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EDITORIJAL



„Ništa nije konačno dok u nama postoji traženje...”

Meša Selimović

Poštovane kolegice i kolege, dragi prijatelji,

Lekari i svi zdravstveni radnici su bili i ostali beskompromisni pregaoci u održavanju zdravlja i života ljudi. U toj večitoj misiji postoji i deo sopstvenog traženja, kao podsticaj da se uvek u saznanju ide dalje i pruži više.

Jedna od grana medicine koja se suočava sa najtežim i najkompleksnijim oboljenjima jeste upravo hematologija. Ovaj broj *Srpskog medicinskog časopisa Lekarske komore* je posvećen hematologiji i svima onima koji će želeći da iz njega saznaju nešto više o hemato-onkološkim oboljenjima, izazovima u njihovoj dijagnostici i savremenim terapijskim modalitetima. Ali, da se malo vratimo u prošlost...

Reč hematologija potiče od grčkih reči *haima* (αἷμα – krv) i *logos* (λόγος – nauka). Proučavanje krvi i njenih sastavnih delova ima veoma dugu istoriju, pošto je primitivni čovek shvatio da je gubitak krvi, ako je dovoljno velik, povezan sa smrću. A u biblijskim referencama, „proliti krv” je bio termin koji se koristio u značenju: „ubiti”. U eri pre mikroskopije, mogao se proučavati samo grubi makroskopski izgled krvi. Kada se posmatrala u staklenoj posudi, videlo se da zgrušana krv formira različite slojeve, a smatralo se da ti slojevi čine supstancu ljudskog tela, te su se zdravlje i bolest posmatrali kao rezultat pravilne mešavine ili pak neravnoteže ovih slojeva. Mikroskopsko ispitivanje krvi, koje su, koristeći prvi mikroskop, sprovedi van Levenhuk i njegovi saradnici u XVII veku, omogućilo je da se teorija i dogma postepeno zamene naučnim saznanjima.

Tokom poslednjih nekoliko decenija, napretkom tehnologije, razvile su se automatizovane molekularno-biološke tehnike, koje omogućavaju elektronsku manipulaciju ćelijama i otkrivanje genetskih mutacija koje leže u osnovi izmenjene strukture i funkcije humanih ćelija i proteina, a kao takve rezultuju u pojavi hematoloških oboljenja.

Septembarski broj Časopisa pokazuje veliko publicističko pregalaštvo i entuzijazam u ovoj dragocenoj grani medicine. Nakon pregleda dostavljenih i za publikaciju prihvaćenih rukopisa, posebno bih se zahvalila svim autorima i saradnicima koji su nam ustupili rezultate svojih originalnih istraživanja, uobličene u veoma kvalitetne radove, a takođe i autorima revijalnih radova i serija interesantnih slučajeva.

Naime, u ovom broju, koji stiže do svojih čitalaca uoči 6. Kongresa hematologa Srbije sa međunarodnim učešćem, nalazi se 17 radova, od toga 7 originalnih, 7 preglednih i tri rada koja prikazuju kazuistiku. U okviru originalnih radova, dva se tematski odnose na akutnu mijeloidnu leukemiju (AML).

Prvi rad pod nazivom: „Prognostički i prediktivni značaj merljive rezidualne bolesti u akutnoj mijeloblastnoj leukemiji” Jovana Rajića i saradnika, analizira određivanje rezidualnih leukemijskih ćelija pomoću merljive rezidualne bolesti (engl. *measurable residual disease – MRD*), prvobitno nazvane minimalna

EDITORIAL

"Nothing is final as long as we continue to search..."

Meša Selimović

Dear colleagues and friends,

Doctors and all healthcare workers have always been uncompromising and tireless in preserving health and life. A part of this never-ending mission is searching within ourselves, which motivates us to learn further and give more.

One of the branches of medicine that deals with the most severe and complex diseases is hematology. This issue of the *Serbian Journal of the Medical Chamber* is dedicated to hematology and to all the readers who would like to learn more about hemato-oncological diseases, the challenges in establishing their diagnosis, and modern therapeutic modalities. But first, let's take a moment to go back in time...

The word hematology originates from the Greek words *haima* (αἷμα – blood) and *logos* (λόγος – science). The study of blood and its components has a lengthy history, as primitive man understood that the loss of blood, if copious enough, was associated with death. Also, in biblical references, "to shed blood" was a term denoting: "to kill." In the period before microscopy, only the gross macroscopic appearance of blood could be studied. When observed in a glass container, the coagulated blood was seen to form different layers, and these layers were thought to make up the substance of the human body, while health and disease were seen as the result of the proper mixture of these layers or an imbalance among them. Microscopic examination of blood, first carried out by van Leeuwenhoek and his colleagues in the 17th century, using the first microscope, made it possible for scientifically proven knowledge to gradually replace theory and dogma.

Over the past few decades, with the advancement of technology, automated molecular biological techniques have been developed, enabling electronic cell manipulation and the detection of genetic mutations which are the underlying cause of the altered structure and function of human cells and proteins, and as such, lead to hematological diseases.

The September issue of the Journal demonstrates how driven and enthusiastic researchers in this crucial branch of medicine are to publish their studies. After reviewing the manuscripts submitted and accepted for publication, I would especially like to thank all the authors of high-quality articles reporting original research results, as well as the authors who have submitted review articles and interesting case series.

This issue, which will reach its readers on the eve of the 6th Congress of Hematologists of Serbia with international participation, presents 17 articles: 7 original articles, 7 review articles, and three case reports. The topic of two of the original articles is acute myeloid leukemia (AML).

The first article, *Prognostic and predictive significance of measurable residual disease in acute myeloblastic leukemia*, by Jovan Rajić et al., analyzes the detection of residual leukemic cells via measurable residual dis-

rezidualna bolest (engl. *minimal residual disease* – MRD). Dokazano je da MRD predstavlja najvažniji prognostički i prediktivni faktor u akutnoj mijeloidnoj leukemiji, te ovaj rad daje još jedan doprinos ovoj problematici. Naime, u ovoj studiji, značajno duže trajanje kompletne remisije (engl. *complete remission* – CR) i duže ukupno preživljavanje (engl. *overall survival* – OS), imali su bolesnici koji su posle pozitivnog MRD statusa (MRD+) nastavili lečenje intenzivnom hemioterapijom. Takođe, CR i OS su bili statistički značajno duži kod bolesnika koji su na kraju lečenja zadržali negativni MRD status.

Drugi je rad Marijane Virijević i saradnika, koji retrospektivno analizira kliničke karakteristike i preživljavanje kod odraslih bolesnika sa akutnom mijeloidnom leukemijom, kroz iskustvo tercijarne ustanove. Autori su prikazali da kod bolesnika lečenih intenzivnom hemioterapijom postoji niska stopa OS, da godine starosti i podtip AML-a, kao i displastične karakteristike, odnosno MRC (engl. *myelodysplasia-related changes*), predstavljaju nepovoljne faktore povezane sa kraćim OS, kao i kraćim preživljavanjem bez znakova bolesti (engl. *disease free survival* – DFS).

Rad Natalije Kecman i saradnika analizira razvoj febrilne neutropenije (FN) kod bolesnika sa hematološkim malignitetima lečenih u uslovima dnevne bolnice tercijarne ustanove. Pokazano je da su veći broj terapijskih linija i ciklusa imunohemioterapije, klinički stadijum bolesti, prisustvo *bulky* tumorske mase i stepen agresivnosti limfoma bili povezani sa razvojem febrilne neutropenije.

Hematološka toksičnost inhibitora ciklin zavisnih kinaza 4 i 6 (CDK4/6i) u lečenju metastatskog karcinoma dojke, kroz iskustvo tercijarnog centra, analizirana je u radu Slobodana Kutića i saradnika. U ovom radu je prikazano da je kod 82.1% pacijentkinja tretiranih pomoću CDK4/6i došlo do pojave jatrogene neutropenije, ali su obustave terapije bile retke. Takođe, palijativna radioterapija koštanih lezija je bila povezana sa češćom doznom redukcijom CDK4/6i, ali redukcija doze ovih lekova nije uticala na preživljavanje.

Nada Vlaisavljević i saradnici pokazali su u svom radu da bi aktivna konkomitantna terapija insulinske rezistencije kod novodijagnostikovanih bolesnika sa multiplim mijelomom (NDMM) mogla da unapredi njihov terapijski odgovor na primenjenu antimijelomsku terapiju. Naime, uočena je statistički značajna negativna korelacija HOMA-IRIn (engl. *homeostatic model assessment-insulin resistance index*, našte), nakon primenjene terapije, i postignutog terapijskog odgovora.

Retrospektivna analiza kliničkih karakteristika bolesnika sa mijeloproliferativnim neoplazmama (MPN), mlađih od 40 godina, predmet je rada Isidore Arsenović i saradnika. Pokazano je da većina bolesnika pripada ELN (engl. *European LeukemiaNet*) grupi niskog rizika, međutim, zbog razvoja tromboze, prisutnih tegoba, stepena trombocitoze i splenomegalije, skoro 50% njih primilo je citoreduktivnu terapiju. Prikazani rezultati ukazuju na potrebu bolje stratifikacije rizika, kao i upotrebe drugačijih metoda za procenu rizika bolesti, kao što je NGS (engl. *next generation sequencing*).

Da hemioterapijski protokol ABVD nije adekvatan za sve pacijente sa uznapredovalim klasičnim Hočkinovim limfomom (UkHL), kroz petogodišnje iskustvo jednog centra, prikazuju Vojin Vuković i saradnici. U ovoj retrospektivnoj studiji ispitivani su kliničko-laboratorijski parametri, lečenje i ishod bolesnika, uz zaključak da značajan broj pacijenata ne može biti izlečen ABVD protokolom, već je za njih neophodno obezbediti intenzivnije lečenje ili inovativne terapijske modalitete.

Prvi u grupi revijalnih radova jeste rad Zorice Cvetković i saradnika, koji prikazuje značaj anemije kod bolesnika sa srčanom slabošću, uz detaljnu analizu njene multifaktorske etiologije, kompleksne patofiziologije, kao i specifičnosti terapijskog pristupa.

Sledeći rad iz ove grupe se odnosi na lekovima indukovane trombocitopenije (LITP) autora Nade Sujvdžić-Vuković i Mirjane Cvetković. Rad se bavi analizom učestalosti i mehanizama nastanka lekovima indukovane trombocitopenije, uz pojedinačni prikaz lekova uzročnika, kao i prikazom vakcinama indukovane trombozne trombocitopenije (VITT).

