5 najčešćih kancera, sa izuzetkom kancera kože koji nisu melanomi (rangirano po slučajevima) *Top 5 most frequent cancers excluding non-melanoma skin cancer (ranked by cases)*	P / *P* Pl / *L* KR / *CR* B/ *B1* Ž/ *S*	D / *Br* KR / *CR* Pl / *L* *CoU / CoU* M / *M*	D / *Br* KR / *CR* KPl / *L* P / *P* B/ *B1*	P / *P* Pl / *L* KR / *CR* B/ *B1* M / *M*	D / *Br* KR / *CR* Pl / *L* M / *M* *CoU / CoU*	P / *P* D / *Br* Pl / *L* KR / *CR* B/ *B1*

Izvor: Globalna opservatorija za rak [4]; Pl – pluća; P – prostata; KR – kolon-rektum; B – bešika; Ž – želudac; *CeU - cervix uteri; CoU- corpus uteri;* D – dojka; M – melanom

Source: Global Cancer Observatory [4]; L – lung; P – prostate; CR – colorectum; Bl – bladder; S – stomach; CeU - *cervix uteri*; CoU - *corpus uteri*; Br – breast

starosti kao i rizik od oboljevanja od kancera opali između 2018. i 2020. godine, zajedno sa indikatorima mortaliteta u Severnoj Makedoniji (Tabela 1a). Dok je stopa incidencije niža, stopa smrtnosti je viša nego u zemljama Evropske regije SZO i zemljama Zapadne Evrope (Tabela 1b).

Tokom petogodišnjeg perioda, ukupno 2.831 pacijent sa GIK-om je lečen na UKG-u, od kojih je 1.484 (53%) imalo kolorektalni kancer, 763 (27%) pacijenata je imalo rak želuca, a 88 (3%) pacijenata je imalo rak jednjaka. Iako su kanceri jetre bili manje zastupljeni, čak preko jedne osmine takvih pacijenata (355 ili 13%) imalo je nespecifični malignitet jetre. Rak jednjaka je dijagnostikovan kod 88 pacijenata, od kojih 73 muškog i 15 ženskog pola, u rasponu starosnih grupa. Sledeći rezultati predstavljaju serije pacijenata na UKG-u, prema lokalizaciji karcinoma, starosti i polu, tokom posmatranog vremenskog perioda (Tabela 2).

2020, along with the mortality indicators in North Macedonia (Table 1a). While the incidence rate is lower, the mortality rate is higher than in the countries of the WHO European Region and Western Europe (Table 1b).

During a 5-year period, a total of 2,831 patients with GIC were treated at the UCG, of which 1,484 (53%) patients had colorectal cancer, 763 (27%) patients had gastric cancer and 88 (3%) patients had esophageal cancer. Although liver cancers were less common, as many as over one eighth of such patients (355 or 13%) had nonspecific liver malignancy. Esophageal cancer was diagnosed in 88 patients, of which 73 male and 15 female, in a range of age groups. The following results present series of patients at the UCG, by carcinoma site, age, and sex, over the observed period (Table 2).

Tabela 2. Broj pacijenata sa rakom jednjaka dijagnostikovanih i lečenih na UKG-u, prikazani prema polu, 2015 – 2019.

Table 2. Number of diagnosed and treated patients with esophageal cancer at the UCG, by sex, 2015 – 2019

Godina *Year*	Br. pacijenata *No. of patients*	Muški pacijenti, br. (%) *Male patients, N (%)*	Ženski pacijenti, br. (%) *Female patients, N (%)*
2015.	20	17 (85%)	3 (15%)
2016.	14	10 (71%)	4 (29%)
2017.	16	12 (75%)	4 (25%)
2018.	19	16 (84%)	3 (16%)
2019.	19	18 (95%)	1 (05%)
Ukupno / *Total*	**88**	**73 (83%)**	**15 (17%)**

Tabela 3. Pacijenti sa rakom želuca dijagnostikovani i lečeni na UKG-u, prikazani prema polu, 2015 – 2019, (br., %).

Table 3. Diagnosed and treated patients with gastric cancer at the UCG, by sex, 2015 – 2019, (n, %)

Godina *Year*	Br. pacijenata *No. of patients*	Muški pacijenti, br. (%) *Male patients, N (%)*	Ženski pacijenti, br. (%) *Female patients, N (%)*
2015.	164	109 (66%)	55 (34%)
2016.	182	132 (73%)	50 (27%)
2017.	142	93 (65%)	49 (35%)
2018.	141	102 (72%)	39 (28%)
2019.	134	96 (72%)	38 (28%)
Ukupno / *Total*	**763**	**532 (70%)**	**231 (30%)**

Evidentno je da je rak jednjaka bio daleko više zastupljen kod muškaraca (85% ukupnog broja slučajeva, raspon: 71 – 95%) nego kod žena (15% ukupnog broja slučajeva, raspon: 5 – 29%). Tokom posmatranog perioda, broj slučajeva sa rakom jednjaka imao je stabilni trend, uz padove u 2016. i 2017. godini.

Tokom analiziranog perioda, na UKG-u je rak želuca otkriven kod 763 pacijenta, od kojih 532 muška pacijenta i 231 ženski pacijent, sa većom distribucijom u starosnoj grupi 60 – 69 godina. Podaci o postojanju ili eradikaciji *Helicobacter pylori*, ili drugi faktori rizika, nisu bili dostupni (Tabela 3).

Podaci o raku želuca otkrivaju da je, i za ovaj tip kancera, broj slučajeva među muškim pacijentima (70% ukupnog broja slučajeva, raspon: 65 – 73%) bio daleko veći nego među ženskim pacijentima (30% ukupnog broja slučajeva, raspon: 27 – 35%). Tokom posmatranog perioda, broj slučajeva raka želuca imao je stabilan trend, sa povećanjem u 2018. godini.

Tokom analiziranog perioda, na UKG je kolorektalni karcinom otkriven kod 1.484 pacijenata, od kojih je 827 bilo muških, a 657 ženskih pacijenata, u starosnoj grupi 60 – 69 godina (Tabela 4). Broj slučajeva sa kolorektalnim karcinomom je imao promenljivi trend tokom posmatranog perioda.

Uočen je trend porasta broja slučajeva karcinoma pankreasa na UKG, tokom analiziranog perioda (Tabela 5). Kod ukupno 528 pacijenata je dijagnostikovan rak pankreasa, od toga 287 muških pacijenata i 241 ženski

It is evident that esophageal carcinoma was far more dominant in men (85% of total cases, range: 71 – 95%) compared to women (15% of total cases, range: 5 – 29%). Over the observed period, the number of cases with esophageal carcinoma had a stable trend with decreases in years 2016 and 2017.

During the analyzed period, at the UCG, gastric cancer was detected in 763 patients, of which 532 male and 231 female, with a higher distribution in the 60 – 69 age group. Data of the existence or eradication of *Helicobacter pylori*, or other risk factors, were not available (Table 3).

The data on gastric cancer reveal that, for this type of cancer, as well, the number of cases in male patients (70% of total cases, range: 65 – 73%) was much higher than in female patients (30% of total cases, range: 27 – 35%). Over the observed period, the number of gastric cancer cases had a stable trend, with an increase in 2018.

During the analyzed period, at the UCG, colorectal carcinoma was detected in 1,484 patients, of which 827 male and 657 female, mainly in the 60 – 69 age group (Table 4). The number of cases with colorectal carcinoma had a variable trend over the observed period.

There was an observed increasing trend of the number of cases with pancreatic carcinoma at the UCG, in the analyzed period (Table 5). A total of 528 patients were diagnosed with pancreatic cancer, of whom 287 male and 241 female, mostly in the 60 – 69

Tabela 4. Pacijenti sa kolorektalnim kancerom dijagnostikovani i lečeni na UKG, prikazani prema polu, 2015 – 2019, (br.,%)

Table 4. Diagnosed and treated patients with colorectal cancer at the UCG, by sex, 2015 – 2019, (n, %)

Godina *Year*	Br. pacijenata *No. of patients*	Muški pacijenti, br. (%) *Male patients, N (%)*	Ženski pacijenti, br. (%) *Female patients, N (%)*
2015.	303	196 (65%)	107 (35%)
2016.	298	119 (40%)	179 (60%)
2017.	280	175 (63%)	105 (38%)
2018.	313	181 (58%)	132 (42%)
2019.	290	156 (54%)	134 (46%)
Ukupno / *Total*	**1.484**	**827 (56%)**	**657 (44%)**

Tabela 5. Pacijenti sa rakom pankreasa dijagnostikovani i lečeni na UKG, prikazani prema polu, 2015 – 2019, (br.,%)

Table 5. Diagnosed and treated patients with pancreatic cancer at the UCG, by sex, 2015 – 2019, (n, %)

Godina *Year*	Br. pacijenata *No. of patients*	Muški pacijenti, br. (%) *Male patients, N (%)*	Ženski pacijenti, br. (%) *Female patients, N (%)*
2015.	89	48 (54%)	41(46%)
2016.	112	64 (57%)	48 (43%)
2017.	81	40 (49%)	41 (51%)
2018.	105	68 (65%)	37 (35%)
2019.	141	67 (48%)	74 (52%)
Ukupno / *Total*	**528**	**287 (54%)**	**241 (46%)**

pacijent, uglavnom starosti između 60 i 69 godina, odnosno 70 i 79 godina. Kod samo dvoje od tih pacijenata (jedan muškog a drugi ženskog pola) je dijagnostikovan *pan-NET*. Podaci o njihovim navikama i drugim faktorima rizika nisu bili dostupni (Tabela 5).

Analizirani podaci sa UKG-a su pokazali da je hepatocelularni karcinom dijagnostikovan kod 48 pacijenata, holangiokarcinom kod 34 pacijenata, a karcinom ekstrahepatičnih žučnih puteva kod 59 pacijenata. Nespecifični malignitet jetre dijagnostikovan je kod 355 pacijenata (Tabela 6). Postojao je značajan trend porasta broja karcinoma sa lokalizacijom na jetri na UKG-u, tokom analiziranog perioda (Table 6).

and 70 – 79 age groups. Only two of them were diagnosed with pan-NET (one male and one female). Data on their habits or other risk factors were not available (Table 5).

The analyzed data from the UCG showed that hepatocellular carcinoma was diagnosed in 48 patients, cholangiocarcinoma in 34 patients, and carcinoma of extrahepatic bile ducts in 59 patents. Nonspecific liver malignancy was diagnosed in 355 patients (Table 6). There was a significant increasing trend of the number of liver site carcinoma cases at the UCG, in the analyzed period (Table 6).

Tabela 6. Pacijenti sa kancerima jetre dijagnostikovani i lečeni na UKG, prikazani prema tipu, 2015 – 2019, (br., %)

Table 6. Diagnosed and treated patients with liver cancers at the UCG, by type, 2015 – 2019, (n, %)

Godina *Year*	Hepatocelularni karcinom *Hepatocellular carcinoma*	Holangiokarcinom *Cholangiocarcinoma*	Hepatoblastom *Hepatoblastoma*	Angioblastom *Angioblastoma*	Karcinom jetre (nespecifični) *Liver carcinoma (nonspecific)*
2015.	7	8	/	/	92
2016.	2	5	/	1	68
2017.	12	8	/	1	45
2018.	11	3	1	/	69
2019.	16	10	/	/	81
Ukupno / *Total*	**48**	**34**	**1**	**2**	**355**

DISKUSIJA

Tokom analiziranog perioda, u seriji pacijenata sa GIK-om koji su lečeni na UKG-u, uočen je značajan trend porasta broja slučajeva karcinoma sa lokalitetom na jetri i pankreasu; broj slučajeva karcinoma želuca i karcinoma jednjaka je imao stabilan trend, dok je broj slučajeva sa kolorektalnim karcinomom imao promenljivi trend. Ovi trendovi približavaju se trendovima podataka o globalnoj incidencije GIK-a. Incidencija i mortalitet od hepatocelularnog karcinoma su u porastu u Severnoj Americi i Evropi [17], a kada su u pitanju kanceri pankreasa, i stope incidencije i stope smrtnosti, u zemljama sa visokim prihodima, pokazale su ili stabilan trend ili blagi porast [18]. Kanceri želuca su pokazali ujednačen pad

DISCUSSION

In the analyzed period, among a series of patients with GIC, treated at the UCG, a considerably increasing trend of the number of carcinoma cases at the site of the liver and the pancreas was observed; the number of cases with gastric and esophageal carcinoma had a stable trend, while the number of cases with colorectal carcinoma had a variable trend. These trends converge towards the trends of GIC global incidence data. The incidence and mortality of hepatocellular carcinoma have been increasing in North America and Europe [17], and, for pancreatic cancers, both incidence and mortality rates in high-income economies have been either stable or slightly increasing [18]. Gastric cancers

u incidencije tokom proteklih decenija, što se pripisuje boljim praksama u čuvanju hrane [18].

Kada je u pitanju histološki tip, na svetskom nivou, skvamozni karcinom je i dalje najčešći tip karcinoma jednjaka, mada je, u zapadnim zemljama, to adenokarcinom. Veruje se da je porast broja slučajeva podtipa adenokarcinoma u vezi sa porastom incidencije gojaznosti, gastroezofagealnog refluksa i Baretovog jednjaka, a da zavisi od genomske nestabilnosti, rase i pola pacijenta [19].

Razvojem dijagnostičkih procedura i antibiotika za lečenje *H. pylori* došlo je do unapređenja u lečenju peptičkih ulkusa [20-22] i pacijenata sa povećanim rizikom od raka želuca [23-27]. Iako eradikacija *H. pylori* može da smanji broj slučajeva raka želuca, broj slučajeva lečenja terapijom eradikacije se povećava [28-32]. Pregledni rad, koji su objavili Satoki Šičijo i Jošihiro Hirata, potvrdio je da postoji potreba za uspostavljanjem programa praćenja pacijenata nakon uspešne eradikacije *H. Pylori,* prema stratifikaciji rizika, odnosno, na osnovu karakteristika i prediktora gastričnog kancera [33].

Kolorektalni kancer je najčešći tip GIK-a u Evropi, sa 342.137 novih slučajeva (14,3% svih kancera) [26,34-41]. Ista incidencija je zabeležena i u Severnoj Makedoniji, što je evidentno iz podataka Direkcije za e-zdravlje i potvrđeno analizom podataka UKG-a. Literatura ukazuje na to da su ishrana i gojaznost među glavnim faktorima rizika za kolorektalni kancer [42]. U Severnoj Makedoniji, nezdrave navike u ishrani su veoma zastupljene, sa prosečnim dnevnim unosom masti od 34,1% (u poređenju sa preporučenim unosom od < 30%), izuzetno visokim unosom natrijuma od 7.883 mg (u poređenju sa preporučenom vrednosti od 500 – 2500 mg), i visokim unosom soli, što su sve posledice visokog stepena konzumacije prerađene hrane [43].

Uprkos napretku u saznanjima o potencijalnim faktorima rizika koji uzrokuju rak pankreasa, kao i novim dostupnim alatima za rano dijagnostikovanje, procenjuje se da će doći do porasta incidencije ove vrste kancera. Iako je uzrok raka pankreasa složen i multifaktorski, pušenje cigareta [10] i porodična istorija su dominantni faktori [11]. Rak pankreasa se uglavnom deli na dva tipa: adenokarcinom pankreasa, koji je najčešći (85% slučajeva) i koji nastaje u egzokrinim žlezdama pankreasa, i neuroendokrini tumor pankreasa (*Pan-NET*), koji je ređi (manje od 5%) i nastaje u endokrinom tkivu pankreasa [44]. Adenokarcinom pankreasa ima veoma lošu prognozu, obično, nakon uspostavljanja dijagnoze, 24% pacijenata ima jednogodišnje, a 9% petogodišnje preživljavanje [44].

Hepatocelularni karcinomi čine 90% primarnih kancera jetre i predstavljaju veliki zdravstveni problem na svetskom nivou. Postoji niz glavnih infektivnih,

have shown a uniform decline in incidence over the decades, which has been attributed to better food preservation practices [18].

With regard to the histological type, globally, squamous cell carcinoma remains the most common type of esophageal carcinoma, although in Western countries, it is adenocarcinoma. It is believed that the rise of the number of adenocarcinoma subtype cases corresponds to a rise in the incidence of obesity, gastro-esophageal reflux disease, and Barrett's esophagus, and depends on the genomic instability, race, and gender of the patient [19].

The development of diagnostic procedures and antibiotics for *H. pylori* have improved the treatment of peptic ulcers [20-22] and patients with increased risk of gastric cancer [23-27]. Even though eradication of *H. pylori* can reduce the number of gastric cancer cases, the number of cases treated with eradication therapy is increasing [28-32]. A review by Satoki Shichijo and Yoshihiro Hirata confirmed the need for the establishment of a surveillance program of patients after a successful H. *Pylori* eradication, according to risk stratification i.e., the characteristics and predictors of gastric cancer [33].

Colorectal cancer is the most common type of GIC in Europe, with 342,137 new cases (14.3% of all cancers) [26,34-41]. The same incidence is in North Macedonia, as evident in data from the Directorate for E-health and confirmed with the analysis of the UCG data. Literature suggests that diet and obesity are among the main risk factors for colorectal cancer [42]. In North Macedonia, unhealthy dietary habits are very prevalent, with average daily intake of fats of 34.1% (compared with < 30% recommended intake), exceptionally high sodium intake of 7,883 mg (compared with the recommended value of 500 – 2500 mg), and high salt intake, which are all the result of a high consumption of processed foods [43].

Despite advancement in the knowledge of potential risk factors that cause pancreatic cancer, as well as newly available tools for early diagnosis, its incidence is estimated to increase. Although the cause of pancreatic cancer is complex and multifactorial, cigarette smoking [10] and family history are dominant [11]. Pancreatic cancer is mainly divided into two types: pancreatic adenocarcinoma, which is the most common (85% of cases) arising in the exocrine glands of the pancreas, and pancreatic neuroendocrine tumor (Pan-NET), which is less common (less than 5%) and occurs in the endocrine tissue of the pancreas [44].

Pancreatic adenocarcinoma has a very poor prognosis, typically after diagnosis, only 24% of people survive 1 year, and 9% live for 5 years [44].

Hepatocellular carcinoma represents about 90% of primary liver cancers and constitutes a major global

metaboličkih i naslednih faktora rizika, kao i faktora rizika vezanih za način života, i kada je u pitanju HCK i kada je u pitanju ICK. Neki od ovih faktora rizika se ili potencijalno mogu sprečiti (na primer, upotreba alkohola i duvana), ili se, u ovom trenutku, mogu lečiti (npr. infektivni hepatitis). U većini slučajeva, molekularni put ili mehanizam putem kojeg ovi etiološki faktori izazivaju primarni kancer jetre, nije dobro definisan [45].

Holangiokarcinom je drugi po učestalosti kancer jetre sa klinički tihim nastankom i napredovanjem i sa rastućom incidencijom na svetskom nivou [46]. Naši rezultati pokazuju da i Severna Makedonija ima sličnu incidenciju i rastući trend. Usled nedostataka markera za rano dijagnostikovanje ovog kancera, većina pacijenata sa holangiokarcinomom bude identifikovana u poodmaklom stadijumu i umire od metastaza [46].

U našem uzorku uočen je vrlo visok broj slučajeva nespecifičnog karcinoma jetre. U većini ovih slučajeva obavljena je histopatološka analiza, ali su rezultati bili nedovoljni da bi obezbedili definitivniju dijagnozu koja bi omogućila jasniju diferencijaciju. Ovo može potencijalno da onemogući izbor adekvatne terapije i obezbeđivanje boljeg ishoda za pacijente [47].

Za preciznu analizu gastrointestinalnih karcinoma, potrebni su detaljniji podaci za celu zemlju, uključujući tu i faktore rizika, lokalizaciju i stadijume. Takođe, ustanovljen je veliki broj slučajeva karcinoma jetre, i, mada je izvestan nivo dijagnostike sproveden, evidentno je da postoji potreba za više dijagnostičkih resursa i alata, kako bi se omogućilo precizno utvrđivanje dijagnoze. Ovo će potencijalno pomoći u utvrđivanju najadekvatnije terapije i lečenja, te će obezbediti bolji kvalitet nege i bolje ishode za pacijente.

Preventiva, međutim, ostaje jedna od najisplativijih intervencija. Kako bi se smanjila incidencija kancera gastrointestinalnog trakta, važno je da postoje dobro organizovani nacionalni programi skrininga, u svrhu prevencije i ranog otkrivanja ovih bolesti, a naročito kolorektalnog kancera. Takođe, pristup terapiji za hepatitis B i C je važan za prevenciju karcinoma jetre.

Na kraju, ali ne i najmanje važno, dugoročna prevencija kancera gastrointestinalnog trakta u velikoj meri zavisi od postojanja i implementacije zdravstvene politike za prevenciju glavnih faktora rizika, kao što su: gojaznost, pušenje, nezdrava ishrana, i fizička neaktivnost. Ovo bi trebalo, između ostalog, da uključuje i promovisanje zdravih životnih navika, kao i smanjenje pristupa nezdravim izborima.

ZAKLJUČAK

Podaci UKG-a ukazuju na trend porasta broja slučajeva gastrointestinalnih kancera, posebno kod muškaraca, pri čemu preovlađuju kolorektalni, i kanceri pankreasa

health problem. There are a number of major infectious, lifestyle, metabolic, and hereditary risk factors for both HCC and ICC. Some of these risk factors are either potentially preventable (e.g., alcohol and tobacco use) or are currently treatable (e.g., hepatitis infection). In most cases, the molecular pathway or mechanism by which these etiological factors cause primary liver cancer, has not been well delineated [45].

Cholangiocarcinoma is the second most common liver cancer with a clinically silent development and an increasing global incidence [46]. Our results show that North Macedonia has a similar incidence and an increasing trend. Due to the absence of early markers for its diagnosis, most cholangiocarcinoma patients are identified at an advanced stage and die of metastases [46].

A very large number of non-specific liver carcinoma cases was observed in our sample. In most of these cases histopathological examination was performed, but the results were insufficient to give a definitive and more differentiating diagnosis. This can potentially impede choosing the appropriate treatment and ensuring a better patient outcome [47].

For a precise analysis of gastrointestinal carcinomas, more detailed data for the entire country are needed, including risk factors, localization, and staging. In addition, a large number of non-specific liver carcinoma cases was observed, and, while some level of diagnostics was performed, it is evident that there is a need for more diagnostic resources and tools, in order to enable a precise determination of the diagnosis. This will potentially assist in determining the most appropriate therapy and treatment and enable better quality of care and patient outcome.

Prevention, however, remains one of the most cost-effective interventions. To decrease the incidence of GI cancers, it is important to have well-organized national screening programs for prevention and early detection, especially of colorectal cancer. In addition, access to therapy for hepatitis B and C is important in the prevention of liver carcinoma.

Last but not the least, prevention of GI cancers in the long term largely depends on having and implementing policies for the prevention of major risk factors, such as obesity, smoking, unhealthy diet and physical inactivity. This should include, among other things, promoting healthy lifestyles and reducing access to unhealthy choices.

CONCLUSION

The UCG data show an increasing trend of the number of GIC cases, especially in men, with a predominance of colorectal, pancreatic and liver cancer. The establishing

i jetre. Uspostavljanje programa praćenja pacijenata sa GIK-om, stratifikovanih prema riziku, nakon lečenja, moglo bi da pomogne u razumevanju načina da se preokrene napredak bolesti. Podaci UKG pokazuju konvergenciju trenda ka globalnim procenama incidencije i prevalencije GIK-a. U budućnosti je važno utvrditi u kojoj meri je skrining doprineo ranom otkrivanju ovih kancera, ali i obezbediti pristup terapiji, kao i dostupnost terapije, za hepatitis B i C.

Sukob interesa: Nije prijavljen.

of a surveillance program of risk stratified patients with GIC, after treatment, could help in understanding the ways to reverse the progress of disease. The UCG data show a convergence trend toward global estimates of GIC incidence and prevalence. In future, it is important to determine how much screening has contributed to the early detection of these cancers and to ensure access to and availability of therapy for hepatitis B and C.

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NEZADOVOLJENE POTREBE ZA STOMATOLOŠKOM ZDRAVSTVENOM ZAŠTITOM U SRBIJI

ORIGINALNI RAD

ORIGINAL ARTICLE

UNMET DENTAL HEALTH CARE NEEDS IN SERBIA

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SAŽETAK

Cilj: Cilj ove studije je bila analiza socijalnih karakteristika i karakteristika zdravstvenog stanja populacije sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom.

Materijal i metode: Ova studija preseka uključivala je 20.069 ispitanika Ankete o prihodima i uslovima života u Republici Srbiji (engl. *Survey on Income and Living Conditions – SILC)* iz 2014. godine.

Rezultati: Gotovo svaki šesti građanin (16,1%) izjavio je da je imao nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Učesnici starosti između 27 i 44 godine (OR: 1,48, 95% CI: 1,21 – 1,82), i između 45 i 64 godine (OR: 1,49, 95% CI: 1,19 – 1,86), učesnici koji su svoje zdravstveno stanje ocenili kao: dobro (OR: 1,91, 95% CI: 1,63 – 2,25), solidno (OR: 3,16, 95% CI: 2,64 – 3,77), loše (OR: 3,65, 95% CI: 2,94 – 4,53), ili jako loše (OR: 4,22, 95% CI: 3,10 – 5,74), imali su veću verovatnoću da prijave nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Najčešće navođeni razlozi za nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom bili su finansijske prepreke pristupačnosti stomatološke zdravstvene zaštite (66,6%) kao i strah od lečenja (15,1%).

Zaključak: Studija je utvrdila povezanost nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom sa socijalnim karakteristikama i karakteristikama zdravstvenog stanja. Zdravstvena politika bi trebalo da primeni multidimenzionalni pristup i ukloni prepreke koje ograničavaju pristupačnost stomatološkoj zdravstvenoj zaštiti.

Ključne reči: nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom, stomatološka zdravstvena zaštita, *SILC*, pristupačnost

ABSTRACT

Aim: The aim of this study was the analysis of the social and health status characteristics of the population with unmet dental health care needs.

Materials and methods: This cross-sectional study included 20,069 respondents from the Survey on Income and Living Conditions (SILC) in the Republic of Serbia in 2014.

Results: Nearly every sixth citizen (16.1%) reported unmet dental health care needs. Participants between the ages of 27 and 44 years (OR: 1.48, 95% CI: 1.21 – 1.82), and between 45 and 64 years (OR: 1.49, 95% CI: 1.19 – 1.86), participants who assessed their health status as: good (OR: 1.91, 95% CI: 1.63 – 2.25), fair (OR: 3.16, 95% CI: 2.64 – 3.77), bad (OR: 3.65, 95% CI: 2.94 – 4.53), or very bad (OR: 4.22, 95% CI: 3.10 – 5.74), had a higher likelihood of reporting unmet dental health care needs. The most frequent reasons for unmet dental health care needs were financial obstacles to the accessibility of dental health care (66.6%) and fear or treatment (15.1%).

Conclusion: The study found associations between unmet dental health care needs and social and health status characteristics. Health policy should adopt a multidimensional approach and eliminate barriers which restrict the accessibility of dental health care.

Key words: unmet dental health care needs, dental health care, SILC, accessibility

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UVOD

Nezadovoljene potrebe pacijenata za zdravstvenom zaštitom su definisane kao „razlika između zdravstvenih usluga koje se smatraju neophodnim za određeni zdravstveni problem i usluga koje su dobijene“ [1]. Nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom mogu biti pokazatelj stepena jednakosti u pristupu stomatološkoj zdravstvenoj zaštiti i povezane su sa lošim oralnim zdravljem [2], što je nadalje povezano sa slabijim opštim zdravstvenim stanjem i kvalitetom života [3]. I subjektivni i objektivni pokazatelji oralnog zdravlja razlikuju se između populacija među kojima postoji nejednakost u pristupu stomatološkoj zdravstvenoj zaštiti [4]. Zabeležena prevalencija nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom varira od 0,3%, u Holandiji, do 24,5% u Južnoj Koreji [5,6]. Nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom mogu biti povezane sa troškovima zaštite [4] i socijalnim karakteristikama učesnika [5-7]. Prevalencija nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom je viša među nezaposlenima i osobama sa nižim stepenom obrazovanja, odnosno sa nižim socijalno-ekonomskim statusom [6,7].

Neke zemlje su preuzele mere sa ciljem da smanje ove nejednakosti i unaprede oralno zdravlje cele populacije kroz smanjenje troškova i unapređenje pristupačnosti stomatološke zdravstvene zaštite [4,8]. Dobar primer sistema zdravstvene zaštite koji je pristupio rešavanju problema nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom jeste Finska, koja je povećala procenat populacije pokrivene zdravstvenim osiguranjem putem ukidanja starosnih ograničenja u pristupu javnim službama stomatološke zdravstvene zaštite, kao i putem šire primene nadoknade troškova lečenja u okviru stomatološke zdravstvene zaštite [8]. Brazil je primenio nacionalni program pod nazivom „Nasmejani Brazil“ [9], što je podrazumevalo i uspostavljanje Tima za unapređenje oralnog zdravlja kao i širu dostupnost stomatološke zdravstvene zaštite za stanovništvo [10].

Stomatološka zdravstvena zaštita je u Srbiji organizovana kao služba koja je dostupna na nivou primarne zdravstvene zaštite, u državnim zdravstvenim ustanovama i u privatnom zdravstvenom sektoru. Tradicionalno su sve usluge bile dostupne svim stanovnicima u Srbiji koji su bili obuhvaćeni obaveznim zdravstvenim osiguranjem. Nakon 2000. godine, započele su reforme u sistemu zdravstvene zaštite u Srbiji, koje su donele sa sobom velike promene u pružanju stomatološke zdravstvene zaštite. Zakon o zdravstvenom osiguranju iz 2005. godine uveo je promene u finansiranje stomatološke zdravstvene zaštite, te je ovaj vid zaštite, bez ikakvog novčanog učešća od strane korisnika, sada dostupan samo maloletnicima, trudnicama i u hitnim

INTRODUCTION

Unmet needs of patients for health care are defined as ‘the difference between health services that are considered necessary for a particular health problem and services that are received’ [1]. Unmet dental health care needs can be an indicator of equity of access to dental health care and are associated with poor oral health [2], which is further associated with poorer general health and quality of life [3]. Both subjective and objective indicators of oral health differ between the populations with disparities in access to dental health care [4]. The reported prevalence of unmet dental health care needs varies from 0.3% in the Netherlands to 24.5% in South Korea [5,6]. Unmet dental health care needs can be associated with the costs of care [4] and social characteristics of the participants [5-7]. The prevalence of unmet dental health care needs is higher among the unemployed and those of lower education, or lower socio-economic status [6,7].

Some countries have taken action with the aim to reduce these disparities and to improve the oral health of the entire population through the reduction of costs of dental health care and an increase in dental health care availability [4,8]. A good example of a healthcare system which has addressed the unmet dental health care needs is Finland, which has increased the percentage of population covered by health insurance through the abolishment of age restrictions to the access to public dental health care services, along with a wider implementation of reimbursement of treatment costs for dental health care [8]. Brazil has implemented a national program called ‘Smiling Brazil’ [9], which has included the establishment of an Oral Health Team and a wider availability of dental services for the population [10].

Dental health care in Serbia is organized as a service that is available at the primary health care level in state-owned public health institutions and in the private sector. Traditionally, all services were available to all residents in Serbia who had mandatory health insurance. After the year 2000, the reforms in the Serbian health care system began, bringing great changes in the provision of dental health care. In 2005, the Health Insurance Law introduced changes in the funding of dental health care, and dental health care without any out-of-pocket payment is now only available to minors, pregnant women and for emergency dental care in the public health care system. The changes introduced in 2010 added college and university students under the age of 26 to the list [11,12]. This has led to a decrease in the use of dental health care services among adults [13]. In the year after the new Health Insurance Law was passed, there was a marked decline in the percentage of adults who used dental health care services at least once a year from 36.8% to 30.7% [13]. Between 2006 and 2013, the number of toothless persons

stomatološkim slučajevima, u okviru državnog sistema zdravstvene zaštite. Promene, uvedene 2010. godine, uključile su i studente do 26 godina starosti u ovu kategoriju [11,12]. Ovo je dovelo do pada u korišćenju stomatoloških zdravstvenih usluga među punoletnim građanima [13]. U godini nakon usvajanja Zakona o zdravstvenom osiguranju, zabeležen je vidan pad u procentu punoletnih građana koji su koristili usluge stomatološke zdravstvene zaštite barem jednom godišnje, sa 36,8 % na 30,7% [13]. U periodu između 2006. i 2013. godine, broj osoba koje su ostale bez svojih zuba porastao je sa 10,4% na 12,4% [13,14]. Umesto da dobiju stomatološku zdravstvenu zaštitu u ranim fazama bolesti, pacijenti su se često opredeljivali da odlože lečenje, te su kasnije morali da se podvrgnu skupljem lečenju od težeg oboljenja [13]. Iako je došlo do očiglednog razvoja privatnog sektora stomatološke zdravstvene zaštite tokom protekle decenije, stomatološke zdravstvene usluge koje se pružaju danas u Srbiji nisu dovoljne da bi se zadovoljile dugoročne potrebe stanovništva [13]. Prema našim saznanjima, nema sprovedenih studija o prevalenciji nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom među punoletnim građanima Srbije, niti o njenoj povezanosti sa socijalnim karakteristikama. Ciljevi studije bili su da se analiziraju socio-demografske, socio-ekonomske, i karakteristike zdravstvenog stanja populacije sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom u Srbiji, kao i da se identifikuju prediktori nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom u Srbiji.

METODE

U ovoj studiji analizirani su podaci iz Ankete o prihodima i uslovima života u Republici Srbiji (*SILC*) koja je sprovedena 2014. godine [15,16]. Uzorkovanje i upitnici koji su korišćeni opisani su na drugom mestu [18]. Stopa odgovora bila je 80,8% (16.220/20.069). Okvir za uzorkovanje uključivao je sve stanovnike Srbije starije od 16 godina.

Zavisna varijabla – nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom, definisana je pitanjem: „Da li je postojao neki trenutak u proteklih 12 meseci kada je trebalo da posetite stomatologa, a niste?“ (Da / Ne). Od onih koji su na navedeno pitanje odgovorili sa ‘Da’ je potom traženo da se izjasne o uzrocima zbog kojih nisu dobili stomatološku zdravstvenu zaštitu.

Posmatrali smo povezanost ishodne varijable sa sledećim: socijalnim varijablama (socio-ekonomskim i socio-demografskim), zdravstvenim stanjem, prisustvom hroničnih bolesti, ograničenjima u dnevnim aktivnostima uzrokovanim nekim postojećim zdravstvenim problemima, raspoloživim prihodom domaćinstva.

Etički komitet Medicinskog fakulteta u Beogradu odobrio je istraživanje (Br. 29/IX-5, 21. 09. 2016).

increased from 10.4% to 12.4% [13,14]. Instead of receiving dental health care at the early stages of illness, patients often opted to wait for the treatment and later had to receive more expensive treatment for a more severe illness [13]. Although there has been a marked development of privately-owned dental health care during the past decade, dental health care services provided in Serbia today are insufficient to meet the long-term needs of the population [13]. To the best of our knowledge, there have been no studies examining the prevalence of unmet dental health care needs among adults in Serbia, or its association with social characteristics. The study aims were to analyze the socio-demographic, socio-economic and health status characteristics of the population with unmet dental health care needs in Serbia and to identify predictors of unmet dental health care needs in Serbia.

METHODS

The data from the Survey on Income and Living Conditions (SILC) in Serbia, conducted in 2014, were analyzed in this study [15,16]. The sampling and the questionnaires used have been described elsewhere [18]. The response rate was 80.8% (16,220/20,069). The sampling frame included all residents of Serbia, above the age of 16 years.

The dependent variable - unmet dental health care needs, was defined by the question: “Was there any time during the past 12 months that you should have visited a dentist, but did not?” (Yes / No). Those who answered ‘Yes’ to the previous questions were then asked to report the causes for not receiving dental health care.

We examined the association of the outcome variable with the following: social variables (socio-economic and socio-demographic), health status, presence of chronic diseases, limitations in daily activities caused by any existing problems with health, household disposable income.

The Ethics Committee of the Faculty of Medicine in Belgrade approved the research (No. 29/IX-5, 21. 09. 2016).

Descriptive and analytical statistics were used, and data were presented as the absolute number and frequency (percentages). Pearson’s chi-square test was used to analyze the differences in general characteristics between respondents with unmet dental health care needs, and those whose dental health care needs had been met, for weighted values. Multicollinearity was examined using the variance inflation factor (VIF).

All variables found to be significant were used as independent variables in the multivariate logistic regression model with the self-perceived unmet dental health care needs as an outcome variable. The analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0.

Tabela 1. Socio-demografske karakteristike populacije koja prijavljuje nezadovoljene i zadovoljene potrebe za stomatološkom zdravstvenom zaštitom

Table 1. Socio-demographic characteristics of the population reporting unmet and met dental health care needs

Varijable	**Populacija koja prijavljuje nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom**	**Populacija koja prijavljuje zadovoljene potrebe za stomatološkom zdravstvenom zaštitom**	**p-vrednost***
Variables	***Population reporting unmet dental health care needs***	***Population reporting met dental health care needs***	***p-value****
	n = (2,599) (16.1%)	n = 13,620 (83.9%)	
Predisponirajući faktori / *Predisposing factors*			
Pol: / *Sex*			<0.001
Muški / *Male*	1,325 (16.9)	6,490 (83.1)	
Ženski / *Female*	1,274 (15.4)	7,130 (84.6)	
Starost: / *Age:*			<0.001
16 – 26	179 (8.1)	2,138 (91.9)	
27 – 44	607 (14.0)	3,761 (86.0)	
45 – 64	1,219 (21.6)	4,549 (78.4)	
65^{+}	594 (15.3)	3,172 (84.7)	
Stepen obrazovanja: / *Education:*			<0.001
Osnovno obrazovanje / *Primary education*	1,121 (21.3)	4,299 (78.7)	
Srednje obrazovanje / *Secondary education*	1,252 (15.4)	7,101 (84.6)	
Tercijarno obrazovanje / *Tertiary education*	226 (9.3)	2,220 (90.7)	
Zaposlenje: / *Employment status:*			<0.001
Zaposleni / *Employed*	885 (15.4)	5,627 (84.6)	
Nezaposleni / *Unemployed*	664 (20.4)	3905 (79.6)	
Penzioneri / *Retired*	706 (15.5)	399 (84.5)	
Neaktivni / *Inactive*	282 (12.0)	289 (88.0)	
Bračno stanje: / *Marital status:*			<0.001
Neoženjeni/neudate / *Unmarried*	494 (11.9)	3,704 (88.1)	
Oženjeni/udate / *Married*	1,554 (17.2)	7,572 (82.8)	
Udovci/udovice / *Widowed*	370 (18.0)	1,683 (82.0)	
Razvedeni / *Divorced*	181 (20.9)	661 (79.1)	
Olakšavajući faktori / *Enabling factors*			
Kvintil prihoda po članu domaćinstva / *Equalized disposable income quintile:*			<0.001
I (0 – 20)	797 (24.5)	2,467 (75.5)	
II (20 – 40)	617 (18.2)	2,631 (81.8)	
III (40 – 60)	504 (15.7)	2,741 (84.3)	
IV (60 – 80)	386 (11.5)	2,835 (88.5)	
V (80 – 100)	295 (9.4)	2,946 (90.6)	
Faktori potreba / *Need factors*			
Zdravstveno stanje: / *Health status:*			<0.001
Vrlo dobro / *Very good*	284 (7.1)	3,778 (92.9)	
Dobro / *Good*	722 (14.2)	4,374 (85.8)	
Solidno / *Fair*	830 (22.4)	3,057 (77.6)	
Loše / *Bad*	638 (23.6)	2,032 (76.4)	
Vrlo loše / *Very bad*	125 (25.0)	379 (75.0)	
Postojanje nekog hroničnog oboljenja: / *Suffering from any chronic condition:*			<0.001
Hronično oboljenje / *Chronic*	1,051 (21.4)	3,807 (78.6)	
Bez hroničnog oboljenja / *No chronic condition*	1,548 (13.9)	9,813 (86.1)	
Ograničenja u dnevnim aktivnostima: / *Limitation in daily activities:*			<0.001
Prilično ograničeno / *Quite limited*	194 (23.9)	617 (76.1)	
Ograničeno / *Limited*	430 (23.2)	1,479 (76.8)	
Bez ograničenja / *Not limited*	1,975 (14.7)	11,524 (85.3)	
Geografsko područje / *Geographical area*			
Region: / *Region:*			<0.001
Grad Beograd / *Belgrade region*	323 (12.6)	2,339 (87.4)	
Region Vojvodine / *Region of Vojvodina*	877 (19.7)	3,587 (80.3)	
Region Šumadije i zapadne Srbije / *Region of Šumadija and Western Ser-bia*	750 (14.8)	4,489 (85.2)	
Region istočne i južne Srbije / *Region of Eastern and Southern Ser-bia*	649 (17.2)	3,205 (82.8)	
Naseljenost: / *Degree of urbanization:*			<0.001
Gusto naseljena oblast / *Densely populated area*	659 (13.1)	4,367 (86.9)	
Srednje naseljena oblast / *Intermediate urbanized area*	708 (16.0)	3,831 (84.0)	
Retko naseljena oblast / *Thinly populated area*	1,232 (19.2)	5,422 (80.8)	

Primenjene su analitičke i deskriptivne statističke metode, a podaci su predstavljeni kao apsolutni broj i učestalost (procenat). Pirsonov hi-kvadratni test primenjen je u analizi razlika u opštim karakteristikama između ispitanika sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom i onih čije su potrebe za stomatološkom zdravstvenom zaštitom bile zadovoljene, za ponderisane vrednosti. Multikolinearnost je ispitivana primenom faktora inflacije varijanse (*VIF*).