Nikica Sablijić i saradnici, u svom radu analiziraju rizik od krvarenja, kao potencijalni problem nakon primene direktnih oralnih antikoagulasa (DOAK), uz prikaz rezultata većine studija koje pokazuju da je rizik od intrakranijalne hemoragije primenom DOAK-a manji, u poređenju sa primenom lekova iz grupe antagonista vitamina K (VKA), kao i da je rizik od major krvarenja isti ili čak i manji primenom DOAK-a. U zaključku, autori navode da se čini da će VKA lekovi postepeno otići u istoriju, odnosno da će se koristiti samo u određenoj, jasno definisanoj populaciji.

ease (MRD), originally named minimal residual disease. It has been proven that MRD represents the most important prognostic and predictive factor in acute myeloid leukemia, and the article further contributes to this topic. Namely, in this study, patients who continued treatment with intensive chemotherapy after a positive MRD status (MRD+) had significantly longer complete remission (CR) and overall survival (OS). Also, CR and OS were statistically significantly longer in patients who maintained a negative MRD status at the end of treatment.

The second article presents the study by Marijana Virijević et al., which retrospectively analyzes clinical characteristics and survival in adult patients with acute myeloid leukemia, through the experience of a tertiary institution. The authors demonstrated a low OS rate in patients treated with intensive chemotherapy. They also showed that age, AML subtype, and dysplastic characteristics, i.e. MRC (myelodysplasia-related changes), were unfavorable factors associated with shorter OS and shorter disease-free survival (DFS).

In their article, Natalija Kecman et al. analyze the development of febrile neutropenia (FN) in patients with hematological malignancies treated at the day-care unit of a tertiary institution. They demonstrated that a higher number of therapeutic lines and cycles of immunochemotherapy, the clinical stage of the disease, the presence of bulky tumor mass, and the degree of lymphoma aggressiveness were associated with the development of febrile neutropenia.

The hematological toxicity of inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6i) in the treatment of metastatic breast cancer, through the experience of a tertiary center, was analyzed in the study by Slobodan Kutić et al. In this article, it was shown that iatrogenic neutropenia occurred in 82.1% of patients treated with CDK4/6i, but discontinuation of therapy was rare. Also, palliative radiotherapy of bone lesions was associated with more frequent CDK4/6i dose reduction, however, the dose reduction of these drugs did not affect survival.

In their study, Nada Vlasisavljević et al. demonstrated that active concomitant therapy of insulin resistance in newly diagnosed patients with multiple myeloma (NDMM) could improve their therapeutic response to the applied antimyeloma treatment. Namely, the authors observed a statistically significant negative correlation between the fasting homeostatic model assessment-insulin resistance index (HOMA-IRIf) after treatment and the achieved therapeutic response.

A retrospective analysis of the clinical characteristics of patients with myeloproliferative neoplasms (MPN) under the age of forty is presented in the article by Isidora Arsenović et al. The study showed that the majority of patients belonged to the European LeukemiaNet (ELN) low-risk group, however, due to the development of thrombosis, existing complaints, and the degree of thrombocytosis and splenomegaly, almost 50% of these patients received cytoreductive therapy. The presented results indicate the need for better risk stratification, as well as the application of different methods of disease risk assessment, such as next-generation sequencing (NGS).

Through the five-year experience of a single center, Vojin Vuković et al. have demonstrated that the ABVD chemotherapy regimen is not adequate for all patients with advanced-stage classical Hodgkin lymphoma (AScHL). This retrospective study examines clinical and laboratory parameters, treatment, and patient outcomes. The authors have concluded that a significant number of patients cannot be cured with the ABVD regimen, which is why it is necessary to provide them with more intensive treatment or innovative therapeutic modalities.

The first among the review articles, submitted by Zorica Cvetković et al., shows the importance of anemia in patients with heart failure and provides a detailed analysis of its multifactorial etiology, complex pathophysiology, and the specificities of the applied therapeutic approach.

The next article in this group, written by Nada Suvajdzic-Vuković and Mirjana Cvetković, focuses on drug-induced thrombocytopenia (DITP). The article analyzes the incidence and mechanisms of drug-induced thrombocytopenia occurrence, providing an individual description of the causative drugs, as well as a description of vaccine-induced thrombotic thrombocytopenia (VITT).

In their article, Nikica Sablijić et al. analyze the risk of bleeding as a potential problem with the use of direct oral anticoagulants (DOACs). They argue that the results of most studies show a lower risk of intracranial hemorrhage with the use of DOACs, as compared to the use of drugs from the vitamin K antagonists (VKA) group, as well as the same or even lower risk of major bleeding with the use of DOACs. In conclusion, the

Andrija Bogdanović i Danijela Leković se u svom revijalnom radu bave analizom literature koja se odnosi na aktuelno lečenje primarne mijelofibroze (PMF). U zaključku navode da je lečenje ove bolesti i dalje izazov, da primena ruxsolutiniba, prvog predstavnika inhibitora Janus kinaze (JAKi) omogućava dobru kontrolu bolesti kod skoro polovine bolesnika, a da novi lekovi treba da omoguće lečenje refraktornih bolesnika ili da poboljšaju terapijski odgovor.

Prikazom kliničkih manifestacija udruženih sa prisustvom antifosfolipidnih (engl. *antiphospholipid* – *aPL*) antitela bave se Predrag Miljić i saradnici. U radu se ističe heterogenost grupe autoantitela usmerenih na anjonske fosfolipide ili proteine koji se vezuju za fosfolipide, kao i njihova udruženost sa brojnim kliničkim manifestacijama u gotovo svim oblastima medicine. Navodi se da je antifosfolipidni sindrom (engl. *antiphospholipid syndrome* – *APS*) najpreciznije definisani entitet koji se karakteriše prisustvom navedenih antitela. Prikazuju se prethodna, ali i najnovija klasifikacija, koje se odnose na kriterijume za postavljanje dijagnoze APS.

Danijela Jovanović i Predrag Đurđević prikazuju odlike retkog entiteta, bolesti taloženja lakih lanaca, kao jednog od oblika obolevanja koji se odnosi na taloženje monoklonskih imunoglobulina. Analiziraju se dijagnostički kriterijumi, klinička prezentacija, od kojih je najčešći nefritički sindrom, kao i terapijski modaliteti, a akcenat se stavlja na multidisciplinarni pristup, najpre nefrologa, hematologa i patologa.

Klinički značaj ferroptoze, koja se prvi put spominje 2012. godine, predmet je rada Danijele Agić i saradnika. Radi se o neapoptotskom, nenekroznom i neautofagnom putu ćelijske smrti koji zavisi od gvožđa i koji zahteva prisustvo lipidnih peroksida, a inhibira se ferostatinom. Autori su sprovedli pretraživanje *Medline* baze podataka sa posebnim osvrtom na radove koji su se bavili značajem ferroptoze u kliničkoj medicini, a prvenstveno u hematologiji.

Interesantni kazusi, u vidu slučajeva sa pregledom literature i serijom slučajeva, doprineli su sveobuhvatnosti ovog broja. Tako su Jelena Ivanović i saradnici dali prikaz bolesnice kod koje je postavljena dijagnoza Hočkinovog limfoma u trudnoći, uz pregled literature koja se bavi ovom problematikom. Autori ističu da lečenje Hočkinovog limfoma tokom trudnoće predstavlja kompleksan izazov koji zahteva koordinisan multidisciplinarni pristup i personalizovani plan lečenja.

Andrijana Kojić i Mila Purić prikazuju dve bolesnice sa multiplim mijelomom kod kojih je dijagnostikovana diseminacija u centralni nervni sistem (CNS-MM). Navedeni entitet je veoma redak i čini manje od 1% ekstramedularnih multiplih mijeloma, a manifestuje se spektrom neuroloških deficita. U zaključku se navodi da, uprkos napretku u dijagnostici i lečenju, prognoza CNS-MM-a ostaje loša zbog kompleksnog i agresivnog kliničkog toka, a zbog niske incidencije i ograničenosti podataka, ukazuje se na potrebu za daljim istraživanjima.

Rad koji se odnosi na progresiju bolesti kod tri pacijenta sa primarnom mijelofibrozom (PMF) niskog rizika dali su Mihailo Smiljanić i saradnici. Iako je medijana preživljavanja ovih pacijenata preko 15 godina, prema "MOST" prospektivnoj studiji, progresiju bolesti ima skoro 60% njih, te je cilj autora bio da kroz prikaze slučajeva predstave tok bolesti i lečenje. U zaključku se, s obzirom na navedeno, ukazuje potreba za istraživanjima koja će pokazati da li rana NGS analiza *non-driver* mutacija i rano uvođenje terapije doprinose promeni toka bolesti.

Nadamo se da će ovaj tematski broj Časopisa, kao vesnik predstojećeg Kongresa hematologa, približiti svim našim čitaocima kompleksnost ove problematike, ukazati na rad i požrtvovanost celog tima stručnjaka, kao i na značaj stalnog usavršavanja. Sve ovo upravo predstavlja to neprestano traženje, kao odraz ličnog i profesionalnog napretka, a na dobrobit svih hematoloških pacijenata.

S poštovanjem,
Profesor Milena Todorović Balint
Pomoćnik glavnog i odgovornog urednika

authors state that it seems that VKA drugs will gradually become obsolete, i.e., they will be used only in a certain, clearly defined population.

The review article, by Andrija Bogdanović and Danijela Leković, analyzes the literature on the current treatment of primary myelofibrosis (PMF). In their conclusion, the authors state that the treatment of this disease remains a challenge, that the use of ruxolitinib (the first representative of the Janus kinase inhibitor – JAKi) enables good control of the disease in almost half of the patients, and that new drugs should be able to treat refractory patients or improve therapeutic response.

Clinical manifestations associated with the presence of antiphospholipid (aPL) antibodies are the focus of the article written by Predrag Miljić et al. The article highlights the heterogeneity of the group of autoantibodies targeting anionic phospholipids or phospholipid-binding proteins, as well as their association with numerous clinical manifestations in almost all areas of medicine. The authors state that antiphospholipid syndrome (APS) is the most precisely defined entity characterized by the presence of said antibodies. They present the previous and the most recent classifications of the criteria for establishing the diagnosis of APS.

Danijela Jovanović and Predrag Đurđević present the characteristics of a rare entity, light chain deposition disease, as one of the forms of monoclonal immunoglobulin deposition disease. The article analyzes the diagnostic criteria, clinical presentation (the most common being nephritic syndrome), and therapeutic modalities, while emphasizing a multidisciplinary approach, primarily by nephrologists, hematologists, and pathologists.

The clinical significance of ferroptosis, which was mentioned for the first time in 2012, is the subject of the article by Danijela Agić et al. It is a non-apoptotic, non-necrotic, and non-autophagic type of cell death dependent on iron, which requires the presence of lipid peroxides and is inhibited by ferrostatin. The authors searched the Medline database with special reference to articles dealing with the significance of ferroptosis in clinical medicine, primarily in hematology.

Interesting case studies, in the form of case reports with a literature review and case series, contributed to the comprehensiveness of this issue. Thus, Jelena Ivanović et al. presented the case of a patient diagnosed with Hodgkin's lymphoma during pregnancy, as well as a review of the existing literature dealing with this issue. The authors point out that the treatment of Hodgkin's lymphoma during pregnancy is a complex challenge that requires a coordinated multidisciplinary approach and a personalized treatment plan.

Andrijana Kojić and Mila Purić present two patients with multiple myeloma diagnosed with central nervous system involvement (CNS-MM). This entity is quite rare, accounting for less than 1% of extramedullary multiple myeloma, and manifests as a variety of neurological deficits. The authors conclude that, despite advances in diagnostics and treatment, the prognosis of CNS-MM remains poor owing to the complex and aggressive clinical course of the disease, at the same emphasizing that further research is necessary, due to low incidence and limited data.

The article reporting on disease progression in three patients with low-risk primary myelofibrosis (PMF) is presented by Mihailo Smiljanić et al. Although the median survival of these patients is more than 15 years, according to the *MOST* prospective study, almost 60% have disease progression, which is why the authors aimed to present the course of the disease and treatment through the case reports. Due to the above-stated characteristics of the disease, the authors conclude that there is a need for further research that would show whether early NGS analysis of non-driver mutations and early initiation of therapy contribute to a change in the course of the disease.

We hope that this themed issue of the Journal, heralding the upcoming Congress of Hematologists, will bring the complexity of the subject of hematology closer to all our readers and highlight the dedicated work of an entire team of experts, as well as the importance of continuous improvement. All this is, in fact, a part of the constant pursuit and a reflection of personal and professional progress, all for the benefit of hematology patients.

Sincerely,
Professor Milena Todorović Balint
Assistant Editor-in-Chief

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PROGNOSTIČKI I PREDIKTIVNI ZNAČAJ MERLJIVE REZIDUALNE BOLESTI U AKUTNOJ MIJELOBLASTNOJ LEUKEMIJI

ORIGINALNI RAD

ORIGINAL ARTICLE

PROGNOSTIC AND PREDICTIVE SIGNIFICANCE OF MEASURABLE RESIDUAL DISEASE IN ACUTE MYELOBLASTIC LEUKEMIA

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SAŽETAK

Uvod: Akutna mijeloblastna leukemija (AML) predstavlja heterogenu grupu klonskih neoplastičnih bolesti hematopoeznih ćelija. Određivanje rezidualnih leukemijskih ćelija (merljiva rezidualna bolest; engl. *measurable residual disease – MRD*) predstavlja najvažniji prognostički i prediktivni faktor u akutnoj mijeloblastnoj leukemiji.

Cilj rada: Cilj rada je analiza efekta primenjene hemioterapije na osnovu rezultata *MRD* testa kod bolesnika sa akutnom mijeloblastnom leukemijom, lečenih na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije.

Materijali i metode: Naša studija je obuhvatila analizu 111 bolesnika sa akutnom mijeloblastnom leukemijom, lečenih u periodu od januara 2020. do januara 2024. godine. Sve dijagnostičke procedure su bazirane na najnovijim preporukama *ELN* (engl. *European LeukemiaNet*) grupe.

Rezultati: Značajno dužu remisiju (engl. *complete remission – CR*) i duže ukupno preživljavanje (engl. *overall survival – OS*) imali su bolesnici koji su posle *MRD*+ nastavili lečenje intenzivnom hemioterapijom (HT), primenom punih doza 3+7 hemioterapija, kao reindukcije. *CR* ($p = 0,004$) i *OS* ($p = 0,019$) su bili statistički značajno duži kod bolesnika koji su na kraju lečenja zadržali negativni *MRD* status. Kod transplantiranih bolesnika, ukupno preživljavanje (*OS*; $p = 0,006$) i trajanje remisije (*CR*; $p = 0,002$) bili su značajno duži (medijana: *OS* 20 meseci; *CR* 21 mesec), u odnosu na grupu netransplantiranih bolesnika (medijana: *OS* 13 meseci; *CR* 8 meseci).

Diskusija: Kao biomarker, merljiva rezidualna bolest (engl. *measurable residual disease – MRD*) može imati prognostički i prediktivni značaj, ali apsolutni merljivi nivo bolesti nije jedina determinanta ishoda bolesnika pošto biologija akutne mijeloblastne leukemije i drugi klinički faktori samog bolesnika (starost, komorbiditeti, različite komplikacije primenjene hemioterapije, naročito infektivne) modifikuju rizik povezan sa rezultatima *MRD* testa.

Zaključak: Studija je pokazala veliki značaj pravovremenog određivanja *MRD*, adekvatnost primene intenzivnije HT kod *MRD* pozitivnih bolesnika, uz nastavak lečenja alogeno transplantacijom matičnih ćelija hematopoeze.

Ključne reči: akutna mijeloblastna leukemija, prognoza, terapija, merljiva rezidualna bolest

ABSTRACT

Introduction: Acute myeloblastic leukemia (AML) is an umbrella term for a heterogeneous group of clonal neoplastic diseases of hematopoietic cells. Detecting residual leukemic cells (measurable residual disease – MRD) is the most important prognostic and predictive factor in AML.

The aim: The study aims to analyze the effect of administered chemotherapy based on the results of MRD testing in patients with AML treated at the University Clinical Center of Serbia (UCCS) Clinic for Hematology.

Materials and methods: Our study included the analysis of 111 AML patients, treated between January 2020 and January 2024. All diagnostic procedures performed were based on the most recent recommendations of *European LeukemiaNet* (ELN).

Results: MRD+ patients who continued treatment with intensive chemotherapy (CHT), using full doses of 3+7 CHT as reinduction therapy, had a significantly longer remission (complete remission – CR) and a longer overall survival (OS). The duration of CR ($p = 0,004$) and OS ($p = 0,019$) were statistically significantly longer in patients who maintained a negative MRD status at the end of treatment. In transplanted patients, overall survival (OS; $p = 0,006$) and duration of remission (CR; $p = 0,002$) were significantly longer (median: OS 20 months; CR 21 months), as compared to the group of non-transplanted patients (median: OS 13 months; CR 8 months).

Discussion: Measurable residual disease (MRD) can be both prognostic and predictive. However, the absolute measurable level of the disease is not the only determinant of the patient's outcome, since the biology of AML, as well as other clinical patient-related factors (age, comorbidities, various complications of applied chemotherapy, especially infections), modify the risk associated with MRD test results.

Conclusion: The study has demonstrated the great importance of timely detection of MRD, as well as the appropriateness of applying more intensive CHT in MRD-positive patients, along with continued treatment with allogeneic hematopoietic stem cell transplantation.

Keywords: acute myeloblastic leukemia, prognosis, therapy, measurable residual disease

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UVOD

Akutna mijeloblastna leukemija (AML) predstavlja heterogenu grupu klonskih neoplastičnih bolesti hematopoeznih ćelija. Primena intenzivne hemioterapije, sa ili bez primene inhibitora malih molekula ili imunoterapije, dovodi do visokog procenta citomorfološke remisije od oko 80%, naročito kod bolesnika mlađih od 60 godina. Problem je visoka stopa relapsa, koja iznosi 30% – 60% unutar dve godine, što predstavlja glavnu barijeru dugotrajnom izlečenju u AML-u [1]. Odavno je poznato da citomorfološka remisija, koja ima nivo osetljivosti od oko 1%, odnosno prisutnost manje od 5% blasta, brojano na 300 do 500 ćelija sa jedrom, nije garancija potpunog izlečenja kada je u pitanju AML [1,2].

Određivanje rezidualnih leukemijskih ćelija, prvobitno nazvano: minimalna rezidualna bolest (engl. *minimal residual disease* – MRD) a zatim merljiva rezidualna bolest, (engl. *measurable residual disease* – MRD), predstavlja najvažniji prognostički i prediktivni faktor u AML-u [3].

U poslednjih pet godina, meta-analize su pokazale da, bez obzira na korišćenu metodologiju određivanja MRD, bolesnici koji su bili pozitivni, bilo u određenom vremenskom trenutku pre alogene transplantacije matičnih ćelija hematopoeze (alloTMČH) ili uopšteno, u bilo kom periodu lečenja akutne mijeloblastne leukemije, imaju značajno kraće preživljavanje u odnosu na MRD negativne bolesnike [4,5].

Postoje tri glavne tehnike određivanja MRD u AML-u: multiparameterska protočna citometrija (engl. – *multiparametric flow-cytometry* – MFC), metoda kvantitativne lančane reakcije polimeraze (engl. – *quantitative polymerase chain reaction* – qPCR) i sekvencioniranje sledeće generacije (engl. – *next-generation sequencing* – NGS) [1,4,5].

Imunofenotipizacija pomoću MFC tehnike je ključna za postavljanje dijagnoze akutne mijeloblastne leukemije, ali i za određivanje MRD. Precizno primenjenim markerima postiže se osetljivost od 10^{-3} . Postoje dva pristupa u ovoj metodi. Prvi se bazira na identifikaciji jednog ili više imunofenotipova povezanih sa leukemijom (engl. – *leukemia associated immunophenotype* – LAIP) pri postavljanju dijagnoze i praćenju tokom celokupnog lečenja. Drugi se fokusira na detekciju ćelijske populacije koja pokazuje odstupanje od obrazaca ekspresije antigena karakterističnih za normalne ili regenerišuće ćelije, takozvane razlike od normalnog (engl. *difference from normal* – DfN). Preporučuje se kombinacija oba pristupa [6,7].