Sve varijable koje su se pokazale značajnim primenjene su kao nezavisne varijable u modelu multivarijantne logističke regresije, u kojem su samoprocenjene nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom predstavljale ishodnu varijablu. Analiza je obavljena primenom softverskog paketa *Statistical Package for Social Sciences* (*SPSS*) *version 22.0*.

REZULTATI

Prema procenama, 16,1% punoletnih građana Srbije izjavilo je da su tokom prethodne godine imali potrebe za stomatološkom zdravstvenom zaštitom ali da nisu mogli da zadovolje te potrebe. Značajno viši procenat muškaraca je prijavio nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom (16,9% naspram 15,4%, $p < 0,001$). Značajno viši procenat učesnika koji su imali samo osnovno obrazovanje je prijavio nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom (21,3%), u poređenju sa učesnicima sa srednjim obrazovanjem (15,4%) i višim ili visokim stepenom obrazovanja (9,3%), $p < 0,001$. Među učesnicima sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom bio je značajno viši procenat onih koji su svoje zdravstveno stanje ocenili kao veoma loše ili loše. Socio-demografske i socio-ekonomske karakteristike ispitanika, koji su prijavili zadovoljene ili nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom, predstavljene su u Tabeli 1.

Najčešće navođen razlog za neodlaženje kod stomatologa tokom prethodne godine odnosio se na

RESULTS

An estimated 16.1% of Serbian adults stated that they needed dental health care but had been unable to obtain it within the previous year. A significantly higher percentage of males reported unmet dental health care need (16.9% vs. 15.4%, $p < 0.001$). A significantly higher percentage of participants with only primary education reported that they had the unmet dental health care needs (21.3%), as compared with participants with secondary education (15.4%) and a college diploma or university degree (9.3%), $p < 0.001$. Among the participants with unmet dental health care needs, there was a significantly higher percentage of those who assessed their health as very bad or bad. Socio-demographic and socio-economic characteristics of the respondents reporting met and unmet dental health care needs are presented in Table 1.

The most common reason for not visiting the dentist during the previous year was related to financial obstacles to the accessibility of dental health care, which was stated by two-thirds of our respondents with unmet dental health care needs (66.6%), followed by the fear of doctors/hospital/testing/treatment, which was stated by 15.1% of participants.

The multivariate logistic regression with self-perceived unmet dental health care needs as an outcome variable showed that females (OR: 0.79, 95% CI: 0.72 – 0.87), participants over 65 years of age (OR: 0.76, 95% CI: 0.59 – 0.98), those with secondary education (OR: 0.79, 95% CI: 0.61 – 0.89), those with a college diploma or university degree (OR: 0.58, 95% CI: 0.49 – 0.69), unemployed, inactive or retired persons (OR: 0.85, 95% CI: 0.76 – 0.95) had a lower likelihood to report unmet dental health care needs. With an increase of equalized disposable income, there was a statistically significant decrease in the likelihood of unmet dental health care needs. Participants between the ages of 27 and 44 years (OR: 1.48, 95% CI: 1.21 – 1.82), and 45 and 64 years (OR: 1.49, 95% CI: 1.19 – 1.86), participants who assessed their health status

Tabela 2. Glavni razlozi zbog kojih ispitanik nije posetio stomatologa

Table 2. The main reasons why the respondent did not visit a dentist

Razlog zbog kojeg ispitanik nije posetio stomatologa	**n**	**%**
Reason why the respondent did not visit a dentist	**n**	**%**
Nisam imao-la za to novca/suviše je skupo. / *Could not afford it/too expensive.*	1,481	66.6
Predaleko se putuje do stomatologa. / *It is too far to travel.*	35	1.4
Postoji lista čekanja. / *There is a waiting list.*	54	2.8
Nisam imao-la vremena zbog posla, brige o deci ili drugima. / *Could not find the time because of work, care of children or others.*	169	8.5
Strah od lekara/bolnice/ispitivanja/lečenja / *Fear of doctors/hospital/testing/treatment*	336	15.1
Hteo-la sam da čekam da vidim da li će mi se popraviti stanje. / *Wanted to wait and see if the situation was going to get better.*	147	5.3
Nisam znao-la dobrog lekara ili specijalistu. / *Did not know of any good doctor or specialist.*	5	0.3
Drugi razlozi / *Other reasons*	-	-

Tabela 3. Modeli multivarijantne logističke regresije u kojima su nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom ishodna varijabla

Table 3. Multivariate logistic regression models with unmet dental health care needs as an outcome variable

Karakteristike / *Characteristics*	**OR (95% CI)**
Pol / *Sex*	
Muški / *Male*	1.0
Ženski / *Female*	**0.79 (0.72 – 0.87)***
Starost / *Age*	
16 – 26	1.0
27 – 44	**1.48 (1.21 – 1.82)***
45 – 64	**1.49 (1.19 – 1.86)***
65+	**0.76 (0.59 – 0.98)***
Stepen obrazovanja: / *Education*	
Osnovno obrazovanje / *Primary education*	1.0
Srednje obrazovanje / *Secondary education*	**0.79 (0.71 – 0.87)***
Tercijarno obrazovanje / *Tertiary education*	**0.58 (0.49– 0.69)***
Zaposlenje: / *Employment status*	
Zaposleni / *Employed*	1.0
Nezaposleni, u penziji, neaktivni / *Unemployed, retired, inactive*	**0.85 (0.76 – 0.95)***
Bračno stanje / *Marital status*	
Oženjeni/Udate / *Married*	1.0
Neoženjeni/neudate / *Unmarried*	0.97 (0.84 – 1.11)
Razvedeni/Udovci-udovice / *Divorced/Widowed*	1.01 (0.89 – 1.14)
Kvintil prihoda po članu domaćinstva / *Equalized disposable income quintile*	
0 – 20%	1.0
20 – 40%	**0.77 (0.68 – 0.87)***
40 – 60%	**0.64 (0.56 – 0.73)***
60 – 80%	**0.50 (0.43 – 0.58)***
80 – 100%	**0.40 (0.34 – 0.47)***
Zdravstveno stanje: / *Health status*	
Vrlo dobro / *Very good*	1.0
Dobro / *Good*	**1.91 (1.63 – 2.25)***
Solidno / *Fair*	**3.16 (2.64 – 3.77)***
Loše / *Bad*	**3.65 (2.94 – 4.53)***
Vrlo loše / *Very bad*	**4.22 (3.10 – 5.74)***
Postojanje nekog hroničnog oboljenja: / *Suffering from any chronic condition*	
Hronično oboljenje / *No chronic condition*	1.0
Bez hroničnog oboljenja / *Chronic*	1.12 (0.99 – 1.27)
Ograničenja u dnevnim aktivnostima / *Limitation in daily activities*	
Nema ograničenja / *Not limited*	1.0
Ima ograničenja / *Limited*	0.98 (0.86 – 1.13)
Naseljenost: / *Degree of urbanization*	
Gusto naseljena oblast / *Densely populated area*	1.0
Srednje naseljena oblast / *Intermediate urbanized area*	0.99 (0.87 – 1.12)
Retko naseljena oblast / *Thinly populated area*	1.11 (0.99 – 1.25)
Region: / *Region*	
Grad Beograd / *Belgrade region*	1.0
Region Vojvodine / *Region of Vojvodina*	0.92 (0.79 – 1.08)
Region Šumadije i zapadne Srbije / *Region of Šumadija and Western Serbia*	**1.31 (1.16 – 1.47)***
Region istočne i južne Srbije / *Region of Eastern and Southern Serbia*	**0.82 (0.73 – 0.93)***

finansijske prepreke pristupačnosti stomatološke zdravstvene zaštite, što je izjavilo dve trećine ispitanika sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom (66,6%), praćen strahom od lekara/bolnice/ispitivanja/lečenja, što je izjavilo 15,1% učesnika.

Multivarijantna logistička regresija, sa samoprocenjenim nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom kao ishodnom varijablom, pokazala je da žene (OR: 0,79, 95% CI: 0,72 – 0,87), učesnici stariji od 65 godina (OR: 0,76, 95% CI: 0,59 – 0,98), ispitanici sa srednjim obrazovanjem (OR: 0,79, 95% CI: 0,61 – 0,89), oni sa višim ili visokim stepenom obrazovanja (OR: 0,58, 95% CI: 0,49 – 0,69), nezaposlena, neaktivna ili penzionisana lica (OR: 0,85, 95% CI: 0,76 – 0,95), imaju manju verovatnoću da prijave nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Sa porastom prihoda po članu domaćinstva, došlo je do statistički značajnog pada verovatnoće nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom. Učesnici starosti između 27 i 44 godine (OR: 1,48, 95% CI: 1,21 – 1,82), 45 i 64 godine (OR: 1,49, 95% CI: 1,19 – 1,86), učesnici koji su svoje zdravstveno stanje ocenili kao dobro (OR: 1,91, 95% CI: 1,63 – 2,25), solidno (OR: 3,16, 95% CI: 2,64 – 3,77), loše (OR: 3,65, 95% CI: 2,94 – 4,53) ili veoma loše (OR: 4,22, 95% CI: 3,10 – 5,74) imali su veću verovatnoću da prijave nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom.

DISKUSIJA

Ova studija je analizirala faktore povezane sa nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom u okviru nacionalno reprezentativnog uzorka Srbije. Naše istraživanje je pokazalo da je 16,1% stanovništva, odnosno svaki šesti stanovnik Srbije, imao nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Prevalencija nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom u Srbiji je druga po visini među evropskim zemljama koje su sprovele *SILC* anketu, odmah iza Litvanije, u kojoj je prevalencija bila 16,8% [5]. Najznačajniji razlog navođen od strane naših ispitanika za njihovu nezadovoljenu potrebu za stomatološkom zdravstvenom zaštitom odnosio se na finansijske prepreke pristupačnosti stomatološke zdravstvene zaštite, praćen strahom od lekara i lečenja, i poteškoćama u organizovanju odlaska kod lekara, usled porodičnih obaveza ili prihvatljivosti stomatološke zdravstvene zaštite. Finansijske prepreke, odnosno 'preskupa' stomatološka zdravstvena zaštita, takođe je bio glavni razlog nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom i u zemljama Evropske unije [5].

Naši rezultati su potvrdili da se neki ispitanici suočavaju sa brojnim i složenim sistemskim barijerama pristupu stomatološkoj zdravstvenoj zaštiti, uključujući tu i

as good (OR: 1.91, 95% CI: 1.63 – 2.25), fair (OR: 3.16, 95% CI: 2.64 – 3.77), bad (OR: 3.65, 95% CI: 2.94 – 4.53) or very bad (OR: 4.22, 95% CI: 3.10 – 5.74) had a higher likelihood of reporting unmet dental health care needs.

DISCUSSION

This study analyzed the factors associated with the unmet dental health care needs within the Serbia national representative sample. Our research showed that 16.1% of the population or nearly every sixth inhabitant of Serbia had unmet dental health care needs. The prevalence of unmet dental health care needs in Serbia is the second highest prevalence among European countries which conducted the SILC survey, immediately after Latvia which had a prevalence of 16.8% [5]. The most significant reason stated by our respondents for their unmet dental health care need was related to the financial obstacles to the accessibility of dental health care, followed by fear of doctors or treatment, and difficulty to arrange the visits due to work and family commitments or acceptability of dental health care. Financial obstacles, i.e., 'too expensive' dental health care was the main reason for unmet dental health care needs in the European Union, as well [5].

Our results have confirmed that some respondents face numerous and complex systemic barriers to accessing dental health care, which include demographic, social, cultural, economic, structural and geographic factors [3]. We found that there were gender differences in experienced unmet dental health care needs, contrary to other studies, where women had a higher likelihood of having unmet dental health care needs [3]. The National Health Survey of the Republic of Serbia showed that a significantly higher percentage of women in Serbia have lost all of their teeth and that women use dentures more often than men (85.7% vs 75.2%) [14]. In our study, the population aged 27 to 44 years, and 45 to 64 years, had a higher likelihood to have unmet dental health care needs. The younger and the older populations are covered with mandatory health insurance, and the dental health care services are available to them. Our findings showed that the unemployed, inactive and retired, who usually belong to the population of older age groups, had a significantly lower probability of having unmet dental health care needs than the working and young age group.

A previous study carried out in Serbia showed the association between the levels of education, income and use of health care services in the private health care sector. The association in this study was positive. Similarly, in our study, participants with higher education levels had a lower likelihood of reporting unmet dental health care needs [17].

demografske, socijalne, kulturne, ekonomske, strukturne, i geografske faktore [3]. Utvrdili smo i postojanje rodnih razlika u iskustvima vezanim za nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom, nasuprot drugim studijama, u kojima su žene imale veću verovatnoću nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom [3]. Nacionalno istraživanje zdravlja stanovništva Srbije pokazalo je da je značajno viši procenat žena ostalo bez svih zuba, te da žene češće koriste zubne proteze od muškaraca (85,7% naspram 75,2%) [14]. U našoj studiji, populacija starosti između 27 i 44 godine, i između 45 i 64 godine, imala je veću verovatnoću da ima nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Mlađa i starija populacija pokrivena je obaveznim zdravstvenim obrazovanjem, te su njima dostupne usluge stomatološke zdravstvene zaštite. Naši nalazi su pokazali da su nezaposlena, neaktivna i penzionisana lica, koja obično pripadaju populaciji starijih uzrasnih grupa, imala značajno nižu verovatnoću da imaju nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom nego grupa radno aktivnih lica i grupa mladih.

Jedna ranija studija sprovedena u Srbiji pokazala je povezanost između stepena obrazovanja, prihoda i korišćenja usluga zdravstvene zaštite u privatnom zdravstvenom sektoru. Povezanost u ovoj studiji je bila pozitivna. Slično tome, u našoj studiji, učesnici višeg nivoa obrazovanja imali su manju verovatnoću da prijave nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom [17].

Naša studija je pokazala da, sa porastom prihoda po domaćinstvu, verovatnoća postojanja nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom opada [3,7,18,19]. U 2013. godini, prosečna plata u Srbiji je iznosila oko 370 evra, te je finansijsko opterećenje plaćanja usluga stomatološke zdravstvene zaštite moglo biti prilično veliko za mnoge porodice u Srbiji. Postojale su takođe i razlike među regionima po pitanju verovatnoće postojanja nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom – najveća verovatnoća da se iskuse nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom bile su u Regionu Šumadije i zapadne Srbije. Razlog ovome može ležati u činjenici da u južnoj, a naročito u istočnoj Srbiji, postoji visok procenat stanovništva koji radi van zemlje, te je finansiranje usluga stomatološke zdravstvene zaštite iz sopstvenih sredstava njima pristupačnije [20]. Takođe, samoprocenjene potrebe za stomatološkim uslugama mogu biti manje u nekim regionima u kojima je viša prevalencija nižeg obrazovanja.

Vrednost ove studije leži u činjenici da ona predstavlja prvo istraživanje o nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom u Srbiji koje je zasnovano na velikom nacionalno reprezentativnom uzorku. Jedina prethodna studija ispitivala je nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom

Our study has shown that, with the increase of income per household, the likelihood of unmet dental health care needs decreases [3,7,18,19]. The average salary in Serbia in 2013 was around 370 euros, and the financial burden of paying for dental health care services could be rather significant for many families in Serbia. There were also regional differences in the likelihood of unmet dental health care needs - the highest probability of experiencing unmet dental health care needs was in the Region of Šumadija and Western Serbia. The reason behind this could be that there is a high percentage of the population from Southern and especially Eastern Serbia working abroad, and out-of-pocket payment of dental health care services is more available to them [20]. Additionally, the self-perceived need for dental services might be lower in some regions with a higher prevalence of lower education.

The strength of this study is that it represents the first survey on unmet dental health care needs in Serbia based on a large nationally representative sample. The only previous study examined the unmet dental health care needs among persons with intellectual disabilities [21]. This study has some limitations. Firstly, this is a cross-sectional study and self-perceived unmet dental health care needs are considerably influenced by personal views, the cultural background, environmental factors and socio-economic status. Secondly, data obtained in this study did not include persons residing in institutions of health and social care and whose health is much more deteriorated than is the case with people living in their own homes. The SILC survey did not include questions about self-perceived oral health, which would have contributed to a better understanding of unmet dental health care needs in Serbia.

CONCLUSION

This study highlights inequalities in self-perceived unmet dental health care needs, according to socio-demographic, socio-economic, health, and regional characteristics of the population, and, consequently, defines the scale of inequalities in Serbia. Male participants, participants aged from 27 to 64 years, participants with a low income and lower educational status, participants with worse self-perceived health, participants living in the Region of Šumadija and Western Serbia, had a higher likelihood of having unmet dental health care needs. A multidimensional approach to health care system organization and elimination of barriers, which restrict the accessibility of dental health care, should be adopted. This approach could reduce inequality in unmet dental health care needs and improve dental health care outcomes.

Conflict of interest: None declared.

među licima sa intelektualnim smetnjama [21]. Studija ima izvesna ograničenja. Prvo, ovo je studija preseka i samoprocenjene nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom su pod velikim uticajem ličnih stavova, kulture, faktora sredine, i socio-ekonomskog statusa. Drugo, podaci u ovoj studiji nisu uključivali lica smeštena u ustanovama zdravstvene i socijalne zaštite, a čije je zdravlje daleko slabije nego kod ljudi koji žive u sopstvenim domovima. *SILC* anketa nije uključivala pitanja o samoprocenjenom oralnom zdravlju, što bi doprinelo boljem razumevanju nezadovoljenih potreba za stomatološkom zdravstvenom zaštitom u Srbiji.

ZAKLJUČAK

Ova studija ističe nejednakosti u samoprocenjenim nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom, prema socio-demografskim, socio-ekonomskim, zdravstvenim, i regionalnim karakteristikama stanovništva, i, sledstveno, definiše skalu nejednakosti u Srbiji. Učesnici muškog pola, učesnici starosti između 27 i 64 godine, učesnici sa nižim prihodom i nižim stepenom obrazovanja, učesnici koji su niže ocenili sopstveno zdravlje, te učesnici iz regiona Šumadije i zapadne Srbije, imali su veću verovatnoću da imaju nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom. Potrebno je primeniti multidimenzionalni pristup organizaciji sistema zdravstvene zaštite i ukloniti prepreke, koje ograničavaju pristupačnost stomatološke zdravstvene zaštite. Ovakav pristup mogao bi da smanji nejednakost u nezadovoljenim potrebama za stomatološkom zdravstvenom zaštitom i unapredi rezultate u stomatološkoj zdravstvenoj zaštiti.

Sukob interesa: Nije prijavljen.

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PREIMPLANTACIONO GENETIČKO TESTIRANJE

PREGLEDNI RAD

REVIEW ARTICLE

PREIMPLANTATION GENETIC TESTING

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SAŽETAK

Primena preimplantacionog genetičkog testiranja (PGT) otpočela je kasnih osamdesetih godina prošlog veka. Preimplantaciono genetičko testiranje, kao najraniji mogući vid prenatalne dijagnostike, omogućava selekciju zdravih embriona sa normalnim kariotipom za embriotransfer.

Upotreba preimplantacionog genetičkog testiranja se pokazala korisnom metodom kod tri grupe naslednih bolesti, i to: monogenskih bolesti, bolesti trinukleotidnih ponovaka i hromozomskih aberacija.

Stopa uspeha vantelesne oplodnje (VTO) značajno je porasla nakon što je PGT uveden u kliničku praksu.

U ovom radu dat je pregled literature, sa ciljem jasnog utvrđivanja uloge PGT-a u selekciji embriona pre embriotransfera, kao i uloge ove vrste testiranja u povećanju stope uspeha VTO-a. Jedan od ciljeva rada je i osvrt na razvoj molekularno genetičkih metoda koje su trenutno, ili su ranije bile, u rutinskoj upotrebi prilikom izvođenja PGT-a.

Aktuelna literatura pokazatelj je razvoja i napretka tehnika molekularne genetike koje se primenjuju u PGT-u. Istovremeno daje mogućnost i podsticaj za dalja opsežna istraživanja koja će dovesti do unapređenja samog preimplantacionog genetičkog testiranja, a samim tim povećati stopu uspeha vantelesne oplodnje.

Ključne reči: preimplantaciono genetičko testiranje – PGT, vantelesna oplodnja – VTO, embrion

ABSTRACT

The application of preimplantation genetic testing (PGT) began in the late 1980s. Preimplantation genetic testing, as the earliest possible method of prenatal diagnosis, enables the selection of embryos with a normal karyotype for embryo transfer.

The use of preimplantation genetic testing has proven to be a useful method in the following three groups of inherited diseases: monogenic disorders (single gene defects), trinucleotide repeat disorders, and chromosomal abnormalities.

The success rate of in vitro fertilization (IVF) has increased significantly since the introduction of PGT into clinical practice.

This paper presents a literature review with the aim of clearly determining the role of PGT in embryo selection before embryo transfer, as well as the role of this type of testing in increasing the success rate of IVF. One of the goals of the paper is also to review the development of molecular genetic methods that are currently, or have once been, in routine use when performing PGT.

The current literature is an indicator of the development and progress of molecular genetics techniques applied in PGT. At the same time, it provides an opportunity and an incentive for further extensive research that will lead to the improvement of preimplantation genetic testing and thus increase the success rate of in vitro fertilization.

Key words: preimplantation genetic testing – PGT, in vitro fertilization – IVF, embryo

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UVOD

Preimplantaciono genetičko testiranje (PGT) obuhvata procedure koje su ranije bile poznate kao preimplantaciona genetička dijagnostika (PGD) i preimplantacioni genetički skrining (PGS). Prema Konzorcijumu *ESHRE* (engl. *European Society for Human Reproduction and Embryology*), PGT se definiše kao test koji se sprovodi kako bi se analizirala DNK iz oocita (polarnih tela) ili embriona (embriona na stadijumu brazdanja ili blastocista) za HLA (humani leukocitni antigen) tipizaciju ili za determinaciju genetičkih abnormalnosti [1].

PGT, kao najraniji mogući oblik prenatalne dijagnostike, primenjuje se kod parova koji nose rizik prenošenja nasledne bolesti na potomstvo [2]. Sve do razvoja PGT-a, kasnih osamdesetih godina prošlog veka, invazivna i neinvazivna prenatalna dijagnostika mogla je samo da konstatuje da plod u utrobi majke ima, ili će razviti neku naslednu bolest. Tada su se parovi suočavali sa jednom od najtežih odluka u životu, da li takvu trudnoću prekinuti ili nastaviti, uprkos saznanju da njihovo dete neće biti zdravo. Pojava PGT-a je ovakvim parovima omogućila dragocenu alternativu [3] budući da se metoda primenjuje na preimplantacionim embrionima. To omogućava da se samo zdravi embrioni sa normalnim kariotipom selektuju za embriotransfer [3].

Preimplantacioni genetički skrining (PGS), koji se često nazivao PGD niskog rizika, primenjivao se kod infertilnih parova koji nose nizak rizik od transmisije nasledne bolesti na potomstvo i koji se podvrgavaju VTO-u, a sa ciljem povećanja stope uspeha u ostvarivanju trudnoće i rađanju zdravog deteta.

Preduslov za PGT je vantelesna oplodnja (VTO). U stimulisanim ciklusima, ona ima za cilj dobijanje što većeg broja jajnih ćelija i embriona. Ovo je veoma važno za parove koji se suočavaju sa problemom steriliteta, jer pruža mogućnost selekcije samo zdravih embriona [3]. Ukoliko postoje indikacije za primenu PGT-a prilikom VTO-a, izbegava se klasična *in vitro* fertilizacija (*IVF*), jer PGT podrazumeva korak DNK amplifikacije [4]. Iz tog razloga pristupa se metodi intracitoplazmatične injekcije spermatozoida (engl. *intracytoplasmic sperm injection – ICSI*). Ovo je metoda prvog izbora jer se njenom primenom isključuje mogućnost kontaminacije neželjenom DNK spermatozoida [2].

PGT je prvi put primenjeno u kliničkoj praksi 1990. godine, prilikom određivanja pola embriona *PCR*-om (engl. *polymerase chain reaction*) usled sumnje na X-vezano oboljenje [2]. Nekoliko godina kasnije, primena fluorescentne *in situ* hibiridizacije (FISH) postaje standardna metoda za selekciju embriona prema polu, za detekciju numeričkih i strukturnih hromozomskih aberacija. Danas se ove metode više ne koriste. Njih su zamenile novije, sofisticiranije, osetljivije i specifičnije

INTRODUCTION

Preimplantation genetic testing (PGT) encompasses procedures, previously known as preimplantation genetic diagnostics (PGD) and preimplantation genetic screening (PGS). According to the PGT Consortium of the ESHRE (European Society for Human Reproduction and Embryology), PGT is defined as a test that is carried out in order to analyze the DNA of oocytes (polar bodies) or embryos (cleavage stage embryos or blastocyst stage embryos) for HLA (human leukocyte antigen) typing or for the determination of genetic abnormalities [1].

PGT, as the earliest possible form of prenatal diagnostics, is applied in couples carrying a risk of passing on a hereditary disease onto their children [2]. Until the late 1980s, when PGT was developed, invasive and noninvasive prenatal diagnostics could only ascertain that the embryo in the mother's womb was indeed afflicted with, or would develop, a hereditary disease. Couples were then faced with one of the most difficult decisions of their lives, whether to terminate or continue with the pregnancy, despite the knowledge that their child would not be healthy. The development of PGT provided a valuable alternative [3], as the method is applied on preimplantation embryos. This provides that only healthy embryos with a normal karyotype are selected for embryo transfer [3].

Preimplantation genetic screening (PGS), previously often referred to as low risk PGD, was applied in infertile couples who were carrying a low risk of passing on a hereditary disease onto their offspring, and who were undergoing IVF, for the purpose of increasing the success rate of achieving pregnancy and producing a healthy child.

The precondition for PGT is in vitro fertilization (IVF). In stimulated cycles, its aim is to produce the maximum number of ova and embryos. This is very important for couples faced with the problem of sterility, as it offers the opportunity of selecting only healthy embryos [3]. If there are indications for the application of PGT during IVF, classic *in vitro* fertilization (IVF) is avoided, as PGT implies the step of DNA amplification [4]. This is why the method of intracytoplasmic sperm injection (ICSI) is employed. This is the method of choice, as its application excludes the possibility of contamination with unwanted sperm DNA [2].

PGT was first applied in clinical practice in 1990, for determining the sex of the embryo, with the aid of PCR (polymerase chain reaction), due to suspected X-linked disease [2]. Several years later, the application of fluorescence *in situ* hybridization (FISH) became the standard method for the selection of embryo by sex, for the purpose of detecting numerical and structural

metode, kao što su *micrroarray CGH* (engl. *comparative genomic hybridization*) i *NGS* (engl. *next generation sequencing*).

Na osnovu podataka iznetih u Uvodu, definisani su ciljevi ovog rada:

1. Utvrđivanje uloge PGT-a u selekciji zdravih euploidnih embriona pre embriotransfera;
2. Utvrđivanje uloge PGT-a u povećanju stope uspeha VTO-a;
3. Pregled korišćenih genetičkih metoda u preimplantacionom genetičkom testiranju.

PREIMPLATACIONO GENETIČKO TESTIRANJE

Prema Konzorcijumu *ESHRE*, PGT delimo na:

1. preimplantaciono genetičko testiranje na aneuploidije (PGT-A)
2. preimplantaciono genetičko testiranje na prisustvo strukturnih hromozomskih rearanžmana (PGT-SR)
3. preimplantaciono genetičko testiranje na prisustvo monogenskih oboljenja (PGT-M) [1].

INDIKACIJE

PGT se primenjuje kod parova koji nose rizik za dobijanje potomstva sa naslednom bolešću ili hromozomskim aberacijama, i to u slučaju: starije životne dobi žene [5], teškog oblika muškog infertiliteta [6], ponovljenih neuspeha implantacije embriona nakon VTO-a [3], rekurentnih pobačaja [5], postojanja već bolesnog deteta ili člana porodice [3], kada je jedan od partnera nosilac monogenskih bolesti, hromozomskih aberacija i mitohondrijalnih DNK mutacija, postojanja genetičke predispozicije za malignitet, i kod HLA tipizacije [2].

STARIJA ŽIVOTNA DOB ŽENE

Poznato je da postoji jasna pozitivna korelacija između numeričkih hromozomskih aberacija, najčešće aneuploidija, sa životnom dobi majke. Studije pokazuju da do 70% oocita žena starijih od 40 godina imaju numeričke hromozomske aberacije [5]. Jedno od objašnjenja niže stope implantacije, uključujući prirodna začeća i VTO, jeste veći procenat embriona sa aneuploidijama. PGT-A embriona je pokazala da su aneuploidije česte i da se njihov procenat značajno povećava sa godinama žene. U takvim slučajevima, moguće je uraditi biopsiju polarnog tela ili biopsiju trofoektoderma blastociste [5].

HLA TIPIZACIJA

PGT je našao primenu pri HLA tipizaciji u reproduktivnoj medicini. Kod para koji ima dete kome je potrebna transplantacija hematopoetskih stem ćelija, PGT-om se vrši selekcija embriona koji nisu nosioci mutacije za

chromosome aberrations. Today, these methods are no longer in use. They have been replaced by newer, more sophisticated, more sensitive and more specific methods, such as micrroarray CGH (comparative genomic hybridization) and NGS (next generation sequencing).

Based on the data depicted in the Introduction, the goals of the present paper have been defined. These are:

1. Determining the role of PGT in the selection of healthy euploid embryos before embryo transfer;
2. Determining the role of PGT in increasing the success rate of IVF;
3. Review of genetic methods applied in preimplantation genetic testing.

PREIMPLANTATION GENETIC TESTING

According to the PGT Consortium of the ESHRE, PGT is classified into the following categories:

1. preimplantation genetic testing for aneuploidies (PGT-A)
2. preimplantation genetic testing for chromosomal structural rearrangements (PGT-SR)
3. preimplantation genetic testing for monogenic disorders (PGT-M) [1].

INDICATIONS

PGT is applied in couples at risk of having children with hereditary disease or chromosome aberrations, in the following cases: older maternal age [5], severe form of male infertility [6], repeated failure of embryo implantation after IVF [3], recurrent miscarriage [5], the occurrence of disease in a child already born to the couple or in a family member [3], when one of the partners is a carrier of a monogenic disorder (single gene defect), chromosome aberrations, and mitochondrial DNA mutations, when there is a genetic predisposition to malignancy, and in HLA typing [2].

OLDER MATERNAL AGE

It is well known that there is a clear positive correlation between numerical chromosome aberrations, most frequently aneuploidies, and the maternal age. Studies have shown that up to 70% of oocytes of women past the age of 40 have numerical chromosome aberrations [5]. One of the explanations for a decreased implantation rate, including both natural conception and IVF, is a higher percentage of embryos with aneuploidies. PGT-A of embryos has shown that aneuploidies are frequent and that their percentage significantly increases with maternal age. In such cases, it is possible to perform polar body biopsy or trophectoderm biopsy of the blastocyst [5].

bolest, a kompatibilni su donori za bolesnog brata ili sestru. Ovaj pristup se prvi put uspešno koristio kod Fankoni anemije [2].

Poznato je da se nasleđuje predispozicija za razvoj nekih tipova maligniteta (tumori dojke i jajnika, tumori želuca i debelog creva, nekih leukemija), te PGT nalazi svoju primenu i u ovoj sferi [7].

GENETIČKI UZROK MUŠKOG INFERTILITETA

Genetički uzrok muškog infertiliteta je vrlo heterogen, te se primenjuju sve tehnike PGT-a. Do sad je identifikovano preko 2.300 gena čije mutacije mogu dovesti do razvoja muškog infertiliteta, dok su hromozomske aberacije potvrđeni uzrok infertiliteta u 20% slučajeva. Oba slučaja praćena su lošim parametrima spermograma [6]. Ovaj deo infertilne populacije ima veliku korist od PGT-a, uz primenu dodatnih urooloških procedura ili bez njih.

Klinefelterov sindrom (47, XXY) karakteriše abonormalna spermatogeneza, a često je prisutna i azoospermija. Pokazano je da se, u bar 50% slučajeva, spermatozoidi mogu dobiti postupkom testikularne ekstrakcije spermatozoida (TESE) [6]. Kod muškaraca sa ovim sindromom postoji povećana učestalost aneuploidija kod njihovog potomstva, pa je primena PGT-A metode neprocenjiva [6].

Zahvaljujući primeni PGT-SR metode, nosioci heterologih Robertsonovih translokacija mogu povećati šanse za dobijanje zdravog potomstva. Kod parova sa ovom indikacijom, PGT-SR omogućava selekciju zdravih embriona kojih je svega 25% [6].

PGT-SR i PGT-M se primenjuju ukoliko postoji mikrodelecija AZF-c regiona Y hromozoma [6], kod pacijenata sa Kartagenerovim sindromom [7], kod pacijenata sa globozoospermijom [8], kod velikog broja numeričkih i strukturnih hromozomskih aberacija i pojedinih monogenskih bolesti.

MITOHONDRIJALNE BOLESTI

Mitohondrijalne bolesti su relativno česti poremećaji metabolizma, a u 15% slučajeva uzrokovane su mutacijama u mitohondrijalnoj DNK (mtDNK) majke [9]. Budući da one dovode do ozbiljne fenotipske ekspresije (gubitak neuroloških funkcija, respiratonih i srčanih problema itd.), primena PGT-M metode kod pacijenata sa ovom indikacijom omogućava selekciju zdravih embriona tokom VTO-a.

Prema Konzoricjumu *ESHRE,* sve patogenetske varijante u mtDNK koje se javljaju u pojedinačnim blastomerama reprezentativne su za ceo embrion, što se i očekuje, budući da se do stadijuma brazdanja, mtDNK ne replikuje. Na stadijumu blastociste počinje mtDNK replikacija, što dovodi do pojave različitih novih varijanti [1].

HLA TYPING

PGT has found its application in HLA typing in reproductive medicine. In a couple who has a child in need of hematopoietic stem cell transplantation, PGT is applied in order to select embryos that are not carriers of the mutation related to disease and are also compatible donors for their sick brother or sister. This approach was first successfully applied in Fanconi anemia [2].

It is a known fact that predisposition towards certain types of malignancy is hereditary (breast and ovarian tumors, tumors of the gaster and colon, certain types of leukemia), which is why PGT is applied in this sphere as well. [7].

GENETIC CAUSE OF MALE INFERTILITY

The genetic cause of male infertility is very heterogenous, which is why all PGT techniques are applied. Thus far, more than 2,300 genes, whose mutations can lead to the development of male infertility, have been identified, while chromosome aberrations have been confirmed as the cause of male infertility in 20% of cases. Both types of cases are characterized by poor semen analysis parameters [6]. This part of the infertile population benefits greatly from PGT, along with additional urological procedures, or without them.

Klinefelter syndrome (47, XXY) is characterized by abnormal spermatogenesis, and azoospermia is also often present. It has been determined that, in at least 50% of cases, spermatozoa can be obtained by means of testicular sperm extraction (TESE) [6]. In men with this syndrome, there is an increased incidence of aneuploidies in their offspring, which is why the application of PGT is invaluable [6].

Owing to the application of the PGT-SR method, carriers of heterologous Robertsonian translocations may increase their chances of having healthy children. In couples with this indication, PGT-SR enables the selection of healthy embryos, of which there are only 25% [6].

PGT-SR and PGT-M are applied in the following cases: if there is a microdeletion of the AZF-c region of the Y chromosome [6], in patients with Kartagener's syndrome [7], in patients with globozoospermia [8], in a large number of numerical and structural chromosome aberrations, and in certain monogenic disorders.

MITOCHONDRIAL DISEASES

Mitochondrial diseases are relatively common metabolic disorders, and, in 15% of cases, they are caused by maternal mitochondrial DNA (mtDNA) mutations [9]. Since they lead to severe phenotypic expression (loss of neurological function, respiratory and cardiac problems, etc.), the use of the PGT-M method in

BIOPSIJA

Biopsija podrazumeva uzimanje ćelija, čiji će genetički materijal biti analiziran, nekom od metoda molekularne dijagnostike. Razlikujemo: biopsiju polarnog tela na stadijumu oocite ili zigota; biopsiju blastomera embriona trećeg dana; biopsiju trofoektoderma embriona petog ili šestog dana tj. biopsiju blastociste [3].

Biopsija počinje ablacijom na glikokaliksnom omotaču (lat. *zona pellucida)*. Nekada se to radilo mehaničkim, zatim hemijskim putem, a od 2003. godine, isključivo primenom lasera. Kad se napravi otvor u zoni pelucidi i oslobodi prolaz, aspirira se polarno telo blastomera ili ćelije trofoektoderma, čiji se genetički materijal analizira [10].

BIOPSIJA POLARNOG TELA

Biopsija polarnog tela je prvi put primenjena 1990. godine za detekciju cistične fibroze. Ova procedura je razvijena sa ciljem da se smanji invazivnost biopsije blastomera. Genetički materijal polarnog tela predstavlja samo DNK iz oocite, pa je biopsija polarnog tela posebno korisna za detekciju maternalno nasleđenih monogenskih bolesti, numeričkih i strukturnih hromozomskih aberacija. Nedostatak ove metode je nemogućnost dobijanja informacija o DNK oca i DNK embriona [10]. Danas se ova metoda uglavnom koristi da bi se prevazišli etički problemi u zemljama gde nije dozvoljena biopsija embriona.

BIOPSIJA BLASTOMERA

Biopsija blastomera embriona trećeg dana izvodi se 66 – 72 sata nakon primene *ICSI* metode, kada embrion ima 6 – 8 blastomera koje su još uvek totipotentne i između njih se jasno uočavaju granice [2].

Nedostaci ove metode su: relevantnost rezultata dobijenih analizom pojedinačne ćelije, imajući u vidu visok procenat mozaicizma, koji se javlja kod embriona, kao i nedostatak informacija o negativnom uticaju uklanjanja blastomere ili blastomera na dalji razvoj embriona [10].

Biopsija koja bi se izvodila na ranijem stadijumu, na nivou četvoroćelijskog embriona, može narušiti odnos buduće unutrašnje ćelijske mase (engl. *inner cell mass – ICM*) i trofoektoderma (TE).

Imajući u vidu sve prethodno navedeno, glavna strategija za primenu ove metode je biopsija embriona trećeg dana, koji u tom momentu ima 6 do 8 blastomera [2]. Problem kod ovog tipa biopsije su blastomere koje mogu lako da liziraju, što bi dovelo do gubitka genetičkog materijala, te bi bila potrebna nova blastomera za analizu.

Kompakcija, koja se dešava na nivou između osmoćelijskog embriona i stadijuma morule, dodatno

patients with this indication enables the selection of healthy embryos during IVF.

According to the PGT Consortium of the ESHRE, all pathogenic mtDNA variants, which occur in individual blastomeres, are representative of the entire embryo, which is to be expected, as mtDNA does not replicate until the cleavage stage. At the stage of the blastocyst, mtDNA replication begins, which leads to the occurrence of different new variants [1].

BIOPSY

Biopsy entails extracting cells, whose genetic material is to be analyzed, by means of one of the molecular diagnostics methods. There are different types of biopsy, namely: biopsy of the polar body at the oocyte or zygote stage; blastomere biopsy on day three of embryo development; trophectoderm biopsy of the embryo on day five or day six of development, i.e., biopsy of the blastocyst [3].

Biopsy begins with ablation on the glycocalyx coat (Lat. *zona pellucida*). In the past, this used to be done mechanically, and then chemically, and as of 2003, it has been done exclusively with the use of the laser. Once an opening is made in the *zona pellucida*, and a pathway is made, the polar body of the blastomere or trophectoderm cell, whose genetic material is analyzed, is aspirated [10].

POLAR BODY BIOPSY

Polar body biopsy was first employed in 1990 for detecting cystic fibrosis. This procedure was developed with an aim to decrease the invasiveness of blastomere biopsy. Genetic material of the polar body represents only the DNA from the oocyte, which is why polar body biopsy is especially useful for the detection of maternally inherited monogenic disorders and numerical and structural chromosome aberrations. The drawback of this method is that there is no possibility of obtaining any information on the DNA of the father or the DNA of the embryo [10]. Today, this method is mainly used to overcome ethical issues and concerns in countries where embryo biopsy is not allowed.

BLASTOMERE BIOPSY

Blastomere biopsy on day three of embryo development is performed between 66 and 72 hours after the application of the ICSI method, when the embryo has 6 – 8 blastomeres, which are still totipotent, and the boundaries between them are clearly visible [2].

The drawbacks of this method are the following: the relevance of the results obtained through the analysis of an individual cell, bearing in mind the high percentage of mosaicism that occurs in embryos; as well as the lack of information on the negative effect that

komplikuje PGT. Za vreme kompakcije, granice između ćelija se gube i nije moguće razlikovati pojedinačne ćelije, te je teško izdvojiti samo jednu blastomeru [11].

Pre same biopsije blastomera, neophodna je inkubacija embriona u medijumima bez kalcijuma i magnezijuma, da bi se usporilo stvaranje međućelijskih veza i olakšala biopsija. Kada se genetički materijal blastomere šalje na *PCR* analizu, preporučena metoda fertilizacije oocita je *ICSI*. U slučaju da je metoda fertilizacije klasičan IVF može doći do kontaminacije i amplifikacije DNK spermatozoida umesto DNK embriona, pa se ova metoda ne preporučuje.

BIOPSIJA TROFOEKTODERMA

Biopsija trofoektoderma (TE) blastociste se može izvoditi petog ili šestog dana od oplodnje. Prednost metode je mogućnost biopsije većeg broja ćelija trofoektoderma (5 do 10 ćelija) [10], bez narušavanja *ICM* [12]. Analiza većeg broja ćelija ima prednost u dijagnostici monogenskih bolesti [2]. Ovaj broj ćelija se može smatrati reprezentativnim za ceo embrion, sem u slučaju placentnog mozaicizma [3], koji je primećen u više od 1% trudnoća [13]. Studije pokazuju da blastocistu karakteriše visok nivo mozaicizma, pa se iz tog razloga ćelije TE ne mogu smatrati pogodnim za PGT analizu [3].

Biopsija blastociste sa krioprezervacijom je već neko vreme postala standard pri izvođenju neke od PGT metoda [2]. Zamrzavanje blastociste metodom vitrifikacije nakon biopsije daje vremena za sve neophodne analize [2]. Glavni problem biopsije blastociste sastoji se u tome što će samo ograničeni broj embriona dostići dati stadijum i odgovarajući kvalitet, uprkos usavršavanju medijuma za kultivaciju. Embrioni koji ne dostignu stadijum blastociste mogu imati visok procenat aneuploidija, koje uključuju hromozome X, Y, 16, 18 i 21 [10].

GENETIČKE ANALIZE

Metode koje su se nekada češće koristile za analizu genetičkog materijala dobijenog biopsijom su: *PCR*, FISH (engl. *fluorescence in situ hybridization*), *CGH*, *SNP* (engl. *single nucleotide polymorphism*), a danas ih zamenjuje metoda *NGS* [10]. Izbor odgovarajuće metode zavisi od medicinskih indikacija.

PCR

PCR metoda se koristila za detekciju mutacija na nivou gena, za detekciju broja trinukleotidnih ponovaka i za određivanje pola embriona [2]. Dva glavna problema *PCR* metode u PGT-M testiranju su: kontaminacija uzorka i *allele dropout* [10].

PCR jedne ćelije je osetljiva metoda budući da postoji opasnost amplifikacije strane DNK (DNK ćelija kumulusa ili DNK spermatozoida). Da bi se ovaj problem

the removal of a blastomere, or blastomeres, may have on the further development of the embryo [10].

Biopsy that would be performed at an earlier stage, at the level of a four-cell embryo, could damage the relation between the future inner cell mass (ICM) and the trophectoderm (TE).

Bearing in mind all of the above, the main strategy for the application of this method is day three embryo biopsy, with the embryo comprising 6 to 8 blastomeres at that moment [2]. The problem with this type of biopsy are the blastomeres which can easily lyse, which would lead to the loss of genetic material, necessitating a new blastomere for analysis.

Compaction occurring at the level between the stage of the eight-cell embryo and the morula stage additionally complicate PGT. During compaction, the cell-cell boundaries disappear, and it becomes impossible to differentiate individual cells, which is why it is difficult to extract only one blastomere [11].

Before the actual blastomere biopsy, the incubation of the embryo in mediums devoid of calcium and magnesium is necessary, in order to slow down the creation of bonds among cells and facilitate the biopsy. When the genetic material of the blastomere is sent for PCR analysis, the recommended method of oocyte fertilization is ICSI. In case the fertilization method is classic IVF, contamination and amplification of the sperm DNA instead of the embryo DNA may occur, which is why this method is not recommended.

TROPHECTODERM BIOPSY

Trophectoderm biopsy (TE) of the blastocyst can be performed on the fifth or sixth day upon fertilization. The advantage of this method is the possibility of performing a biopsy of a higher number of cells of the trophectoderm (5 to 10 cells) [10], without damaging the ICM [12]. Analysis of a greater number of cells is the preferred method in the diagnostics of monogenic disorders [2]. This number of cells can be considered representative for the entire embryo, except in the case of placental mosaicism [3], which has been observed in over 1% of pregnancies [13]. Studies have shown that blastocytes are characterized by a high level of mosaicism, which is why TE cells cannot be considered suitable for PGT analysis [3].

Blastocyst biopsy with cryopreservation has been the standard for performing some of the PGT methods for a certain period of time now [2]. Blastocyst freezing by means of the vitrification method upon biopsy provides time for all the necessary analyses [2]. The main problem of blastocyst biopsy is the fact that only a limited number of embryos reaches this stage and the appropriate quality, despite the refined cultivation

izbegao, procedura se izvodi u posebnoj *PCR* sobi sa pozitivnim pritiskom, a metoda fertilizacije je isključivo *ICSI* [2]. Rešenje ovog problema bi bio multipleks *PCR* kojim bi se identifikovala sva 4 parentalna alela, čime bi se osiguralo da ampifikovana DNK bude isključivo embrionskog porekla [2].

Drugi problem je DNK *allele dropou*t ili preferencijalna amplifikacija, pri čemu se jedan od dva alela preferencijalno amplifikuje u odnosu na drugi, što kod dominantnih heterozigota može dati lažno negativan ili lažno pozitivan rezultat [10].

FLUORESCENTNA *IN SITU* HIBRIDIZACIJA - FISH

Fluorescentna *in-situ* hibridizacija je prvi put u PGT-u počela da se primenjuje 1991. godine, za analizu hromozoma embriona radi određivanja pola i hromozomskih aberacija, pre svega aneuploidija [2].

Za razliku od *PCR* metode, kod FISH-a ne postoji rizik od kontaminacije uzorka i nismo ograničeni samo na *ICSI* metodu pri fertilizaciji oocita. Hromozomi koji se najčešće analiziraju su X, Y, 13, 16, 18, 21, i 22 [7]. Ponavljanjem ciklusa i uključivanjem većeg broja hromozoma u analizu smanjuje se efikasnost procedure i povećava verovatnoća lažno pozitivnih i lažno negativnih rezultata. Kako se FISH-om mogu analizirati samo određeni hromozomi i kako je broj prijavljenih lažno pozitivnih i lažno negativnih rezultata visok, ova metoda se više ne koristi u preimplantacionom genetičkom testiranju [14].

KOMPARATIVNA GENOMSKA HIBRIDIZACIJA *I MICROARRAY-CGH*

U međuvremenu su se razvijale nove genetičke metode koje omogućavaju simultanu analizu svih hromozoma sa daleko većom preciznošću. Jedna od tih metoda je metafazna komparativna genomska hibridizacija (engl. *metaphase comparative genomic hybridization – mCGH*) [2]. Iako je ova molekularna citogenetička metoda pouzdano detektovala aneuploidije, nedostatak je bilo vreme neophodno za analizu (3 do 5 dana) te se embriotransfer nije mogao vršiti u vremenski predviđenom intervalu. Prelazak sa tadašnjeg metoda zamrzavanja, sporog zamrzavanja (engl. *slow freezing*) na usavršen metod vitrifikacije koji se i danas koristi (preživljavanje blastocista nakon odmrzavanja preko 96%) [15] omogućilo je primenu *mCGH* . Vitrifikacija nakon biopsije obezbeđuje dovoljno vremena za genetičku analizu i tumačenje rezultata i dozvoljava da se embriotransfer uradi u trenutku optimalne receptivnosti endometrijuma [14].

Kasnije je *mCGH* zamenjen metodom *microarray-CGH*. Prednost ove metode u odnosu na prethodne je smanjenje vremena analize na jedan dan, povećanje broja hromozoma koji se analizira, kao i preciznija detekcija aneuploidija [2].

mediums. The embryos that do not reach the blastocyst stage may have a high percentage of aneuploidies, which include chromosomes X, Y, 16, 18 and 21 [10].

GENETIC ANALYSES

Methods which were more frequently used in the past for the analysis of genetic material obtained through biopsy are the following: PCR, FISH (fluorescence *in situ* hybridization), CGH, SNP (single nucleotide polymorphism), while today these are being replaced by the NGS method [10]. The selection of the appropriate method depends on medical indications.

PCR

The PCR method was used for the detection of gene-level mutations, for the detection of the number of trinucleotide repeats, and for determining the sex of the embryo [2]. The two main problems of the PCR method in PGT-M testing are the following: sample contamination and allele dropout [10].

PCR of a single cell is a sensitive method as there is the danger of the amplification of foreign DNA (DNA of the cumulus cells or sperm DNA). In order to avoid this problem, the procedure is carried out in a special PCR room with positive air pressure, and the method of fertilization is exclusively ICSI [2]. The solution to this problem would be multiplex PCR which would identify all 4 parental alleles, which would, in turn, ensure that the amplified DNA is exclusively of embryonic origin [2].

The second problem is DNA allele dropout or preferential amplification, whereby one of the two alleles is preferentially amplified in relation to the other, which, in dominant heterozygotes, may produce a falsely negative or falsely positive result [10].

FLUORESCENCE *IN SITU* HYBRIDIZATION – FISH

Fluorescence *in situ* hybridization (FISH) was first used in PGT in 1991 for the purpose of analyzing embryonic chromosomes in order to determine the sex of the embryo and chromosome aberrations, primarily aneuploidies [2].

As opposed to the PCR method, in FISH, there is no risk of sample contamination and we are therefore not limited only to the ICSI method in oocyte fertilization. The chromosomes most commonly analyzed are X, Y, 13, 16, 18, 21, and 22 [7]. By repeating the cycles and including a higher number of analyses, the efficiency of the procedure is diminished, and the probability of false positive and false negative results is increased. As FISH can be used to analyze only certain chromosomes, and since the number of reported false positive and false negative results is high, this method is no longer used in preimplantation genetic testing [14].

SNP i NGS

Metoda *SNP* otkriva ne samo aneuploidije, već i duplikacije i delecije, a može dati informaciju o poreklu hromozoma od oca i od majke kod uniparentalne dizomije (UPD) [2,16].

Danas se u kliničkoj praksi kao standard koristi *NGS*. Ova metoda uključuje prethodnu amplifikaciju celog genoma (engl. *whole genome amplification*), što omogućava da se uradi veći broj analiza u isto vreme, na samo jednoj ćeliji. *NGS*-om se detektuju mutacije, visoko polimorfne sekvence, aneuploidije, kao i epigenetički profil [17]. Ciljana *NGS* strategija, koja se fokusira na amplifikaciju i analizu specifičnih sekvenci, pokazala je mnogo veću moć detektovanja mozaicizma u odnosu na sve prethodne metode [8].

PRIMENA

Tri grupe naslednih bolesti mogu biti dijagnostikovane uz pomoć PGT-a [3] :

- monogenske bolesti (engl. *single gene defects*),
- bolesti trinukleotidnih ponovaka,
- hromozomske aberacije.

MONOGENSKE BOLESTI

Monogenske bolesti se mogu nasleđivati autozomno dominantno, autozomno recesivno, kao i X vezano.

Prve autozomno dominantne bolesti koje su dijagnostikovane primenom PGT-M metode su: Marfanov sindrom, familijarna adenomatoza, Hantigtonova bolest, miotonična distrofija i bolest krhkih kostiju (lat. *Osteogenesis imperfecta)*. Danas se PGT-M primenjuje za većinu autozomno dominantnih bolesti [3].

Neke od autozomno recesivnih bolesti koje se mogu dijagnostikovati pomoću PGT-M metode su: cistična fibroza, srpasta anemija, *Tay Sachs*-ova bolest, spinalna mišićna distrofija, ß talasemija, adrenogenitalni sindrom, i hipofosfatemija.

Beta talasemija je izazvana mutacijom u beta globinskom genu. Međutim, postoji veliki broj različitih mutacija u okviru beta globinskog gena, pogotovo između različitih etničkih grupa, što dodatno komplikuje PGT-M [3].

Imajući u vidu da je cistična fibroza najučestalije monogensko autozomno recesivno oboljenje kod ljudi bele rase, opravdana je najčešća upotreba PGT-M, u slučajevima kada se sumnja na ovu bolest. Ono što ovu, gotovo rutinsku proceduru, može otežati jeste postojanje 800 mutacija koje se dovode u vezu sa razvojem ovog patološkog stanja [3].

Zahvaljujući primeni PGT-M, prilikom sumnje na X vezana oboljenja moguće je izvršiti selekciju embriona za embriotransfer, koji nisu nosioci mutacije, bez obzira na pol – zdravi muški i ženski embrioni sa normalnim kariotipom [3].

COMPARATIVE GENOMIC HYBRIDIZATION AND MICROARRAY- CGH

In the meantime, new genetic methods have been developed, enabling simultaneous analysis of all chromosomes with far greater precision. One of these methods is metaphase comparative genomic hybridization - mCGH [2]. Although this molecular cytogenetic method could reliably detect aneuploids, its weakness lay in the time necessary for analysis (3 to 5 days) which is why embryo transfer could not be performed within the required time interval. Abandoning the previous freezing method (slow freezing) and employing the more sophisticated vitrification method, which is still in use nowadays (survival of blastocysts upon thawing is over 96%) [15], enabled the application of mCGH. Vitrification upon biopsy provides enough time for genetic analysis and interpretation of results and allows for embryo transfer to be performed at a time of optimal endometrial receptivity [14].

Subsequently, mCGH was substituted with the microarray-CGH method. The advantage of this technique over other methods lies in the shortening of the analysis time to one day, in increasing the number of chromosomes analyzed, as well as in a more precise detection of aneuploidies [2].

SNP and *NGS*

The SNP method discovers, not only aneuploidies, but also duplications and deletions, and it can also provide information on the origin of the chromosomes from the father and the mother in uniparental disomy (UPD) [2,16].

Today, NGS is used as the standard in clinical practice. This method includes previous whole genome amplification, which enables performing multiple analyses, at the same time, on only one cell. NGS detects mutations, highly polymorphic sequences, aneuploidies, as well as the epigenetic profile [17]. Targeted NGS strategy, focused on the amplification and analysis of specific sequences, has shown a much greater power of detecting mosaicism than all the previous methods [8].

APPLICATION

Three groups of hereditary diseases can be diagnosed with the use of PGT [3]:

- monogenic disorders, i.e., single gene defects,
- trinucleotide repeat disorders,
- chromosome aberrations.

MONOGENIC DISORDERS

Monogenic disorders (single gene defects) can have autosomal dominant inheritance, autosomal recessive inheritance and X-linked inheritance.

BOLESTI TRINUKLEOTIDNIH PONOVAKA

Bolesti trinukleodinih ponovaka koje nastaju prisustvom dinamičkih mutacija, moguće je dijagnostikovati primenom PGT-M metode.

Broj ponavljanih tripleta se povećava iz generacije u generaciju, što za posledicu ima težu kliničku sliku i raniju pojavu bolesti u narednoj generaciji [14].

Hantingtonova bolest i sindrom fragilnog X hromozoma su prve u grupi bolesti trinukleotidnih ponovaka, koje su dijagnostikovane zahvaljujući PGT-M metodi. [3].

Za sve bolesti trinukleotidnih ponovaka postoje definisane granične vrednosti (enlg. *cut-off*). PGT-M omogućava razlikovanje embriona koji će razviti bolest izazvanu mutacijom od onih koji su zdravi.

HROMOZOMSKE ABERACIJE

Treća grupa bolesti koje se dijagnostikuju PGT-om su hromozomske aberacije. Hromozomske aberacije mogu biti numeričke i strukturne.

Numeričke hromozomske aberacije autozoma su uglavnom letalne, sa izuzetkom trizomija 13, 18 i 21, dok kod polnih hromozoma neke od aneuploidija su kompatibilne sa životom (Tarnerov sindrom - 45, X0; Klinerfelterov sindrom - 47, XXY; trizomija X hromozoma - 47, XXX; Jakobsov sindrom - 47, XYY) i moguće ih je detektovati primenom PGT-A metode.

Strukturne hromozomske aberacije mogu biti balansirane i nebalansirane. PGT-SR dijagnostika otkriva nosioce balansiranih hromozomskih rearanžmana od kojih su najčešće u populaciji balansirane translokacije. Nosioce karakteriše normalan fenotip, međutim često se javlja problem infertiliteta, rekurentnih pobačaja i rođenja deteta sa hromozomskim anomalijama [6].

ETIČKA RAZMATRANJA PGT-a

Kada govorimo o PGT-u, neophodno je detaljno razmotriti etičke i moralne aspekte. Prilikom sprovođenja procesa VTO može se dobiti veći broj embriona, ali embrioni sa genetičkim opterećenjem ostaće neiskorišćeni. Nameće se pitanje: šta se dešava sa tim preostalim embrionima? [18]. Stav koji je trenutno zastupljen u američkom pravosuđu i zdravstvenoj politici je da je odbacivanje embriona na ovom stadijumu daleko više etički opravdan postupak u odnosu na uništavanje fetusa prilikom abortusa [18].

Sa druge strane, postoje izvesna protivljenja PGT-u koja proističu iz istih etičkih razloga kao i protivljenja genskoj terapiji i genetičkom inženjeringu. Selektivna implantacija nedvosmisleno vodi ka sprečavanju postojanja određenih genotipova, čime se ugrožava genetička raznovrsnost i razvija izvesni oblik diskriminacije invaliditeta. Zagovornici ovog stava pozivaju se

The first autosomal dominant diseases to be diagnosed with the PGT-M method were: Marfan syndrome, familial adenomatosis, myotonic dystrophy, and brittle bone disease (lat. *Osteogenesis imperfecta)*. Nowadays, PGT-M is used for diagnosing most autosomal dominant diseases [3].

Some of the autosomal recessive diseases that can be diagnosed with the PGT-M method are the following: cystic fibrosis, sickle cell anemia, Tay-Sachs disease, spinal muscular atrophy, beta thalassemia, adrenogenital syndrome, and hypophosphatemia.

Beta thalassemia is caused by mutation in the beta-globin gene. However, there is a large number of different mutations within the beta-globin gene, especially among different ethnic groups, which additionally complicates PGT-M [3].

Bearing in mind the fact that cystic fibrosis is the most common single gene autosomal recessive disorder in Caucasians, the predominant use of PGT-M is justified, in cases where this disease is suspected. What may impede this practically routine procedure is the existence of 800 different mutations which are linked to the development of this pathological state [3].

Thanks to the application of PGT-M, when there is suspicion of X-linked disorders, it is possible to perform the selection of embryos that are not carriers of the mutation for embryo transfer, regardless of sex – healthy male and female embryos with a normal karyotype [3].

TRINUCLEOTIDE REPEAT DISORDERS

It is possible to diagnose trinucleotide repeat disorders occurring in the presence of dynamic mutations with the application of the PGT-M method.

The number of triplet repeats increases from generation to generation, which results in a more severe clinical presentation and an earlier onset of the disease in the next generation [14].

Huntington's disease and the fragile X syndrome were the first diseases in the group of trinucleotide repeat disorders to be diagnosed with the PGT-M method [3].

There are cut-off values defined for all trinucleotide repeat disorders. PGT-M enables the differentiation of the embryos that will develop disease caused by mutation from the healthy embryos.

CHROMOSOME ABERRATIONS

The third group of diseases diagnosed by PGT are chromosome aberrations. Chromosome aberrations can be numerical and structural.

Numerical autosomal chromosome aberrations are mostly lethal, with the exception of trisomy 13, 18, and 21, while in sex chromosomes some of the aneuploidies are compatible with life (Turner syndrome - 45, XO;

na ismevanje pravog značenja roditeljstva, lišavanja mogućnosti roditelja i dece za lični i moralni rast koji se ostvaruje iskorišćavanjem maksimalnih potencijala onoga što im je priroda podarila [18].

Umanjivanje genetske raznolikosti, kao problem od izuzetnog značaja, izneli su predstavnici osoba sa invaliditetom. Kao argument, navode da složeni skupi i neprirodni postupci za odabir embriona bez nepravilnosti u genomu prenose poruku o postojanju diskriminacije prema osobama sa invaliditetom. Iako se smislenost ove tvrdnje ne može dovesti u pitanje, ne postoji način da se ograniče reproduktivne slobode parova koji žele da smanje rizik od rađanja deteta sa invaliditetom [18].

Sve tehnike, koje su trenutno u upotrebi, razvijene su sa ciljem da favorizuju zdravlje u odnosu na bolest. Mogućnost zloupotrebe ovih tehnika za odabir embriona prema polu ili na osnovu drugih osobina, koje nisu u vezi sa zdravljem, nedvosmisleno postoji. Upravo to je jedan od razloga zabrinutosti bioetičara [18].

Opravdan i prihvatljiv razlog za izbor pola deteta jeste postojanje visokog rizika za razvoj poremećaja koja se nasleđuju X-vezano ili Y-vezano. Selekcija u odnosu na pol, radi uravnoteženja porodice, u smislu broja muške ili ženske dece, ne nailazi na odobravanje [19].

U porodicama u kojima postoji već rođeno dete sa teškim monogenskim oboljenjem ili ako postoji visok rizik za nastanak aneuploidija, korišćenje PGT, u etičkom smislu, nailazi na odobravanje, jer se izbegava abortus ili rana smrt novorođenčeta i omogućava dobijanje zdravog potomstva. Takođe, korišćenje PGT u svrhu donacije stem ćelija ili tkiva bolesnom bratu/sestri, tzv. preimplantaciono tipiziranje tkiva, u većini zemalja Evropske Unije nailazi na odobravanje [20].

Izbor osobina koje se povezuju sa razvojem nekog talenta, određenih fizičkih atributa ili bilo kakvih osobina koje nisu u direktnoj vezi sa zdravljem nailazi na oštre osude [19]. Dejvid King izražava zabrinutost u vezi sa takvim načinom odabira potomstva jer bi on značio determinizam koji je daleko snažniji od samih gena. On smatra da bi se na taj način ugrozio genofond uticajem privremenih kulturoloških koncepata koji imaju za cilj stvaranje savršene jedinke [19].

ZAKLJUČAK

PGT omogućava precizniju selekciju najkvalitetnijih embriona, sa jasnim, moralnim ciljem – rođenje zdravog euploidnog deteta.

Ako imamo u vidu senzitivnost i rezoluciju metode *NGS,* koja se danas koristi kao standard u dijagnostici, jasno je zašto primena *NGS*-a u PGT-u, u odnosu na ranije korišćene metode, značajno povećava stopu uspešnosti začeća u VTO.

Klinefelter syndrome - 47, XXY; trisomy X - 47, XXX; Jacob's syndrome - 47, XYY), and they can all be detected with PGT methods.

Structural chromosome aberrations may be balanced and unbalanced. PGT-SR diagnostics discovers the carriers of balanced chromosomal rearrangements, of which the most common ones in the population are balanced translocations. The carriers are characterized by a normal phenotype, however, the problem of infertility, recurrent miscarriages, and birth of children with chromosomal anomalies often occur [6].

ETHICAL CONSIDERATIONS OF PGT

When speaking of PGT, it is necessary to consider the ethical and moral aspects in detail. In the process of IVF, multiple embryos may be obtained, however, embryos with a genetic load will remain unused. The question remains as to what happens with the residual embryos [18]. The current prevailing attitude within the American judiciary and health policy systems is that discarding embryos at this stadium is far more ethically acceptable than the destruction of the fetus during abortion [18].

On the other hand, there are certain oppositions to PGT, stemming from the same ethical reasons as the opposition to gene therapy and genetic engineering. Selective implantation unequivocally leads to the prevention of the existence of certain genotypes, whereby genetic diversity is jeopardized and a certain type of disability discrimination is developed. The proponents of this attitude suggest that this is a mockery of the true meaning of parenthood, depriving the parents and the children of an opportunity for personal and moral growth, which is achieved through maximal utilization of the potentials provided by nature [18].

Decreasing genetic diversity, as a problem of utmost importance, has been brought forward by representatives of persons with disability. They state, as a key argument, the fact that expensive and unnatural procedures of selecting embryos without abnormalities in the genome, send a message of discrimination towards persons with disabilities. Although the reasonableness of this claim is not to be disputed, there is no way to limit the reproductive liberties of couples who seek to reduce the risk of giving birth to a child with disability [18].

All the techniques currently in use have been developed with the aim of favoring health over disease. The possibility of abusing these techniques for the purpose of choosing the sex of the embryo, or favoring any other trait, which is not connected with health issues, is undoubtedly present. This is the very reason for concern expressed by bioethicians [18].

Dosadašnji podaci iz literature daju nam nedvosmislenu informaciju o napretku metoda koje se koriste u preimplantacionom genetičkom testiranju, ali otvaraju mogućnost za razvoj novih, manje invazivnih i neinvazivnih metoda analize i procene kvaliteta embriona, a sve u cilju dobijanja zdravog potomstva.

Značaj i korisnost razvoja PGT-a nije moguće osporiti. Ipak, u svakom trenutku, treba sagledati što širu sliku, imajući u vidu potencijalnu zloupotrebu do sada razvijenih metoda i svest o etičkim i moralnim ciljevima PGT-a.

SPISAK SKRAĆENICA

CGH – komparativna genomska hibridizacija (engl. *comparative genomic hybridization*)

DNK – dezoksiribonukleinska kiselina

ESHRE – Evropsko udruženje za humanu reprodukciju i embriologiju (engl. *European Society for Human Reproduction and Embryology*)

FISH – fluorescentna *in situ* hibridizacija (engl. *fluorescence in situ hybridization*)

HLA tipizacija – humana leukocitarna antigen tipizacija (engl. *human leukocyte antigen typing*)

ICM – unutrašnja ćelijska masa (engl. *inner cell mass*)

ICSI – intracitoplazmatično injektiranje spermatozoida (engl. *intracytoplasmic sperm injection*)

IVF – *in vitro* fertilizacija

mCGH – metafazna komparativna genomska hibridizacija (engl. *metaphase comparative genomic hybridization*)

mtDNK – mitohondrijalna DNK

NGS – sekvenciranje nove generacije (engl. *next generation sequencing*)

PCR – lančana rekacija polimeraze (engl. *polymerase chain reaction*)

PGD – preimplantaciona genetička dijagnostika

PGS – preimplantacioni genetički skrining

PGT – preimplantaciono genetičko testiranje

PGT-A – preimplantaciono genetičko testiranje na aneuploidije

PGT-SR – preimplantaciono genetičko testiranje na prisustvo strukturnih hromozomskih rearanžmana

PGT-M – preimplantaciono genetičko testiranje na prisustvo monogenskih oboljenja

SNP – polimorfizmi pojedinačnih nukleotida (engl. *single nucleotide polymorphism*)

TE – trofoektoderm

TESE – testikularna ekstrakcija spermatozoida

UPD – uniparentalna dizomija

VTO – vantelesna oplodnja

Sukob interesa: Nije prijavljen.

A justified and acceptable reason for selecting the sex of a child is the existence of a high risk of developing a disorder that is X-linked or Y-linked. Selection of the baby's sex for the purpose of balancing the family ratio of boys and girls has not met with approval [19].

In families where there is already a child with a severe monogenic disorder or in couples at high risk of the occurrence of aneuploidies, the use of PGT, ethically speaking, is being met with approval, as it enables avoiding abortion or early death of the newborn and enables couples to have healthy children. Additionally, the use of PGT for the purpose of donating stem cells or tissue to a sick brother or sister, the so-called preimplantation tissue typing, is also being met with approval in most countries of the European Union [20].

The selection of traits linked to the development of certain talents, certain physical properties, or any other traits that are not directly linked to health, is being met with strong disapproval [19]. David King expresses concern regarding this type of offspring selection, as it would imply a determinism that would be far stronger than the genes themselves. He feels that the gene pool would thereby be jeopardized, since it would be influenced by temporary culturological concepts aimed at producing the perfect human being [19].

CONCLUSION

PGT enables a more precise selection of embryos of the best quality, with a clear moral goal – the birth of a healthy euploid child.

Bearing in mind the sensitivity and the resolution of the NGS method, which is used today as the diagnostic standard, it is clear why the application of NGS in PGT, when compared to methods employed earlier, significantly increases the success rate of conception in IVF.

The data currently available in literature provide us with clear information on the advancement of the methods applied in preimplantation genetic testing, however, they also open the possibility for the development of new, less invasive, and noninvasive methods of analyzing and assessing the quality of embryos, all for the purpose of producing healthy offspring.

The importance and benefit of developing PGT is undeniable. However, we must always bear in mind the broader picture, in order to preempt potential abuse of the methods developed so far, and not lose sight of the ethical and moral goals of PGT.

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LIST OF ACRONYMS AND ABBREVIATIONS

CGH – comparative genomic hybridization
DNA – deoxyribonucleic acid
ESHRE – European Society for Human Reproduction and Embryology
FISH – fluorescence *in situ* hybridization
HLA typing – human leukocyte antigen typing
ICM – inner cell mass
ICSI – intracytoplasmic sperm injection
IVF – in vitro fertilization
mCGH – metaphase comparative genomic hybridization
mtDNA – mitochondrial DNA
NGS – next generation sequencing
PCR – polymerase chain reaction
PGD – preimplantation genetic diagnostics
PGS – preimplantation genetic screening
PGT – preimplantation genetic testing
PGT-A – preimplantation genetic testing for aneuploidies
PGT-SR – preimplantation genetic testing for chromosomal structural rearrangements
PGT-M – preimplantation genetic testing for monogenic disorders (single gene defects)
SNP – single nucleotide polymorphism
TE – trophectoderm
TESE – testicular sperm extraction
UPD – uniparental disomy

Conflict of interest: None declared.

PO IZBORU UREDNIKA

Poštovane koleginice i kolege,

Veliko nam je zadovoljstvo da vam u Srpskom medicinskom časopisu Lekarske komore predstavimo istraživačke radove studenata Medicinskog fakulteta u Beogradu, realizovane na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije.

U ovom broju biće predstavljeno 5 stručnih radova iz oblasti hematologije, koji se bave širokim spektrom dijagnostike i lečenja različitih hematoloških oboljenja.

U radu „Karakteristike bolesnika sa sekundarnom eritrocitozom u odnosu na bolesnike sa policitemijom verom", autora Milice Jeremić i saradnika, prikazana je serija bolesnika, jasno su definisani parametri diferencijalne dijagnoze između dva pomenuta entiteta, i istaknut je značaj ovih parametara u daljem terapijskom pristupu.

Dva rada se bave problematikom akutnih leukemija. Autor Anka Poštić je u radu „Prognostički faktori kod starijih bolesnika sa akutnom mijeloidnom leukemijom" prikazala seriju bolesnika starijih od 65 godina, obolelih od akutne mijeloidne leukemije, i dala analizu faktora koji utiču na preživljavanje.

Rad Mirjane Cvetković „Diseminovana intravaskularna koagulopatija u akutnoj nepromijelocitnoj mijeloidnoj leukemiji – učestalost, kliničko-laboratorijske karakteristike i prognostički značaj" imao je za cilj da prikaže analizu učestalosti diseminovane intravaskularne koagulopatije i njenih kliničko-laboratorijskih karakteristika, kao i da istakne uticaj ovih karakteristika na preživljavanje i ranu smrtnost bolesnika.

Dva rada se bave značajem dijagnostike, profilakse i terapije citomegalovirusne (CMV) infekcije, odnosno gljivičnih infekcija, kod bolesnika u programu alogene transplantacije matičnih ćelija.

Rad Jovane Line Kessler „Citomegalovirusna reaktivacija u procesu alogene transplantacije matičnih ćelija hematopoeze" analizira učestalost CMV reaktivacije, kao i predisponirajuće faktore, te ističe njihov značaj za slabo funkcionisanje transplantata, ali i za njegovo odbacivanje.

Jelena Cakić u radu „Uticaj antigljivične profilakse na pojavu gljivičnih infekcija kod bolesnika u programu alogene transplantacije" analizira problematiku dijagnostike gljivičnih infekcija, akcentuje značaj profilaktične terapije, te analizira značaj prisustva invazivnih gljivičnih infekcija na uspeh same transplantacije.

Dva rada iz oblasti transplantacione medicine daju svoj doprinos analizi parametara koji utiču na uspeh same procedure lečenja.

Nadamo se da će upoznavanje sa navedenim radovima biti od interesovanja za vaš dalji rad.

Srdačan pozdrav,
Prof. dr Milena Todorović Balint

EDITOR'S CHOICE

Dear Colleagues,

It is with great pleasure that we present to you, in the Serbian Journal of the Medical Chamber, research papers written by the students of the Faculty of Medicine in Belgrade, which have been developed at the Clinic for Hematology of the Clinical Center of Serbia.

In this issue of the Journal, five professional papers in the field of hematology will be presented, which deal with a broad spectrum of diagnostics and treatment issues related to different hematological diseases.

The paper *Characteristics of patients with secondary erythrocytosis in relation to patients with polycythemia vera*, written by Milica Jeremić et al., presents a series of patients, clearly defines the parameters of differential diagnosis between the two entities mentioned in the title, and emphasizes the significance of these parameters in further treatment procedure.

Two papers deal with the problem of acute leukemias. In her paper *Prognostic factors in elderly patients with acute myeloid leukemia*, the author, Anka Poštić, presents a series of patients older than 65 years, suffering from acute myeloid leukemia, and offers an analysis of the factors affecting the overall survival of these patients.

The aim of the paper, written by Mirjana Cvetković, *Disseminated intravascular coagulopathy in non-promyelocytic acute myeloid leukemia – incidence, clinical and laboratory features and prognostic significance,* was to analyze the frequency of disseminated intravascular coagulopathy and its clinical and laboratory characteristics, as well as to emphasize the effects of these features on the survival and early mortality of patients.

Two papers deal with the significance of diagnostics, prophylaxis, and therapy in cytomegaloviral (CMV) infections, i.e., fungal infections, in patients submitted to the program of allogenic stem-cell transplantation.

The paper written by Jovana Lina Kessler *Cytomegalovirus reactivation in patients treated with allogeneic hematopoietic stem cell transplantation* analyzes the frequency of CMV reactivation, as well as the predisposing factors, and also emphasizes their importance in the poor functioning and the rejection of grafts.

In her paper *Influence of antifungal prophylaxis on the occurrence of fungal infections in patients undergoing allogenic transplantation,* Jelena Cakić analyzes the issue of diagnostics in fungal infections, emphasizes the importance of prophylaxis, and analyzes the significance of the presence of invasive fungal infections for the success of transplantation.

These two papers related to the field of transplantation medicine contribute to the analysis of parameters influencing the success of this treatment procedure.

We hope that you will find these papers to be of interest for your future work.

Sincerely, Professor Milena Todorović Balint, PhD

PROGNOSTIČKI FAKTORI KOD STARIJIH BOLESNIKA SA AKUTNOM MIJELOIDNOM LEUKEMIJOM

ORIGINALNI RAD

ORIGINAL ARTICLE

PROGNOSTIC FACTORS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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SAŽETAK

Uvod: Akutna mijeloidna leukemija (AML) predstavlja patološku proliferaciju ćelija mijeloidne loze. Predominantno se javlja kod pacijenata starijih od 60 godina, sa značajno lošijim ishodom lečenja u poređenju sa mlađima.

Cilj: Cilj rada bio je da se izvrši analiza kliničkih karakteristika starijih bolesnika sa AML-om, kao i uticaj tih karakteristika na: postizanje kompletne remisije (KR), ukupno preživljavanje (engl. *overall survival – OS*), ranu smrtnost (engl. *early mortality – EM*), i recidiv bolesti.

Materijal i metode: Ovo je retrospektivna studija koja je obuhvatila 94 pacijenta sa AML-om, nakon primene hemioterapije i palijativne terapije, čiji podaci su preuzeti iz istorija bolesti. Ispitivani faktori rizika za *OS*, KR, recidiv bolesti i *EM* bili su: leukociti, nivo serumske laktat dehidrogenaze (LDH), opšte funkcionalno stanje prema *ECOG (Eastern Cooperative Oncology Group) skali, European LeukemiaNet* togenetska grupa rizika, komorbiditetni indeks- *HCT-CI* (*hematopoietic cell transplantation - comorbidity index*) i *NPM1/FLT3-ITD* (engl. *nucleophosmin 1/FLT3-internal tandem mutation*) molekularni status. Za identifikaciju prognostičkih faktora korišćena je Koksova regresiona analiza.