Metoda lančane reakcije polimeraze (engl. *polymerase chain reaction* – PCR) ima senzitivnost od 10^{-4} do 10^{-5} . Ovom metodom se mogu otkriti mutacije DNK

INTRODUCTION

Acute myeloblastic leukemia (AML) is an umbrella term for a heterogeneous group of clonal neoplastic diseases of hematopoietic cells. The use of intensive chemotherapy, with or without the use of small molecule inhibitors or immunotherapy, leads to a high percentage of cytomorphological remission of about 80%, especially in patients younger than 60 years. The problem is the high rate of relapse, which is 30% – 60% within two years, which represents the main barrier to achieving complete cure in AML [1]. It has long been known that cytomorphological remission, which has a sensitivity level of about 1%, i.e. the presence of less than 5% blasts, counted in 300 to 500 nucleated cells, is not a guarantee of complete cure when it comes to AML [1,2].

Detecting residual leukemic cells, originally referred to as minimal residual disease (MRD) and subsequently measurable residual disease (MRD), is the most important prognostic and predictive factor in AML [3].

In the last five years, meta-analyses have shown that, regardless of the methodology used to detect MRD, patients who were positive, either at a certain point in time before allogeneic hematopoietic stem cell transplantation (alloTMHC) or in general, during any period of treatment for acute myeloblastic leukemia, have significantly shorter survival compared to MRD negative patients [4,5].

There are three main techniques for detecting MRD in AML: multiparametric flow-cytometry (MFC), quantitative polymerase chain reaction (qPCR), and next-generation sequencing (NGS) [1,4,5].

Immunophenotyping by MFC is crucial for the diagnosis of acute myeloblastic leukemia, but also for MRD detection. Precisely applied markers achieve a sensitivity of 10^{-3} . There are two approaches to this method. The first approach is based on the identification of one or more immunophenotypes associated with leukemia (leukemia-associated immunophenotype – LAIP) at diagnosis and monitoring throughout the entire treatment. The second approach focuses on the detection of cell populations deviating from antigen expression patterns characteristic of normal or regenerating cells, the so-called difference from normal (DfN). A combination of both approaches is recommended [6,7].

The polymerase chain reaction (PCR) method has a sensitivity of 10^{-4} to 10^{-5} . This method can detect DNA mutations at very sensitive levels. Polymerase chain reaction represents a more sensitive and standardized way of quantifying a specific mutation, which seems to be an ideal method for the detection of low levels of residual leukemic cells. However, this method also has several limitations. Perhaps the most important lim-

na veoma osetljivim nivoima. Lančana reakcija polimeraze predstavlja osetljiviji i standardizovaniji način kvantifikacije specifične mutacije, što izgleda kao idealna metoda za detekciju niskog nivoa rezidualnih leukemijskih ćelija. Međutim, i ova metoda ima nekoliko ograničenja. Možda je najvažnije ograničenje heterogenost same akutne mijeloblastne leukemije, jer je, uprkos brojnim citogenetskim i molekularnim mutacijama, genom relativno stabilan u AML-u. Izuzetak su kasni stadijumi AML-a, kao i postojanje mutacije TP53 ili kompleksnog kariotipa [8,9].

Sekvencioniranje sledeće generacije DNK i RNK je najosetljivija tehnika (do 10^{-7} senzitivnosti), ali se standardi ove tehnike još uvek uspostavljaju. Glavno ograničenje ove tehnike, kada je u pitanju određivanje MRD, jeste činjenica da je AML „tornado“ niskog mutacionog statusa, gde često mutacije nisu u jednoj tački, već su u pitanju fuzije gena ili druge strukturne varijacije, koje je teže otkriti aktuelnim NGS pristupom. Takođe, još jedno ograničenje NGS-a je što se ne mogu detektovati varijante sa učestalošću manjom od 1%, zbog stope greške konvencionalnog NGS-a, dajući mogućnost lažno negativnog rezultata [10-12].

Posle 60 godina relativne stagnacije u osavremenjivanju kriterijuma odgovora u AML-u, Evropska mreža za leukemiju (engl. *European LeukemiaNet – ELN*) je, 2017. godine, uvela novu kategoriju MRD-negativnih kompletnih remisija [13]. Zatim je, 2018. godine, usledilo saopštenje prvog ELN konsenzusa o standardnim smernicama za lečenje, koji sadrži detaljna uputstva za primenu protočne citometrije, molekularnog testiranja i kliničkih tumačenja MRD u AML-u [14].

ELN AML MRD smernice su ažurirane (uz pretpostavku da će ove smernice zasnovane na dokazima biti ažurirane svake dve do četiri godine). One sadrže skoro 60 različitih preporuka koje obuhvataju niz metoda detekcije MRD, kao što je multiparameterska protočna citometrija (MFC) MRD, potom molekularni MRD, kao i smernice za kliničku upotrebu MRD i uputstva za budući napredak u sferi MRD [15].

CILJ RADA

Svesni velikog prognostičkog i prediktivnog značaja merljive rezidualne bolesti, analizirali smo njen značaj kod bolesnika sa AML-om lečenih na Klinici za hematologiju UKCS, sa ciljem da prikazemo strategiju testiranja MRD, vidove terapije koje smo primenjivali, posebno kod MRD pozitivnih (MRD⁺) bolesnika, i preživljavanje bolesnika sa AML-om u odnosu na vrednost MRD.

MATERIJALI I METODE

Dijagnoza je uspostavljena pomoću mijelograma. Određivanje imunofenotipskih karakteristika AML-a je

itation is the heterogeneity of acute myeloblastic leukemia itself since, despite numerous cytogenetic and molecular mutations, the genome is relatively stable in AML. Exceptions are late stages of AML, as well as the presence of a TP53 mutation or a complex karyotype [8,9].

Next-generation DNA and RNA sequencing is the most sensitive technique (up to 10^{-7} sensitivity), but standards for this technique are still being established. The main limitation of this technique, when it comes to detecting MRD, is the fact that AML is a “tornado” of low mutational status, where mutations are often not in one point, but gene fusions or other structural variations, which are more difficult to detect with the current NGS approach. Also, another limitation of NGS is that variants with a frequency of less than 1% cannot be detected, due to the error rate of conventional NGS, thus opening the possibility of a false negative result [10-12].

After 60 years of relative inactivity in updating response criteria in AML, in 2017, the European Leukemia Network (ELN) introduced a new category of MRD-negative complete remissions [13]. In 2018, this was followed by the announcement of the first ELN consensus on standard treatment guidelines, which contains detailed instructions for the application of flow cytometry, molecular testing, and clinical interpretations of MRD in AML [14].

The ELN AML MRD guidelines have been updated (with the expectation that these evidence-based guidelines will be updated every two to four years). They contain almost 60 different recommendations covering a range of MRD detection methods, such as MRD by multiparametric flow cytometry and molecular MRD detection, as well as guidelines for the clinical use of MRD and instructions for future developments in the field of MRD [15].

STUDY AIM

Aware of the great prognostic and predictive significance of measurable residual disease, we have analyzed the significance of MRD in patients with AML treated at the University Clinical Center of Serbia (UCCS) Clinic for Hematology, with the aim of presenting the MRD testing strategy, the modalities of the therapy applied, especially in MRD-positive (MRD⁺) patients, and the survival of patients with AML in relation to the MRD values.

MATERIALS AND METHODS

The diagnosis was established using a myelogram. The immunophenotypic characteristics of AML were determined at diagnosis; cytogenetic/molecular testing

izvršeno pri dijagnozi bolesti; urađeno je citogenetsko/molekularno ispitivanje; stratifikacija rizika bolesnika sa AML-om je izvršena na osnovu *ELN* kriterijuma [16]. Za određivanje *MRD* statusa, korišćena je metoda multiparametarske protočne citometrije, na osmokolornom protočnom citometru [17,18].

Statistička analiza

U zavisnosti od od tipa varijabli i normalnosti raspodele, deskripcija podataka je prikazana kao *n* (%) ili medijana (min-maks). Za analizu preživljavanja korišćena je Kaplan-Majerova metoda. Za procenu funkcije preživljavanja ovih pacijenata u zavisnosti od transplantacije i pozitivne *MRD* posle tri ili više ciklusa hemioterapije korišćen je log-rank test, a za procenu nezavisnog prediktora smrtnog ishoda primenjen je Koksov proporcionalni regresioni model sa 95% intervalom poverenja. Podaci su cenzorisani iz sledećih razloga: ispitanik je preživeo celokupan period praćenja ili je izgubljen iz evidencije. Statističke hipoteze su testirane na nivou statističke značajnosti (alfa nivo) od 0,05. Svi podaci su obrađeni u *IBM SPSS Statistics 24* (SPSS Inc., Chicago, IL, USA) softverskom paketu ili u R programskom okruženju (R Core Team, 2023).

REZULTATI

Naša studija je obuhvatila analizu 111 bolesnika sa AML-om, lečenih na Klinici za hematologiju UKCS, u periodu od januara 2020. do januara 2024. Svi bolesnici su primili najmanje dva ili više ciklusa hemioterapije (HT). Procena *MRD* je rađena nakon dva ciklusa hemioterapije (indukcija i reindukcija ili indukcija i prva konsolidacija) i na kraju lečenja hemioterapijom. Medijana starosti ispitivanih bolesnika je bila 55 godina (18-75 godina). Od 111 bolesnika, 71 (64%) bolesnik je bio starosti 18-60 godina, dok je 40 (36%) bolesnika bilo starijeg životnog doba, iznad 60 godina. Od ukupnog broja, 64 (57,7%) bolesnika je bilo ženskog pola, dok je 47 (42,3%) pacijenata bilo muškog pola. Najveći broj bolesnika, ukupno 59 (53,2%), imalo je *ECOG* performans status (PS) 1. Neuroleukemiju je imalo 29 (26,1%) bolesnika. Prema *ELN* klasifikaciji, najviše bolesnika je svrstano u grupu intermedijarnog rizika, odnosno 67 (60,4%) bolesnika, povoljan oblik je imalo 20 (18,0%) pacijenata, dok je nepovoljan oblik bio prisutan kod 24 (21,6%) pacijenta. *FLT3-ITD* (engl. *FMS-like tyrosine kinase 3 internal tandem duplication*) pozitivnost je bila prisutna kod 15 (13,5%) pacijenata, dok je *NPM1* (engl. *nucleophosmin 1*) mutacija bila pozitivna kod 27 (24,3%) bolesnika. Dati rezultati su prikazani u Tabeli 1.