Rezultati: Prosečna starost pacijenata iznosila je 69 godina (opseg: 65 – 87). KR je postiglo 23 (46%) od 50 pacijenata (53,2%) koji su primili intenzivnu hemioterapiju, pri čemu je do relapsa došlo kod 17/23 pacijenata (73,9%). *EM* je zabeležena kod 17 pacijenata (18,1%). Pacijenti sa *ECOG PS* $\geq$ 2 imali su statistički značajno lošije *OS* u odnosu na pacijente sa *ECOG PS* < 2 ($p = 0{,}030$). Pacijenti sa *HCT-CI* $\geq$ 3 imali su lošiji *OS* u odnosu na pacijente sa *HCT-CI* < 3 ($p = 0{,}040$). Nivo LDH $\geq$ 450 U/l pokazao se kao loš prognostički faktor za *OS* u odnosu na LDH < 450 U/l ($p = 0{,}044$).

Zaključak: Zaključak je da stariji pacijenti sa AML-om, koji imaju lošije funkcionalno opšte stanje po *ECOG* skali, visok *HCT-CI* i povećan nivo LDH, imaju lošije *OS*.

Ključne reči: akutna mijeloidna leukemija, stariji pacijenti, ukupno preživljavanje, prognostički faktori

ABSTRACT

Introduction: Acute myeloid leukemia (AML) is characterized by pathological proliferation of myeloid lineages. It predominantly occurs in patients over 60 years of age, whose outcome is considerably worse, as compared to younger patients.

Aim: The aim of the study was the analysis of the clinical characteristics of older patients with AML and their impact on the following: achieving complete remission (CR), overall survival (OS), early mortality (EM), and relapse.

Materials and methods: This retrospective study included 94 patients with AML, treated with chemotherapy and palliative treatment, whose information was taken from their medical histories, upon treatment. The following clinical features were analyzed as risk factors for OS, CR, relapse and EM: leukocytes, the level of serum lactate dehydrogenase (LDH), performance status on the ECOG (Eastern Cooperative Oncology Group) scale, the European LeukemiaNet cytoplasmic risk group, the HCT-CI (hematopoietic cell transplantation - comorbidity index) and the NPM1/FLT3-ITD (nucleophosmin 1/FLT3-internal tandem mutation) molecular status. For the identification of prognostic factors, the Cox regression analysis was used.

Results: The average age of the patients was 69 years (range: 65 – 87). CR was achieved in 23 (46%) of the 50 patients (53.2%) who received intensive chemotherapy, with relapse occurring in 17/23 patients (73.9%). EM was reported in 17 patients (18.1%). Patients with ECOG PS $\geq$ 2 had a statistically significantly lower OS than patients with ECOG PS < 2 ($p = 0.030$). Patients with HCT-CI $\geq$ 3 had a poorer OS than patients with HCT-CI < 3 ($p = 0.040$). Serum LDH $\geq$ 450 U/l was found to be a factor, i.e., marker of unfavorable prognosis for the OS, as compared to LDH < 450U/l ($p = 0.044$).

Conclusion: The conclusion is that older AML patients with poorer ECOG PS, high HCT-CI, increased LDH levels have a poorer OS.

Key words: acute myeloid leukemia, elderly patients, overall survival, prognostic factors

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UVOD

Akutne leukemije (AL) su maligne klonalne bolesti matične ćelije hematopoeze koje nastaju usled poremećaja genoma matične ćelije sa posledičnom nekontrolisanom proliferacijom i infiltracijom različitih tkiva [1].

Akutna mijeloidna leukemija (AML) odlikuje se patološkom proliferacijom ćelija mijeloidne loze [1]. AML je bolest koja se predominantno javlja kod pacijenata starijih od 60 godina, odnosno pacijenata starije životne dobi [2-4]. Incidencija AML-a kod pacijenata starijih od 75 godina iznosi preko 15 na 100.000 stanovnika, dok incidencija kod pacijenata mlađih od 40 godina iznosi oko 4 na 100.000 stanovnika [5,6].

Ishod lečenja AML-a je značajno lošiji kod starijih pacijenata, u odnosu na pacijente mlađe od 60 godina [7,8]. Loš ishod kod starijih pacijenata povezan je sa mnogobrojnim faktorima, koji mogu biti vezani za samu bolest – AML, ili se odnose na samog pacijenta [8]. Faktori, za koje se smatra da utiču na loš ishod terapije AML-a, kod starijih, a odnose se na same pacijente su: komorbiditeti, farmakodinamske osobine, smanjenje funkcije organa usled starosti, slabiji odgovor na sistemske bakterijske i gljivične infekcije usled slabije funkcije imunog sistema, kao i lošije opšte funkcionalno stanje procenjeno prema *ECOG* (*Eastern Cooperative Oncology Group*) skali. Sa druge strane, loši prognostički faktori kod akutne mijelodine leukemije, kod starijih pacijenata, koji se odnose na samu bolest, jesu: veća zastupljenost sekundarnih AML-a i AML-a nastalih posle primene citotoksične terapije, kao i nepovoljniji genetsko-mutacioni profil, što je sve povezano sa rezistencijom na lečenje [7-9,11].

Takođe, stariji pacijenti lošije tolerišu standardnu intenzivnu terapiju, zbog čega se često pribegava primeni manje intenzivne ili palijativne terapije [10-12]. Rana smrtnost u toku standardne intenzivne terapije jeste jedan od razloga lošeg ishoda lečenja starijih pacijenata ovim vidom terapije [11].

Međutim, rezultati koji pokazuju prognozu starijih pacijenata sa AML-om u mnogim dostupnim studijama, nisu u tolikoj meri zastupljeni, zbog selektivne pristrasnosti većine studija da izbacuju veoma stare i visoko rizične pacijente [8,11].

Cilj ovog rada bila je analiza kliničkih karakteristika kod starijih bolesnika sa AML-om i analiza njihovog uticaja na: postizanje kompletne remisije (KR), ukupno preživljavanje, ranu smrt, i recidiv bolesti.

MATERIJALI I METODE

Istraživanje je sprovedeno u vidu retrospektivne studije na osnovu baze podataka Klinike za hematologiju, Kliničkog Centra Srbije, a uključilo je 94 pacijenta sa AML-om, starijih od 65 godina, koji su

INTRODUCTION

Acute leukemias (AL) are malignant clonal diseases of the hematopoietic stem cell, occurring due to anomaly in the stem cell genome, and resulting in uncontrolled proliferation and infiltration of different tissues [1].

Acute myeloid leukemia (AML) is characterized by pathological proliferation of the cells of the myeloid lineage [1]. AML is a disease predominantly affecting patients above the age of 60 years, i.e., elderly patients [2-4]. The incidence of AML in patients older than 75 years is more than 15 per 100,000 population, while the incidence of this disease in patients younger than 40 years is approximately 4 per 100,000 population [5,6].

The outcome of AML treatment is significantly poorer in elderly patients, as compared to patients younger than 60 years [7,8]. An unfavorable outcome in elderly patients is connected to numerous factors, which can be connected to AML itself, or related to the individual characteristics of the patient [8]. The factors believed to affect the unfavorable outcome of AML treatment in elderly patients, which are related to the individual characteristics of the patients, are the following: existing comorbidities, pharmacodynamic features, organ function weakening due to old age, weaker response to systemic bacterial and fungal infections - as a result of the weakened function of the immune system, as well as poorer general performance status assessed with the ECOG (Eastern Cooperative Oncology Group) scale. On the other hand, markers, i.e., factors predicting an unfavorable outcome in acute myeloid leukemia in elderly patients, which are related to the disease itself, are the following: higher incidence of secondary AML and AML developing after the application of cytotoxic therapy, as well as a less favorable genetic mutation profile, which is all related to resistance to treatment [7-9,11].

Also, elderly patients are worse at tolerating standard intensive treatment, which is why less intensive and palliative treatment are frequently resorted to [10-12]. Early mortality during standard intensive treatment is one of the reasons of unfavorable outcome in the treatment of elderly patients with this form of therapy [11].

However, results showing the prognosis of elderly patients with AML in many available studies, are not frequently presented, due to selection bias in most of these studies, wherein very old and high-risk patients are excluded from the analysis [8,11].

The aim of the study was the analysis of the clinical characteristics of older patients with AML, and their impact on the following: achieving complete remission (CR), overall survival (OS), early mortality (EM), and relapse.

dijagnostikovani i lečeni u periodu od novembra 2013. do novembra 2018.

Kod bolesnika su, pri dijagnozi, evidentirane demografske i kliničko-laboratorijske karakteristike: pol, starost, opšte funkcionalno stanje prema *ECOG* skali [13], kompletna krvna slika (hemoglobin, broj leukocita, trombocita, leukocitarna formula), nivo serumske laktat dehidrogenaze (LDH), procenat blasta u perifernoj krvi i koštanoj srži. Procena komorbiditeta je vršena na osnovu komorbiditetnog indeksa (engl. *hematopoietic cell transplantation- comorbidity index* (*HCT-CI*)) koji se koristi za transplantaciju matičnih ćelija hematopoeze [14]. Citogenetski stepen rizika određen je prema preporukama međunarodnog ekspertskog panela za leukemiju, izrađenih za *European LeukemiaNet* (*ELN*) [15].

U okviru hematološke dijagnostike učinjena je:

1. Citološka analiza – obavljena je na razmazima koji su bojeni Mej-Grinvald- Gimza (MGG) metodom, uz dopunska bojenja (*MPO, SBB, PAS, NSE*);
2. Imunofenotipizacija protočnom citometrijom, metodom direktne višekolorne imunofluorescencije [17];
3. Klasična citogenetska analiza, metodom HG traka, prema Međunarodnom sistemu za humanu citogenetsku nomenklaturu [18];
4. Molekularno-genetska istraživanja – testirana je koštana srž bolesnika na prisustvo genskih mutacija – *nucleophosmin/FLT3-internal tandem mutations* (*NPM1/FLT3-ITD*).

Pacijenti su lečeni hemioterapijskim protokolima za bolesnike starije od 60 godina, u skladu sa *ELN* preporukama [15]. U zavisnosti od *ECOG* i *HCT-CI,* primenjivana je intenzivna hemioterapija, terapija niskog intenziteta ili palijativna terapija. Hemioterapiju visokog intenziteta primili su pacijenti koji su imali *ECOG* ≤ *2, HCT-CI* < *3* po šemi '*3+7 light*', u sastavu: daunorubicin – u dozi od 45 mg/m^2 na dan 1, 2, 3, u kombinaciji sa citarabinom – u dozi od 100 mg/m^2 dnevno, kontinuirano intravenskom (iv) infuzijom, 7 dana. Pacijenti koji su imali *ECOG* > *2, HCT-CI* ≥ *3,* lečeni su po šemi '2+5', u sastavu: daunorubicin – u dozi 30 mg/m^2 iv D 1, 3, i citarabin – u dozi 100 mg/m^2 iv kontinuirano D 1-5.

Hemioterapija niskog inteziteta primenjena je kod pacijenta sa *ECOG* > 2, *HCT-CI* ≥ 3, koji nisu bilo nepovoljnog citogenetskog rizika prema *ELN* klasifikaciji. Podrazumevala je primenu niskih doza citarabina (20 mg, s.c., na 12 h, D 1-10), i monoterapiju vepezidom amp. 100 mg D 1-5. Palijativna terapija se sastojala iz primene citoreduktivne terapije (Litalir kapsule) i suportivne terapije, u vidu primene transfuzije derivata krvi. Primenjena je kod pacijenta koji nisu mogli da tolerišu nikakvu antileukemijsku terapiju, ili nisu želeli da se leče.

MATERIALS AND METHODS

This is a retrospective study carried out on the basis of the database of the Clinic for Hematology of the Clinical Center of Serbia, which included 94 patients with AML, older than 65 years, who were diagnosed and treated in the period between November 2013 and November 2018.

The following demographic, clinical and laboratory characteristic of the patients were recorded at diagnosis: sex, age, general performance status according to the ECOG scale [13], complete blood count (hemoglobin, white blood cell count, platelet count, WBC differential), level of serum lactate dehydrogenase (LDH), and the percentage of blasts in peripheral blood and bone marrow. The assessment of comorbidities was performed on the basis of the hematopoietic cell transplantation-comorbidity index (HCT-CI), used in hematopoietic stem-cell transplantation [14]. The cytogenetic risk level was determined on the basis of the recommendations of an international leukemia expert panel, given on behalf of European LeukemiaNet (ELN) [15].

The following was performed as part of the hematological diagnostics:

1. Cytological analysis – performed on smears stained with the use of the May- Grünwald Giemsa (MGG) method, with additional staining (MPO, SBB, PAS, NSE);
2. Immunophenotypization by means of flow cytometry with the use of the direct multicolor immunofluorescence method [17];
3. Classical cytogenetic analysis with the application of the HG-banding technique, in keeping with the International System for Human Cytogenetic Nomenclature [18];
4. Molecular genetic research - patient bone marrow was tested for the presence of genetic mutations - nucleophosmin/FLT3-internal tandem mutations (NPM1/FLT3-ITD).

Patients were treated with chemotherapeutic protocols for patients older than 60 years, in keeping with ELN recommendations [15]. Depending on the ECOG and HCT-CI, intensive chemotherapy, low-intensity therapy, or palliative treatment were applied. Patients with ECOG ≤ 2, HCT-CI < 3 received high-intensity chemotherapy, following the '3+7 light' regimen; the therapy included: daunorubicin - in the dosage of 45 mg/m^2 per day 1, 2, 3 in combination with cytarabine – in the dosage of 100 mg/m^2 per day, continuously via iv infusion, for 7 days. Patients with ECOG > 2, HCT-CI ≥ 3, were treated under the '2+5' regimen, which included: daunorubicin – in the dosage of 30 mg/m^2, iv, D 1, 3, and cytarabine – in the dosage of 100 mg/m^2, iv, continuously, D 1-5.

Procena efikasnosti lečenja sprovođena je na kraju indukcionog lečenja prema opšte prihvaćenim kliničkim kriterijumima Međunarodne radne grupe za AML [19]. Pod refraktornom bolešću podrazumeva se nepostizanje KR, za pacijente koji su preživeli ≥ 7 dana od završetka indukcije. Ukupno preživljavanje (engl. *overall survival – OS*) je definisano kao vreme proteklo od dijagnoze do smrti ili datuma poslednjeg praćenja. Rana smrt je definisana kao smrt u periodu od 28 dana od otpočinjanja indukcione hemioterapije [20].

Statistička analiza rađena je pomoću podataka iz otpusnih lista uzetih iz Registara Klinike za hematologiju, Kliničkog centra Srbije, korišćenjem programa *Microsoft Excel*. Zavisno od tipa varijabli i normalnosti raspodele, deskripcija podataka prikazana je kao n (%) ili medijana (opseg, min-max). Za pronalaženje nezavisnog prediktora smrtnog ishoda kod starijih bolesnika sa AML-om primenjen je univarijantni Koksov regresioni model sa 95%-tnim intervalom poverenja. Statističke hipoteze su testirane na nivou statističke značajnosti (alfa nivo) od 0,05.

REZULTATI

Istraživanjem je obuhvaćeno 94 pacijenta, od toga 56 muškaraca (59,6%) i 38 žena (40,4%). Prosečna starost pacijenata iznosila je 69 godina (opseg: 65 – 87 godina). Starijih od 70 godina bilo je 36/94 pacijenata (38,3%), dok je najveći broj, 58 bolesnika (61,7%), bio starosti između 65 i 70 godina.

Pri dijagnozi, veći broj bolesnika, njih 49 (53,3%), bio je dobrog opšteg funkcionalnog stanja, *ECOG* skor < 2, dok je 43 bolesnika (46,81%) imalo *ECOG* skor ≥ 2.

Visok *HCT-CI* skor ≥ 3, pri dijagnozi je imalo 26 pacijenata (28,6%), dok je 65 bolesnika (71,4%) imalo *HCT-CI* skor < 3.

Prema *ELN* citogenetsko-molekularnoj klasifikaciji stepena rizika, 5 pacijenata (5,3%) imalo je povoljan, 58 pacijenata (61,7%) intermedijarni, i 31 pacijent (33%) nepovoljni rizik.

Prema vrednostima LDH u serumu, pacijenti su klasifikovani u grupu sa vrednostima LDH < 450 U/l, kojih je bilo 56 (60,9%), i vrednostima LDH ≥ 450 U/l, kojih je bilo 36 (39,1%).

Pacijenata sa brojem leukocita Le < $30x10^9$/l bilo je 66 (71%), dok je pacijenata sa vrednostima Le ≥ $30x10^9$/l bilo 27 (29%).

Pacijenta sa *de novo* AML-om je bilo 81 (86,2%), dok je slučajeva sekundarnih AML-a, kao transformacija druge hematološke bolesti, bilo 13 (13,8%).

NPM1/FLT3-ITD status određen je kod 19/94 pacijenata, od kojih je sa *NPM1-/FLT3-ITD-* statusom bilo 15 pacijenata (78,9%), a *NPM1+/FLT3-ITD-* status je imalo 4 pacijenta (21,1%).

Low-intensity chemotherapy was applied in patients with ECOG > 2, HCT-CI ≥ 3, whose cytogenetic risk, according to the ELN classification, was not unfavorable. This therapy involved the administration of low doses of cytarabine (20 mg, SC, per 12 h, D 1-10), and monotherapy with VePesid infusion vials, 100 mg, D 1-5. Palliative treatment included administering cytoreductive therapy (Litalir capsules) and supportive therapy, in the form of the transfusion of blood products. It was applied in patients who could not tolerate any kind of antileukemia therapy, or patients who refused such treatment.

The assessment of treatment efficacy was made at the end of induction treatment, in keeping with the widely accepted clinical criteria of the International Working Group for AML [19]. The disease is considered refractory if CR has not been achieved, in patients who survived ≥ 7 days after the completion of induction therapy. Overall survival (OS) is defined as the time that has elapsed from diagnosis until death or until the day of the last follow-up. Early mortality is defined as death within 28 days of the initiation of induction chemotherapy [20].

Statistical analysis was performed on the basis of data found in patient discharge papers taken from the Records of the Clinic for Hematology of the Clinical Center of Serbia, with the use of Microsoft Excel. Depending on the types of variables and the normality of distribution, the description of data is presented as n (%) or the median value (range, min-max). The univariate Cox regression model with a 95% confidence interval was used for finding the independent predictor of mortality in elderly patients with AML. Statistical hypotheses were tested at the level of statistical significance (alpha level) of 0.05.

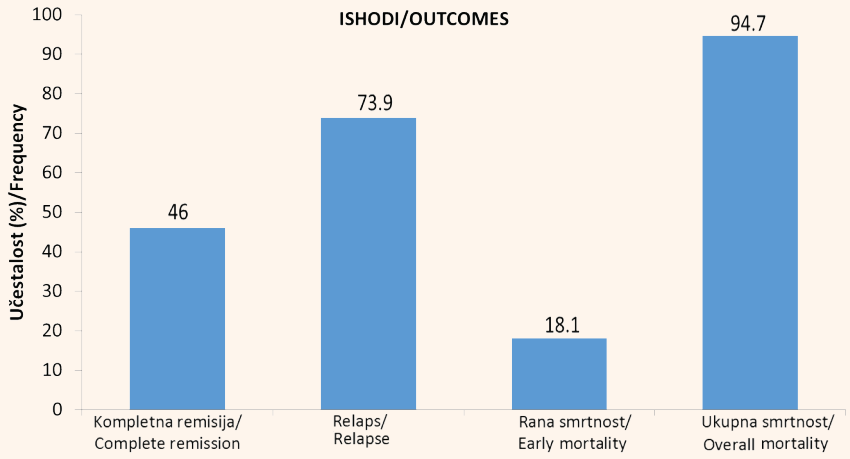
RESULTS

The study included 94 patients, 56 men (59.6%) and 38 women (40.4%). The average age of the patients was 69 years (range: 65 – 87 years). There were 36/94 patients older than 70 years (38.3%), while the largest number of patients, 58 (61.7%), were between 65 and 70 years old.

More patients, 49 of them (53.3%), had a good performance status, and their ECOG score was < 2, while 43 patients (46.81%) had an ECOG score ≥ 2.

At diagnosis, 26 patients (28.6%) had a high HCT-CI score ≥ 3, while 65 patients (71.4%) had an HCT-CI score < 3.

According to the ELN cytogenetic and molecular risk level classification, 5 patients (5.3%) had a favorable, 58 patients (61.7%) had an intermediate, and 31 patients (33%) had an unfavorable level of risk.



Grafikon 1. Učestalost ishoda pacijenata obuhvaćenih studijom

Figure 1. Frequency of outcomes in patients included in the study.

Procenat blasta u perifernoj krvi (PK) < 50% imalo je 73 pacijenta (80,2%), dok je procenat blasta u PK ≥ 50% imalo 18 pacijenata (19,8%). Procenat blasta u koštanoj srži < 50% imalo je 43 pacijenta (46,7%), dok je procenat blasta u koštanoj srži ≥ 50% imalo 49 pacijenata (53,3%).

Od ukupno 50 pacijenata (53,2%) u studiji, koji su primili intenzivnu hemioterapiju, kompletnu remisiju (KR) postiglo je 23 pacijenta (46%).

Tabela 1. Uticaj prognostičkih faktora na *OS*

Table 1. Influence of prognostic factors on OS

Prediktor OS / *Predictor of OS*	***HR***	**95%*CI HR***	**p**
Godine starosti, > 70 / *Age, > 70*	0.929	0.61 – 1.42	0.734
Pol, ženski / *Sex, female*	1.270	0.83 – 1.95	0.273
ECOG PS ≥ 2	**1.607**	**1.05 – 2.46**	**0.030**
HCT-CI ≥ 3	**1.638**	**1.02 – 2.63**	**0.040**
Stepen rizika, nepovoljan / *Degree of risk, unfavorable*	1.433	0.98 – 2.10	0.065
LDH ≥ 450U/L / *LDH ≥ 450U/L*	**1.552**	**1.01 – 2.38**	**0.044**
Le ≥ 30x109/L / *Le ≥ 30x109/L*	1.425	0.90 – 2.26	0.132
NPM1/FLT3 status, *NPM1-/FLT3ITD+* / *NPM1/FLT3 status, NPM1-/FLT3ITD+*	1.031	0.70 – 1.51	0.874
Status bolesti, sekundarna / *Disease status, secondary*	1.472	0.81 – 2.67	0.204
% blasta u PK ≥ 50 / *% of blasts in PB ≥ 50*	1.319	0.78 – 2.22	0.299
% blasta u KS ≥ 50/ / *% of blasts in BM ≥ 50*	1.189	0.78 – 1.82	0.423

OS – Ukupno preživljavanje (engl. *overall survival*)
HR – Odnos hazarda (engl. *hazard ratio*)
CI – Interval poverenja (engl. *confidence interval*)
HCT-CI – Indeks komorbiditeta (engl. *hematopoetic cell transplantation-comorbidity index*)
ECOG PS – Opšte funkcionalno stanje prema: *Eastern Cooperative Oncology Group Performance Score*
LDH- Laktat dehidrogenaza / *Lactate Dehydrogenase*
PK- Periferna krv / *Peripheral blood*
KS- Koštana srž / *Bone marrow*

Patients were classified, according to the level of serum LDH, in the LDH < 450 U/l group, with 56 patients (60.9%), and the LDH ≥ 450 U/l group, with 36 patients (39.1%).

There were 66 patients (71%) whose WBC count (Le count) was Le < 30x109/l, while 27 patients (29%) had a leukocyte count of Le > 30x109/l.

There were 81 (86.2%) patients with *de novo* AML, while 13 cases (13.8%) were secondary AML, occurring as a transformation of a different hematological disease.

NPM1/FLT3-ITD status was determined in 19/94 patients, of whom 15 patients (78.9%) had an NPM1-/FLT3-ITD- status, while 4 patients (21.1%) had an NPM1+/FLT3-ITD- status.

The percentage of blasts in peripheral blood (PB) < 50% was present in 73 patients (80.2%), while the percentage of blasts in PB > 50% was present in 18 patients (19.8%). The percentage of blasts in bone marrow < 50% was present in 43 patients (46.7%), while the percentage of blasts in bone marrow > 50% was present in 49 patients (53.3%).

Of a total of 50 patients (53.2%) in the study who had received intensive chemotherapy, complete remission (CR) was achieved by 23 patients (46%).

Relapse occurred in 17 patients (73.9%) who had achieved CR. The median duration of CR was 7 months (range: 1 – 24).

Early mortality was registered in 17 patients (18.1%) involved in the study.

The median overall survival of AML-NK patients was three months (0.1 – 38 months), two-year OS was 5.3%, while median overall survival without signs of illness was 8 months. Throughout the duration of the study a total of 89 patients (94.7%) died (Figure 1).

Univariate Cox analysis revealed factors significant for patient OS, namely: ECOG PS, HCT-CI, and LDH. Patients with ECOG PS > 2 had statistically significantly

Do relapsa je došlo kod 17 pacijenata (73,9%) koji su postigli KR. Medijana trajanja KR je bila 7 meseci (opseg: 1 – 24).

Rana smrtnost registrovana je kod 17 pacijenata (18,1%) u studiji.

Medijana preživljavanja pacijenata obolelih od AML-NK-a je bila tri meseca (0,1 – 38 meseci), dvogodišnji *OS* je bio 5,3%, dok je medijana preživljavanja bez znakova bolesti bila 8 meseci. Tokom studijskog perioda umrlo je ukupno 89 bolesnika (94,7%) (Grafikon 1).

Univarijantna Koksova analiza je pokazala faktore značajne za *OS* pacijenata, i to su: *ECOG PS, HCT-CI* i LDH. Pacijenti sa *ECOG PS* $\geq$ 2 imali su statistički značajno lošije *OS* u odnosu na pacijente sa *ECOG PS* < 2 (p = 0,030). Takođe, pacijenti iz grupe sa *HCT-CI* $\geq$3 imali su lošije *OS* u odnosu na pacijente iz grupe sa *HCT-CI* < 3 (p = 0,040). Serumski nivo LDH $\geq$ 450 U/l pokazao se kao loš prognostički faktor za *OS* u odnosu na LDH < 450 U/l (p = 0,044) (Tabela 1).

Nijedan od navedenih parametara nije bio statistički značajan prediktor postizanja KR, pojave recidiva i rane smrtnosti (p > 0,05).

DISKUSIJA

Studije koje ispituju uticaj prognostičkih faktora kod starijih bolesnika imaju stroge kriterijume za uključivanje pacijenata, tako da mali broj pacijenata sa velikim brojem mogućih udruženih faktora rizika biva uključen u te studije [8,11]. Takođe, stariji pacijenti oboleli od akutne mijeloidne leukemije, koji su uključivani u studije ovog tipa zbog brzog toka bolesti i značajnih komorbiditeta, su uglavnom imali smrtni ishod pre procene odgovora na terapiju [7,11,21]. U našoj studiji smrtni ishod imalo je 89 pacijenata (94,7%), a povoljan 5 pacijenata (5,3%), što je u saglasnosti sa podacima iz literature [24].

Bolest se približno jednako javlja i kod muškaraca i kod žena (59,6% naspram 40,4%), kako u našoj studiji, tako i u literaturi [9]. Uticaj pola, kao faktora koji utiče na *OS*, KR, ranu smrtnost, ili pojavu relapsa, nije dokazan.

Prosečna starost pacijenata uključenih u našu studiju iznosila je 69 godina (opseg: 65 – 87). Najviše pacijenata bilo je u grupi starosti od 65 do 70 godina (61,7%).

U prospektivnoj AML96 studiji, koja je obuhvatila 909 pacijenata obolelih od akutne mijeloidne leukemije, starosti 61 – 87 godina, prosečna starost obolelih je iznosila 67 godina [9]. Starost od preko 70 godina, kao pojedinačni prognostički faktor u ishodu AML-a, nije dokazan kao statistički značajan parametar (p = 0,734) u ovoj studiji, dok podaci u literaturi govore suprotno [8]. U AML96 studiji, dokazano je da starost od preko 65 godina ima statistički značajan uticaj na kraće *OS* [9].

lower OS, as compared to patients with ECOG PS < 2 (p = 0.030). Also, patients from the HCT-CI >3 group had a lower OS, as compared to patients from the HCT-CI < 3 group (p = 0.040). The serum level of LDH > 450 U/l proved to be a marker of unfavorable prognosis for OS, as compared to LDH < 450 U/l (p = 0.044) (Table 1).

None of the abovementioned parameters was a statistically significant predictor for the following: achieving CR, the occurrence of relapse, early mortality (p > 0.05).

DISCUSSION

Studies analyzing the effect of prognostic factors, i.e., markers in elderly patients have strict inclusion criteria, which is why a small number of patients with a large number of possible associated risk factors are included in these studies [8,11]. Also, elderly patients suffering from acute myeloid leukemia, included in this type of study, usually had a lethal outcome before assessment on the response to treatment could be made, due to a rapid progression of the illness and significant comorbidities [7,11,21]. In our study, 89 patients (94.7%) had a lethal outcome, while 5 patients (5.3%) had a favorable outcome, which is in keeping with the data available in literature [24].

The disease occurs approximately equally in both men and women (59.6% versus 40.4%), not only in our study, but in literature as well [9]. The effect of the sex of the patient, as a factor influencing OS, CR, early mortality, or relapse of the illness, has not been proven.

The average age of the patients included in our study was 69 years (range: 65 – 87). Most of the patients belonged to the 65 – 75 age group (61.7%).

In the prospective AML96 study, which included 909 patients suffering from acute myeloid leukemia, aged 61 – 87 years, the average age was 67 years [9]. Age above 70 years, as an individual prognostic factor in the outcome of AML, was not proven as a statistically significant parameter (p = 0.734) in this study, while data from literature state the opposite [8]. The AML96 study demonstrated that age above 65 years had a statistically significant effect on shorter OS [9].

The average age of the patients with AML included in a multicentric Italian study, which covered 1,005 patients, was 69 years. The study analyzed the effect of intensive and non-intensive treatment on the overall survival of elderly patients and did not demonstrate better survival in patients treated with intensive chemotherapy. A smaller number of patients older than 70 years, as compared to younger patients, received intensive therapy, which lead to an unfavorable outcome [8].

In other studies, which analyzed the prognostic markers related to the outcome of AML in elderly patients,

Prosečna starost pacijenata sa AML-om uključenih u multicentričnu italijansku studiju koja je obuhvatila 1.005 pacijenata, bila je 69 godina. Studija je analizirala uticaj intenzivne i ne-intenzivne terapije na preživljavanje starijih pacijenata, i nije pokazala da su bolje preživljavanje imali pacijenti koji su lečeni intenzivnom hemioterapijom. Manji broj pacijenata starijih od 70 godina je, u odnosu na mlađe pacijente, primio intenzivnu terapiju, što je dovelo do lošeg ishoda [8].

U drugim studijama koje su se bavile analizom prognostičkih faktora na ishod AML-a kod starijih pacijenata, dokazan je uticaj nepovoljnog *ECOG PS* na veću smrtnost, postizanje KR i kraće *OS*, kod pacijenata starijih od 65 godina [7,11]. U našoj studiji je pokazano da je *ECOG PS* imao statistički značajan uticaj na pojavu rane smrti (p = 0,030). Uticaj *ECOG PS* na postizanje KR, ranu smrtnost i pojavu relapsa nije dokazan u našoj studiji. U studiji *Southwestern Oncology Group* (*SWOG*) u kojoj su analizirani pacijenti starosti ≥ 56 godina, pokazan je značajan uticaj nepovoljnog *ECOG PS* na loš ishod AML-a [23].

Takođe, rezultati dobijeni analizom uticaja *HCT-CI* na *OS* pokazali su statističku značajnost u našoj studiji (p = 0,040), što je u skladu sa rezultatima drugih studija [10,21,25,26]. U studiji sprovedenoj na Klinici za hematologiju Kliničkog centra Srbije, 2011. godine, koja se bavila ispitivanjem *HCT-CI* kao prognostičkog faktora za *OS* i koja je pomagala u donošenju odluke o primeni intenzivne terapije kod starijih pacijenata sa AML-om, dokazana je statistički značajna povezanost između *HCT-CI* ≥ 3 i *OS* [7]. Međutim, uticaj *HCT-CI* na postizanje KR, ranu smrtnost i pojavu relapsa, u našoj studiji, nije dokazan.

Povišene vrednosti LDH (LDH ≥ 450 U/l) su se pokazale kao značajan prognostički faktor *OS* (p = 0,044) u našoj studiji, što je u skladu sa podacima iz ranije objavljenih studija [6,21,25]. Uticaj povišene vrednosti LDH u serumu na postizanje KR, ranu smrtnost i pojavu relapsa, nismo dokazali. U prospektivnoj AML96 studiji, koja je obuhvatila 909 pacijenata obolelih od AML-a, vrednosti LDH ≥ 700 U/l pokazale su značajan uticaj na kraće *OS* [9]. Takođe, u studiji sprovedenoj na Klinici za hematologiju Kliničkog centra Srbije, 2011. godine, koja se bavila ispitivanjem komorbiditeta kao prognostičkog faktora za *OS* obolelih od AML-a, pokazan je nepovoljan uticaj povišenih vrednosti LDH kako na *OS*, tako i na KR [7].

Pacijenti sa intermedijarnim i nepovoljnim rizikom, prema *ELN* klasifikaciji, bili su zastupljeniji u našoj studiji, u odnosu na pacijente sa povoljnim rizikom (61,7% i 33% naspram 5,3%), što je u skladu sa literaturnim podacima [7]. Međutim, u ovoj studiji nije pokazana statistička povezanost nepovoljnog stepena rizika prema *ELN*-u sa lošijim vrednostima *OS* i KR, češćom pojavom relapsa i češćom ranom smrtnošću, što se može

the effect of unfavorable ECOG PS on higher mortality, achieving CR and shorter OS, in patients older than 65 years, has been proven [7,11]. Our study showed that ECOG PS had a statistically significant effect on the occurrence of early mortality (p = 0.030). The effect of ECOG PS on the following: achieving CR, early mortality, and the occurrence of relapse, was not proven in our study. In the study carried out by the Southwestern Oncology Group (SWOG), where patients aged ≥ 56 years were analyzed, a significant effect of unfavorable ECOG PS on unfavorable outcome of AML was demonstrated [23].

Also, the results obtained from the analysis of the effect of HCT-CI on OS showed statistical significance in our study (p = 0.040), which is in keeping with the results of other studies [10,21,25,26]. In a study carried out at the Clinic for Hematology of the Clinical Center of Serbia, in 2011, which analyzed HCT-CI as a prognostic factor of OS, and which assisted in making decisions on the application of intensive therapy in elderly patients with AML, a statistically significant connection between HCT-CI ≥ 3 and OS was proven [7]. However, the effect of HCT-CI on the following: achieving CR, early mortality, the occurrence of relapse, was not proven in our study.

In our study, elevated levels of LDH (LDH ≥ 450 U/l) proved to be a significant prognostic marker of OS (p = 0.044), which is in keeping with data from previous studies [6,21,25]. The effect of elevated LDH serum levels on CR, early mortality, and the occurrence of relapse, were not proven in our study. In the prospective AML96 study, involving 909 patients suffering from AML, the values of LDH ≥ 700 U/l showed a significant effect on shorter OS [9]. Also, in a study carried out at the Clinic for Hematology of the Clinical Center of Serbia, in 2011, which analyzed comorbidities as a prognostic marker of the OS of patients suffering from AML, a negative effect of elevated LDH levels on both OS and CR was demonstrated [7].

There were more patients with intermediate and unfavorable risk levels, according to the ELN classification, as compared to the patients with a favorable risk level (61.7% and 33% vs. 5.3%), in our study, which is in keeping with data that can be found in literature [7]. However, the study did not demonstrate a statistical connection between unfavorable risk levels, according to ELN, and unfavorable values of OS and CR, a more frequent occurrence of relapse, and a more frequent occurrence of early mortality, which can be explained by the small number of patients in our study. A study carried out by the American Society of Hematology indicates the possible connection between unfavorable karyotype, i.e., risk level, and a more unfavorable outcome, due to a greater association of resistant disease with the said karyotype [11].

objasniti malim brojem pacijenata u studiji. Studija Američkog udruženja hematologa ukazuje na moguću povezanost nepovoljnog kariotipa, odnosno stepena rizika, sa lošijim ishodom, usled veće udruženosti rezistentne bolesti sa navedenim kariotipom [11].

NPM1/FLT3 status određivan je kod 19 pacijenata u našoj studiji. Kod četiri pacijenta (21,1%) sa *NPM1-/FLT3ITD+* statusom, zbog malog broja pacijenata nismo mogli da dokažemo prognostički značaj u pogledu *OS*, KR, rane smrtnosti i relapsa. U prospektivnoj AML96 studiji, koja je obuhvatila 909 pacijenata, analiza 663 pacijenta na *NPM1* i *FLT3* status pokazala je da postoji statistički značajna povezanost pozitivnog *NPM1*, ali ne i pozitivnog *FLT-3ITD* statusa, sa boljim preživljavanjem [9].

Analizom svih pacijenata uključenih u studiju, otkriveno je da se AML češće javljala kao *de novo* bolest, u odnosu na progresiju prethodno postojeće hematološke bolesti (86,2% naspram 13,8%). Ispitivanjem uticaja sekundarno nastale AML na *OS*, KR, ranu smrtnost i pojavu relapsa, nismo registrovali povezanost, moguće je zbog malog broja pacijenata. U *SWOG* studiji, zastupljenost sekundarno nastalih AML-a kretala se od 22% do 24% [23]. U nemačkoj AML HD98-B studiji, zastupljenost sekundarnih AML-a iznosila je 33% [22].

U našoj studiji, bilo je više pacijenata u grupi sa brojem Le < $30x10^9$/l u odnosu na grupu sa brojem Le ≥ $30x10^9$/l (71% naspram 29%), pri čemu veći broj Le u krvi nije imao uticaja na *OS*, KR, ranu smrtnost i pojavu relapsa.

Neke studije ukazuju na uticaj većeg broja Le na ishod AML-a, dok druge studije negiraju navedenu povezanost. Studija sprovedena na Klinici za hematologiju, koja se bavila ispitivanjem komorbiditeta kao prognostičkog faktora za *OS* kod starijih pacijenata sa AML-om, ukazala je na značajnu povezanost povišenog broja Le (Le > $30x10^9$/l) i *OS* [7].

Nije pokazalo da je procenat blasta ≥ 50% u periferној krvi, kao i u koštanoj srži, povezan sa *OS*, KR, ranom smrtnošću i pojavom relapsa, kako u našoj tako i u ranije objavljenim studijama [9].

ZAKLJUČAK

Kao zaključak ovog istraživanja možemo da istaknemo da opšte funkcionalno stanje pacijenta izraženo putem *ECOG* skora, zatim prisustvo komorbiditeta označeno putem *HCT-CI* skora, kao i povišene vrednosti LDH u serumu, imaju uticaja na *OS* starijih pacijenata obolelih od AML-a. Međutim, naša studija nije dokazala značaj ovih, kao ni drugih parametara koje smo pratili, za učestalost KR, relapsa i rane smrtnosti.

Sukob interesa: Nije prijavljen.

The NPM1/FLT3 status was determined in 19 patients, in our study. In four patients (21.1%) with NPM1-/FLT3ITD+ status, due to a small number of patients, it was not possible to prove the prognostic significance in relation to OS, CR, early mortality, and relapse. In the prospective AML96 study, which included 909 patients, analysis of 663 patients as to their NPM1 and FLT3 status showed a statistically significant connection of positive NPM1, but not of positive FLT-3ITD status, to better OS [9].

Analysis of all patients included in the study showed that AML occurred more frequently as a *de novo* disease, as compared to progression of an existing hematological disease (86.2% versus 13.8%). Analysis of the effect of secondary AML on OS, Cr, early mortality, and the occurrence of relapse, did not register any connection, possibly due to a small number of such patients. In the SWOG study, the participation of secondary AMLs was between 22% and 24%. In the German AML HD98-B study, the participation of secondary AMLs was 33% [22].

In our study, there were more patients belonging to the group with Le < $30x10^9$/l as compared to those belonging to the group with Le ≥ $30x10^9$/l (71% vs. 29%), while a higher Le blood count did not affect OS, CR, early mortality, and occurrence of relapse.

Some studies indicate that a higher Le count does have an effect on AML outcome, while other studies negate such a connection. A study carried out at the Clinic for Hematology, which analyzed the existence of comorbidities as a prognostic factor for OS in elderly patients with AML, indicated a significant connection between an elevated Le blood count (Le > $30x10^9$/l) and OS [7].

The percentage of blasts ≥ 50% in peripheral blood, as well as in bone marrow, did not prove to be connected with OS, CR, early mortality, and occurrence of relapse, either in our study or in earlier studies [9].

CONCLUSION

As a conclusion to this study, we can state that the general performance status of patients expressed as the score on the ECOG scale, the existence of comorbidities marked as the HCT-CI score, as well as elevated serum levels of LDH, do have an effect on the OS of elderly patients suffering from AML. However, our study did not prove the significance of these, or other parameters that we monitored, in relation to the frequency of CR, relapse, or early mortality.

Conflict of interest: None declared.

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KARAKTERISTIKE BOLESNIKA SA SEKUNDARNOM ERITROCITOZOM U ODNOSU NA BOLESNIKE SA POLICITEMIJOM VEROM

ORIGINALNI RAD

ORIGINAL ARTICLE

CHARACTERISTICS OF PATIENTS WITH SECONDARY ERYTHROCYTOSIS IN RELATION TO PATIENTS WITH POLYCYTHEMIA VERA

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SAŽETAK:

Uvod: Eritrocitoza predstavlja povišene vrednosti hemoglobina i hematokrita iznad opsega normalnih vrednosti. Primarnu eritrocitozu - policitemiju veru, karakteriše poremećaj na nivou multipotentne matične ćelije hematopoeze koštane srži koja dovodi do povećane produkcije eritrocita. Sa druge strane, sekundarna eritrocitoza (SE) je posledica stimulacije koštane srži spoljašnjim faktorom.

Cilj: Cilj našeg istraživanja bio je da se utvrde parametri koji su značajni u diferencijalnoj dijagnozi SE u odnosu na primarnu eritrocitozu – policitemiju veru (PV).

Materijal i metode: Ovo je retrospektivno istraživanje koje je obuhvatilo 108 bolesnika sa SE-om i 111 bolesnika sa PV-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju, Univerzitetskog kliničkog centra Srbije (UKCS), u periodu: decembar 2005 – novembar 2018. Iz medicinske dokumentacije su prikupljeni podaci o demografskim i laboratorijskim parametrima, veličini jetre i slezine, celokupnoj masi eritrocita, prisustvu prethodnika hematopoeze (spontani rast *BFU-E* kolonija) i vrednosti eritropoetina (EPO) u serumu.

Rezultati: Bolesnici sa SE-om bili su mlađeg uzrasta, uz češću pojavu kod muškaraca, sa značajno višim vrednostima serumskog EPO-a, u odnosu na bolesnike sa PV-om. Bolesnici sa PV-om su imali značajno više vrednosti broja leukocita, broja trombocita, veličine slezine i vrednosti laktatne dehidrogenaze (LDH) kao i više vrednosti prethodnika eritrocitopoeze (BFU-E), u odnosu na bolesnike sa SE-om. Celokupna masa eritrocita nije pokazala diferencijalno dijagnostički značaj.

Zaključak: Normalna veličina slezine, normalne vrednosti broja leukocita, trombocita, i serumske LDH i povišena vrednost EPO-a, kod pacijenata, govore u prilog sekundarne eritrocitoze, dok nalaz splenomegalije, leukocitoze, trombocitoze, povišene vrednosti serumske LDH, nalaz snižene vrednosti EPO-a i prisutne spontane *BFU-E* kolonije, govore u prilog policitemije vere.

Ključne reči: sekundarna eritrocitoza, policitemija vera, diferencijalna dijagnoza

ABSTRACT:

Introduction: Erythrocytosis represents elevated hemoglobin and hematocrit levels above the range of normal values. Primary erythrocytosis - polycythemia vera, is characterized by increased erythrocyte production, due to a disorder at the level of the multipotent stem cell in the bone marrow. On the other hand, secondary erythrocytosis (SE) is the result of bone marrow stimulation by an external factor.

Aim: The aim of our study was to determine parameters which are significant in differentiating SE from primary erythrocytosis - polycythemia vera (PV).

Materials and methods: This is a retrospective study involving 108 patients with SE and 111 patients with PV, who were diagnosed and treated at the Clinic of Hematology of the Clinical Center of Serbia (CCS), in the period: December 2005 – November 2018. From the patient records, the following data were extracted: demographic characteristics, laboratory parameters, spleen size, total red cell mass, serum erythropoietin (EPO) level, and spontaneous growth of the BFU-E colony.

Results: Patients with SE were younger, with a predominance of the male gender and with significantly higher serum EPO values than patients with PV. Patients with PV had significantly higher values of BFU-E, leukocyte and platelet count, spleen size, and LDH level than patients with SE. Total red cell mass analysis did not show a differential diagnostic significance.

Conclusion: Findings of normal spleen size, normal leukocyte and platelet count, normal serum LDH level, and elevated EPO, in patients, refer to the diagnosis of secondary erythrocytosis, while the findings of splenomegaly, leukocytosis, thrombocytosis, elevated serum LDH level, decreased EPO, and the presence of spontaneous BFU-E colony speak in favor of the diagnosis of polycythemia vera.

Key words: secondary erythrocytosis, polycythemia vera, differential diagnosis

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UVOD

Eritrocitoza predstavlja povišene vrednosti hemoglobina i hematokrita iznad opsega normalnih referentnih vrednosti. Regulacija eritropoeze je kompleksan proces koji uključuje osetljivost koštane srži na kiseonik i eritropoetin (EPO). Pokazatelji eritrocitoze su broj eritrocita u perifernoj krvi, vrednost hemoglobina (Hb) i zapreminski odnos – hematokrit (Hct).

Apsolutna eritrocitoza je definisana kada je celokupna masa eritrocita veća od 125% u odnosu na referentne vrednosti predviđene za pol i telesnu masu, analizirano visoko specijalizovanim nuklearnim testom [1]. Analizom celokupne mase eritrocita može se diferencirati apsolutna od relativne eritrocitoze, koju karakteriše normalna masa eritrocita ali smanjen volumen plazme [2]. Eritrocitoza se deli na primarnu i sekundarnu. Primarnu eritrocitozu karakteriše poremećaj na nivou multipotentne matične ćelije hematopoeze koštane srži koja dovodi do povećane produkcije eritrocita [3]. Tada dolazi do spontanog rasta prethodnika eritropoeze u odsustvu eritropoetina (EPO) [3]. Primarna eritrocitoza, poznata kao policitemija vera – PV, jeste posledica pojave stečene mutacije u JAK2 genu [4,5]. JAK2-V617F mutacija je opisana kod 95% bolesnika, dok je JAK2 *exon* 12 mutacija, prisutna kod 3% bolesnika sa PV-om [4,5]. Ove mutacije dovode do stvaranja konstitutivno aktivne tirozin kinaze, koja aktivira eritropoetinski receptor i JAK-STAT signalni put, koji povećava produkciju eritrocita i često prateću produkciju leukocita i trombocita.

Sa druge strane, sekundarna eritrocitoza (SE) nastaje kao posledica stimulacije koštane srži spoljašnjim faktorom. Najčešća stanja koja dovode do sekundarne eritrocitoze su: hipoksemija usled pušenja, respiratornih ili kardiovaskularnih bolesti, kao i policistične bolesti bubrega ili ektopična sekrecija eritropoetina. Izražena gojaznost se navodi kao faktor rizika za sekundarnu eritrocitozu, u slučaju razvoja *spleep apnea*-e tj. hipoksemije tokom spavanja. Kada nijedan uzrok ne može biti definisan, takvo stanje se naziva idiopatskom eritrocitozom.

Cilj našeg istraživanja bio je da se utvrde parametri koji su značajni u diferencijaciji sekundarne eritrocitoze (SE) u odnosu na primarnu eritrocitozu – policitemiju veru (PV).

MATERIJAL I METODE

Ovo je retrospektivno istraživanje koje je obuhvatilo 108 bolesnika sa dijagnozom sekundarne eritrocitoze (SE) i 111 bolesnika sa dijagnozom policitemije vere (PV), koji su dijagnostikovani, praćeni i lečeni na Klinici za hematologiju, Univerzitetskog kliničkog centra Srbije, u periodu od decembra 2005. do novembra 2018.

INTRODUCTION

Erythrocytosis represents elevated hemoglobin and hematocrit levels above the range of normal values. The regulation of erythropoiesis is a complex process which includes bone marrow sensitivity to oxygen and erythropoietin (EPO). The indicators of erythrocytosis are the following: the number of erythrocytes in peripheral blood, the hemoglobin (Hb) level, and the volume ratio – hematocrit (Hct).

Absolute erythrocytosis is defined as the state where the total red blood cell mass is above 125%, as compared to the reference range defined for sex and body mass, as analyzed by a highly specialized nuclear test [1]. Analysis of the total red blood cell mass enables differentiation between absolute and relative erythrocytosis, which is characterized by normal red blood cell mass, but a decreased plasma volume [2]. Erythrocytosis can be primary and secondary. Primary erythrocytosis is characterized by increased erythrocyte production, due to a disorder at the level of the multipotent stem cell in the bone marrow [3]. This is when spontaneous growth of the erythropoiesis precursor occurs, in the absence of erythropoietin (EPO) [3]. Primary erythrocytosis, also known as polycythemia vera – PV, is the result of the occurrence of an acquired mutation on the JAK2 gene [4,5]. The JAK2-V617F mutation is described in 95% of patients, while the JAK2 exon 12 mutation is present in 3% of the patients with PV [4,5]. These mutations lead to the production of constitutively active tyrosine kinase, which activates the erythropoietin receptor and the JAK-STAT signaling pathway, which, in turn, increases the production of erythrocytes, and often the accompanying production of leucocytes and thrombocytes.

On the other hand, secondary erythrocytosis (SE) happens as the result of the stimulation of bone marrow by an external factor. The most common states leading to secondary erythrocytosis are the following: hypoxemia caused by smoking, respiratory or cardiovascular diseases, as well as polycystic kidney disease or ectopic production of erythropoietin. Marked obesity is stated as a risk factor for secondary erythrocytosis, in case of the development of sleep apnea, i.e., sleep hypoxemia. When no cause can be defined, such a state is called idiopathic erythrocytosis.

The goal of our research was to determine the parameters relevant for the differentiation of secondary erythrocytosis (SE) from primary erythrocytosis – polycythemia vera (PV).

MATERIALS AND METHODS

This is a retrospective study involving 108 patients with the diagnosis of secondary erythrocytosis (SE) and 111 patients with the diagnosis of polycythemia vera (PV),

godine. Kod bolesnika sa PV-om, dijagnoza je postavljena prema kriterijumima Svetske zdravstvene organizacije (SZO) iz 2016. godine [7], (Tabela 1).

Iz medicinske dokumentacije su prikupljeni sledeći podaci: 1) demografske karakteristike bolesnika; 2) parametri kompletne krvne slike i leukocitarne formule; 3) laboratorijske analize laktat dehidrogenaze; 4) veličina slezine i jetre (određene na osnovu ultrazvuka – UZ abdomena); 5) volumen eritrocita; 6) prisustvo prethodnika hematopoeze u koštanoj srži ili perifernoj krvi; 6) nivo eritropoetina (EPO) u serumu; 7) status JAK2V617F mutacije.

Medijana praćenja za bolesnike sa SE-om je bila 32 meseca (raspon: 1 – 151), dok je kod bolesnika sa PV-om bila 17 meseci (raspon: 1 – 62).

U statističkoj obradi podataka korišćene su metode deskriptivne statistike, zatim, u zavisnosti od distribucije podataka, za procenu značajanosti razlike između analiziranih podataka, korišćene su parametrijske (Studentov T test i analiza varijanse – ANOVA) i neparametrijske metode (Hi-kvadrat test, Man Vitnijev test i Kraskal Volisov test). Univarijantnim i multivarijantnim *Cox*-ovim regresionim modelom indentifikovani su prediktori sekundarne eritorcitoze u odnosu na policitemiju veru.

REZULTATI

Bolesnici sa sekundarnom eritrocitozom (SE)

Kod analiziranih 108 bolesnika sa SE-om bilo je 89 osoba muškog (82,4%) i 19 osoba ženskog pola (17,6%)

who were diagnosed, monitored and treated at the Clinic of Hematology of the Clinical Center of Serbia, in the period between December 2005 and November 2018. In patients with PV, diagnosis was established on the basis of the criteria of the World Health Organization (WHO) from 2016 [7], (Table 1).

The following data was collected from medical records: 1) patient demographic characteristics; 2) complete blood count and white blood cell count parameters; 3) lactate dehydrogenase laboratory analyses; 4) spleen and liver size (determined with abdominal ultrasound – US); 5) erythrocyte volume; 6) presence of the erythropoiesis precursor in bone marrow or peripheral blood; 6) serum level of erythropoietin (EPO); 7) JAK2V617F mutation status.

The median follow-up for patients with SE was 32 months (range: 1 – 151), while in patients with PV it was 17 months (range: 1 – 62).

In statistical data processing, descriptive statistics methods were employed, and, depending on data distribution, parametric (Student's t-test and analysis of variance – ANOVA) and nonparametric (the chi-squared test, the Mann-Whiteny test, the Kruskal-Wallis test) methods were employed for assessing the significance of the difference among the analyzed data. The univariate and multivariate Cox regression models were used to identify the predictors of secondary erythrocytosis in relation to polycythemia vera.

Tabela 1. SZO kriterijumi (2016) za dijagnozu PV*

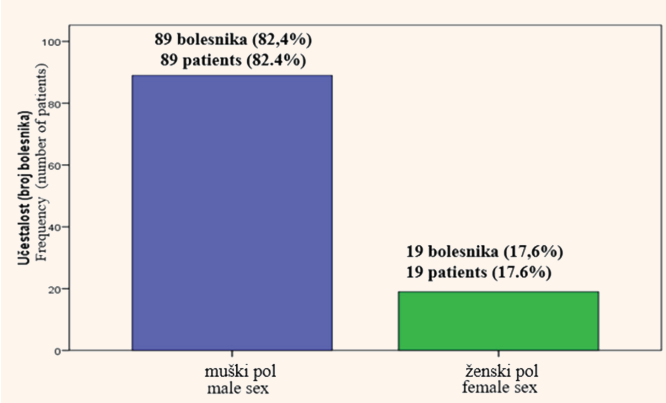
MAJOR KRITERIJUMI	*MINOR* KRITERIJUMI
1) Vrednost hemoglobina >165g/L kod muškaraca; vrednost hemoglobina > 160g/L kod žena ili vrednost hematokrita > 49% kod muškaraca ili vrednost hematokrita > 48% kod žena ili povećana masa eritrocita (više od 25% od prosečne normalne vrednosti)	**1)** Snižena serumska vrednost eritropoetina
2) Patohistološki nalaz bioptata koštane srži pokazuje hipercelularnost u odnosu na očekivanu za uzrast, sa trilinijskom proliferacijom (panmijelozom) uključujući eritroidnu, granulocitnu i megakariocitnu proliferaciju sa pleomorfnim, zrelim megakariocitima (razlike u veličini)	
3) Prisustvo *JAK2* ili *JAK2 exon* 12 mutacije	

Dijagnoza PV: prisustvo sva tri major kriterijuma ili prva dva major i minor kriterijuma
* Preuzeto iz: Barbui T, Thiele J, Gisslinger H, et al. Blood Cancer J. 2018;8(2):15.

Table 1. WHO criteria (2016) for PV diagnosis*

MAJOR CRITERIA	MINOR CRITERIA
1) Hemoglobin value > 165g / L in men; hemoglobin value > 160g / L in women or hematocrit value > 49% in men or hematocrit value > 48% in women or increased erythrocyte mass (more than 25% of the average normal value)	**1)** Decreased erythropoietin value
2) Pathohistological finding of bone marrow biopsy shows hypercellularity, as expected for the given age, with trilinear proliferation (panmyelosis), including erythroid, granulocyte, and megakaryocyte proliferation with pleomorphic, mature megakaryocytes (differences in size)	
3) The presence of the JAK2 or JAK2 exon 12 mutation	

PV diagnosis: presence of all three major criteria or first two major and minor criteria
* Taken from: Barbui T, Thiele J, Gisslinger H, et al. Blood Cancer J. 2018;8(2):15.



Grafikon 1. Raspodela bolesnika sa sekundarnom eritrocitozom prema polu (muški/ženski)

Figure 1. Distribution of patients with secondary erythrocytosis by sex (male/female)

(M:Ž = 4,7:1), (Grafikon 1). U celoj grupi ispitivanih bolesnika, medijana uzrasta bolesnika, u momentu postavljanja dijagnoze, bila je 55 godina (raspon: 19 – 88).

Medijana promera jetre je iznosila 135 mm (raspon: 130 – 174), dok je medijana promera slezine iznosila 100 mm (raspon: 90 – 150). Celokupna masa eritrocita je iznosila 136% (raspon: 105 – 269), (Grafikon 3). Povišena celokupna masa eritrocita od preko 125%, koja je karakteristična za policitemiju veru, bila je prisutna kod 90 bolesnika (83%). Medijana saturacije kiseonikom (SO2) je iznosila 96% (raspon: 89,4 – 99). Sniženu SO2 (< 92%) je imalo 5 bolesnika. Srednja vrednost EPO-a u serumu je iznosila 9,3 IU/mL (raspon: 3,3 – 32,4). Od 108 bolesnika sa SE-om, analiza JAK2V617F mutacije je urađena kod 42 bolesnika (38,8%), većinom tokom njihovog praćenja, i kod svih analiziranih bolesnika je bila negativna.

Bolesnici sa policitemijom verom (PV)

Ispitivanjima je obuhvaćeno ukupno 111 bolesnika sa policitemijom verom (PV), među kojima je bilo 65 osoba ženskog pola (58,5%) i 46 osoba muškog pola (41,5%) (Ž:M = 1,4:1), (Grafikon 2). U celoj grupi ispitivanih bolesnika, medijana uzrasta bolesnika u momentu postavljanje dijagnoze bila je 62 godine (raspon 24 – 85). Medijana promera jetre je iznosila 135 mm (raspon 120 – 180), dok je medijana promera slezine iznosila 121 mm (raspon: 90 – 255). Hepatomegaliju je imalo 15 bolesnika (13,3%), dok je splenomegaliju imalo 52 bolesnika (42%).

Srednja vrednost EPO-a u serumu je bila snižena i iznosila je 2 mU/mL (raspon: 0,06 – 31,8). Od 101 ispitivanog bolesnika, kod 95% je detektovano prisustvo JAK2V617F mutacije, dok 5% bolesnika nije imalo ovu mutaciju. Celokupna masa eritrocita ukazivala je na postojanje apsolutne eritrocitoze od 137% (raspon: 102 – 258), (Grafikon 3). Prisustvo apsolutne

RESULTS

Patients with secondary erythrocytosis (SE)

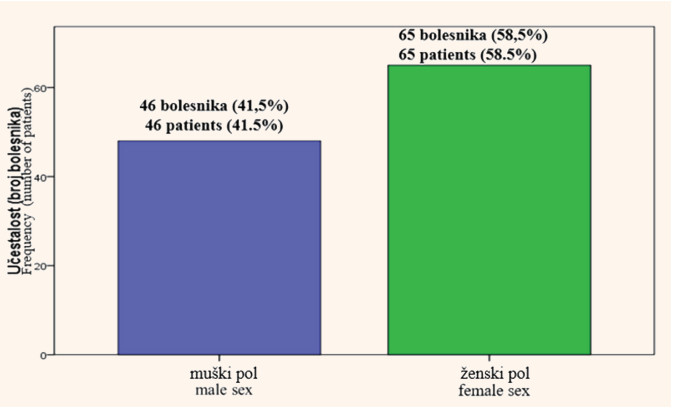
Among the 108 analyzed patients with SE, 89 were male subjects (82.4%), and 19 were female subjects (17.6%), (M:F = 4.7:1), (Figure 1). In the entire group of analyzed patients, the median age at the moment of diagnosis was 55 years (range: 19 – 88).

The median dimeter of the liver was 135 mm (range: 130 – 174), while the median diameter of the spleen was 100 mm (range: 90 – 150). The total erythrocyte mass was 136% (range: 105 – 269), (Figure 3). An elevated total erythrocyte mass of over 125%, which is characteristic of polycythemia vera, was present in 90 patients (83%). The median value of the oxygen saturation level (SO2) was 96% (range: 89.4 – 99). Below-normal SO2 (<92%) was noted in 5 patients. The median value of serum EPO was 9.3 IU/mL (range: 3.3 – 32.4). Among the 108 SE patients, analysis of the JAK2V617F mutation was carried out in 42 patients (38.8%), mostly during follow-up, and it was negative in all of the analyzed patients.

Patients with polycythemia vera (PV)

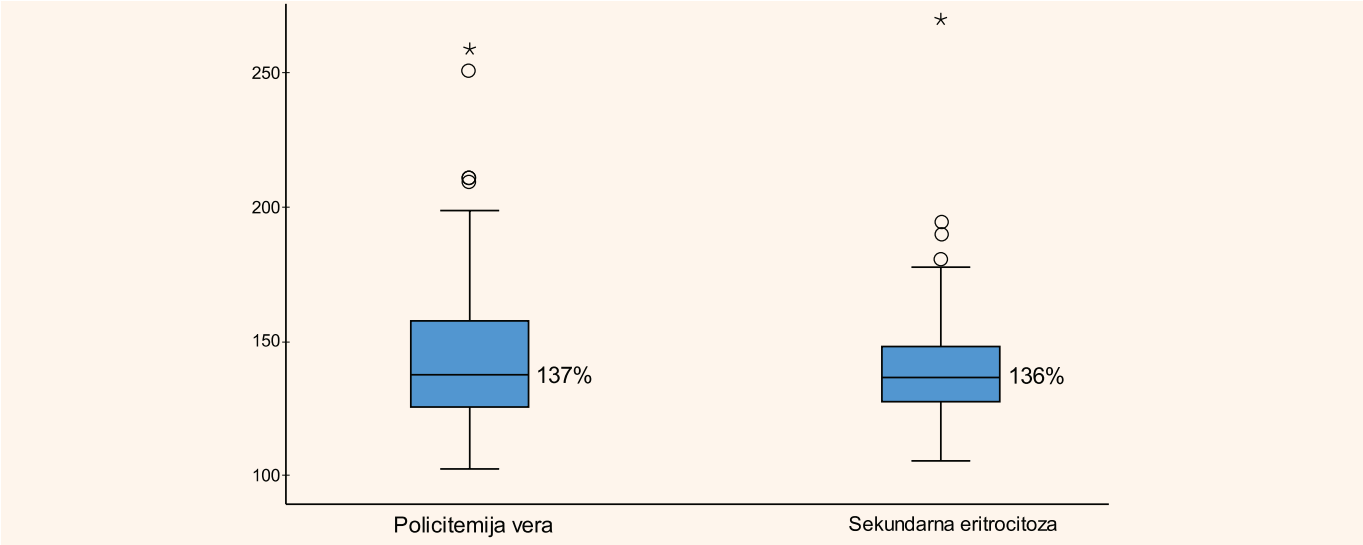
Among the 111 patients with polycythemia vera (PV) included in the study, 65 were female (58.5%) and 46 were male subjects (41.5%), (F:M = 1.4:1), (Figure2). In the entire group of analyzed patients, the median age at the moment of diagnosis was 62 years (range: 24 – 85). The median diameter of the liver was 135 mm (range 120 – 180), while the median diameter of the spleen was 121 mm (range: 90 – 255). A total of 15 patients (13.3%) were found to have hepatomegaly, while 52 patients (42%) had splenomegaly.

The median value of serum EPO was below-normal and amounted to 2 mU/mL (range: 0.06 – 31.8). Of the 101 analyzed patients, the JAK2V617F mutation was



Grafikon 2. Raspodela bolesnika sa policitemijom verom prema polu (muški/ženski)

Figure 2. Distribution of patients with polycythemia vera by gender (male/female)



Grafikon 3. Celokupna masa eritrocita kod bolesnika sa policitemijom verom i kod bolesnika sa sekundarnom eritrocitozom (%)

Figure 3. Total red cell mass in patients with polycythemia vera and in patients with secondary erythrocytosis (%)

eritrocitoze, tipične za policitemiju veru, potvrđeno je kod 88 bolesnika (79,2%).

Bolesnici sa PV-om, u odnosu na bolesnike sa SE-om, bili su značajno stariji (p = 0,001), sa većim promerom slezine (p < 0,001), većim brojem leukocita (12 ± 5,2x10^9/L naspram 7,7 ± 2,8 x10^9/L, p < 0,001), većim brojem trombocita (718 ± 354x10^9/L naspram 221 ± 61x10^9/L, p < 0,001), povišenom LDH u serumu (559 ± 183 U/L naspram 360 ± 64 U/L, p < 0,001), sniženim eritropoetinom (EPO) u serumu (2,5 ± 0,4 ml/mL naspram 13,8 ± 1,3 ml/mL, p < 0,001) i prisutnim spontanim *BFU-E* kolonijama (45±12 naspram 0, p = 0,011).

DISKUSIJA

Ova studija je analizirala demografske, kliničke i laboratorijske karakteristike bolesnika sa SE-om i PV-om. Rezultati su pokazali da bolesnici sa PV-om imaju značajno veći broj leukocita, veći broj trombocita, veće LDH vrednosti, veću veličinu slezine i prisutne spontane *BFU-E* kolonije, u odnosu na bolesnike sa SE-om. Kod bolesnika sa eritrocitozom, koji imaju uredan broj leukocita i trombocita, uredan nalaz LDH i normalnu veličinu slezine, savetuje se prvo isključivanje uzroka sekundarne eritrocitoze.

U našoj studiji, celokupna masa eritrocita nije pokazala statistički značajnu razliku između bolesnika sa SE-om u odnosu na pacijente sa PV-om, i najverovatnije se značaj celokupne mase eritrocita ogleda u diferencijaciji apsolutne i relativne eritrocitoze [1,3]. Vrednosti EPO-a u serumu kod bolesnika sa SE-om su normalne ili povišene, dok su kod bolesnika sa PV-om obično snižene, *što ukazuje* na značaj EPO analize u diferencijaciji sekundarne eritrocitoze od policitemije vere. Snižena vrednost EPO-a u serumu predstavlja *minor* kriterijum za postavljanje dijagnoze PV-a [6]. Kod bolesnika

detected in 95% of the subjects, while 5% of the patients did not have this mutation. The total erythrocyte mass indicated the presence of absolute erythrocytosis amounting to 137% (range: 102 – 258), (Figure 3). The presence of absolute erythrocytosis, typical for polycythemia vera, was confirmed in 88 patients (79.2%).

As compared to SE patients, patients with PV were significantly older (p = 0.001), they had a larger spleen diameter (p < 0.001), a higher leukocyte count (12 ± 5.2x10^9/L vs. 7.7 ± 2.8 x10^9/L, p < 0.001), a higher platelet count (718 ± 354x10^9/L vs. 221 ± 61x10^9/L, p < 0.001), elevated serum LDH (559 ± 183 U/L vs. 360 ± 64 U/L, p < 0,001), lower serum erythropoietin (EPO) (2.5 ± 0.4 ml/mL vs. 13.8 ± 1.3 ml/mL, p < 0.001) and the presence of spontaneous BFU-E colonies (45±12 vs. 0, p = 0.011).

DISCUSSION

The present study analyzed demographic, clinical and laboratory characteristics of patients with SE and PV. The results have shown that patients with PV have a significantly higher white blood cell count, higher platelet count, higher LDH values, larger spleen size, and the presence of spontaneous BFU-E colonies, as compared to patients with SE. In patients with erythrocytosis, with a normal white blood cell count and platelet count, with a normal LDH level and a normally sized spleen, it is advised that the causes of secondary erythrocytosis should first be excluded.

In our study, the total erythrocyte mass did not demonstrate a statistically significant difference between SE patients and PV patients, and the significance of the total erythrocyte mass is reflected, most probably, in the differentiation between absolute and relative erythrocytosis [1,3]. Serum EPO values in patients with SE are either normal or above-normal, while in patients with PV

sa eritrocitozom savetuje se da se prvo isključe uzroci sekundarne eritrocitoze. Međutim, ukoliko je bolesnik nepušač i nema prateće komorbiditete, a ima leukocitozu i/ili trombocitozu, kao i povišenu LDH vrednost, može se razmotriti i inicijalna analiza EPO-a u serumu.

Vrednosti *BFU-E* kolonija su bile prisutne kod bolesnika sa PV-om, u odnosu na bolesnike sa SE-om, kod kojih su bile odsutne. JAK2V617F mutacija je detektovana kod 95% bolesnika sa PV-om, što je u skladu sa prethodno objavljenim literaturnim podacima, dok kod bolesnika sa SE-om, kod kojih je analizirana ova mutacija, ona nije detektovana, što ukazuje na to da njena analiza može imati značaja u diferencijaciji policitemije vere od sekundarne eritrocitoze [4]. Međutim, u našoj studiji, JAK2V617F mutacija je analizirana kod 39% bolesnika, što predstavlja ograničavajući faktor za donošenje definitivnog zaključka.

ZAKLJUČAK

Celokupna masa eritrocita nije pokazala dijagnostički značaj u diferencijaciji sekundarne u odnosu na primarnu eritritrocitozu. Nalaz splenomegalije, leukocitoze, trombocitoze, povišene serumske LDH vrednosti i snižene vrednosti EPO-a u serumu govore u prilog policitemije vere. Sa druge strane, normalna veličina slezine, normalan broj leukocita i trombocita, normalne vrednosti serumske LDH i povišene vrednosti EPO-a, govore u prilog sekundarne eritrocitoze. Prema tome, kod bolesnika sa eritrocitozom, koji imaju uredan broj leukocita i trombocita, urednu LDH vrednost i normalnu veličinu slezine, savetuje se da se prvo isključe uzroci sekundarne eritrocitoze, a da se zatim uradi analiza EPO-a u serumu i razmotri potreba za daljom hematološkom obradom u pravcu policitemije vere.

SPISAK SKRAĆENICA:

BFU-E – burst forming unit-erythroid
EPO – eritropoetin
Hb – hemoglobin
Hct – hematokrit
LDH – laktat dehidrogenaza
PV – policitemija vera
SE – sekundarna eritrocitoza
SO2 – saturacija kiseonikom
SZO – Svetska zdravstvena organizacija
UZ – ultrazvuk
UKCS – Univerzitetski klinički centar Srbije

Sukob interesa: Nije prijavljen.

these values are typically below-normal, which indicates the significance of EPO analysis in the differentiation between secondary erythrocytosis and polycythemia vera. Below-normal serum EPO value is a minor criterion for diagnosing PV [6]. In patients with erythrocytosis it is recommended that secondary erythrocytosis causes should first be excluded. However, if the patient is a non-smoker without associated comorbidities, but has leukocytosis and/or thrombocytosis, as well as an above-normal LDH level, initial analysis of serum EPO can also be considered.

BFU-E colony values were present in patients with PV, as compared to SE patients, where these values were absent. The JAK2V617F mutation was detected in 95% of the patients with PV, which is in keeping with the above stated data from literature, while in SE patients, in whom this mutation was tested, it was not detected, which indicates that the analysis of this mutation can be significant in the differentiation between polycythemia vera and secondary erythrocytosis [4]. However, in our study, the JAK2V617F mutation was analyzed in 39% of the patients, which is a limiting factor for reaching a definitive conclusion.

CONCLUSION

Total erythrocyte mass did not demonstrate diagnostic significance in the differentiation of between primary and secondary erythrocytosis. The findings of splenomegaly, leukocytosis, thrombocytosis, above-normal serum LDH levels and below-normal serum EPO values, speak in favor of polycythemia vera. On the other hand, a normally sized spleen, a normal white blood cell count and platelet count, normal values of serum LDH and elevated EPO values, indicate secondary erythrocytosis. Therefore, in patients with erythrocytosis, who have a normal white blood cell and platelet count, a normal LDH value and a normally sized spleen, it is recommended that causes of secondary erythrocytosis should be excluded first, upon which serum EPO analysis should be performed, and the need for further hematological analysis considered, in relation to polycythemia vera.

LIST OF ABBREVIATIONS AND ACRONYMS:

BFU-E – burst forming unit-erythroid
EPO – erythropoietin
Hb – hemoglobin
Hct – hematocrit
LDH – lactate dehydrogenase
PV – polycythemia vera
SE – secondary erythrocytosis
SO2 – oxygen saturation
WHO – World Health Organization
US – ultrasound
KCS – Clinical Center of Serbia

Conflict of interest: None declared.

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CITOMEGALOVIRUSNA REAKTIVACIJA KOD PACIJENATA U PROCESU ALOGENE TRANSPLANTACIJE MATIČNH ĆELIJA HEMATOPOEZE

ORIGINALNI RAD

ORIGINAL ARTICLE

CYTOMEGALOVIRUS REACTIVATION IN PATIENTS TREATED WITH ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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SAŽETAK

Uvod: Oportunistička CMV reaktivacija je najčešća virusna komplikacija nakon alogene transplantacije matičnih ćelija hematopoeze (AloTMĆH).

Cilj: Cilj rada je da se ispita učestalost CMV reaktivacije u odnosu na serostatus donora i recipijenta, kao i korelacija sa danom postizanja engraftmenta leukocita (Le) i trombocita (Tr). Analizirano je da li je veća učestalost CMV reaktivacije kod *MAC (myeloablative conditioning)* ili *RIC* (*reduced intensity conditioning*) režima i da li je učestalost veća kod srodne (*MRD, match related donor*) ili nesrodne (*MUD, match unrelated donor*) aloTMĆH. Analizirali smo da li CMV reaktivacija utiče na preživljavanje nakon alogene TMĆH.

Materijal i metode: U retrospektivnoj kohortnoj studiji, ispitivana su 42 bolesnika, starosti preko 18 godina, lečenih na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije (UKCS) od decembra 2017. do novembra 2019. godine.

Rezultati: Najveća učestalost reaktivacije je bila u grupi u kojoj je recipijent (R) bio seropozitivan a donor (D) seronegativan (R+/D- = 60,0%). Broj kopija CMV DNK korelira sa danom engraftmenta Le ($p = 0,031$), ali ne i sa engraftmentom Tr ($p = 0,598$). Učestalost reaktivacije kod pacijenata podvrgnutih *RIC*-u je 25,0%, a 63,5% kod pacijenata podvrgnutih *MAC*-u. Jačina režima korelira sa brojem kopija CMV DNK ($p = 0,025$). Korelacija između tipa transplantacije (*MRD* ili *MUD*) i reaktivacije CMV infekcije nije utvrđena ($p = 0,515$). Sveukupno preživljavanje je bilo 36,39 meseci (95% CI 26,0 – 46,78). Srednja vrednost preživljavanja nakon transplantacije, ukoliko se desila reaktivacija, iznosilo je 7,39 meseci (95% CI 5,72 – 9,06), ali nismo dokazali da CMV reaktivacija utiče na preživljavanje ($p = 0,527$).

Zaključak: CMV reaktivacija nije povezana sa povećanjem mortaliteta u ispitivanoj grupi bolesnika nakon aloTMĆH i bila je najčešća u kombinaciji R+D-.

Ključne reči: alogena transplantacija matičnih ćelija hematopoeze, CMV reaktivacija

ABSTRACT

Introduction: Opportunistic CMV reactivation is the most common viral complication after allogenic hematopoietic stem cell transplantation (allo-HSCT).

Aim: The aim of our study is to evaluate the frequency of CMV reactivation in relation to the serostatus od the donor and the recipient, and the correlation with the day of leukocyte (Le) and thrombocyte (Tr) engraftment. We compared the frequency of CMV reactivation in myeloablative conditioning (MAC) versus reduced intensity conditioning (RIC), as well as in match related donor (MRD) versus match unrelated donor (MUD) allo-HSCT. We analyzed whether CMV reactivation affected the overall survival (OS) after allo-HSCT.

Materials and methods: In a retrospective cohort study, we inspected 42 patients over the age of 18 years, who were treated at the Clinic for Hematology of the Clinical Center of Serbia, from December 2017 to November 2019.

Results: Most CMV reactivations were noticed if the recipient (R) was seropositive, and the donor (D) was seronegative (R+/D– = 60.0%). The number of CMV DNA copies corelated with the day of leukocyte engraftment of ($p = 0.031$), but not of thrombocyte engraftment ($p = 0.598$). The frequency of reactivation in patients treated with RIC was 25.0%, and it was 63.5%, if they were treated with MAC. The intensity of the conditioning regimen corelated with the number of CMV DNA copies ($p = 0.025\%$). There was no correlation found between the type of transplantation (MRD or MUD) and CMV reactivation ($p = 0.515$). OS after allo-HSCT was 36.39 months (95% CI 26,0 – 46,78). The mean OS in patients with CMV reactivation was 7.39 months (95% CI 5,72 – 9,06), but we did not prove that CMV reactivation had an impact on OS ($p = 0.527$).

Conclusion: CMV reactivation was most common in the R+/D– group. CMV reactivation did not affect OS after allo-HSCT in our group of patients.

Key words: allogeneic hematopoietic stem cell transplantation, CMV reactivation

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UVOD

Citomegalovirus (CMV) je ubikvitarni herpes virus, koji je naziv dobio po tome što su inficirane ćelije uvećane, odnosno citomegalične.

Prevalencija CMV seropozitivnosti varira u svetu od 60% do 100%. Do primarne infekcije najčešće dolazi u ranom detinjstvu, i u najvećem broju slučajeva je asimptomatska, a može se ispoljiti i u formi atipične mononukleoze [1]. Nakon primarne infekcije, genom CMV virusa perzistira u genomu ćelija domaćina, ne produkujući virusne partikule, odnosno ostaje u latentnom stanju, zadržavajući mogućnost reaktivacije usled imunokompromitovanosti domaćina.

Alogena transplantacija matičnih ćelija hematopoeze (aloTMĆH) podrazumeva zamenu i repopulaciju matičnih ćelija hematopoeze primaoca, matičnim ćelijama donora. Najčešće indikacije za sprovođenje ove procedure lečenja su akutne leukemije, mijelodisplastični sindromi, aplastična anemija, urođene bolesti metabolizma i autoimunske bolesti. Donori su HLA identični (rođeni brat i sestra) ili haploidentični srodnici, ili davalac može biti nesrodan HLA podudaran ili delimično podudaran [2,3].