Najčešći primenjivani protokol indukcione hemioterapije je bio 3+7 i primenjen je kod 61 (55%) bolesnika. Sedam (6,3%) pacijenata je uz 3+7 primilo i *FLT3*

was performed; risk stratification of AML patients was carried out based on *ELN* criteria [16]. To determine the *MRD* status, the method of multiparametric flow cytometry was applied, using an eight-color flow cytometer [17,18].

Statistical analysis

Depending on the type of variables and the normality of distribution, data is presented as *n* (%) or median (min-max). The Kaplan-Meier method was used for survival analysis. The log-rank test was used to evaluate the survival function of these patients depending on transplantation and positive *MRD* after three or more cycles of chemotherapy, and the Cox proportional regression model with a 95% confidence interval was used to evaluate the independent predictor – fatal outcome. Data were censored for the following reasons: the subject survived the entire follow-up period or was lost from the records. Statistical hypotheses were tested at a statistical significance level (alpha level) of 0.05. All data were processed with the *IBM SPSS Statistics 24* (SPSS Inc., Chicago, IL, USA) software package or in the R programming environment (R Core Team, 2023).

RESULTS

Our study analyzed 111 AML patients, treated at the UCCS Clinic for Hematology, between January 2020 to January 2024. All patients received at least two or more cycles of chemotherapy (CHT). *MRD* assessment was performed after two cycles of chemotherapy (induction and reinduction or induction and first consolidation) and at the end of chemotherapy treatment. The median age of the examined patients was 55 years (18-75 years). Out of 111 patients, 71 (64%) patients were aged 18-60 years, while 40 (36%) patients were older than 60 years. Out of the total number, 64 (57.7%) patients were female, while 47 (42.3%) were male. The largest number of patients, 59 (53.2%) in total, had *ECOG* performance status (PS) 1. Neuroleukemia was present in 29 (26.1%) patients. According to the *ELN* classification, most patients were classified in the intermediate risk group, i.e. 67 (60.4%) patients, 20 (18.0%) patients were in the favorable risk group, and 24 (21.6%) patients were in the adverse risk group. *FMS*-like tyrosine kinase 3 internal tandem duplication (*FLT3-ITD*) positivity was present in 15 (13.5%) patients, while the nucleophosmin 1 (*NPM1*) mutation was positive in 27 (24.3%) patients. These results are presented in Table 1.

The 3+7 regimen was the most frequently applied induction chemotherapy regimen. It was used in 61 (55%) patients. Seven (6.3%) patients received the *FMS*-like tyrosine kinase 3 (*FLT3*) inhibitor (midostaurin) in addition to the 3+7 regimen, while 25 (22.5%)

Tabela 1. Inicijalne kliničke karakteristike ispitivanih bolesnika

		Učestalost / Frequency	Procenat / Percentage
Starost / Age	18-60	71	64.0
	> 60	40	36.0
Pol / Gender	Muški / Male	47	42.3
	Ženski / Female	64	57.7
ECOG / ECOG	0	38	34.2
	1	59	53.2
	2	11	9.9
	3	3	2.7
ELN / ELN	Povoljan / Favorable	20	18.0
	Intermedijaran / Intermediate	67	60.4
	Nepovoljan / Adverse	24	21.6
Neuroleukemija / Neuroleukemia	Da / Yes	29	26.1
	Ne / No	82	73.9
FLT3 / FLT3	Pozitivan / Positive	15	13.5
	Negativan / Negative	96	86.5
NPM1 / NPM1	Pozitivan / Positive	27	24.3
	Negativan / Negative	84	75.7
Ukupno / Total		111	100.0

Legenda: ECOG – engl. Eastern Cooperative Oncology Group; ELN – engl. European Leukemia Network; FLT3 – engl. FMS-like tyrosine kinase 3; NPM1 – engl. nucleophosmin 1

Table 1. Initial clinical characteristics of the examined patients

Legend: ECOG – Eastern Cooperative Oncology Group; ELN – European Leukemia Network; FLT3 – FMS-like tyrosine kinase 3; NPM1 – nucleophosmin 1

(FMS-like tyrosine kinase 3) inhibitor (midostaurin), dok je 25 (22,5%) bolesnika primalo 3+7 light, odnosno 18 (16,2%) bolesnika je primilo 2+5 protokol.

Citomorfološku remisiju nakon indukcione HT postiglo je 64 (57,7%) bolesnika. Citomorfološka remisija nakon primene reindukcije HT je postignuta kod još 20 (18%) pacijenata, dok je preostalih 27 (24,3%) bolesnika bilo refraktorno na primenjenu HT. Dakle, u citomorfološkoj remisiji je, posle indukcije i reindukcije, bilo 84 (75,7%) bolesnika.

Nakon primene dva ciklusa HT, MRD negativnost je ostvarena kod 35 (31,5%) bolesnika i svi su u nastavku lečeni IDAC konsolidacionom terapijom. Pozitivnu vrednost MRD je imalo 76 (68,5%) bolesnika koji su lečeni različitim HT protokolima: Mitox-VP je primao 31 (40,8%) bolesnik, IDAC+DA je primilo 19 (25%) pacijenata, IDA-VP je dobilo 8 (10,5%) bolesnika, MEC je primilo 7 (9,2%) pacijenata, 3+7 je dobilo 7 (9,2%) bolesnika, IDAC su dobila tri (4,0%) bolesnika, dok je FLAG IDA primio jedan (1,3%) pacijent (Grafikon 1).

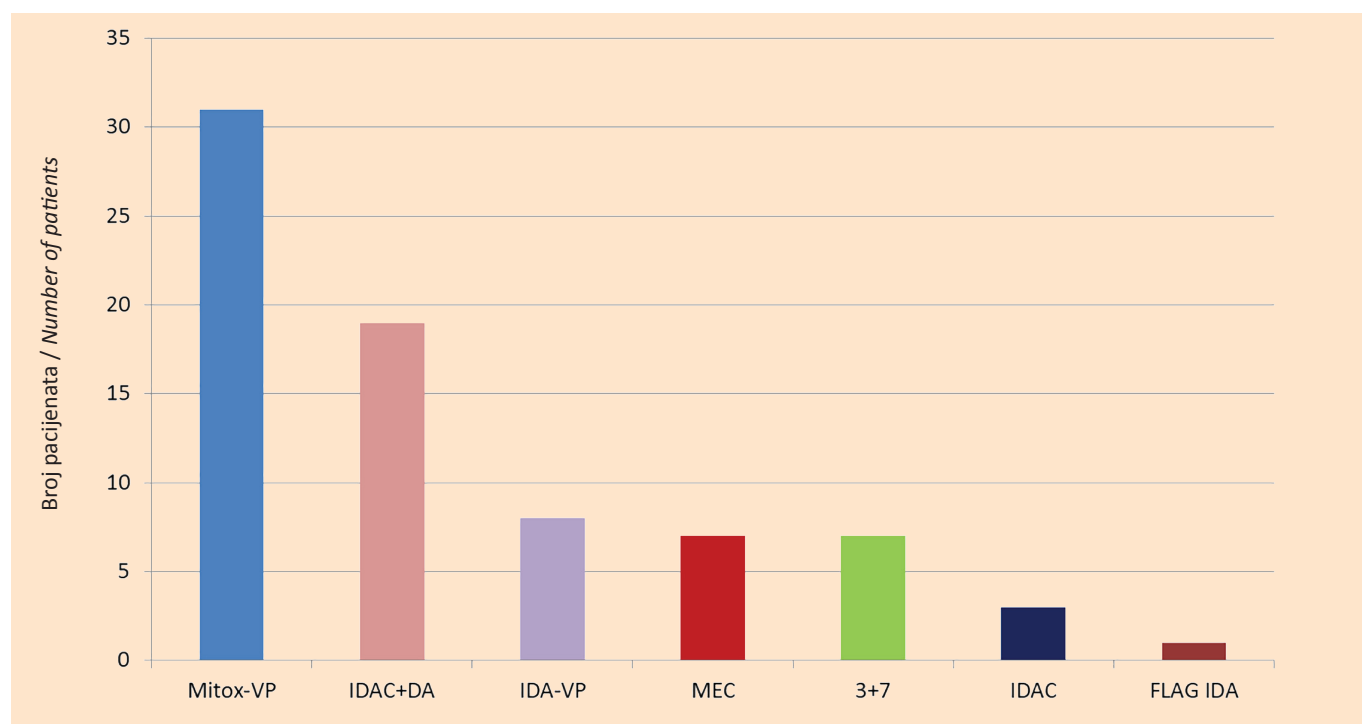
Kod 81 (73%) pacijenta je rađen i MRD na kraju lečenja, od kojih je 46 imalo negativan MRD, dok je 35 bolesnika imalo pozitivan MRD. Takođe, od 50 bolesnika koji su imali MRD pozitivnost nakon drugog ciklusa HT, 28 (56%) pacijenata je ostvarilo negativan MRD na kraju lečenja. Relaps bolesti je ustanovljen kod 43 (37,8%) pacijenta.

patients received the 3+7 light regimen, and 18 (16.2%) patients received the 2+5 regimen.

Cytomorphological remission after induction CHT was achieved by 64 (57.7%) patients. Cytomorphological remission after reinduction CHT was achieved in another 20 (18%) patients, while the remaining 27 (24.3%) patients were refractory to the applied CHT. Thus, after induction and reinduction, there were 84 (75.7%) patients in cytomorphological remission.

After two cycles of CHT were administered, MRD negativity was achieved in 35 (31.5%) patients, who were all subsequently treated with IDAC consolidation therapy. Seventy-six (68.5%) patients who were treated with various CHT protocols had a positive MRD value: 31 (40.8%) patients received Mitox-VP, 19 (25%) patients received IDAC+DA, IDA-VP was administered to 8 (10.5%) patients, MEC was received by 7 (9.2%) patients, 7 (9.2%) patients received the 3+7 regimen, IDAC was given to three (4.0%) patients, while FLAG IDA was received by one (1.3%) patient (Graph 1).

In 81 (73%) patients, MRD testing was also performed at the end of treatment. Of these patients, 46 were MRD-negative, while 35 patients were MRD-positive. Also, out of 50 patients who demonstrated MRD positivity after the second cycle of CHT, 28 (56%) patients achieved MRD negativity at the end of treatment. Disease relapse occurred in 43 (37.8%) patients.