Morbiditet i mortalitet nakon aloTMĆH zavise od stope relapsa bolesti, kao i od mortaliteta nevezanog za relaps bolesti (*non-relapse mortality – NRM*). Sve su značajniji uzroci *NRM*-a nakon alogene TMĆH, koji iznosi 10 – 30% [4,5], a u njih spadaju infekcije, disfunkcija organa, kao i bolest kalema protiv domaćina (engl. *graft versus host disease – GvDH*) [6]. Najčešće infekcije su infekcije krvi sa incidencijom koja može da varira od 20% – 70% [7].

Imunska reaktivnost primalaca koštane srži ili organa je veštački suprimirana imunosupresivnom terapijom kako bi se sprečilo odbacivanje grafta, tako da su oni u visokom riziku za reaktivaciju različitih latentnih virusnih infekcija. Oportunistička CMV reaktivacija je najčešća virusna komplikacija nakon aloTMĆH, zbog čega se rutinski određuje DNK viremija kvantitativnom *PCR* metodom, jednom do dva puta nedeljno, u prvih 100 dana nakon transplantacije [8]. Verovatnoća i učestalost reaktivacije zavisi od serostatusa donora (D) i recipijenta (R). Reaktivacija se dešava kod 60% seronegativnih i 10% seropozitivnih recipijenata, koji su graft dobili od seropozitivnih donora [8,9]. Međutim, najveća incidencija reaktivacije se beleži kod seropozitivnih recipijenata, koji su graft dobili od seronegativnog donora [8,10]. Osim serostatusa donora i recipijenta, kao faktor rizika navode se i starija životna dob pacijenata, HLA nepodudarnost ili nesrodni donor, deplecija T ćelija, bolest kalema protiv domaćina (*GvHD*) i visoke doze kortikosteroida u terapiji *GvHD* [8]. Manifestacije CMV bolesti su: pneumonija, hepatitis, gastroenteritis,

INTRODUCTION

The cytomegalovirus (CMV) is a ubiquitous herpes virus, whose name stems from the enlargement of the infected cells, i.e., they are cytomegalic.

The prevalence of CMV seropositivity varies in the world from 60% do 100%. Primary infection usually occurs in early childhood, and in most cases, it is asymptomatic, though it can manifest in the form of atypical mononucleosis [1]. After primary infection, the genome of the CMV virus persists in the genome of the host, without producing viral particles, i.e., it remains latent, maintaining the possibility of reactivation, if the host is immunocompromised.

Allogenic hematopoietic stem cell transplantation (allo-HSCT) is the replacement and repopulation of the recipient's hematopoietic stem cells with the stem cells of the donor. The most common indications for carrying out this therapeutic procedure are acute leukemias, myelodysplastic syndromes, aplastic anemia, congenital metabolic disorders, and autoimmune diseases. The donors are HLA identical (brother or sister), or haploidentical relatives, or the donor can be unrelated HLA-matching or partially matching [2,3].

Morbidity and mortality upon allo-HSCT depend on the relapse rate of the disease, as well as on mortality unrelated to the relapse of the disease (non-relapse mortality – NRM). There is a growing significance of the causes of NRM after allo-HSCT, which is 10 – 30% [4,5], and amongst these causes are infections, organ dysfunction, as well as graft versus host disease (GvDH) [6]. The most common infections are blood infections with an incidence that can vary from 20% – 70% [7].

Immune reactivity of recipients of bone marrow or organs is artificially suppressed with immunosuppressive therapy, in order to prevent graft rejection, which is why these patients are at high risk of the reactivation of different latent viral infections. Opportunistic CMV reactivation is the most common viral complication after allo-HSCT, which is why DNA viremia is determined with the quantitative PCR method, once to twice a week, in the first 100 days after transplantation [8]. The probability and frequency of reactivation depends on the serostatus of the donor (D) and the recipient (R). Reactivation happens in 60% of seronegative and 10% of seropositive recipients who received their graft from seropositive donors [8,9]. However, the highest reactivation incidence is recorded in seropositive recipients who received their graft from a seronegative donor [8,10]. Apart from the serostatus of the donor and recipient, the following are also described as risk factors: older age of the patients, unmatching HLA or unrelated donor, T-cell depletion, graft versus host disease (GvHD) and high doses of corticosteroids in GvHD

retinitis i encefalitis, pri čemu se bolest može razviti kao rana ili kasna komplikacija nakon procedure [11].

Sve veći uspeh antivirusne terapije je smanjio incidenciju CMV bolesti na približno 10% u prvoj godini nakon transplantacije, ali se zato uvećala incidencija kasnih CMV reaktivacija, zato što je utvrđeno da antivirusna terapija usporava oporavljanje T specifičnog odgovora na CMV. Mortalitet od CMV pneumonije je i dalje visok, oko 70%, dok se CMV bolest gastrointestinalnog trakta može ispoljiti bez detektabilne viremije, zbog čega se teško razlikuje od *GvHD* gastrointestinalnog trakta [12].

U literaturi su navedeni podaci o zaštitnom dejstvu rane CMV reaktivacije od relapsa mijeloidne leukemije, ali isto nije dokazano za druge hematološke neoplazme [13].

U ovom radu smo upoređivali CMV reaktivaciju u odnosu na serostatus donora i recipijenta (D/R) i ispitali da li postoji korelacija CMV statusa sa danom postizanja engraftmenta Le i Tr. Analizirano je da li je veća učestalost CMV reaktivacije kod *MAC* (*myeloablative conditioning*) ili *RIC (reduced intensity conditioning)* režima i da li je veća učestalost kod srodne *(match related donor, MRD)* ili nesrodne *(match unrelated donor, MUD)* aloTMĆH. Cilj nam je takođe bio da utvrdimo da li CMV reaktivacija utiče na preživljavanje nakon aloTMĆH.

METODE

U retrospektivnoj kohortnoj studiji, ispitivana su 42 bolesnika, starosti preko 18 godina, sa dijagnozom: akutna mijeloidna leukemija (AML), akutna limfocitna leukemija (ALL), hronična limfocitna leukemija (HLL), mijelodisplastična/mijeloproliferativna neoplazma (MDS/MPN), Hočkinov limfom (HL), Nehočkinov limfom (NHL), koji su lečeni na Klinici za hematologiju UKCS, od decembra 2017. do novembra 2019. godine. Podaci o pacijentima dobijeni su uvidom u medicinsku dokumentaciju (Tabela 1).

Mikrobiološke metode

Nakon aloTMĆH, reaktivacija CMV je dokazivana kvantitativnom *PCR* metodom, gde je određivan broj CMV DNK kopija po 1 ml krvi.

Terapija reaktivacije

Reaktivacija CMV je definisana kao bilo koja vrednost viremije (broj kopija/1 ml krvi), dokazana kvantitativnom *PCR* metodom, pri čemu su svi naši pacijenti imali preko 100 kopija CMV DNK na 1 ml krvi. Reaktivacija CMV je lečena terapijskim dozama valganciklovira (Valcyte^{R}) 2x900 mg po dve nedelje, uz smanjenje doza na 2x450 mg u narednom toku, ili ganciklovirom (Cymevene^{R}) 2x500mg iv dve nedelje, uz davanje anti CMV imunoglobulina (Cytotect^{R}) 50mg iv, na 2 nedelje, do negativizacije broja kopija CMV DNK.

therapy [8]. The manifestations of CMV disease are the following: pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis, while the disease can develop as an early or late complication after the procedure [11].

The increasing success of antiviral therapy has decreased the incidence of CMV disease to approximately 10% in the first year after transplantation, however, the incidence of late CMV reactivation has increased, since it has been established that antiviral therapy slows down the restoration of CMV-specific T-cell response. Mortality from CMV pneumonia is still high, approximately 70%, while gastrointestinal CMV disease may manifest without detectible viremia, which makes it difficult to distinguish from gastrointestinal GvHD [12].

There is data in literature related to the protective role of early CMV reactivation against the relapse of myeloid leukemia, however, the same has not been proven for other hematologic neoplasms [13].

In this paper, we have compared CMV reactivation in relation to the serostatus of the donor and the recipient (D/R), and we have analyzed whether there is a correlation between CMV status and the day of Le and Tr engraftment. We have also analyzed whether there is a greater frequency of CMV reactivation in the MAC (myeloablative conditioning) or in the RIC (reduced intensity conditioning) regimen, and whether there is greater frequency in match related donor (MRD) or match unrelated donor (MUD) allo-HSCT. The goal is also to determine whether CMV reactivation affects survival after allo-HSCT.

METHODS

This retrospective cohort study included 42 patients, above the age of 18 years, with one of the following diagnoses: acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), myelodysplastic/myeloproliferative neoplasm (MDS/MPN), Hodgkin lymphoma (HL), and Non-Hodgkin lymphoma (NHL), who were treated at the Clinic for Hematology of the Clinical center of Serbia, from December 2017 to November 2019. The data on the patients have been obtained from patient medical records (Table 1).

Microbiology methods

After allo-HSCT, CMV reactivation was proven with the quantitative PCR method, determining the number of CMV DNA copies per 1 ml of blood.

Reactivation therapy

CMV reactivation is defined as any value of viremia (number of copies/1 ml of blood) proven with the quantitative PCR method. All of our patients, however, had more than 100 copies of CMV DNA per 1 ml of blood. CMV reactivation was treated with therapeutic doses of

Tabela 1. Kliničke forme oboljenja

Table1. Clinical forms of diseases

Dijagnoza *Diagnosis*	**Broj pacijenata** *Number of patients*
Hočkinov limfom *Hodgkin lymphoma*	9 (21.43%)
Akutna mijeloidna leukemija *Acute myeloid leukemia*	14 (33.33%)
Akutna limfocitna leukemij *Acute lymphocytic leukemia*	15 (35.71%)
Mijelodisplastične/mijeloproliferativne neoplazme *Myelodysplastic/myeloproliferative neoplasms*	2 (4.76%)
Hronična mijeloidna leukemija *Chronic myeloid leukemia*	1 (2.38%)
Nehočkinov limfom *Non-Hodgkin lymphoma*	1 (2.38%)
Ukupno / *Total*	**42 (100%)**

Statistička obrada

Za statističku obradu podataka korišćen je softverski paket *SPSS (Statistical Package for Social Sciences)* za *Windows* , verzija 23.0. Statistička obrada je obuhvatila formiranje baze podataka sa grupisanjem i tabeliranjem podataka pacijenata koji su bili značajni za studiju. Deskriptivni statistički parametri su izraženi kroz: aritmetičku sredinu sa merama disperzije (SD, SE), medijanu, MOD i raspodele relevantnih frekvencija. Ukupno preživljavanje bolesnika je obuhvatilo period od momenta dijagnoze do smrtnog ishoda ili zaključno sa novembrom 2019. godine, kod živih pacijenata. Preživljavanje bolesnika u odnosu na lečenje je računato *Kaplan-Meier*-ovom metodom, a razlike u preživljavanju u kontekstu ispitivanih parametara ispitivani su *Log Rank* testom.

Na početku statističkog testiranja definisan je nivo značajnosti od 0,05. Vrednosti koje su iznosile manje od 0,05 su smatrane statistički značajnim.

REZULTATI

Studijom je obuhvaćeno 42 pacijenta, prosečne starosti 37,83±11,36 godina, u rasponu od 19 do 57 godina, u vreme sprovođenja procedure aloTMĆH. U vreme dijagnoze, prosečna starost je iznosila 35,19±12,02 godina. Od 42 pacijenta, 21 je bio muškog pola i 21 ženskog pola (Tabela 2).

Bitan je bio odnos serostatusa recipijenta i donora (R/D), i utvrđeno je da je najučestalija kombinacija bila kada su i recipijent i donor bili CMV seropozitivni, što je utvrđeno kod 28 pacijenata (68,3%). Kombinacija u kojoj je recipijent bio pozitivan, a donor negativan bilo je 10 (24,4%). Kombinacija u kojoj je recipijent bio

valganciclovir (Valcyte^{R}) 2x900 mg for two weeks, with a lowering of the doses to 2x450 mg during further treatment, or with ganciclovir (Cymevene^{R}) 2x500mg iv, for two weeks, with the administering of anti-CMV immunoglobulin (Cytotect^{R}) 50mg iv, every two weeks, until the nullification of the number of CMV DNA copies.

Statistical analysis

The SPSS (Statistical Package for Social Sciences) for Windows, version 23.0 was used for statistical analysis. Statistical analysis included the formation of a database with the grouping and tabular presentation of patient data relevant to the study. Descriptive statistical parameters have been expressed through the following: arithmetic mean with measure of dispersion (SD, SE), median, MOD, and the distribution of relevant frequencies. The overall survival (OS) of patients covered the period from the establishing of the diagnosis to the lethal outcome, or ending with November 2019, in living patients. Overall survival in relation to treatment was calculated with the Kaplan-Meier method, while the differences in survival in the context of the analyzed parameters were analyzed with the use of the Log Rank test.

At the beginning of statistical testing, the level of significance was defined at 0.05. Values below 0.05 were considered statistically significant.

RESULTS

The study included 42 patients of the average age 37.83±11.36 years, in the range of 19 to 57 years, at the time of the allo-HSCT procedure. At the time of diagnosis, the average age was 35.19±12.02 years. Out of 42 patients, 21 were male and 21 were female patients (Table 2).

The relationship (ratio) between the serostatus of the recipient and donor (R/D ratio) was important, and it was determined that the most common combination was when both the recipient and the donor were CMV seropositive, which was found in 28 patients (68.3%). There were 10 combinations of positive recipient and negative donor 10 (24.4%). There were two

Tabela 2. Demografske karakteristike pacijenata

Table2. Demographic characteristics of patients

Ukupan broj pacijenata *Total number of patients*	**42**
Pol / *Sex*	
Žene / *Women*	21 (50%)
Muškarci / *Men*	21 (50%)
Starost / *Age*	37,83±11,36 (19 – 57) years
Sveukupno preživljavanje / *Overall survival*	36,39±5,30 months

Tabela 3. Odnos serostatusa recipijenta (R) i donora (D) i učestalost CMV reaktivacije u odnosu na serostatus R/D

Table3. Recipient-donor serostatus ratio and frequency of CMV reactivation in relation to the serostatus of the R/D

Serostatus *Serostatus R/Ds*	**Učestalost** *Frequency*	**Procenat** *Percentage*
IgG +/+	28	68,3%
IgG +/–	10	24,4%
IgG –/+	2	4,9%
IgM+IgG+/+	1	2,4%
Ukupno / *Total*	41	100%
Serostatus R/D *Serostatus R/Ds*	**Došlo do reaktivacije** *Reactivation occurred*	**Nije došlo do reaktivacije** *Reactivation did not occur*
IgG +/+	15 (53.6%)	13 (46.4%)
IgG +/–	6 (60.0%)	4 (40.0%)
IgG –/+	1 (50%)	1 (50.0%)
IgM+IgG+/+	0 (0.0%)	1 (50.0%)
Ukupno / *Total*	22 (53.7%)	19 (46.3%)

negativan, a donor pozitivan bilo je 2 (4,9%). U jednom slučaju je recipijent bio pozitivan i na IgM i na IgG antitela na CMV, pri čemu je i donor bio IgG pozitivan. U jednom slučaju nedostajali su podaci o serostatusu recipijenta i donora (Tabela 3).

Napravili smo korelaciju između CMV reaktivacije sa odnosom serostatusa recipijenta i donora (R/D). U slučaju R+/D+, reaktivacija se dogodila kod 15 pacijenata (53,6%). Kada je CMV serostatus bio R+/D–, reaktivacija se dogodila kod 6 pacijenata (60,0%). Kod odnosa CMV statusa R–/D+, kod jednog pacijenta je došlo do reaktivacije, dok kod drugog nije. U onom jednom slučaju gde je dokazana pozitivnost na anti-CMV IgM antitela kod pacijenta, nije došlo do reaktivacije, odnosno nije detektovana virusna DNK (Tabela 3).

combinations where the recipient was negative, and the donor was positive 2 (4.9%). In one case, the recipient was positive for both IgM and IgG CMV antibodies, while the donor was IgG positive. In one case, the data on the serostatus of the recipient and donor were missing (Table 3).

We established a correlation between CMV reactivation and the serostatus ratio between the recipient and donor (R/D). In case of R+/D+, reactivation occurred in 15 patients (53.6%). When the CMV serostatus was R+/D–, reactivation occurred in 6 patients (60.0%). When the CMV status relationship was R–/D+, reactivation occurred in one patient, while it did not occur in the other patient from this group. In the one case where anti-CMV IgM antibody positivity was proven, reactivation did not occur, i.e., viral DNA was not detected (Table 3).

Correlation between the number of CMV copies and Le engraftment ($p = 0.031$) was established, but not the correlation between the number of CMV copies and Tr engraftment ($p = 0.598$).

Twelve patients underwent the RIC conditioning regimen (28.6%), while 30 patients (71.4%) underwent the MAC conditioning regimen. Of the 12 patients on the RIC regimen, reactivation occurred in 3 patients (25.0%), while of the 30 patients on the MAC regimen, reactivation of the latent CMV infection occurred in 9 patients (63.5%). Correlation was determined between the intensity of the conditioning regimen and CMV reactivation. Namely, reactivation in patients under the more intensive regimen, i.e., MAC was significantly more frequent ($p = 0.025$) (Table 4).

Of the 15 patients who had undergone MRD transplantation, reactivation occurred in 8 patients. In the patient subgroup with related allo-HSCT, in 8 patients, related haploidentical allo-HSCT was performed, but, due to the small number of patients in this group, we did not separate it from the rest of the MRD transplantations. MUD transplantation was performed in 19 patients, and, again, in 8 of them, reactivation of the CMV

Tabela 4. Učestalost reaktivacije CMV infekcije u odnosu na jačinu kondicionog režima i na vrstu alogene transplantacije: MRD naspram MUD

Table4. Frequency of CMV reactivation in relation to the conditioning regimen and the type of allogeneic transplantation (MRD vs. MUD)

Režim *Conditioning Regimen*	**Došlo do reaktivacije** *Reactivation occurred*	**Nije došlo do reaktivacije** *Reactivation did not occur*	**Ukupno** / *Total*
MAC	19 (63,3%)	11 (36,7%)	30
RIC	3 (25,0%)	9 (75,0%)	12
Tip alogene transplantacije *Type of allogeneic transplantation*	**Došlo do reaktivacije** *Reactivation occurred*	**Nije došlo do reaktivacije** *Reactivation did not occur*	
MRD	8 (53,3%)	7 (46,7%)	15
MUD	8 (42,1%)	11 (57,9%)	19

Utvrđeno je da broj CMV kopija korelira sa engraftmentom Le (p = 0,031), ali korelacija sa engraftmentom Tr nije utvrđena (p = 0,598).

RIC kondicionom režimu je podvrgnuto 12 pacijenata (28,6%), a režimu *MAC* 30 pacijenata (71,4%). Od 12 pacijenata koji su podvrgnuti *RIC*-u, kod 3 pacijenta (25,0%) je došlo do reaktivacije, a od 30 pacijenata podvrgnutih *MAC*-u, kod 19 (63,5%) je došlo do reaktivacije latentne CMV infekcije. Utvrđena je korelacija između intenziteta kondicionog režima i CMV reaktivacije. Naime, značajno češće je dolazilo do reaktivacije kod pacijenata koji su bili podvrgnuti jačem režimu, odnosno *MAC*-u (p = 0,025) (Tabela 4).

Od 15 pacijenata, kod kojih je obavljena *MRD* transplantacija, kod njih 8 je došlo do reaktivacije. U podgrupi pacijenata sa srodnom aloTMĆH, kod 8 pacijenata je urađena srodna haploidentična aloTMĆH, ali tu grupu, zbog malog broja pacijenata, nismo izdvajali od ostalih *MRD* transplantacija. *MUD* transplantacija je obavljena kod 19 pacijenata i opet je kod 8 došlo do reaktivacije CMV infekcije, iako korelacija između tipa transplantacije (*MRD* ili *MUD)* sa reaktivacijom CMV infekcije nije utvrđena (p = 0,515) (Tabela 4).

Sveukupno preživljavanje nakon aloTMĆH izračunato na osnovu *Kaplan-Meier*-ove studije na uzorku od 42 pacijenta je bilo 36,39 meseci (95% CI 26,0 – 46,78) (Grafikon 1). Srednja vrednost preživljavanja, ukoliko nije došlo do reaktivacije, je bila 39,57 meseci, a ukoliko je do nje došlo, srednja vrednost preživljavanja je bila 7,39 meseci (95% CI 5,72 – 9,06) (Grafikon 2). Na osnovu *Log Rank* testa, nije utvrđeno da CMV reaktivacija utiče na preživljavanje (p = 0,527).

DISKUSIJA

U našoj studiji ispitivana su 42 pacijenta, od toga je bilo 21 žena i 21 muškarac, čija je prosečna starost u vreme sprovođenja procedure aloTMĆH iznosila 37,83±11,36 godina. Distribucija dijagnoza je bila takva da je najviše pacijenata bilo obolelo od ALL, odnosno njih 15 (35,71%), od AML 14 (33,33%), od HL 9 (21,43%), od MDS/MPN 2 (4,76%), a po 1 pacijent od NHL i HLL (po 2,38%).

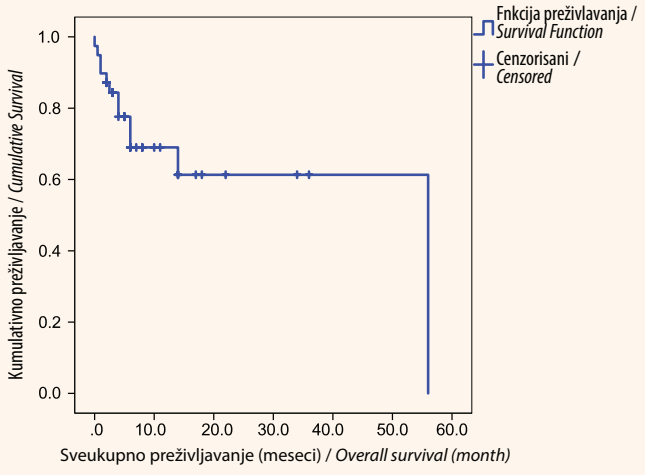
Što se tiče distribucije CMV serostatusa, najviše je bilo R+/D+ kombinacija (68,3%), 24,4% je bilo R+/D- kombinacija, 4,9% je bilo R-/D+ kombinacija, i bio je jedan slučaj pacijenta pozitivnog i na IgM i na IgG antitela, pri čemu je i donor bio pozitivan na IgG, ali u tom slučaju nije detektovana virusna DNK. Kada smo upoređivali učestalost reaktivacije sa CMV serostatusom recipijenata i donora, rezultati su pokazali sledeće: u slučaju R+/D+, reaktivacija se dogodila kod 15 pacijenata (53,6%), kod R+/D-, reaktivacija se dogodila kod 6 pacijenata (60,0%), u slučaju R-/D+, kod jednog pacijenta je došlo do reaktivacije. Iz toga zaključujemo da je najveća učeinfection occurred, although correlation between the type of transplantation (MRD or MUD*)* and the reactivation of CMV was not established (p = 0.515) (Table 4).

The overall survival after allo-HSCT, calculated on the basis of the Kaplan-Meier study, on a sample of 42 patients, was 36.39 months (95% CI 26.0 – 46.78). The mean value for survival was 39.57 months, if there had been no reactivation, and it was 7.39 months, if reactivation had occurred (95% CI 5.72 – 9.06) (Figure 1). On the basis of the Log Rank test, it was not determined that CMV reactivation affected survival (p = 0.527).

DISCUSSION

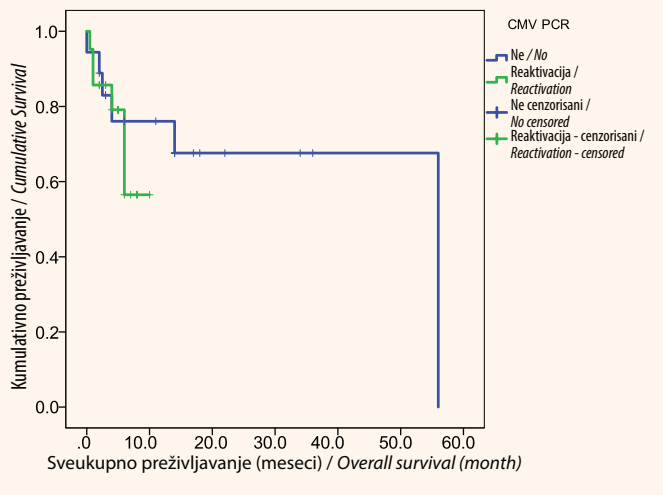
Our study analyzed 42 patients, 21 female and 21 male patients, whose average age at the time of the allo-HSCT procedure was 37.83±11.36 years. The distribution of the diagnoses was such that the greatest number of patients was affected by ALL, i.e., 15 of them (35.71%), 14 suffered from AML (33.33%), 9 were afflicted with HL (21.43%), two were MDS/MPN patients (4.76%), and there was one patient suffering from NHL (2.38%) and one affected by CLL (2.38%).

As far as the distribution of the CMV serostatus is concerned, most of the combinations were R+/D+ (68.3%), 24.4% were R+/D– combinations, 4.9% were R–/D+ combinations, and there was one case of a patient who was positive for both IgM and IgG antibodies, while the donor was also IgG positive, though, in this case, viral DNA was not detected. When we compared reactivation frequency with the CMV serostatus of the recipients and donors, the results showed the



Grafikon 1. Sveukupno preživljavanje nakon alogene transplantacije matičnih ćelija hematopoeze

Figure 1. Overall survival after allogenic hematopoietic stem cell transplantation



Grafikon 2. Preživljavanje u odnosu na CMV reaktivaciju

Figure 2. Survival in relation to CMV reactivation

stalost reaktivacije u slučaju kada je kombinacija R+/D–. Slične rezultate su dobili Džordž i saradnici, koji su svojih 315 pacijenata podelili u 3 grupe: grupu niskog rizika (R–/D–), grupu srednjeg rizika (R–/D+), i grupu visokog rizika za reaktivaciju (R+/D- ili R+/D+) [9]. Utvrdili su da je najveća incidencija bila u grupi visokog rizika, i iznosila je 53,3% (11 pacijenata). Stern i saradnici visoku incidenciju reaktivacije u grupi sa R+/D+ objašnjavaju ili reaktivacijom latentne CMV infekcije u ćelijama recipijenata ili/i reaktivacijom iz inficiranih ćelija donora prenetih graftom matičnih ćelija [8]. Najveća incidencija reaktivacije u grupi R+/D– se objašnjava odloženim CMV specifičnim imunskim odgovorom, zbog nedostatka T specifičnih ćelija u graftu [8,14].

Naša studija je takođe ispitivala da li CMV reaktivacija utiče na engraftment Le i Tr. Utvrdili smo da postoji korelacija između broja kopija CMV DNK i engraftmenta Le (p = 0,031), ali korelacija sa engraftmentom Tr nije utvrđena (p = 0,598), što znači da reaktivacija CMV, ovde definisana preko detektovane viremije kvantitativnim *PCR*-om, odlaže prihvatanje kalema i rekonstituciju alogene hematopoeze u slučaju Le.

RIC kondicioni režim je alternativa za pacijente kojima je aloTMĆH neophodna, ali postoje kontraindikacije za jači režim (*MAC*), zbog komorbiditeta ili starije životne dobi [15]. U našoj studiji, pre transplantacije, *MAC* kondicionom režimu je bilo podvrgnuto 30 pacijenata (71,4%), a *RIC* režimu 12 pacijenata (28,6%). Kod naših pacijenata, reaktivacija se dogodila kod 19 pacijenata koji su bili podvrgnuti *MAC*-u i kod 3 pacijenta koji su bili podvrgnuti *RIC*-u, čime smo pokazali da se CMV reaktivacija statistički značajno češće dešava ukoliko se koriste jači kondicioni režimi (*MAC*), odnosno da jačina kondicionog režima korelira sa brojem kopija virusne DNK. Studija koju su sproveli Pinjana i saradnici following: in case of R+/D+, reactivation occurred in 15 patients (53.6%), in R+/D–, reactivation happened in 6 patients (60.0%), in the case of R-/D+, reactivation occurred in one patient. This leads us to the conclusion that the highest reactivation frequency is in case of the R+/D– combination. Similar results were obtained by George et al., who divided their 315 patients, regarding the risk of CMV reactivation, into the following three groups: the low-risk group (R–/D–), the medium-risk group (R–/D+), and the high-risk group (R+/D– or R+/D+) [9]. They established that the highest incidence of reactivation was in the high-risk group, amounting to 53.3% (11 patients). Stern et al. explained the high incidence of reactivation in the R+/D+ group with either the reactivation of the latent CMV infection in the cells of the recipient or/and with reactivation from infected donor cells transplanted in the stem cell graft [8]. The highest incidence of reactivation in the R+/D– group is explained by the delayed CMV-specific immune response, due to the lack of T-specific cells in the graft [8,14].

Our study also analyzed whether CMV reactivation affected Le and Tr engraftment. We found that there was a correlation between the number of CMV DNA copies and Le engraftment (p = 0.031), but correlation with Tr engraftment was not found (p = 0.598), which means that CMV reactivation, defined here through viremia detected with quantitative PCR, delays graft acceptance and the reconstruction of allogenic hematopoiesis in the case of Le.

The RIC conditioning regimen is an alternative for patients who need allo-HSCT, but who have contraindications for the more intensive regimen (MAC), due to comorbidities or a more advanced age [15]. In our study, prior to transplantation, 30 patients (71.4%) underwent the MAC conditioning regimen, while 12 patients underwent the RIC conditioning regimen (28.6%). Amongst our patients, reactivation occurred in 19 patients who had undergone MAC and three patients who had undergone RIC, whereby we demonstrated that CMV reactivation occurs statistically significantly more often when more intensive conditioning regimens are applied (MAC), i.e., that the intensity of the conditioning regimen correlates with the number of viral DNA copies. A study by Piñana et al. examined the effect of the RIC regimen on CMV reactivation and disease development in 195 patients. They established that reactivation occurred in 36.0% of patients [15].

In their cohort study, Nakamae et al. compared the incidence of reactivation against the MAC and RIC conditioning regimen and came up with different results. In fact, they demonstrated that, in conditioning regimens of reduced intensity, there was a lesser inci-

je ispitivala uticaj *RIC* režima na CMV reaktivaciju i razvoj bolesti kod 195 pacijenata. Utvrdili su da je kod 36,0% došlo do reaktivacije [15].

Nakamae i saradnici su u svojoj kohortnoj studiji upoređivali incidenciju reaktivacije u odnosu na *MAC* ili *RIC* kondicioni režim i došli su do različitih rezultata. Zapravo su pokazali da kod kondicionih režima slabijeg intenziteta postoji manja incidencija visokog *viral load*-a u odnosu na *MAC,* u prvih 100 dana nakon transplantacije, kao i da se CMV bolest kasnije javlja, ali značajne razlike u samoj incidenciji reaktivacije ili CMV bolesti između dva režima nije bilo. Oni pretpostavljaju da rezidualne T memorijske ćelije kod recipijenata koji su bili seropozitivni deluju zaštitno, u smislu da sprečavaju visoki *viral load*, sve dok ne dođe do potpunog himerizma, kada se više ne zapaža njihov zaštitni efekat [16].

Zlatni standard za aloTMĆH su HLA identični srodnici, što ovde označava *MRD* transplantacija. Ukoliko je graft uzet od HLA podudarnog, ali nesrodnog donora, radi se o *MUD*-u. Od 15 pacijenata kod kojih je obavljena *MRD* transplantacija, kod njih 8 je došlo do reaktivacije. *MUD* transplantacija je obavljena kod 19 pacijenata, i opet je kod 8 došlo do reaktivacije CMV infekcije, iako korelacija između tipa transplantacije (*MRD* ili *MUD*) sa reaktivacijom CMV infekcije nije utvrđena ($p = 0,515$), u našoj studiji.

Do istih zaključaka došli su Jaskula i saradnici, u svojoj studiji u kojoj su upoređivali CMV reaktivaciju kod 71 pacijenta koji su graft dobili od nesrodnog donora, ali sa HLA podudarnošću 10/10 i kod 78 pacijenata koji su graft dobili od srodnika. U grupi nesrodnika, do reaktivacije je došlo kod 19 pacijenata, a u grupi srodnika kod 17 pacijenata, i nije nađena statistički značajna korelacija, ali, ukoliko je HLA podudarnost bila manja od 10/10, povećavala se incidencija reaktivacije ($p < 0,08$) [17].

Naša studija nije pokazala da CMV reaktivacija utiče na sveukupno preživljavanje. U stranoj literaturi se pominje zaštitni efekat CMV reaktivacije na relaps, posebno u slučaju AML, ali to ne utiče na poboljšanje sveukupnog preživljavanja [18]. Sa druge strane, retrospektivna studija Souse i saradnika je pokazala da je CMV infekcija značajno smanjila preživljavanje nakon transplantacije, te je medijana preživljavanja bila 16 meseci, ukoliko je došlo do reaktivacije, i 36 meseci, ako do reaktivacije nije došlo ($p = 0,002$) [19]. To se delimično objašnjava većom incidencijom infekcije usled mijelosupresije indukovane antivirusnom terapijom i usled akutnog *GvHD* [18].

Naša studija je imala više ograničenja. Obuhvatila je mali broj pacijenata, svega 42, sa heterogenim dijagnozama, a sama analiza je vršena retrospektivno.

dence of high *viral load* in relation to MAC, in the first 100 days upon transplantation, and that CMV disease occurred later, but that there was no significant difference in the incidence of reactivation itself, nor in CMV disease incidence, between the two regimens. They propose that residual memory T-cells act protectively in recipients who were seropositive, in the sense that they prevent high *viral load*, until complete chimerism occurs, after which their protective effect is no longer apparent [16].

The golden standard for allo-HSCT are HLA identical relatives, marked here as MRD transplantation. If the graft is taken from an HLA-matching, but unrelated donor, this is MUD. Out of 15 patients who underwent MRD transplantation, reactivation occurred in 8 of them. MUD transplantation was performed in 19 patients, and, again, reactivation of the CMV infection occurred in 8 of them, although correlation between the type of transplantation (MRD or MUD) and the reactivation of CMV infection was not established in our study ($p = 0.15$).

Jaskula et al. came to the same conclusion, in their study wherein they compared CMV reactivation in 71 patients who had received a graft from an unrelated donor, but with an HLA match of 10/10, with 78 patients, who had received a graft from a relative. In the unrelated group, reactivation occurred in 19 patients, whereas in the related group it occurred in 17 patients, and statistically significant correlation was not found, but, if the HLA match was below 10/10, the incidence of reactivation increased ($p < 0.08$) [17].

Our study did not show that CMV reactivation affected overall survival. International literature mentions the protective effect of CMV reactivation on relapse, especially in case of AML, but this has no influence on the improvement of overall survival [18]. On the other hand, a retrospective study by Sousa et al. showed that CMV infection significantly decreased survival after transplantation, so the median survival was 16 months, if reactivation occurred, and it was 36 months, if there was no reactivation ($p = 0.002$) [19]. This is partially explained by a higher incidence of infection due to myelosuppression induced by antiviral therapy and due to acute GvHD [18].

Our study had a number of limitations. It included a small number of patients, only 42, who had heterogenous diagnoses, and the analysis itself was performed retrospectively.

CONCLUSION

CMV reactivation after allo-HSCT is still a frequent complication of this procedure, and is affected, to a great extent, by the serostatus ratio of the donor and

ZAKLJUČAK CMV reaktivacija nakon aloTMĆH je i dalje česta komplikacija ove procedure i na nju u velikoj meri utiče serostatus donora i recipijenta (D/R). Najveća incidencija je u kombinaciji R+/D-, tako da izbor između seropozitivnog i seronegativnog donora jeste značajan za seropozitivnog recipijenta. CMV reaktivacija usporava engraftment Le, odnosno odlaže trenutak rekonstitucije alogene hematopoeze, tako da je odgovarajuća preventivna terapija, kod pacijenata koji su pod rizikom, neophodna (letermovir). Ista korelacija za Tr nije utvrđena. Izbor između *MUD* i *MRD* transplantacije nije značajan, u smislu reaktivacije latentne CMV infekcije. *RIC* kondicioni režim je u našoj studiji bio povezan sa manjom incidencijom reaktivacije. Takođe, nije utvrđen uticaj reaktivacije latentne CMV infekcije na sveukupno preživljavanje pacijenata.

SPISAK SKRAĆENICA

CMV- citomegalovirus

aloTMĆH- alogena transplantacija matičnih ćelija hematopoeze

HLA – human leukocyte antigens

GvDH (*graft versus host disease*) – bolest kalema protiv domaćina

MAC – myeloablative conditioning

RIC – reduced intensity conditioning

MRD – match related donor

MUD – match unrelated donor

AML – akutna mijeloidna leukemija

ALL – akutna limfocitna leukemija

HLL – hronična limfocitna leukemija

MDS/MPN mijelodisplastične/mijeloproliferativne neoplazme

HL – Hočkinov limfom

NHL – Nehočkinov limfom

Sukob interesa: Nije prijavljen.

the recipient (D/R). The highest incidence is in the R+/D– combination, which is why the choice between a seropositive and a seronegative donor is significant for a seropositive recipient. CMV reactivation slows down Le engraftment, i.e., it delays the moment of the reconstitution of allogenic hematopoiesis, which is why appropriate preventive therapy, in patients at risk, is necessary (letermovir). The same correlation was not established for Tr. The choice between MUD and MRD transplantation is not significant, in the sense of the reactivation of latent CMV infection. In our study, the RIC conditioning regimen was connected with a lesser reactivation incidence. Also, the effect of latent CMV infection reactivation on the overall survival of patients was not established.

LIST OF ABBREVIATIONS AND ACRONYMS

CMV – cytomegalovirus

allo-HSCT – allogenic hematopoietic stem cell transplantation

HLA – human leukocyte antigens

GvDH (graft versus host disease)

MAC – myeloablative conditioning

RIC – reduced intensity conditioning

MRD – match related donor

MUD – match unrelated donor

AML – acute myeloid leukemia

ALL – acute lymphocytic leukemia

CLL – chronic lymphocytic leukemia

MDS/MPN – myelodysplastic/myeloproliferative neoplasm

HL – Hodgkin lymphoma

NHL – Non-Hodgkin lymphoma

Conflict of interest: None declared.

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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 (aloTMĆ) 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 aloTMĆ-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 aloTMĆ-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 allergenic 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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UVOD

S pojavom poboljšanih hemioterapijskih režima, mogućnosti alogene transplantacije matičnih ćelija (aloTMĆ), 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 neprimenjena 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].