Grafikon 1. Vrsta primenjene terapije kod MRD pozitivnih bolesnika (nakon II ciklusa inicijalne terapije)

Nakon primene dva ciklusa HT, MRD negativnost je ostvarena kod 35 (31,5%) bolesnika i svi su u nastavku lečeni konsolidacionom IDAC terapijom. Pozitivnu vrednost MRD-a je imalo 76 (68,5%) bolesnika, koji su lečeni različitim HT protokolima: Mitox-VP je primio 31 (40,8%) pacijent; IDAC+DA je primilo 19 (25%) pacijenata; IDA-VP je primilo 8 (10,5%) bolesnika; MEC je dobilo 7 (9,2%) pacijenata; 3+7 je primilo 7 (9,2%) bolesnika; IDAC je dobilo tri (4,0%) bolesnika; FLAG IDA je primio jedan (1,3%) pacijent.

Graph 1. Type of therapy used in MRD-positive patients (after the second cycle of initial therapy)

After two cycles of CHT were administered, MRD negativity was achieved in 35 (31.5%) patients and all of them were subsequently treated with consolidation IDAC therapy. Seventy-six (68.5%) patients had a positive MRD and were treated with different CHT protocols: 31 (40.8%) patients received Mitox-VP; 19 (25%) patients received IDAC+DA; 8 (10.5%) patients received IDA-VP; 7 (9.2%) patients received MEC; 3+7 was received by 7 (9.2%) patients; three (4.0%) patients received IDAC; FLAG IDA was received by one (1.3%) patient.

Medijana ukupnog preživljavanja (engl. *overall survival* – OS) je kod naših bolesnika iznosila 13 meseci (2-69). Medijana dužine citomorfološke remisije je kod naših bolesnika iznosila 9 meseci (0-64). Medijana preživljavanja je kod mlađih bolesnika (18-60 godina) iznosila 18,0 meseci (95% CI = 13,3-22,7), dok je kod starijih (> 60 godina) pacijenata iznosila 13,0 meseci (95% CI = 7,5-18,5), što je statistički značajna razlika (log-rank = 3,722; $p = 0,054$). Dužina remisije (engl. *complete remission* – CR) je bila značajno veća u starijoj grupi 18-60 godina u odnosu na grupu > 60 godina ($p = 0,02$, HR = 0,568, 95% CI = 0,352-0,915).

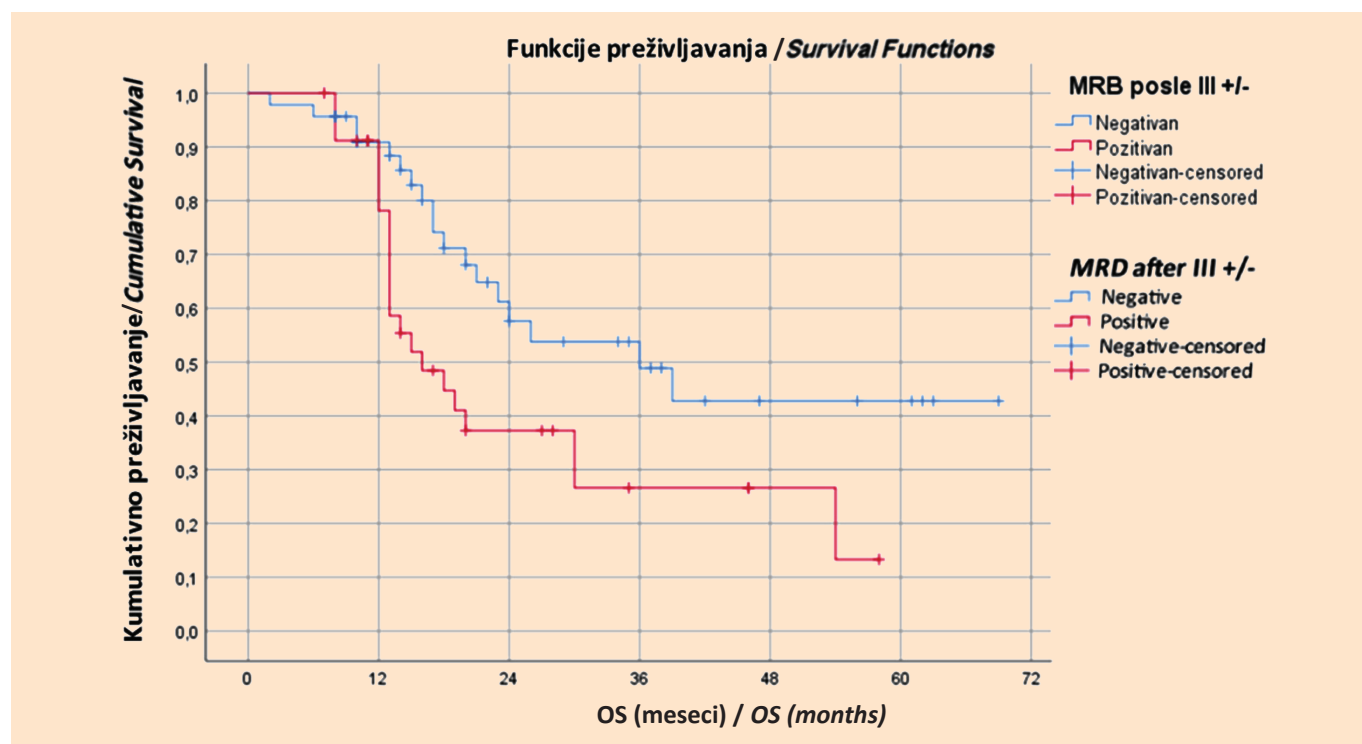
Nije postojala statistički značajna razlika u ukupnom preživljavanju (OS) i dužini remisije (CR) u odnosu na pol ($p = 0,245$, HR = 1,321, 95% CI = 0,826-2,114). Nije utvđena statistički značajna razlika u ukupnom preživljavanju (OS) i dužini CR u odnosu na MRD I status (posle prve konsolidacije ili nakon dva ciklusa uvodne terapije reindukcije), ($p = 0,380$; $p = 0,288$).

Međutim, medijana preživljavanja kod bolesnika sa pozitivnim MRD testom na kraju lečenja je iznosila 16,0 meseci (95% CI = 9,3-22,7), dok je kod MRD negativnih pacijenata iznosila 36,0 meseci (95% CI = 17,6-54,4), što je statistički značajna razlika ($p = 0,019$). Ispitanici sa po-

Median overall survival (OS) in our patients was 13 months (2-69). The median length of cytomorphological remission in our patients was 9 months (0-64). Median survival in younger patients (18-60 years) was 18.0 months (95% CI = 13.3-22.7), while in older (> 60 years) patients it was 13.0 months (95% CI = 7.5-18.5), which is a statistically significant difference (log-rank = 3.722; $p = 0.054$). The length of remission (complete remission – CR) was significantly longer in the age group 18-60 years, as compared to the group > 60 years ($p = 0.02$, HR = 0.568, 95% CI = 0.352-0.915).

There was no statistically significant difference in overall survival (OS) and length of remission (CR) in relation to gender ($p = 0.245$, HR = 1.321, 95% CI = 0.826-2.114). No statistically significant difference was found in overall survival (OS) and length of CR in relation to MRD I status (after the first consolidation or after two cycles of initial reinduction therapy), ($p = 0.380$; $p = 0.288$).

However, median survival in patients with a positive MRD test at the end of treatment was 16.0 months (95% CI = 9.3-22.7), while in MRD-negative patients it was 36.0 months (95% CI = 17.6-54.4), which is a statistically significant difference ($p = 0.019$). Subjects



Grafikon 2. Kaplan-Majerova kriva ukupnog preživljavanja (engl. overall survival – OS) u odnosu na MRD status na kraju lečenja

Medijana preživljavanja kod bolesnika sa pozitivnim MRD-om na kraju lečenja iznosila je 16,0 meseci (95% CI = 9,3-22,7), dok je kod pacijenata sa negativnim MRD-om iznosila 36,0 meseci (95% CI = 17,6-54,4), što je statistički značajna razlika ($p = 0,019$). Ispitanici sa pozitivnim MRD-om na kraju lečenja imali su za preko dva puta veći hazard za nastanak smrtnog ishoda ($HR = 2,06$, 95% CI = 1,10-3,85; $p = 0,024$).

Graph 2. Kaplan-Meier curve of overall survival (OS) in relation to the MRD status at the end of treatment

Median survival in patients with a positive MRD at the end of treatment was 16.0 months (95% CI = 9.3-22.7), while it was 36.0 months (95% CI = 17.6-54.4) in patients with a negative MRD, which is a statistically significant difference ($p = 0.019$). Subjects with a positive MRD at the end of treatment had more than twice the hazard for a fatal outcome ($HR = 2.06$, 95% CI = 1.10-3.85; $p = 0.024$).

zitivnim MRD na kraju lečenja imali su više od dva puta veći hazard za nastanak smrtnog ishoda ($HR = 2,06$, 95% CI = 1,10-3,85; $p = 0,024$), (Grafikon 2). Takođe, dužina CR je bila statistički značajno veća kod bolesnika sa negativnim MRD na kraju lečenja ($p = 0,004$, $HR = 2.517$, 95% CI = 1,342-4,720).

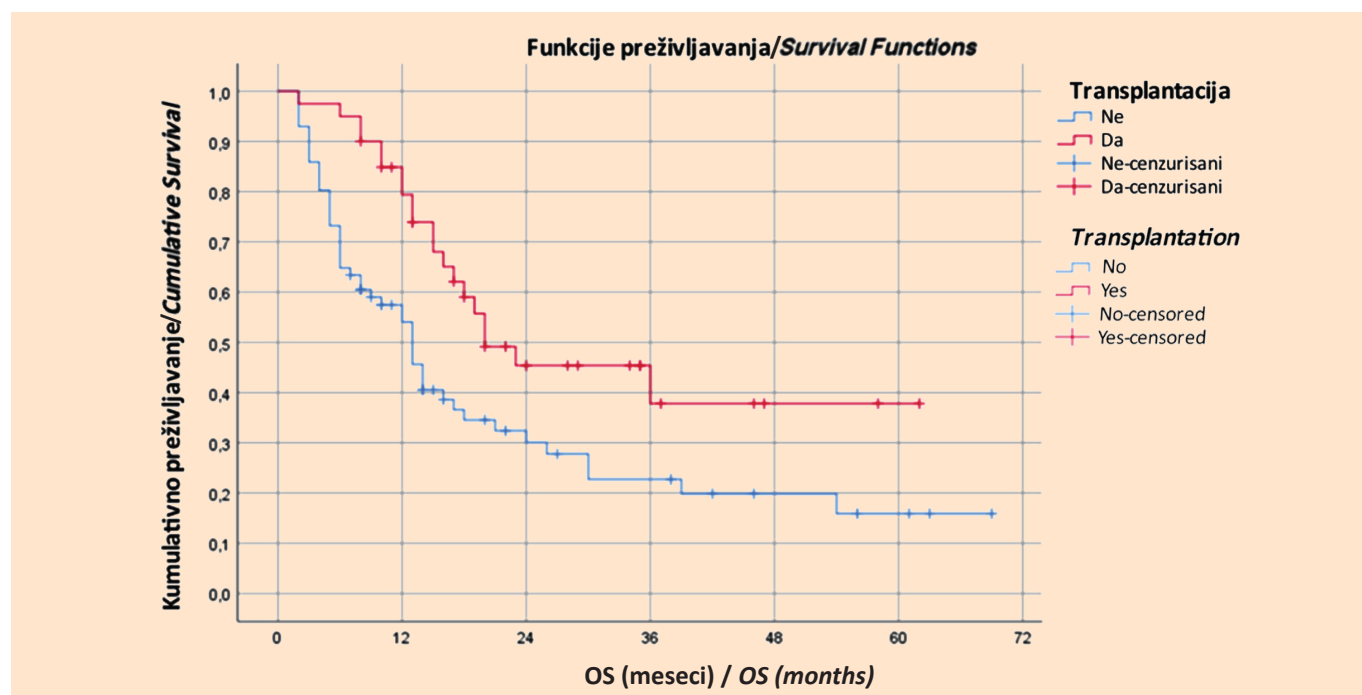
Ukupno preživljavanje (OS) je bilo značajno duže u grupi sa intermedijarnim rizikom leukemije (medijana 18 meseci) prema ELN klasifikaciji, u odnosu na grupu sa povoljnim (medijana 13 meseci) i nepovoljnim (medijana 6 meseci) rizikom ($p = 0,013$). Takođe, dužina trajanja remisije (CR) je bila značajno duža u grupi bolesnika sa intermedijarnim rizikom (medijana 14 meseci), u odnosu na povoljni (medijana 11 meseci) i nepovoljni rizik (medijana 5 meseci), ($p = 0,014$, $HR = 0,503$, 95% CI = 0,291-0,871).

Ukupno preživljavanje (OS) je bilo značajno duže kod bolesnika kod kojih je primenjena reindukcija punim dozama protokola 3+7 nakon pozitivnog MRD (medijana preživljavanja 35 meseci), dok je preživljavanje kod primene drugih protokola bilo značajno manje: IDAC (medijana 15,5 meseci), IDAC+DA (medijana 13 meseci), MEC (medijana 10 meseci), Mitox-VP (medijana 10 meseci), IDA-VP (medijana 7,5 meseci).

with positive MRD at the end of treatment had more than twice the hazard ($HR = 2.06$, 95% CI = 1.10-3.85; $p = 0.024$), (Graph 2). Also, the length of CR was statistically significantly longer in patients with negative MRD at the end of treatment ($p = 0.004$, $HR = 2.517$, 95% CI = 1.342-4.720).

Overall survival (OS) was significantly longer in the group with intermediate risk of leukemia (median 18 months) according to the ELN classification, compared to the group with favorable (median 13 months) and adverse (median 6 months) risk ($p = 0.013$). Also, the duration of remission (CR) was significantly longer in the group of patients with intermediate risk (median 14 months), compared to favorable (median 11 months) and adverse risk (median 5 months), ($p = 0.014$, $HR = 0.503$, 95% CI = 0.291-0.871).

Overall survival (OS) was significantly longer in patients in whom reinduction with full doses of the 3+7 regimen was applied after a positive MRD test (median survival 35 months), while survival was significantly shorter with the application of other protocols: IDAC (median 15.5 months), IDAC+DA (median 13 months), MEC (median 10 months), Mitox-VP (median 10 months), IDA-VP (median 7.5 months).



Grafikon 3. Kaplan-Majerova kriva ukupnog preživljavanja (engl. *overall survival – OS*) kod bolesnika na hemioterapiji u odnosu na transplantirane pacijente. Medijana preživljavanja kod transplantiranih bolesnika iznosila je 20 meseci (95% CI = 4,0-36,0), dok je kod netransplantiranih pacijenata iznosila 13 meseci (95% CI = 10,1-15,9), što je statistički značajna razlika ($p = 0,006$). Ispitanici sa transplantacijom su imali za 50% manji hazard za nastanak smrtnog ishoda ($HR = 0,498$, 95% CI = 0,296-0,839; $p = 0,009$).

Graph 3. Kaplan-Meier curve of overall survival (OS) in patients on chemotherapy in relation to transplant patients

The median survival in transplant patients was 20 months (95% CI = 4.0-36.0), while in non-transplant patients it was 13 months (95% CI = 10.1-15.9), which is a statistically significant difference ($p = 0.006$). Subjects who had received a transplant had a 50% lower hazard for the fatal outcome ($HR = 0.498$, 95% CI = 0.296-0.839; $p = 0.009$).

Transplantacija je izvršena kod 40 bolesnika (36%). Indikacije za transplantaciju su bile sledeće: nepovoljan rizik (ELN) kod šest (15%) pacijenata, relaps bolesti kod 12 (30%) pacijenata, pozitivan MRD kod 22 (55%) bolesnika. Medijana preživljavanja kod transplantiranih bolesnika je iznosila 20 meseci (95% CI = 4,0-36,0), dok je kod netransplantiranih pacijenata iznosila 13 meseci (95% CI = 10,1-15,9), što je statistički značajna razlika ($p = 0,006$). Ispitanici sa transplantacijom su imali za 50% manji hazard za nastanak smrtnog ishoda ($HR = 0,498$, 95% CI = 0,296-0,839; $p = 0,009$), (Grafikon 3). Trajanje remisije (CR) je bilo značajno duže (21 mesec) kod transplantiranih, u odnosu na grupu netransplantiranih bolesnika (8 meseci), ($p = 0,002$, $HR = 0,441$, 95% CI = 0,261-0,745).

DISKUSIJA

Kao biomarker, merljiva rezidualna bolest (MRD) može imati i prognostički, kao i prediktivni značaj, ali apsolutni merljivi nivo bolesti nije jedina determinanta ishoda bolesnika pošto biologija AML i drugi klinički faktori samog bolesnika (starost, komorbiditeti, različite komplikacije primenjene HT, naročito infektivne) modifikuju rizik povezan sa rezultatima MRD testa [19-21].

Jasno je da sve mutacije detektovane pri dijagnozi AML-a neće biti od jednake kliničke koristi za praćenje

Transplantation was performed in 40 patients (36%). The indications for transplantation were as follows: adverse risk (ELN), found in six (15%) patients, disease relapse, found in 12 (30%) patients, and a positive MRD test, which was found in 22 (55%) patients. Median survival in transplanted patients was 20 months (95% CI = 4.0-36.0), while in non-transplanted patients it was 13 months (95% CI = 10.1-15.9), which is a statistically significant difference ($p = 0.006$). Transplant subjects had a 50% lower risk of death ($HR = 0.498$, 95% CI = 0.296-0.839; $p = 0.009$), (Chart 3). The duration of remission (CR) was significantly longer (21 months) in transplanted patients, compared to the group of non-transplanted patients (8 months), ($p = 0.002$, $HR = 0.441$, 95% CI = 0.261-0.745).

DISCUSSION

As a biomarker, measurable residual disease (MRD) can be both prognostic and predictive. However, the absolute measurable level of the disease is not the only determinant of the patient's outcome, since the biology of AML, as well as other clinical patient-related factors (age, comorbidities, various complications of applied chemotherapy, especially infections), modify the risk associated with MRD test results [19-21].

Clearly, not all mutations detected at AML diagnosis will be equally clinically useful for MRD monitoring.

MRD. Optimalni ciljevi za molekularno merenje **MRD** takođe nisu definisani. Izolovana detekcija mutacija uobičajenih u klonskoj hematopoezi povezanoj sa starenjem (engl. *age-related clonal hematopoiesis*), npr. *DNMT3A*, *TET2*, ili *ASXL1*, ili kod sindroma nasledne predispozicije (engl. *germline predisposition syndromes*), npr. *DDKS41*, *RUNKS1* ili *GATA2*, nužno ne predstavljaju rezidualnu AML [17-20].

U našoj studiji smo pokazali da nije bilo značajne razlike u dužini **CR** i u **OS** između bolesnika u odnosu na status **MRD** posle dva ciklusa HT. Sa druge strane, vrsta primenjene HT kod **MRD+** bolesnika je imala značajnog uticaja na dužinu **CR**, kao i na **OS**, sa značajno dužom **CR** i dužim **OS** kod bolesnika koji su posle **MRD+** nastavili lečenje punim dozama 3+7 HT, kao reindukcije. Takođe, **CR** i **OS** su bili statistički značajno duži kod bolesnika koji su na kraju lečenja zadržali negativan **MRD** status.

U odnosu na **ELN** stratifikaciju rizika, najduže **OS** su imali bolesnici intermedijarne grupe rizika. Jedno od mogućih objašnjenja je da su bolesnici iz povoljne grupe bolesnika, za koje se očekivalo da imaju najduže **OS** imali određene genske mutacije koje nismo detektovali usled toga što, za sada, testiranje na njih nije deo standardnog vida ispitivanja na našoj klinici.

Bolesnici lečeni alogenom TMČH su imali statistički duže trajanje **CR** i **OS**, u odnosu na netransplantirane bolesnike, što je pokazano u nekoliko velikih studija [22-24].

ZAKLJUČAK

Analizom rezultata ove studije, kao i podataka iz literature, izvedeno je nekoliko zaključaka.

Postojeći podaci ukazuju na to da su mutacije u genima signalnog puta (*FLT3*, *KIT*, *RAS*, i dr.) korisne kada su pozitivne, ali njihova negativnost u kasnijem praćenju ne mora obavezno značiti molekularnu remisiju, posebno kada je mutacija terapijski targetovana (ciljana) adekvatnim agensima. Takođe, pitanje je da li negativnost **MRD** ima isti prognostički značaj ako se postigne nakon intenzivne u odnosu na neintenzivnu terapiju.