Prevencija i lečenje invazivnih gljivičnih infekcija kod bolesnika u programu aloTMĆ-a predstavlja veliki izazov. Stoga su u protekle dve decenije učinjeni veliki napori 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 dijagonoza 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 kompijuterizovana 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 χ^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 aloTMĆ-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 preforming 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 aloTMĆ-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

Table1. 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 (Hočkinov limfom); NHL (Nehočkinov 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 redukovanog 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 χ^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*

Table2. 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 aloTMĆ-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 (aloTMĆ). 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 aloTMĆ-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 aloTMĆ-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 mikafungina, 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 aloTMĆ-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 aloTMĆ-a, u vidu primene posakonazola *per os* (POS), i njegove kombinacije tj. premošćavanja (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, ispitivali 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 aloTMĆ-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 aloTMĆ-a i razvoja klinički manifestne aspergiloze. Ovaj nalaz je očekivan jer slabost kalema prolongira imunokompromitovanost bolesnika nakon aloTMĆ-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 from 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,

aloTMĆ-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 aloTMĆ-a bude začajno 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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DISEMINOVANA INTRAVASKULARNA KOAGULOPATIJA U AKUTNOJ NEPROMIJELOCITNOJ MIJELOIDNOJ LEUKEMIJI – UČESTALOST, KLINIČKO-LABORATORIJSKE KARAKTERISTIKE I PROGNOSTIČKI ZNAČAJ

ORIGINALNI RAD

ORIGINAL ARTICLE

DISSEMINATED INTRAVASCULAR COAGULOPATHY IN NON-PROMYELOCYTIC ACUTE MYELOID LEUKEMIA – INCIDENCE, CLINICAL AND LABORATORY FEATURES AND PROGNOSTIC SIGNIFICANCE

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SAŽETAK

Uvod: Diseminovana intravaskularna koagulopatija (DIK) je prisutna kod 90% bolesnika sa akutnom promijelocitnom leukemijom (APL). Učestalost DIK-a kod ostalih tipova akutnih mijeloidnih leukemija (ne-APL AML) je znatno manja (10-40%) i do sada ne postoje studije koje su ispitivale uticaj DIK-a na ranu smrt kod ovih bolesnika.

Cilj: Cilj rada bio je da se izvrši analiza učestalosti diseminovane intravaskularne koagulopatije, njenih kliničko-laboratorijskih karakteristika, kao i uticaj na preživljavanje i ranu smrt bolesnika sa ne-APL AML-om.

Materijal i metode: Retrospektivnom analizom je obuhvaćeno 176 bolesnika sa ne-APL AML-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju Univerzitetskog Kliničkog centra Srbije (UKCS) u periodu od 2015. do 2020. godine. Dijagnoza DIK-a je postavljena na osnovu *ISTH* (engl. *International Society on Thrombosis and Haemostasias*) kriterijuma.

Rezultati: Prosečna starost bolesnika iznosila je 53,8±14,5 godina, uz prevalenciju muškog pola (99/176; 56,2%). Manifestna diseminovana intravaskularna koagulopatija konstatovana je kod 74/176 bolesnika (42%), koji su značajno češće imali hemoragijski sindrom ($p = 0,01$). Faktori rizika za nastanak DIK-a bili su: starije životno doba ($p < 0,01$), prisustvo komorbiditeta ($p = 0,01$), leukocitoza ($p < 0,001$) i visoka koncentracija LDH ($p < 0,001$). FAB (engl. French, American and British) podtip ne-APL AML-a, citogenetska grupa rizika i ekspresija CD56 (engl. cluster of differentiation) nisu uticali na nastanak DIK-a ($p > 0,05$). Nije utvrđena razlika u ranoj smrtnosti, ishodu i preživljavanju ne-APL AML bolesnika, sa i bez DIK-a ($p > 0,05$).

Zaključak: Starije životno doba, prisustvo komorbiditeta, leukocitoza i visoke koncentracije LDH nose značajan rizik za razvoj DIK-a, kod bolesnika sa ne-APL AML-om. Prisustvo manifestne diseminovane intravaskularne koagulopatije ne utiče negativno na ranu smrtnost, ishod i preživljavanje bolesnika sa ne-APL AML-om, ukoliko se dijagnoza DIK-a postavi na vreme i preduzme neodložna, adekvatna i intenzivna primena suportivne terapije derivatima i komponentama krvi.

Ključne reči: akutna mijeloidna leukemija, diseminovana intravaskularna koagulopatija, ishod, preživljavanje

ABSTRACT

Introduction: Acute promyelocytic leukemia (APL) has the highest risk for overt disseminated intravascular coagulopathy (DIC), with reported incidence of DIC of up to 90%, as compared to 10-40% in other AML types. The influence of DIC on early death in non-APL AML patients has not been evaluated so far.

Aim: The aim of our study was to analyze the incidence of DIC, its clinical and laboratory characteristics, and the impact on the survival and early death of patients with non-APL AML.

Materials and methods: A total of 176 patients with non-APL AML, diagnosed and treated at the Clinic for Hematology of the Clinical Center of Serbia, between 2015 and 2020, were evaluated retrospectively. The diagnosis of DIC was made on the basis of ISTH (International Society on Thrombosis and Haemostasias) criteria.

Results: The mean age of our patients was 53.8 ± 14.6 years, with 99/176 patients being men (56.2%). DIC was present in 74/176 patients (42.05%), who had a significant prevalence of the hemorrhagic syndrome ($p = 0.01$). The risk factors for overt DIC were the following: older age ($p < 0.01$), comorbidities ($p = 0.01$), leukocytosis ($p < 0.001$) and a high level of LDH ($p < 0.001$). The FAB (French, American and British) type of non-APL AML, the cytogenetic risk group, and CD56 (cluster of differentiation) had no influence on overt DIC ($p > 0.05$). No difference was found in early mortality, outcome, and the survival of non-APL AML patients, with and without DIC ($p > 0.05$).

Conclusion: Older age at diagnosis, comorbidities, leukocytosis, and high LDH concentrations are found to be adverse risk factors for overt DIC in non-APL AML patients. If treated promptly, with immediate, adequate and intensive use of blood derivates and components, DIC has no negative impact on early mortality, outcome, and survival.

Key words: acute myeloid leukemia, disseminated intravascular coagulopathy, outcome, survival

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UVOD

Akutne mijeloidne leukemije (AML) su heterogena grupa malignih bolesti krvi koje karakteriše klonalna ekspanzija mijeloblasta u koštanoj srži (≥20%), perifernoj krvi i/ili drugim tkivima [1]. AML se ubraja u retke bolesti i čini 1,1% svih malignih bolesti. AML je najčešći tip akutnih leukemija adultnog doba i javlja se sa godišnjom incidencijom od 4,3/100.000 stanovnika, nešto češće kod osoba muškog pola (M:Ž = 5,2/100.000 : 3,6/100.000). AML je bolest starih – prilikom postavljanja dijagnoze, 54% bolesnika ima 65 ili više godina, sa medijanom životnog doba od 68 – 71 godine [2-4]. I pored savremenog lečenja, preživljavanje obolelih od AML-a je veoma kratko (petogodišnje preživljavanje = 24%) [2].

Nastanku AML-a može doprineti prethodna primena hemioterapije, radioterapije i imunosupresivnih lekova, za lečenje malignih ili autoimunih bolesti – kada govorimo o *therapy-related AML* (*t-AML*). Nastanku AML-a takođe može doprineti i okupaciono izlaganje ili izlaganje agensima iz životne sredine, koji oštećuju DNK (dezoksiribonukleinska kiselina). AML može biti i sekundarna, tj. nastati evolucijom hroničnih mijeloproliferativnih neoplazmi (MPN) ili mijelodisplaznih sindroma (MDS). Takođe, utvrđena je i genetska predispozicija za nastanak AML-a (Fankonijeva anemija, Daunov sindrom, Švahman-Dajmondov sindrom, sindromi kongenitalne neutropenije) [1,5]. Međutim, etiologija većine AML-a je nepoznata, kada govorimo o *de novo* AML-u.

U AML-u postoji čitav spektar različitih hromozomskih promena, kao što je translokacija t(15;17) (*PML-RARA),* koja je karakteristična za akutnu promijelocitnu leukemiju (APL). Takođe postoji i niz genetskih mutacija, koje utiču na: signalne puteve (kao što su *FLT3-ITD, KIT, MLL, KRAS, NRAS*), nukleofozmin (*NPM1*), transkripcione faktore (kao što su *CEBPA, RUNX1, GATA-2),* i tumorsku supresiju (*TP53, WT1*). Postoji i niz epigenetskih mutacija, koje dovode do metilacije DNK i modifikacije hromatina (kao što su *TET, IDH1, IDH2, MLL*) [5,6].

Savremena klasifikacija akutnih leukemija Svetske zdravstvene organizacije – SZO (*World Health Organization – WHO*), iz 2016. godine, kao i preporuke *ELN* (*European LeukemiaNet*), upravo se i zasnivaju na molekularnim karakteristikama AML-a, budući da one imaju i prognostički i terapijski značaj [7,8], mada se u svakodnevnoj praksi i dalje koristi *FAB* (*French, American and British*) klasifikacioni sistem koji se zasniva na morfološkim i imunofenotipskim karakteristikama leukemijskih ćelija [9,10].

Klinički, AML nastaje iz „punog zdravlja" i manifestuje se povišenom telesnom temperaturom, anemijom, krvarenjem i rekurentnim infekcijama. Bolesnici sa AML-om često imaju trombocitopeniju i poremećaje hemostaze, tj. koagulopatije, koji značajno komplikuju lečenje i doprinose ranoj smrtnosti ovih bolesnika [11].

INTRODUCTION

Acute myeloid leukemias (AML) are a heterogenous group of malignant diseases of the blood characterized by clonal expansion of myeloblasts in the bone marrow (≥20%), in peripheral blood, and/or in other tissues [1]. AML is a rare disease and accounts for 1.1% of all malignant diseases. AML is the most frequently occurring type of acute leukemia of adult age and has an annual incidence of 4.3 per 100,000 population, occurring somewhat more frequently in men (m : f = 5.2/100,000 : 3.6/100,000). AML is a disease of the elderly – at diagnosis, 54% of the patients are 65 years old or above, with the median age being 68 – 71 years [2-4]. Despite modern treatment, survival of AML patients is very short (five-year survival = 24%) [2].

As far as therapy-related AML (t-AML) is concerned, previous chemotherapy, radiotherapy, and immunosuppressive drugs, for the treatment of malignant and autoimmune diseases, can contribute to its development. Occupational exposure, as well as exposure to agents from the environment, which damage DNA (deoxyribonucleic acid), can also contribute to the occurrence of AML. AML can develop as a secondary disease, i.e., it can occur as the result of the evolution of chronic myeloproliferative neoplasms (MPN) or myelodysplastic syndromes (MDS). Also, a genetic predisposition for the development of AML (Fanconi anemia, Down syndrome, Shwachman-Diamond syndrome, congenital neutropenia syndromes) has been confirmed [1,5]. However, as far as *de novo* AML is concerned, the etiology of most AMLs remains unknown.

In AML, there is a whole array of different chromosomal alterations, such as the translocation t(15;17) (PML-RARA), which is characteristic of acute promyelocytic leukemia (APL). There are also a number of genetic mutations, which affect signal pathways (such as FLT3-ITD, KIT, MLL, KRAS, NRAS), nucleophosmin (NPM1), transcription factors (such as CEBPA, RUNX1, GATA-2), and tumor suppression (TP53, WT1). In AML, there are also epigenetic mutations which lead to the methylation of DNA and the modification of chromatin (such as TET, IDH1, IDH2, MLL) [5,6].

The contemporary classification of acute leukemias, issued by the World health Organization (WHO) in 2016, as well as the European LeukemiaNet (ELN) recommendations are, in fact, based on the molecular characteristics of AML, since they have both prognostic and therapeutic significance [7,8], although, in everyday clinical practice, the French, American and British (FAB) classification system, which is based on morphological and immunophenotypical characteristics of leukemia cells is still in use [9,10].

Diseminovana intravaskularna koagulopatija (DIK) je stečeni sindrom koji karakteriše sistemska intravaskularna aktivacija koagulacije, koja može dovesti do multiorganske disfunkcije, tromboze i/ili ekscesivnog krvarenja [12,13]. Najčešća stanja koja dovode do DIK-a su sepsa, šok, solidni tumori i maligne bolesti krvi – akutne leukemije i Nehočkinovi limfomi. Pod dejstvom proinflamatornih citokina, mononuklearne i endotelne ćelije eksprimiraju tkivni faktor (TF). Kontaktom TF-a sa faktorima koagulacije u krvi, započinje koagulaciona kaskada, koja dovodi do generacije trombina i konverzije fibrinogena u fibrin. Istovremeno, interakcija između trombocita i zida krvnog suda doprinosi stvaranju vaskularnih (ili mikrovaskularnih) ugrušaka. P-selektin iz aktivisanih trombocita dodatno pojačava ekspresiju TF-a. Vezivanje TF-a, trombina i drugih aktivisanih faktora koagulacije (proteaza) za specifične proteaza-aktivirane receptore (PAR), i vezivanje fibrina za *toll-like receptor 4* (*TLR4*) na inflamatornim ćelijama, utiče na inflamaciju posledičnim oslobađanjem pro-inflamatornih citokina i hemokina, što dalje modulira koagulaciju i fibrinolizu [14].

Međunarodno društvo za trombozu i hemostazu (*ISTH – International Society on Thrombosis and Hemostasis*) preporučilo je sistem bodovanja, koji se upotrebljava kod bolesnika koji imaju neki osnovni poremećaj, za koji se zna da je povezan sa razvojem DIK-a, i u kojem se prate četiri laboratorijska parametra: broj trombocita (Tr), protrombinsko vreme (*PT*), koncentracija fibrinogena, i nivo D-dimera [12]. Diseminovana intravaskularna koagulopatija je prisutna kod čak 90% bolesnika sa APL-om [15], dok je učestalost DIK-a kod ostalih tipova AML-a znatno manja, i kreće se od 10% do 40% [16]. Ne postoje studije koje su pratile uticaj vrednosti *ISTH* DIK skora na ranu smrt kod bolesnika sa AML-om.

Cilj našeg rada bilo je prikupljanje i analiza podatka o: učestalosti DIK-a u grupi bolesnika sa ne-APL AML-om, kliničkoj slici, kliničko-laboratorijskim parametrima, odnosno učestalosti krvarenja, trombozi, i ranoj smrti ne-APL AML bolesnika sa DIK-om.

METODE

Rađena je retrospektivna analiza 176 uzastopnih bolesnika sa ne-APL AML-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju Kliničkog centra Srbije, u periodu između 2015. i 2020. godine. Dijagnoza AML-a je postavljena na osnovu citomorfoloških, imunofenotipskih, citogenetskih, i molekularnih karakteristika ćelija koštane srži ili periferne krvi, a u skladu sa preporukama Svetske zdravstvene organizacije, iz 2016. godine [7]. Morfološka dijagnoza je postavljena na osnovu *FAB* klasifikacije [9], a prilikom imunofenotipizacije, tehnikom protočne citometrije, sem standardnih monoklonskih antitela [10], primenjeno je

Clinically, AML develops from "full health" and presents with elevated body temperature, anemia, bleeding and recurrent infections. AML patients often have thrombocytopenia and hemostasis disorders, i.e., coagulopathies, which significantly complicate treatment and contribute to early mortality of these patients [11].

Disseminated intravascular coagulopathy (DIC) is an acquired syndrome characterized by systemic intravascular activation of coagulation, which can lead to multiorgan dysfunction, thrombosis, and/or excessive bleeding [12,13]. The most common conditions leading to DIC, are the following: sepsis, shock, solid tumors, and malignant diseases of the blood – acute leukemias and non-Hodgkin lymphomas. Under the influence of proinflammatory cytokines, mononuclear and endothelial cells express the tissue factor (TF). Through the contact of TF with coagulation factors in the blood, the coagulation cascade is initiated, which leads to the generation of thrombin and the conversion of fibrinogen into fibrin. At the same time, interaction between thrombocytes and the blood vessel wall contributes to the creation of vascular (or microvascular) thrombi. P-selectin from activated thrombocytes additionally intensifies the expression of TF. The binding of TF, thrombin, and other activated coagulation factors (proteases) to specific protease-activated receptors (PAR), and the binding of fibrin to toll-like receptor 4 (TLR4) on inflammatory cells, affects inflammation through the consequential release of pro-inflammatory cytokines and chemokines, which further modulates coagulation and fibrinolysis [14].

The International Society on Thrombosis and Hemostasis (ISTH) has recommended a scoring system, which is applied in patients with an underlying disorder, known to be linked to the development of DIC, where the following four laboratory parameters are monitored: platelet (thrombocyte) count (Tr), prothrombin time (PT), fibrinogen concentration, and the D-dimer level [12]. Disseminated intravascular coagulopathy is present in as many as 90% of the patients with APL [15], while the frequency of DIC in other types of AML is significantly lower, and ranges from 10% do 40% [16]. There are no studies analyzing the effect of the ISTH DIC on early mortality in patients with AML.

The aim of our study was to collect and analyze data on the following: the incidence of DIC in a group of patients with non-APL AML, the clinical presentation, clinical and laboratory parameters, i.e., the frequency of bleeding, thrombosis, and early death of non-APL AML patients with DIC.

METHODS

A retrospective analysis was performed, involving 176 consecutive patients with non-APL AML, diagnosed

i monoklonsko antitelo za *CD56* (engl. *cluster of differentiation*), karakteristično za *NK* (engl. *natural killer*) ćelije. Citogenetska procena rizika (povoljna, intermedijarna, nepovoljna) je izvršena prema *ELN* preporukama [8] određivanjem kariotipa – pomoću konvencionalne citogenetike, i molekularnih karakteristika – korišćenjem *PCR* (engl. *polymerase chain reaction*) metode. Dijagnoza t-AML-a je postavljena bolesnicima koji su imali pozitivnu ličnu anamnezu i medicinsku dokumentaciju o prethodnoj primeni hemioterapije, radioterapije i imunosupresivnih lekova, za lečenje malignih ili autoimunskih bolesti. Prilikom postavljanja dijagnoze određivan je i značaj postojećih pridruženih bolesti, tj. komorbiditeta, na osnovu *HCT-CI* (engl. *Hematopoietic cell transplantation specific comorbidity index*) skora [17].

Kod svih bolesnika analizirani su sledeći laboratorijski parametri: hemoglobin-Hb (g/l), broj leukocita – Le (x10^9/l), broj Tr (x10^9/l), procenat mijeloblasta u perifernoj krvi, koncentracija laktat dehidrogenaze – LDH (U/l), PT, aktivisano parcijalno tromboplastinsko vreme (aPTT), fibrinogen i D-dimer. Normalne vrednosti za LDH bile su 220 – 460 U/l, dok su za parametre hemostaze bili: 75 – 120%, za PT; 25 – 35 s, za aPTT; 2 – 4 g/l, za fibrinogen; <0,5 μg/ml, za D-dimer. Dijagnoza DIK-a je postavljena na osnovu *ISTH* kriterijuma: broj trombocita (x10^9/l) : >100x10^9/l = 0,<100 = 1, <50 = 2; D-dimer: normalan = 0, umereno (2 – 4 puta) povećan = 2, izrazito visok (≥5 puta) = 3; PT: >75 % = 0, 50 – 75 % = 1, <50 % = 2; fibrinogen: >1 g/l = 0,<1 g/l = 1. Ukupan skor ≥5 ukazuje na manifestnu diseminovanu intravaskularnu koagulopatiju. Kod svih bolesnika određivano je da li su imali kliničke znake prisustva hemoragijskog sindroma prilikom postavljanja dijagnoze.

Svi bolesnici su primali indukcionu kombinovanu citostatsku terapiju (doksorubicin i citozin-arabinozid po šemi '3+7' ili '2+5', u zavisnosti od opšteg funkcionalnog stanja i komorbiditetnog indeksa), i potom konsolidaciju, primenom citozin-arabinozida. Uporedo sa lečenjem AML-a, bolesnici sa menifestnom diseminovanom intravaskularnom koagulopatijom su lečeni derivatima i komponentama krvi, prema važećim preporukama. Transfuzije trombocita su primenjivane pri vrednostima trombocita <50x10^9/l, kod bolesnika sa manifestnim krvarenjem, a u odsustvu krvarenja, ukoliko su trombociti bili <20x10^9/l. Kod bolesnika sa hemoragijskim oblikom DIK-a i produženim PT-om i aPTT-om, primenjivana je zamrznuta sveža plazma (ZSP) u dozi od 15ml/kg. Bolesnici sa teškom hipofibrinogenemijom (<1g/l), koja se nastavljala i pored primene ZSP-a, primali su i krioprecipitat [13]. Praćeni su: ishod lečenja (živ/umro), rana smrtnost (smrtni ishod od prvog dana hospitalizacije do završetka indukcionog lečenja, odnosno otpusta), ukupno preživljavanje

and treated at the Clinic for Hematology of the Clinical Center of Serbia, between 2015 and 2020. AML diagnosis was established on the basis of cytomorphological, immunophenotypical, cytogenetic, and molecular characteristics of bone marrow cells or peripheral blood cells, in keeping with the recommendations of the World Health Organization from 2016 [7]. The morphological diagnosis was based on the FAB classification [9]. During immunophenotypization by means of flow cytometry, in addition to the standard monoclonal antibodies [10], the monoclonal antibody for CD56 (cluster of differentiation), which is characteristic of NK (natural killer) cells, was also applied. The cytogenetic risk assessment (favorable, intermediate, unfavorable) was carried out in keeping with the ELN recommendations [8] through the determination of the karyotype – by means of conventional cytogenetics and molecular characteristics – with the use of the polymerase chain reaction (PCR) method. The diagnosis of t-AML was established in patients with a positive personal anamnesis and medical documentation on previous application of chemotherapy, radiotherapy, and immunosuppressive drugs, for treating malignant or autoimmune diseases. When establishing the diagnosis, the significance of existing associated diseases, i.e., comorbidities was determined, on the basis of the HCT-CI (Hematopoietic cell transplantation specific comorbidity index) score [17].

In all patients, the following laboratory parameters were analyzed: hemoglobin-Hb (g/l), white blood cell count (WBC), i.e., leukocyte count – Le (x10^9/l), platelet count, i.e., thrombocyte count - Tr (x10^9/l), percentage of myeloblasts in peripheral blood, concentration of lactate dehydrogenase – LDH (U/l), PT, activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer. The normal values for LDH were 220 – 460 U/l, while the normal values for hemostasis parameters were: 75 – 120%, for PT; 25 – 35 s, for aPTT; 2 – 4 g/l, for fibrinogen; <0.5 μg/ml, for D-dimer. The diagnosis of DIC was established on the basis of the ISTH criteria: platelet count (x10^9/l) : >100x10^9/l = 0,<100 = 1, <50 = 2; D-dimer: normal = 0, moderately elevated (2 – 4 times) = 2, very high (≥5 times) = 3; PT: >75 % = 0, 50 – 75 % = 1, <50 % = 2; fibrinogen: >1 g/l = 0,<1 g/l = 1. The total score of ≥5 indicates overt disseminated intravascular coagulopathy. In all of the patients, it was assessed whether they had clinical signs of hemorrhagic syndrome at the time of diagnosis.

All of the patients received combined induction cytostatic therapy (doxorubicin and cytosine arabinoside, following the '3+7' and '2+5' regimen, depending on the general performance status and the comorbidity index), and then consolidation, with the application of cytosine arabinoside. Parallel to the treatment of AML, patients with overt disseminated intravascular coagulopathy

svih bolesnika (engl. *overall survival – OS*), kao i da li je prisustvo DIK-a imalo uticaja na ishod i *OS*.

Prilikom statističke analize korišćene su metode deskriptivne statistike: a) za kontinuirane varijable - aritmetička sredina i standardna devijacija (SD), odnosno medijana i opseg i b) za kategoričke varijable – učestalost, izražena u apsolutnim brojevima i procentima. Za određivanje razlike između dve grupe, korišćeni su odgovarajući statistički testovi: parametarski Studentov T-test za dva nezavisna uzorka, odnosno njegova neparametarska paralela – test sume rangova (Man Vitnijev U test). Za ispitivanje razlike učestalosti, korišćeni su Hi kvadrat test, odnosno Fišerov test tačne verovatnoće. Za analizu preživljavanja, korišćena je Kaplan Majerova metoda, kao i log-rank test za poređenje preživljavanja među ispitivanim grupama. Vrednosti $p < 0,05$ smatrane su statistički značajnim.

REZULTATI

U studiju je uključeno 176 bolesnika sa ne-APL AML-om, 99 muškaraca (56,2%) i 77 žena (43,7%) (M:Ž = 1,29), prosečne starosti 53,8 ± 14,6 godina. Demografske, laboratorijske i kliničke karakteristike bolesnika prikazane su u Tabeli 1.

Prilikom postavljanja dijagnoze, hemoragijski sindrom je bio prisutan kod 72/176 bolesnika (40,9%). Analiza parametara krvne slike pokazala je da su bolesnici, u proseku, imali anemiju umerenog stepena (97,3 ± 18,4 g/l), trombocitopeniju gr III (medijana: 44 x10^9/l; raspon: 1 – 421) i leukocitozu (medijana: 18,5 x10^9/l; raspon: 0,6 – 473,2) sa prisustvom blasta u perifernoj krvi (medijana: 16%, raspon: 0 – 99). Vrednost LDH je, u proseku, bila povišena (medijana: 450 U/l, raspon: 102 – 8.840). Parametri hemostaze su pokazali produženo PT (70 ± 18%) i veoma visok D-dimer (medijana: 3,0 µg/ml, raspon: 0,19 – 138). Kriterijume za manifestnu diseminovanu intravaskularnu koagulopatiju je ispunjavalo 74/176 bolesnika (42%), i oni su imali značajno viši *ISTH* skor (grupa I – *ISTH,* medijana: 5, raspon: 5 – 7; grupa II – *ISTH*, medijana: 3, raspon: 0 – 3) (p < 0,001).

Najveći broj bolesnika je imao AML *FAB* tip M4 (n = 66, 37,5%) i pripadao je *ELN* grupi intermedijarnog citogenetskog rizika (n = 93, 52,8%). Pozitivnost *CD*56 je utvrđena kod 62 bolesnika (35,2%). Bolesnici su imali i značajne pridružene bolesti (*HCT-CI* skor, medijana: 1, raspon: 0 – 8).

Bolesnici sa DIK-om su bili značajno stariji (57,4 ± 12,4 godina) u odnosu na bolesnike koji nisu imali DIK (51,2±15,5 godina), $p = 0,006$. Hemoragijski sindrom prilikom postavljanja dijagnoze je bio značajno češći u grupi bolesnika sa DIK-om, 39/74 (52,7%) u odnosu na bolesnike koji nisu imali DIK, 33/102 (32,4%), $p = 0,01$. U pogledu laboratorijskih parametara, were treated with blood derivatives and blood components, as per the current recommendations. Transfusions of thrombocytes were applied when the thrombocyte count was <50 x10^9/l, in patients with manifest bleeding, and when bleeding was absent, in cases when the thrombocyte count was <20 x10^9/l. In patients with the hemorrhagic form of DIC and prolonged PT and aPTT, frozen fresh plasma (FFP) was applied, in the dose of 15ml/kg. Patients with severe hypofibrinogenemia (<1g/l), which persisted despite the application of FFP, also received cryoprecipitate [13]. The following were monitored: treatment outcome (living/deceased), early mortality (lethal outcome occurring between the first day of hospitalization and the conclusion of induction treatment, i.e., discharge from hospital), overall survival of all the patients (OS), as well as whether the existence of DIC had any effect on the outcome and OS.

In statistical analysis, the following methods of descriptive statistics were applied: a) for continuous variables – the arithmetic mean and the standard deviation (SD), i.e., the median and the range, and b) for categorical variables – frequency, expressed in absolute values, and percentages. For determining the difference between the two groups, the appropriate statistical tests were applied, namely: the parametric Student's T-Test for two independent samples, i.e., its non-parametric parallel – the rank sum test (Mann–Whitney U test). For testing the difference in frequency, the chi-square test, i.e., Fisher's exact probability test were used. For analyzing survival, the Kaplan Meier method, as well as the log-rank test for comparing survival amongst the tested groups, were applied. The values $p < 0.05$ were believed statistically significant.

RESULTS

The study included 176 patients with non-APL AML, 99 men (56.2%) and 77 women (43.7%) (M : Ž = 1.29), whose average age was 53.8 ± 14.6 years. The demographic, laboratory and clinical characteristics of the patients are presented in Table 1.

At the time of diagnosis, hemorrhagic syndrome was present in 72/176 patients (40.9%). The analysis of the blood count parameters showed that the patients, on average, had moderate anemia (97.3 ± 18.4 g/l), grade 3 thrombocytopenia (median: 44 x10^9/l; range: 1 – 421), and leukocytosis (median: 18.5 x10^9/l; range: 0.6 – 473.2), with the presence of blasts in peripheral blood (median: 16%, range: 0 – 99). On average, the LDH level was elevated (median: 450 U/l, range: 102 – 8,840). The parameters of hemostasis showed prolonged PT (70 ± 18%) and very high D-dimer (median: 3.0 µg/ml, range: 0.19 – 138). The criteria for overt disseminated intravascular coagulopathy were met

Tabela 1. Modeli multivarijantne logističke regresije u kojima su nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom ishodna varijabla

Table 1. Multivariate logistic regression models with unmet dental health care needs as an outcome variable

Parametar / *Parameter*	**Vrednost / *Value***	**Manifestna DIK (*ISTH* DIK skor ≥5) / *Overt DIC (ISTH DIC score ≥5)***		**P vrednost / *P value***
		Grupa I - DIK da / *Group I - DIC yes* (n = 74/176; 42%)	**Grupa II - DIK ne / *Group II - DIC no*** (n = 102/176; 58%)	
Pol – n, % / *Sex – n, %*				
Muški / *Male*	99 (56.25)	47 (63.5)	52 (51)	
Ženski / *Female*	77(43.75)	27 (36.5)	50 (49)	0.124
Starost- srednja vrednost (godine), SD / *Age – mean (years), SD*	53.8 ± 14.6	57.4 ± 12.4	51.2 ± 15.5	0.006
Le – medijana ($x10^9$/l), raspon / *WBC – median ($x10^9$/L), range*	18.5 (0.6-473.2)	32.1 (0.6-451)	13.6 (1.09-473.2)	0.001
Hb – medijana (g/l), SD / *Hb – median (g/L), SD*	97.3 ± 18.4	97.1 ± 17.4	97.6 ± 19.2	0.874
Tr – medijana ($x10^9$/l), raspon / *Plt – median ($x10^9$/L), range*	44 (1 – 421)	32.5 (1 – 151)	61.5 (2 – 421)	0.001
% blasta u perifernoj krvi – medijana, raspon / *% peripheral blood blasts- median, range*	16 (0 – 99)	15.5 (0 – 97)	17 (0 – 99)	0.741
LDH – medijana (U/l), raspon / *LDH – median (U/L), range*	450 (102 – 8,840)	591.5 (102 – 5,786)	383 (108 – 8,840)	0.001
PT – srednja vrednost (%), SD / *PT – mean (%), SD*	70 ± 18	61 ± 16	78 ± 15	0.001
aPTT – srednja vrednost (s), SD / *aPTT – mean (s), SD*	29.6 ± 5.9	30.2 ± 6.1	29.2 ± 5.8	0.175
Fibrinogen– srednja vrednost (g/l), SD / *Fibrinogen – mean (g/L), SD*	5.4 ± 1.9	5.2 ± 2.0	5.6 ± 1.8	0.270
D-dimer – medijana (µg/ml), raspon / *D-dimer – median (µg/mL), range*	3.0 (0.2 – 138)	6.2 (0.8 – 138)	1.32 (0.2 – 74)	0.001
Tip AML (n,%) / *AML type (n, %)*				
FAB M0	10 (5.7)	5 (6.8)	5 (4.9)	
FAB M1	23 (13.1)	10 (13.5)	13 (2.7)	
FAB M2	34 (19.3)	13 (17.6)	21 (20.6)	
FAB M4	66 (37,5)	33 (44.6)	33 (33.4)	0.228
FAB M5	21 (11.9)	9 (12.2)	12 (10.8)	
FAB M6	0	0	0	
FAB M7	0	0	0	
t-AML	22 (12.5)	4 (5.3)	18 (17.8)	
Citogenetska grupa rizika (n,%) / *Cytogenetic risk group (n, %)*				
Povoljna / *Favorable*	18 (10.2)			
Intermedijarna / *Intermediate*	93 (52.8)	5 (8.3)	13 (13.5)	
Nepovoljna / *Unfavorable*	45 (25.6)	37 (61.7)	56 (58.3)	0.612
Nema podataka / *Data missing*	20 (11.4)	18 (30)	27 (28.2)	
CD56 (n,%) /				
Da / *Yes*	62 (35.2)	28 (45.9)	34 (41)	
Ne / *No*	82 (46.6)	33 (54.1)	49 (59)	0.674
Nema podataka / *Data missing*	32 (18.8)			
Hemoragijski sindrom (n,%) /				
Da / *Yes*	72 (40.9)	39 (52.7)	33 (32.4)	0.01
Ne / *No*	104 (59.1)	35 (47.3)	69 (67.6)	
HCT-CI (medijana, raspon) / *HCT-CI (median, range)*	1 (0 – 8)	2 (0 – 8)	1 (0 – 6)	0.01
Konačan ishod (n,%) / *Outcome (n, %) / Bleeding-hemorrhagic syndrome (no, %)*				
Živ / *Living*	48 (27.3)	18 (24.3)	30 (29.4)	
Umro / *Deceased*	124 (70.4)	54 (73)	70 (68.6)	
Nema podataka / *Data missing*	4 (2.3)	2 (2.70)	2 (12)	0.496
Rana smrt (n, %) /				
Da / *Yes*	41 (23.3)	20 (27)	21 (20.6)	
Ne / *No*	128 (72.7)	50 (67.6)	78 (76.4)	
Nema podataka / *Data missing*	7 (4)	4 (5.4)	3 (3)	0.281

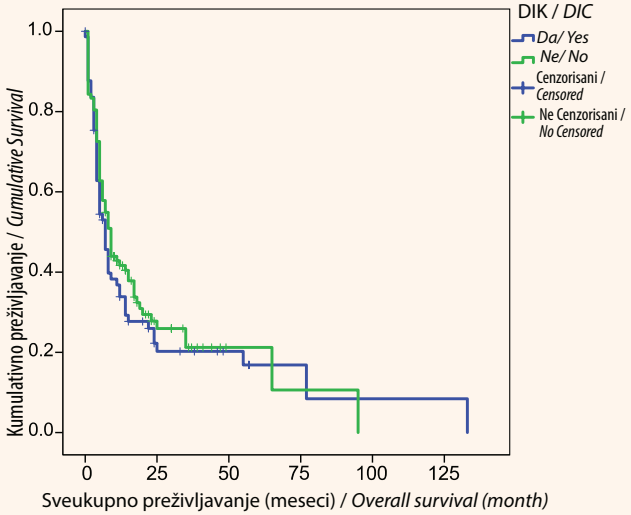
Legenda: APL – akutna promijelocitna leukemija; DIK – diseminovana intravaskularna koagulopatija; Hb – hemoglobin,; Le – leukociti; Tr – trombociti, LDH – laktat dehidrogenaza, PT – protrombinsko vreme; aPTT- aktivisano parcijalno tromboplastinsko vreme; ISTH – International Society for Thrombosis and Haemostasias; FAB – French, American and Bristish; HCT CI – Hematopoietic cell transplantation specific comorbidity index

Legend: *APL – acute promyelocytic leukemia; DIC – disseminated intravascular coagulation; Hb –hemoglobin; WBC – leukocyte count; Plt – platelets, LDH – lactate dehydrogenase, PT – prothrombin time; aPTT – activated partial thromboplastin time; ISTH – International Society for Thrombosis and Haemostasias; FAB – French, American and British; HCT CI – hematopoietic cell transplantation specific comorbidity index*

bolesnici sa DIK-om su imali značajno niži broj Tr (grupa I – medijana: 32,5 x10^9/l, raspon: 1 –151, u odnosu na grupu II – medijana: 61,5, raspon: 2 – 421; $p < 0,001$), značajno više vrednosti LDH (grupa I – medijana: 591,5 U/l, raspon: 102 – 5.786; grupa II – medijana: 383 U/l, raspon: 108 – 8.840; $p < 0,001$), značajno duže PT (grupa I: 61,6 ± 16%; grupa II: 78 ± 15%; $p < 0,001$), i značajno veće vrednosti D-dimera (grupa I – 6,2 µg/ml, raspon: 0,82 – 138; grupa II – 1,32 µg/ml, raspon: 0,2 – 74; $p < 0,001$). Bolesnici sa DIK-om su imali veći broj komorbiditeta (grupa I – prosečan *HCT-CI* skor: 2, raspon: 0 – 8) u odnosu na one koji nisu imali DIK (grupa II – prosečan *HCT-CI* skor: 1, raspon: 0 – 6), $p = 0,01$. Tip AML-a, *ELN* citogenetska grupa rizika, i pozitivnost *CD56* nisu uticali na razvoj manifestne diseminovane intravaskularne koagulopatije ($p > 0,05$).

U pogledu ishoda, od 176 ispitivanih bolesnika, do kraja praćenja živih je bilo 48 ne-APL AML bolesnika (27,7%). Prisustvo DIK-a nije uticalo na ishod (živi, grupa I – 18/74 (24,3%); živi, grupa II – 30/102 (29,4%), $p = 0,496$). Indukciona smrt je zabeležena kod 41/176 bolesnika (23,3%), i nije se značajno razlikovala između dve ispitivane grupe (grupa I – n = 20/74 (27%); grupa II – n = 21/102 (27%); $p = 0,291$).

Ukupno preživljavanje svih bolesnika iznosilo je 7 meseci (raspon: 0 – 57), i premda je bilo kraće kod bolesnika sa manifestnom diseminovanom intravaskularnom koagulopatijom (grupa I – 5 meseci, raspon: 0 – 57), u odnosu na bolesnike bez koagulopatije (grupa II – 7 meseci, raspon: 0 – 49), ova razlika nije pokazala statističku značajnost (log-rank: 0,518), što je i prikazano Kaplan Majerovom krivom preživljavanja (Grafikon 1).



Grafikon 1. Preživljavanje (meseci) 176 bolesnika sa ne-APL AML-om, u odnosu na prisustvo manifestne diseminovane intravaskularne koagulopatije (DIK)

Figure 1. Survival (months) of 176 patients with non-APL AML, in relation to overt DIC

by 74/176 patients (42%), and they had a significantly higher ISTH score (group I – ISTH, median: 5, range: 5 – 7; group II – ISTH, median: 3, range: 0 – 3) ($p < 0.001$).

The greatest number of patients had AML FAB type M4 (n = 66, 37.5%) and belonged to the ELN group of intermediate cytogenetic risk (n = 93, 52.8%). CD56 positivity was established in 62 patients (35.2%). Patients also had significant associated diseases (HCT-CI score, median: 1, range: 0 – 8).

Patients with DIC were significantly older (57.4 ± 12.4 years), as compared to patients without DIC (51.2 ± 15.5 years), $p = 0.006$. Hemorrhagic syndrome at the time of diagnosis was significantly more common in the group of patients with DIC, 39/74 (52.7%), as compared to patients without DIC, 33/102 (32.4%), $p = 0.01$. As to laboratory parameters, patients with DIC had a significantly lower platelet count (group I – median: 32.5x10^9/l, range: 1 – 151, as compared to group II – median: 61.5, range: 2 – 421; $p < 0.001$), significantly higher levels of LDH (group I – median: 591.5 U/l, range: 102 – 5,786; group II – median: 383 U/l, range: 108 – 8,840; $p < 0.001$), significantly longer PT (group I: 61.6±16%; group II: 78±15%; $p < 0.001$), and significantly higher levels of D-dimer (group I – 6.2 µg/ml, range: 0.82 – 138; group II – 1.32 µg/ml, range: 0.2 – 74; $p < 0.001$). Patients with DIC had a greater number of comorbidities (group I – average HCT-CI score: 2, range: 0 – 8), as compared to the patients without DIC (group II – average HCT-CI score: 1, range: 0 – 6), $p = 0.01$. The type of AML, the ELN cytogenetic risk group, and CD56 positivity, did not affect the development of overt disseminated intravascular coagulopathy ($p > 0.05$).

As to the outcome, by the end of the follow-up period, of the 176 subjects, there were 48 living non-APL AML patients (27.7%). The occurrence of DIC did not affect the outcome (living, group I – 18/74 (24.3%); living, group II – 30/102 (29.4%), $p = 0.496$). Induction death was registered in 41/176 patients (23.3%), and it did not significantly differ between the two analyzed groups (group I – n = 20/74 (27%); group II – n = 21/102 (27%); $p = 0.291$).

The overall survival of all patients was 7 months (range: 0 – 57), and though it was shorter in patients with overt disseminated intravascular coagulopathy (group I – 5 months, range: 0 – 57), as compared to patients without coagulopathy (group II – 7 months, range: 0 – 49), this difference did not show statistical significance (log-rank: 0.518), which has been presented with the Kaplan Meier survival curve (Figure 1).

DISCUSSION

The pathophysiological mechanism of DIC development in acute leukemias is complex, and it simultaneously includes the following: a) coagulation activation caused by the exposure of the tissue factor (TF) to blood;

DISKUSIJA

Patofiziološki mehanizam nastanka DIK-a u akutnim leukemijama je kompleksan, i podrazumeva istovremeno: a) aktivaciju koagulacije uzrokovanu izlaganjem tkivnog faktora (TF) krvi; b) poremećaj kontrole antikoagulantih mehanizama, i c) supresiju fibrinolize putem povećane ekspresije *PAI-1* (*plasminogen activator inhibitor-1*). Ove promene udruženo uzrokuju endotelijalnu disfunkciju i mikrovaskularne tromboze, koje dovode do disfunkcije organa i značajno negativno utiču na prognozu osnovne bolesti [14]. Leukemijske ćelije oslobađaju TF, a takođe sekretuju i proinflamatorne citokine, pre svega IL-6 (interleukin) i *TNF* alfa (*tumor necrosis factor*), koji oštećuju endotel krvnih sudova. Oštećenje endotela, s jedne strane, dovodi do povećane ekspresije TF-a, kao i *PAI-1*, a sa druge strane dovodi do smanjene ekspresije trombomodulina (TM) koji konvertuje protein C (PC) u aktivisani PC (APC), i inhibira koagulaciju. Leukemijske ćelije oslobađaju mnoge mikropartikule, koje, osim TF-a, sadrže i kancer-prokoagulant, koji ima aktivnost serin proteaze, i koji direktno, aktivacijom faktora X (FX) započinje koagulacionu kaskadu i generisanje trombina.

DIK karakteriše i poremećaj fibrinolize, koji, u fiziološkim uslovima, ima za cilj da degradacijom fibrinskih depozita spečava insuficijenciju periferne cirkulacije. U akutnim leukemijama, proinflamatorni citokini uzrokuju povećanu eksperesiju *PAI-1*, koji inhibicijom aktivatora plazminogena sprečava nastanak plazmina (sekundarna fibrinoliza). U akutnim leukemijama, veoma je pojačana i primarna fibrinoliza. Sve ovo dovodi do izražene hipofibrinogenemije i porasta fibrinogen/fibrin degradacionih proizvoda (FDP) i D-dimera [18]. Oko 15% bolesnika sa ne-APL AML-om dodatno razvije DIK u toku indukciono-remisione terapije. Maligne ćelije, pod dejstvom citotoksičnih lekova, podležu apoptozi, uz oslobađanje intranuklearnih proteina, poput histona H3 i *HMGB1* (*high-mobility group box-1*), što doprinosi nastanku DIK-a i sindroma lize tumora (engl. *tumor lysis syndrome – TLS*) [19].

Incidencija DIK-a u akutnim leukemijama je varijabilna. Najveća je kod APL-a, a najmanja kod B-ćelijske akutne limfoblastne leukemije [16]. Diseminovana intravaskularna koagulopatija je veoma ispitivana kod APL-a, ali su podaci o njenoj učestalosti i značaju u ne-APL AML-u veoma oskudni i raznoliki. Tako se saopštena učestalost DIK-a, na osnovu *ISTH* kriterijuma, kod bolesnika sa ne-APL AML-om, prilikom postavljanja dijagnoze, kreće od 6,4% [20] do 25,2% [16]. Liburel i saradnici [21] su saopštili da je učestalost diseminovane intravaskularne koagulopatije, određene prema *ISTH* kriterijumima, bila veća kod mlađih bolesnika (18 – 65 godina) (8,5%), u odnosu na starije bolesnike sa ne-APL

b) the disruption of the anticoagulant mechanism control, and c) the suppression of fibrinolysis through increased expression of PAI-1 (plasminogen activator inhibitor-1). These changes jointly cause endothelial disfunction and microvascular thromboses, which lead to organ dysfunction, and have a significantly negative effect on the prognosis of the underlying disease [14]. Leukemia cells release TF, and they also secrete proinflammatory cytokines, primarily IL-6 (interleukin) and TNF alfa (tumor necrosis factor), which damage the endothelium of blood vessels. Endothelial damage, on the one hand, leads to increased expression of TF and PAI-1, and, on the other hand, it causes decreased expression of thrombomodulin (TM), which converts protein C (PC) into activated PC (APC), and inhibits coagulation. Leukemia cells release many microparticles, which, in addition to TF, also contain cancer procoagulant, which has the activity of serine proteas, and which initiates the coagulation cascade as well as the generating of thrombin through direct activation of factor X (FX).

DIC is characterized by fibrinolysis disruption, whose purpose, in physiological conditions, is to prevent insufficiency in peripheral circulation through the degradation of fibrin deposits. In acute leukemias, proinflammatory cytokines cause the increased expression of PAI-1, which, through the inhibition of plasminogen activators, prevents the synthesis of plasmin (secondary fibrinolysis). In acute leukemias, primary fibrinolysis is also very intensified. All of this leads to marked hyperfibrinogenemia and the increase in fibrinogen/fibrin degradation products (FDP) and D-dimer [18]. Around 15% of patients with non-APL AML additionally develop DIC during induction remission therapy. Malignant cells, under the influence of cytotoxic drugs, undergo apoptosis, releasing intranuclear proteins, such as histone H3 and HMGB1 (high-mobility group box-1), which contributes to the development of DIC and the tumor lysis syndrome (TLS) [19].

The incidence of DIC in acute leukemias is variable. The highest incidence is in APL, and the lowest incidence is in B-cell acute lymphoblastic leukemia [16]. Disseminated intravascular coagulopathy has been intensively tested and analyzed in APL, however, the data on its frequency and significance in non-APL AML are very limited and varied. Hence, reported frequency of DIC, based on ISTH criteria, in patients with non-APL AML, at diagnosis, ranges from 6.4% [20] to 25.2% [16]. Libourel et al. [21] reported that the frequency of disseminated intravascular coagulopathy, determined according to the ISTH criteria, was higher in younger patients (18 – 65 years) (8.5%), as compared to older patients with non-APL AML (6.3%), while older patients significantly more often had hemorrhagic syndrome (13%). The frequency

AML-om (6,3%), dok su stariji bolesnici značajno češće imali hemoragijski sindrom (13%). Učestalost DIK-a kod bolesnika u našoj studiji iznosila je 42%, znatno više, u poređenju sa gorenavedenim podacima. Uz to, naši bolesnici su znatno češće imali manifestno krvarenje prilikom postavljanja dijagnoze, preko 50% bolesnika sa DIK-om (39/74) imalo je hemoragijski oblik DIK-a.

Leukocitoza (>20x10^9/l) nosi sa sobom veći rizik za razvoj DIK-a u AML-u. Leukemijske ćelije koje, za razliku od eritrocita, nisu elastične i savitljive, stvaraju agregate u mikrocirkulaciji, što, sa jedne strane, dovodi do vaskularne okluzije i dodatnog oštećenja endotela, a sa druge, do pojačanog oslobađanja citokina, mikropartikula i intranuklearnih proteina iz agregiranih blasta [16,22]. Sem izražene leukocitoze, u grupi naših bolesnika sa DIK-om, registrovane su i značajno više vrednosti LDH. Visoka koncentracija LDH je prediktor visokog rizika od krvarenja [23].

Važno je istaći da su bolesnici sa DIK-om bili znatno stariji i imali su značajno više komorbiditeta, u odnosu na bolesnike sa ne-APL AML-om koji nisu razvili DIK. Samo po sebi, starije životno doba se smatra stanjem hronične inflamacije [24], a prisutne pridružene bolesti dodatno mogu uticati na razvoj DIK-a. Analizu uticaju komorbiditeta na razvoj DIK-a nismo našli u dostupnoj literaturi.

Bolesnici sa DIK-om, iz naše studije, imali su teži stepen trombocitopenije, značajno duže PT i viši D-dimer, u odnosu na bolesnike koji nisu imali DIK, a to su i parametri koji se prate u *ISTH* DIK skoru. Postavljanje adekvatne dijagnoze DIK-a u AML često predstavlja izazov, s obzirom na samu prirodu bolesti [11]. Kako bolesnici sa akutnim leukemijama veoma često imaju trombocitopenije, i u odsustvu DIK-a, usled infiltracije koštane srži i primene citotoksične terapije, preporučen je novi *JMWH* (*Japanese Ministry of Health and Welfare*) sistem bodovnja, za dijagnozu DIK-a, u kom se boduje i osnovna bolest (akutne leukemije nose 1 bod), a modifikovani su bodovni kriterijumi za trombocitopeniju, koncentraciju fibrinogena i FDP-a [25].

U pogledu bioloških karakteristika ne-APL AML-a i rizika za nastanak DIK-a, kod naših bolesnika nije utvrđena razlika između *FAB* podtipova AML-a i citogenetske grupe rizika, a ni ekspresija *CD56*, za koju je saopšteno da nosi lošu prognozu u AML-u [26], nije uticala na razvoj DIK-a. Naši rezultati se razlikuju od rezultata Guo i saradnika [16], koji su utvrdili da je prevalencija DIK-a značajno veća kod bolesnika sa normalnim kariotipom i mutacijama *NPM1* i/ili *FLT3-ITD*. Liburel i saradnici [21] su objavili najveću učestalost DIK-a u *FAB* tipu M5. Interesantno je da su mutacije *NPM*1 i *FLT3-ITD* [27], kao i AML tip *FAB*5 [28] povezani sa hiperleukocitozom.

of DIC in the patients from our study was 42%, which is significantly higher, as compared to the abovementioned data. Additionally, our patients more often had manifest bleeding at diagnosis, more than 50% of the patients with DIC (39/74) had hemorrhagic syndrome.

Leukocytosis (>20x10^9/l) carries a higher risk for the development of DIC in AML. Leukemia cells, which, conversely to erythrocytes, are not elastic and flexible, create accumulations in the microcirculation, which, on one hand, leads to vascular occlusion and additional damage to the endothelium, and, on the other hand, it leads to increased release of cytokines, microparticles, and intranuclear proteins from aggregated blasts [16,22]. In addition to marked leukocytosis, in our group of patients with DIC, significantly higher levels of LDH were also registered. A high concentration of LDH is a predictor of a high risk of bleeding [23].

It is important to emphasize that patients with DIC were significantly older and that they had significantly more comorbidities, as compared to patients with non-APL AML who did did not develop DIC. Elderly age, in itself, is considered to be a state of chronic inflammation [24], while present associated diseases can additionally affect the development of DC. We were not able to find analyses of the influence of comorbidities on the development of DIC in available literature.

The patients with DIC, from our study, had a more severe degree of thrombocytopenia, significantly longer PT, and a higher D-dimer, as compared to patients without DIC, and these are the parameters that are monitored on the ISTH DIC score. Establishing the correct diagnosis of DIC in AML is often a challenge, bearing in mind the nature of the disease itself [11]. As patients with leukemia very often suffer from thrombocytopenia, even in the absence of DIC, due to the infiltration of bone marrow and the application of cytotoxic therapy, a new JMWH (Japanese Ministry of Health and Welfare) scoring system has been proposed for DIC diagnosis. This system scores the underlying disease, as well (acute leukemias are scored with one point), however, the scoring criteria for thrombocytopenia, fibrinogen concentration, and FDP have been modified [25].

As far as the biological characteristics of non-APL AML and the risks for the development of DIC are concerned, in our patients, no difference was determined between the FAB subtypes of AML and the cytogenetic group of risks. Additionally, the expression of CD56, which has been reported to carry an unfavorable prognosis in AML [26], did not affect the development of DIC. Our results differ from the results obtained in the study by Guo et al. [16], who found that the prevalence of DIC was significantly higher in patients with a normal karyotype and the mutations NPM1 and/or FLT3-ITD. Libourel et al. [21] reported the highest frequency of DIC

Udruženost DIK-a i AML-a nosi lošu prognozu [11,15,18-22]. Bolesnici sa DIK-om iz naše studije su, uporedo sa lečenjem osnovne bolesti, primali i suportivnu terapiju derivatima i komponentama krvi [11,13]. Primena antifibrinolitka u lečenju DIK-a u AML-u se ne preporučuje, s obzirom na opasnost od promocije stvaranja fibrinskih depozita [11]. U Japanu je, za lečenja DIK-a u akutnim leukemijama, odobrena primena rekombinantnog solubilnog trombomodulina (rTM), koji vezivanjem za trombin, inaktivira koagulaciju [29]. Ishod, rana smrtnost i preživljavanje kod naših ne-APL AML bolesnika sa prisutnim DIK-om, nisu se značajno razlikovali u odnosu na bolesnike koji nisu imali DIK, što je posledica opisanog terapijskog pristupa.

ZAKLJUČAK

Na osnovu sprovedenog istraživanja, možemo zaključiti da starije životno doba, prisustvo komorbiditeta, leukocitoza, i visoke koncentracije LDH, nose značajan rizik za razvoj DIK-a kod bolesnika sa ne-APL AML-om. Prisustvo manifestne diseminovane intravaskularne koagulopatije ne utiče negativno na ranu smrtnost, ishod i preživljavanje bolesnika sa ne-APL AML-om, ukoliko se dijagnoza DIK-a postavi na vreme i preduzme neodložna, adekvatna i intenzivna primena suportivne terapije derivatima i komponentama krvi.

Sukob interesa: Nije prijavljen.

in the FAB type M5. It is interesting to note that the mutations NPM1 and FLT3-ITD [27], as well as the AML type FAB5 [28] have been linked to hyperleukocytosis.

The joint occurrence of DIC and AML carries an unfavorable prognosis [11,15,18-22]. The patients with DIC, from our study, received, not only treatment for their underlying disease, but were simultaneously also given supportive blood derivatives and components therapy [11,13]. The application of antifibrinolytics in the treatment of DIC in AML is not recommended, due to the danger of promoting the creation of fibrin deposits [11]. In Japan, the application of recombinant soluble thrombomodulin (rTM) has been approved, for treating DIC in acute leukemias, as it inactivates coagulation by binding to thrombin [29]. The outcome, early mortality, and survival in our non-APL AML patients with DIC did not significantly differ from the same parameters in our patients without DIC, which is the consequence of the above-described treatment approach.

CONCLUSION

Based on the research conducted within this study, we can conclude that older age, the presence of comorbidities, leukocytosis, and high levels of LDH, carry a significant risk of DIC development in patients with non-APL AML. The occurrence of overt disseminated intravascular coagulopathy does not negatively affect early mortality, the outcome, and overall survival of patients with non-APL AML, if the diagnosis of DIC is established on time, and timely, appropriate and intensive supportive therapy with blood derivatives and components is administered promptly.

Conflict of interest: None declared.

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MEDICINSKA STATISTIKA U *R* PROGRAMSKOM OKRUŽENJU

PRIKAZ KNJIGE

BOOK REVIEW

MEDICAL STATISTICS IN THE *R* SOFTWARE ENVIRONMENT

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Izdavač: CIBID, Medicinski fakultet, Univerzitet u Beogradu, Beograd, 2019.
ISNB 978-86-7117-572-2

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ISNB 978-86-7117-572-2

Knjiga „Medicinska statistika u *R* programskom okruženju" autora Trajković G. i Bukumirić Z., u izdanju Medicinskog fakulteta Univerziteta u Beogradu, jeste nezaobilazna publikacija u poslediplomskom usavršavanju i istraživačkim naporima u oblasti medicine i zdravstvenih nauka. Na stručan i jednostavan način, autori koji su dokazani eksperti u oblasti medicinske statistike i informatike, omogućavaju čitaocima aktivno učenje uz rešavanje realnih problema, korak po korak, u *R* programskom okruženju. Osnovna poglavlja obuhvataju *Medicinsku statistiku* (opis različitih statističkih metoda, objašnjavanje analize i interpretacije rezultata) i *R programsko okruženje* (svi primeri se izvode u *R*-u, uz detaljna objašnjenja za pisanje kôda i vizuelizaciju, koja obezbeđuje jednostavno korišćenje). Cilj autora nije bio da prikažu sve mogućnosti *R*-a, već njegovu

The book *Medical Statistics in the R Software Environment*, written by Trajković G. and Bukumirić Z., published by the Faculty of Medicine of the University of Belgrade, is a publication of essential value for graduate education as well as for research efforts in the area of medicine and health sciences. In a professional and straightforward manner, the authors, who are recognized experts in the area of medical statistics and informatics, provide the readers with an opportunity for active learning whilst resolving real problems, step by step, in the *R* software environment. The main chapters are - *Medical Statistics* (describing different statistical methods, explaining results analysis and interpretation) and *R Software Environment* (all examples are carried out in *R* software, with detailed explanations for code writing and visualization, which enables easy

praktičnu primenu u rešavanju realnih problema iz oblasti medicinske statistike i grafičke prezentacije podataka.

Statistička metodologija je u savremenom životu deo opšteg obrazovanja, može se reći i opšte kulture, jer svaka struka susreće se sa situacijama gde je neophodno poznavanje određenih osnovnih ključnih statističkih pojmova, a pogotovo statističkog načina mišljenja. Upotreba različitih statističkih softverskih alata pomaže nam u primeni statističkih metoda. Jedan od sve češće primenjivanih statističkih softverskih alata u medicini je *R* programski jezik i okruženje, koji predstavlja najsveobuhvatniji statistički softverski alat, sa mogućnošću analize klasičnih i naprednih tehnika, i koji ima najnaprednije grafičke mogućnosti za vizuelizaciju kompleksnih podataka. Ova publikacija je namenjena, kako polaznicima koji se po prvi put susreću sa okruženjem programskog jezika *R*, ali i sa primenom statističkih metoda, tako i iskusnim korisnicima.

Poglavlja knjige osmišljena su tako da obuhvataju teorijske osnove i praktičnu primenu statističkih procedura kroz *R* programsko okruženje:

- Poglavlje *R programsko okruženje* sadrži uputstva o instaliranju i osnovama korišćenja ovog statističkog softverskog alata.
- U poglavlju *Razvoj i dokumentovanje baze podataka*, sadržane su informacije o načinu organizacije prikupljenih podataka u formu podesnu za kasniju analizu u *R* programskom okruženju.
- Slede poglavlja koja se odnose na statističku deskripciju i analizu podataka: *Deskriptivna statistika*; *Analiza empirijskih raspodela*; *Grafičko prikazivanje podataka i rezultata primenjenih statističkih analiza*; *Ocenjivanje populacionih parametara na osnovu uzorka*; *Testiranje hipoteza za raspodele*, *aritmetičke sredine i medijane*.
- U knjizi su sadržana i poglavlja koja se odnose na složenije statističke analize, odnosno statističko modelovanje, i način njihovog izvođenja u ovom statističkom programu: *Ispitivanje odnosa između varijabli*; *Generalni linearni modeli*; i *Kontrola pridruženosti*.
- Pojmovi valjanosti i pouzdanosti merenja, kao i performanse dijagnostičkih testova obrađeni su u okviru poglavlja *Adekvatnost merenja*.
- U okviru poglavlja *Meta-analiza*, prikazane su procedure objedinjavanja publikovanih rezultata, kao i njihovo modelovanje kroz meta-regresiju.

Poseban kvalitet publikacije je njen koncept i specifičan dizajn. Autori su uz publikaciju ponudili i baze podataka i *R* skriptove sa komandama za rešavanje svih

use). The authors' goal was not to present all the possibilities of the *R* software, rather its practical application in the resolution of real problems in the area of medical statistics and graphic data representation.

In contemporary life, statistical methodology is a part of general education, one could say general knowledge, as, in every profession, one encounters situations where knowledge of certain basic key statistical concepts, and especially of the statistical way of thinking, is necessary. The application of different statistical software tools helps us in the application of statistical methods. One of the statistical software tools applied more and more frequently in medicine is the *R* programming language and environment, which is the most comprehensive statistical software tool that enables the analysis of standard and advanced techniques, and which has the most advanced graphic solutions for the visualization of complex data. This publication is intended, not only for people encountering the *R* programming language environment, as well as the application of statistical methods, for the first time, but also for experienced users.

The chapters of the book have been designed to cover the theoretical bases as well as the practical application of statistical procedures through the *R* software environment:

- The chapter *R Software Environment* contains instructions on the installation and the bases for the application of this statistical software tool.
- The chapter *The Development and Documenting of the Database* contains information on the method of organizing collected data into a form appropriate for subsequent analysis in the *R* software environment.
- The chapters that follow are related to statistical description and data analysis: *Descriptive Statistics*; *Analysis of Empirical Distributions*; *Graphic Presentation of Data and Results of Applied Statistical Analyses*; *Sample Based Assessment of Population Parameters*; *Testing Hypotheses for Distributions*, *Arithmetic Means and Medians*.
- The book also includes chapters related to more complex statistical analyses, i.e., statistical modelling, and the method of their execution in this statistical program: *Testing Relationships amongst Variables*; *General Linear Models*; and *Association Control*.
- The terms of validity and reliability of measurement, as well as the performance of diagnostic tests, are dealt with within the chapter *Measurement Adequacy*.
- Within the chapter *Meta-analysis*, the procedures of collating the published results, as well as their modelling, through meta-regression, are presented.

primera. Na ovaj način, korisnici mogu ponoviti analizu, korak po korak, i imati pristup kompletnim izlaznim rezultatima u digitalnom – elektronskom okruženju. Baze podataka i *R* skriptovi mogu se preuzeti sa internet stranice knjige: *http://cibid.med.bg.ac.rs/publikacije/med.stat.R*.

Takođe, autori su postigli i dodatnu vrednost primenjujući koncizan, razumljiv i jasan stil pisanja, a postojeća saznanja o primeni statističkih metoda i njihovo izvođenje uz upotrebu *R* programskog okruženja, izneli su sistematično i logičnim redosledom. Tekstovi autora predstavljaju rezultat njihovog višegodišnjeg naučnog i stručnog rada, proverenog kroz više generacija studenata na više fakulteta. U publikaciji se ogledaju originalnost i naučni i stručni doprinos što je čini nezaobilaznim delom svake stručne biblioteke i značajnom literaturom na koju se uvek vraćaju, ne samo studenti osnovnih i poslediplomskih studija, već i istraživači medicinskih i zdravstvenih nauka, kao i lekari svih specijalnosti.

Publikacija je štampana nakon iznenadne smrti prof. dr Gorana Trajkovića, jednog od autora, koji i na ovaj način nastavlja da nesebično prenosi svoje znanje drugima.

U Beogradu 25.05.2021.

Recenzenti
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A particular quality of the publication is its concept and its distinctive design. Within this publication, the authors have also offered databases and *R* scripts with commands for solving all the examples. In this way, the users can repeat analysis, step by step, and have access to complete output results in the digital – electronic environment. Databases and *R* scripts can be downloaded from the publication webpage: *http://cibid.med.bg.ac.rs/publikacije/med.stat.R*.

Furthermore, the authors have achieved additional value with their concise, understandable and clear writing style, while the existing knowledge on the application of statistical methods and their execution with the use of the *R* software environment has been presented systematically, in logical order. The author's texts are the result of their many years of research and professional work, which has been tested by multiple generations of students at a number of different faculties. The publication is original and its scientific and expert contribution is evident, which is what makes it an essential part of any specialized library and an important reference which, not only university students at the undergraduate and graduate levels, but also researchers in the field of medicine and health sciences, as well as doctors of all specialties, can always go back to and rely on.

The publication came out of print after the sudden death of one of the authors, Professor Goran Trajković, PhD, who continues, in this way, to generously share his knowledge.

Belgrade, 25.05.2021.

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UPUTSTVO AUTORIMA ZA PRIPREMU RADOVA

Srpski medicinski časopis Lekarske komore objavljuje do sada neobjavljene originalne stručne i naučne radove, pregledne članke, kratka saopštenja, uvodnike, pisma uredniku, meta-analize, prikaze slučajeva, aktuelne teme, prikaze knjiga, radove iz istorije medicine i drugo, iz svih oblasti medicine, farmacije i stomatologije, čime doprinosi promociji i razvoju struke i nauke. Časopis se objavljuje u papirnom i elektronskom obliku četiri puta godišnje.

Da bi se rad razmatrao za publikovanje neophodno je da bude pripremljen prema propozicijama ovog Časopisa. U suprotnom Urednik može da ga odbije, bez daljih objašnjenja. Objavljivanje radova se ne honorariše. Prilikom prihvatanja rada za publikovanje svi autori prenose svoja autorska prava na izdavača časopisa.

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OPŠTA UPUTSTVA

Tekst rada treba da bude napisan latinicom u programu za obradu teksta *Word*, fontom *Times New Roman* i veličinom slova 12 tačaka (12 pt). Sve četiri margine treba podesiti na 25 mm, a veličinu stranice na format A4. Prored treba da bude 1,5, sa levim poravnanjem i uvlačenjem svakog pasusa za 10 mm. Ne treba da bude podeljenih reči (hifenacije). Ne treba koristiti tabulatore i uzastopne prazne karaktere (spejsove) radi poravnanja teksta, već alatke za kontrolu poravnanja u tulbaru. Za prelazak na novu stranu dokumenta koristi se isključivo *Page break*. Posle svakog znaka interpunkcije treba staviti samo jedan prazan karakter (spejs). Ako se u tekstu koriste specijalni znaci (simboli), koristite font iz paketa *Symbol*. Za nazive lekova koristiti isključivo generička imena. Uređaji (aparati) se označavaju fabričkim nazivima, a ime i mesto proizvođača treba navesti u oblim zagradama. Ukoliko se u tekstu koriste oznake koje su spoj slova i brojeva potrebno je precizno napisati broj koji se javlja u superskriptu ili supskriptu (na pr. 99Tc, IL-6, O2, B12, CD8 itd.). Ukoliko se nešto uobičajeno piše kurzivom (*italic*) tako se i navodi (npr. nazivi gena). Neophodno je koristiti međunarodni sistem mernih jedinica (SI), uz izuzetak temperature (°C) i krvnog pritiska (mmHg). Ukoliko je rad deo magistarske teze, odnosno doktorske disertacije, ili je urađen u okviru naučnog projekta, to treba posebno naznačiti u Napomeni na kraju teksta.

Za izradu grafičkih priloga koristite standardne grafičke programe za vindovs (*Windows*), najbolje iz programskih paketa mikrosoft ofisa (*Excel, Word Graph*). Izbegavajte upotrebu boja, naročito prelaze boja i senčenje.

PRIPREMA RADA

Delovi rada su: naslovna strana, sažetak sa ključnim rečima, tekst rada, zahvalnost (po želji), literatura i prilozi.

NASLOVNA STRANA (prva strana) sadrži naslov rada bez skraćenica, predlog kratkog naslova rada, puna imena i prezimena autora (bez titula) indeksirana brojevima, zvaničan naziv ustanova u kojima autori rade, mesto i državu (redosledom koji odgovara indeksiranim brojevima autora). Na dnu stranice navesti ime i prezime, adresu za kontakt, broj telefona, faksa i imejl adresu autora zaduženog za korespondenciju. Naslovna strana treba da bude na spskom i engleskom jeziku (druga strana).

SAŽETAK treba da ima do 250 reči, uključujući skraćenice (treća strana). Za originalne radove, prethodno i kratko saopštenje, meta-analize i pregledne radove, sažetak treba da ima sledeću strukturu: Uvod, Cilj, Metode, Rezultati, Zaključak. Svaki od navedenih segmenata treba da bude napisan kao poseban pasus. Za prikaze slučajeva sažetak treba da ima sledeće delove: Uvod, Prikaz bolesnika, Zaključak. Svaki deo, takođe, pisati kao poseban pasus. Za ostale vrste radova sažetak nema posebnu strukturu. Ispod Sažetka navesti od tri do šest ključnih reči ili izraza. Ne tre-

INSTRUCTIONS FOR AUTHORS

The **Serbian Journal of the Medical Chamber** publishes previously unpublished, original professional and scientific papers, reviews, short communications, editorials, letters to the editor, meta-analyses, case reports, current topics, book reviews, papers on the history of medicine and more, from all fields of medicine, pharmacy and dentistry, therefore contributing to the promotion and development of the profession and science. The journal is published in print and electronic form four times per year.

In order for a paper to be considered for publication, it is necessary to prepare it according to the Journal's guidelines, otherwise the editor may reject it without further explanation. The publication of papers is not paid. When accepting a paper for publication, all authors transfer their copyrights to the Journal publisher.

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The paper should be submitted as a Microsoft Word document (.docx, .doc), using the Times New Roman font, size 12 pt. All four margins should be set to 25 mm and the page size to A4. The line spacing should be 1.5, with left alignment and 10 mm indentation of each paragraph. There should be no end-of-line hyphenation. The alignment tools should be used for aligning the text, not tabs or multiple spaces. Only the page-break should be used for a new page in the document. A single space should follow each punctuation mark. If special characters (symbols) are used in the text, use the font from the Symbol package. Use only generic names for drugs. Devices (equipment) should be identified using factory names, and the name and location of the manufacturer should be indicated in parentheses. If the text uses labels that are a combination of letters and numbers, it is necessary to appropriately write the number that appears in superscript or subscript (e.g. ^{99}Tc, IL-6, O_2, B12, CD8, etc.). If something is typically written in italic, it should be written so (e.g. gene names). International System of Units (SI) units should be used, with the exception of temperature (°C) and blood pressure (mmHg). If the paper is part of a master's thesis or doctoral dissertation, or was created within a scientific project, this should be specifically indicated in the Notes section, at the end of the text.

Graphics attachments should be created using standard graphics software for MS Windows, preferably from the MS Office suite (MS Excel, MS Word Graph). Use of colors should be avoided, especially gradients and shading.

PREPARING THE PAPER

The sections of the paper are: the cover page, the abstract and keywords, the text of the paper, acknowledgements (optional), literature, and attachments.

COVER PAGE (first page) should include the title of the paper, without acronyms, a suggested short title of the paper, the full names of the authors (without titles) indexed using numbers, the official name of the institutions where the authors work, place and country (in the order corresponding to the indexed numbers of the authors). At the bottom of the page, provide the name, contact address, phone number, fax number and email address of the corresponding author. The cover page should be in Serbian and English language (second page).

ABSTRACT should be up to 250 words, including acronyms (third page). For original papers, preliminary and short communications, meta-analyses and review papers, the abstract should have the following structure: Introduction, Aim, Methods, Results, Conclusion. Each of these segments should be written as a separate paragraph. For case report, the summary should have the following sections: Introduction, Case Report, Conclusion; each part should be written as a separate paragraph. For other types of papers, the abstract does not have a special structure. List three to six key words or phrases below the abstract. Words from the

ba da se ponavljaju reči iz naslova, a ključne reči treba da budu relevantne i opisne. U izboru ključnih reči koristiti *Medical Subject Headings* – MeSH (*http://www. nlm.nih. gov/mesh*). Sažetak treba da bude napisan na srpskom i engleskom jeziku.

Na četvrtoj **strani** treba da se nalazi prevod teksta koji je na trećoj strani (sažetak). Ova strana mora da ima identičnu strukturu trećoj strani.

Od pete strane broji se broj strana predviđen za odgovarajuću strukturu rada.

STRUKTURA RADA. Svi podnaslovi se pišu velikim masnim slovima (**bold**). Originalni rad, meta-analiza, prethodno i kratko saopštenje obavezno treba da imaju sledeće podnaslove: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Metode rada, Rezultati, Diskusija (koncizna, jasna, predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi), Zaključak (mora proisteći isključivo iz rezultata istraživanja rada, Spisak skraćenica (ukoliko su korišćene u tekstu), Zahvalnica, Literatura (navodi se arapskim brojevima redosledom kojim je u tekstu navedena u uglastim zagradama, vidi dalje). Struktura rada kod pregleda literature sastoji se od Uvoda, Većeg broja podnaslova, Zaključka, Literature. Autori preglednog rada treba da navedu bar tri rada (kao autori ili koautori) publikovanih u časopisima s recenzijom. Struktura rada kod prikaza jednog ili više slučajeva čine: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Prikaz bolesnika, Diskusija, Literatura. Ne treba koristiti imena bolesnika, inicijale, niti brojeve istorija bolesti, naročito u ilustracijama. Prikazi slučajeva ne smeju imati više od pet autora.

STATISTIČKE METODE, PRIKAZ I INTERPRETACIJA REZULTATA

Ukoliko je moguće, sve numeričke vrednosti zaokružiti na jedno decimalno mesto. Ne duplirati prikaz rezultata u tabelama i grafikonima. Za prikaz rezultata u tabelama i tekstu preporuka je da se: numerički podaci sa normalnom raspodelom i bez ekstremnih vrednosti prikazuju kao aritmetička sredina ± standardna devijacija; numerički podaci čija raspodela odstupa od normalnosti ili kada postoje ekstremne vrednosti, i ordinalni podaci prikazuju kao medijana i opseg (minimalna – maksimalna vrednost); nominalni podaci i ordinalni podaci sa malim brojem kategorija prikazuju kao n (%).

Prilikom opisa primenjenih statističkih metoda, prikaza i interpretacije rezultata u radu pridržavati se *SAMPL Guidelines (Lang TA, Altman DG. Basic statistical reporting for articles published in biomedical journals: the "Statistical Analyses and Methods in the Published Literature" or the SAMPL Guidelines. Int J Nurs Stud 2015;52(1):5-9.).* Primenjene statističke metode treba opisati dovoljno detaljno da ih čitalac može ponoviti na svojim podacima. Navesti korišćeni statistički test i nivo značajnosti. Saopštiti tačnu *p*-vrednost na 3 decimalna mesta.

PRILOGE (tabele, grafikone, slike itd.) postaviti na kraj rukopisa, a u samom tekstu jasno naznačiti mesto koje se odnosi na dati prilog. Krajnja pozicija priloga biće određena u toku pripreme rada za publikovanje.

SKRAĆENICE. Koristiti samo kada je neophodno, i to za veoma dugačke nazive hemijskih jedinjenja, odnosno nazive koji su kao skraćenice već prepoznatljivi (standardne skraćenice, kao npr. DNK, sida, HIV). Za svaku skraćenicu pun termin treba navesti pri prvom navođenju u tekstu, sem ako nije standardna jedinica mere. U naslovi se ne koriste skraćenice. Izbegavati korišćenje skraćenica u sažetku, ali ako su neophodne, svaku skraćenicu objasniti pri prvom navođenju u tekstu.

DECIMALNI BROJEVI. U tekstu rada na engleskom jeziku, u tabelama, na grafikonima i drugim prilozima decimalne brojeve pisati sa tačkom (npr. 12.5 ± 3.8), a u tekstu na srpskom jeziku sa zarezom (npr. 12,5 ± 3,8). Kad god je to moguće, broj zaokružiti na jednu decimalu.

JEDINICE MERA. Dužinu, visinu, težinu i zapreminu izražavati u metričkim jedinicama (metar – m, kilogram (gram) – kg (g), litar – l) ili njihovim delovima. Temperaturu izražavati u stepenima Celzijusa (°C), količinu supstance u molima

title should not be repeated, and the key words should be relevant and descriptive. The Medical Subject Headings - MeSH thesaurus (http://www.nlm.nih.gov/mesh) should be used for selecting the key word. The abstract should be submitted in both Serbian and English language.

The **fourth page** should contain the translation of the text from the third page (abstract). This page must have the identical structure as the third page.

The number of pages provided for the appropriate structure of the paper is counted from the fifth page.

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If possible, all numbers should be rounded to one decimal place. The display of results in tables and graphs should not be repeated. When presenting results in tables and text, it is recommended that numerical data with normal distribution and without extreme values be presented as arithmetic mean ± standard deviation; that numerical data whose distribution deviates from normal distribution or when there are extreme values, and ordinal data, be presented as the median and range (minimum–maximum value); that nominal data and ordinal data with a small number of categories be displayed as n (%).

When describing the applied statistical methods, presentation and interpretation of results in the paper, follow the SAMPL Guidelines (Lang TA, Altman DG. Basic statistical reporting for articles published in biomedical journals: The "Statistical Analyses and Methods in the Published Literature" or the SAMPL Guidelines. Int J Nurs Stud 2015; 52(1):5-9.). The applied statistical methods should be described in sufficient detail so that the reader may repeat them using own data. Indicate the statistical test used and the level of significance. Report the correct *p*-value to the third decimal place.

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ACRONYMS should be used only when necessary, i.e. for very long names of chemical compounds and names that are already commonly used as acronyms (standard acronyms, such as DNA, AIDS, HIV). For each acronym, the full term should be given at the first appearance in the text, unless it is a standard unit of measure. Acronym should not be used in the title. Avoid using acronyms in the abstract, but if necessary, explain each acronym at the first appearance in the text.

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(mol), a pritisak krvi u milimetrima živinog stuba (mm Hg). Sve rezultate hematoloških, kliničkih i biohemijskih merenja navoditi u metričkom sistemu prema Međunarodnom sistemu jedinica (SI).

PRILOZI su tabele, slike (fotografije, crteži, sheme, grafikoni), do šest po radu. Svaki prilog se dostavlja kao poseban dokument, obeležen na isti način kao u tekstu. Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje

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SLIKE. Slike su svi oblici grafičkih priloga. Objavljuju se fotografije, crteži, sheme i grafikoni. Slike se označavaju arapskim brojevima prema redosledu navođenja u tekstu. Primaju se isključivo digitalne fotografije (crno-bele ili u boji) rezolucije najmanje 300 *dpi* i formata zapisa *tiff* ili *jpg* (male, mutne i slike lošeg kvaliteta neće se prihvatati za štampanje!). Ukoliko autori nemaju ili nisu u mogućnosti da dostave digitalne fotografije, onda originalne slike treba skenirati u rezoluciji 300 *dpi* i u originalnoj veličini. Ukoliko je rad neophodno ilustrovati sa više slika, u radu će ih biti objavljeno nekoliko, a ostale će biti u e-verziji članka kao *PowerPoint* prezentacija (svaka slika mora biti numerisana i imati legendu). U legendi slika treba napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu. Potrebno je priložiti nazive slika i legendu na srpskom i engleskom jeziku.

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ali još nisu objavljeni, treba označiti sa *in press* i priložiti dokaz o prihvatanju rada za objavljivanje.

Podaci o korišćenoj literaturi u tekstu označavaju se arapskim brojevima u uglastim zagradama – npr. [1,2], i to redosledom kojim se pojavljuju u tekstu. Reference se citiraju prema **Vankuverskom stilu** (uniformisanim zahtevima za rukopise koji se predaju biomedicinskim časopisima), koji je uspostavio Međunarodni komitet urednika medicinskih časopisa (*http://www.icmje.org*), čiji format koriste *U.S. National Library of Medicine* i baze naučnih publikacija. Primeri navođenja publikacija (članaka, knjiga i drugih monografija, elektronskog, neobjavljenog i drugog objavljenog materijala) mogu se pronaći na internet-stranici *http://www.nlm.nih.gov/bsd/uniform_ requirements.html.* Prilikom navođenja literature veoma je važno pridržavati se pomenutog standarda, jer je to jedan od najbitnijih faktora za indeksiranje prilikom klasifikacije naučnih časopisa. Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

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