Kao biomarker, merljiva rezidualna bolest (**MRD**) može imati i prognostički i prediktivni značaj, ali apsolutni merljivi nivo bolesti nije jedina determinanta ishoda bolesnika, pošto biologija AML-a i drugi klinički faktori modifikuju rizik povezan sa rezultatima **MRD** testa.

Naša studija je pokazala veliki značaj pravovremenog određivanja **MRD**, te adekvatnost primene intenzivnije HT kod **MRD** pozitivnih bolesnika, uz nastavak lečenja alogenom transplantacijom matičnih ćelija hematopoeze.

Sukob interesa: Nije prijavljen.

Optimal targets for molecular **MRD** measurement have also not been defined.

Isolated detection of mutations common in age-related clonal hematopoiesis, e.g. *DNMT3A*, *TET2*, or *ASXL1*, or in germline predisposition syndromes, e.g. *DDKS41*, *RUNKS1*, or *GATA2*, do not necessarily constitute residual AML [17-20].

Our study has demonstrated no significant difference in **CR** and **OS** length among patients, with regard to **MRD** status after two cycles of CHT. On the other hand, the type of CHT used in **MRD+** patients had a significant impact on the length of **CR**, as well as on **OS**, with significantly longer **CR** and **OS** in patients who, after **MRD+**, continued treatment with full doses of 3+7 CHT, as reinduction therapy. Also, **CR** and **OS** were statistically significantly longer in patients who maintained a negative **MRD** status at the end of treatment.

In relation to the **ELN** risk stratification, patients in the intermediate-risk group had the longest **OS**. One of the possible explanations is that the patients from the favorable-risk group, who were expected to have the longest **OS**, had certain gene mutations that we did not detect since, for now, testing for these mutations is not part of the standard tests performed at our clinic.

Patients treated with allogeneic HSCT had a statistically longer duration of **CR** and **OS**, as compared to non-transplanted patients, as demonstrated in several large studies [22-24].

CONCLUSION

Analysis of the results of this study, as well as of the data from literature, has yielded several conclusions.

Existing data indicate that mutations in signaling pathway genes (*FLT3*, *KIT*, *RAS*, etc.) are useful when they are positive, but their negativity in later follow-up does not necessarily mean molecular remission, especially when the mutation is therapeutically targeted with the appropriate agents. Also, it is debatable whether **MRD** negativity has the same prognostic significance if it is achieved after intensive versus non-intensive therapy.

As a biomarker, measurable residual disease (**MRD**) can have both prognostic and predictive value. However, the absolute measurable level of disease is not the only determinant of patient outcome, as AML biology and other clinical factors modify the risk associated with **MRD** test results.

Our study emphasizes the great importance of timely detection of **MRD**, as well as the appropriateness of applying more intensive CHT in **MRD**-positive patients, along with continued treatment with allogeneic hematopoietic stem cell transplantation.

Conflict of interest: None declared.

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KLINIČKE KARAKTERISTIKE I PREŽIVLJAVANJE ODRASLIH PACIJENATA OBOLELIH OD AKUTNE MIJELOIDNE LEUKEMIJE: ISKUSTVO JEDNOG CENTRA

ORIGINALNI RAD

ORIGINAL ARTICLE

CLINICAL CHARACTERISTICS AND SURVIVAL OUTCOMES IN ACUTE MYELOID LEUKEMIA PATIENTS: SINGLE CENTER EXPERIENCE

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SAŽETAK

Uvod/Cilj: Akutna mijeloidna leukemija (AML) je heterogena bolest, po svojim biološkim karakteristikama i po odgovoru na terapiju. Brojni prognostički faktori pri dijagnozi koji su u vezi sa samom bolešću kod pacijenta, terapija i odgovor na terapiju utiču na ishod AML-a. Uprkos velikoj stopi postizanja kompletne remisije, ukupno preživljavanje (engl. *overall survival* – OS) je i dalje loše. Cilj ovog istraživanja je da se odredi kliničko-biološki profil bolesnika sa AML-om i njegov prognostički uticaj na stopu OS i preživljavanje bez znakova bolesti (engl. *disease-free survival* – DFS).

Materijali i metode: Retrospektivna analiza obuhvatila je 271 bolesnika sa dijagnozom akutne mijeloidne leukemije postavljenom na Klinici za hematologiju, Univerzitetskog kliničkog centra Srbije (UKCS), između januara 2018. i januara 2023. godine. Analizirani su demografski parametri, ECOG PS (engl. *Eastern Cooperative Oncology Group performance status*), komorbiditeti, parametri laboratorijske analize i hematološke dijagnostike AML-a, kao potencijalni faktori rizika za OS i DFS, uz korišćenje univarijantnog Koksovog regresionog modela.

Rezultati: Od ukupno 271 pacijenta, 206 (76%) je lečeno intenzivnom hemioterapijom, od kojih je bilo 108 muškaraca i 98 žena. Prosečna starost ovih ispitanika je bila 50,3 godine. Prema klasifikaciji rizika Evropske mreže za leukemiju (engl. *European LeukemiaNet* – ELN), najviše je bilo bolesnika intermedijarnog stepena rizika, odnosno 128 (62,1%) pacijenata. Najzastupljeniji podtip AML-a je bio NOS AML (engl. *AML not otherwise specified*), prisutan kod 123 (59,7%) bolesnika. Univarijantna analiza je pokazala da su starost od ≥ 60 godina ($p = 0,009$) i vrednost $Le \geq 30 \times 10^9/l$ ($p = 0,031$) nepovoljni prognostički parametri za OS. Podtip AML-a, MRC (engl. *myelodysplasia-related changes*) bio je najznačajniji faktor rizika za kraće preživljavanje bez znakova bolesti ($p = 0,027$).

Zaključak: U analiziranoj grupi bolesnika sa AML-om lečenih intenzivnom terapijom, pokazali smo nisku stopu OS, kao i da godine starosti i MRC podtip AML-a predstavljaju nepovoljne faktore rizika za kraće ukupno preživljavanje, odnosno preživljavanje bez znakova bolesti.

Ključne reči: akutna mijeloidna leukemija, prognostički faktori, preživljavanje

ABSTRACT

Introduction/Aim: Acute myeloid leukemia (AML) is a heterogeneous disease, in terms of its biological characteristics and response to therapy. Numerous prognostic factors at diagnosis related to the disease itself, the treatment, and the response to treatment influence the outcome of AML. Despite the high rate of complete remission, overall survival (OS) is still poor. This study aims to determine the clinical and biological profile of patients with AML and its prognostic impact on the OS rate and disease-free survival (DFS).

Materials and methods: The retrospective analysis included 271 patients diagnosed with acute myeloid leukemia at the University Clinical Center of Serbia (UCCS) Hematology Clinic, between January 2018 and January 2023. Demographic parameters, the Eastern Cooperative Oncology Group performance status (ECOG PS), comorbidities, and the parameters of laboratory analysis and hematological diagnosis of AML were analyzed as potential risk factors for OS and DFS, using the univariate Cox regression model.

Results: Out of a total of 271 patients, 206 (76%) were treated with intensive chemotherapy, of whom 108 were men and 98 were women. The average age of these respondents was 50.3 years. According to the European Leukemia Network (ELN) risk classification, the patients were mostly in the group with intermediate risk, i.e. 128 (62.1%) patients. The most common subtype of AML was AML NOS (AML not otherwise specified), present in 123 (59.7%) patients. Univariate analysis showed that age ≥ 60 years ($p = 0.009$) and $WBC \geq 30 \times 10^9/l$ ($p = 0.031$) were unfavorable prognostic parameters for OS. AML subtype, MRC (myelodysplasia-related changes) was the most significant risk factor for shorter disease-free survival ($p = 0.027$).

Conclusion: In the analyzed group of AML patients treated with intensive therapy, we demonstrated a low OS rate and showed that age and the MRC subtype of AML represent unfavorable risk factors for shorter OS and DFS, respectively.

Keywords: acute myeloid leukemia, prognostic factors, survival

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UVOD

Akutna mijeloidna leukemija (AML) je heterogena maligna bolest hematopoeznog sistema koju karakteriše klonaska proliferacija nediferentovanih mijeloidnih prekursora tj. blasta [1]. Usled nakupljanja nezrelih ćelija dolazi do potiskivanja i oštećenja normalnih ćelija hematopoeze, što se posledično klinički manifestuje kao infekcija, anemija i krvarenje [2]. Povremeno, AML može da ima ekstramedularnu prezentaciju, uključujući i zahvaćenost centralnog nervnog sistema (CNS) [3].

AML je bolest starijeg životnog doba – prosečna starosti pacijenata u trenutku postavljanja dijagnoze je 69 godina. Spada u retke bolesti, sa godišnjom incidencijom od 4,2 /100.000 stanovnika, i češća je kod muškaraca (M : Ž = 5,1/100.000 : 3,5/100.000) [4].

Poznato je nekoliko faktora rizika koji se dovode u vezu sa nastankom AML-a: izloženost mutagenim faktorima spoljašnje sredine, prethodna hemioterapija i druge hematološke bolesti [2]. Novija istraživanja su pokazala povezanost AML-a i urođenog nasleđivanja u okviru porodice [5,6].

Tehnološki napredak u molekularnoj dijagnostici doveo je do novih saznanja o biologiji AML-a. Prethodna klasifikacija AML-a Svetske zdravstvene organizacije (SZO), iz 2016. godine, predviđala je $\geq 20\%$ blasta kao dijagnostički kriterijum, sa izuzetkom AML-a sa rekurentnim citogenetskim abnormalnostima (RCA) i obuhvatala je četiri glavne grupe: AML sa RCA, AML sa mijelodisplastičnim karakteristikama (engl. *AML with myelodysplasia-related changes* – MRC), AML nastala posle prethodnog lečenja (engl. *therapy-related AML* – t-AML) i NOS AML (engl. *AML not otherwise specified*) [5]. Međunarodna konsenzus klasifikacija (engl. *International Consensus Classification* – ICC) i klasifikacija SZO, iz 2022. godine, unapredile su prethodnu klasifikaciju AML-a, uvođenjem novih genetski definisanih entiteta za koje je granična vrednost procenta blasta snižena [6,7]. Obe klasifikacije iz 2022. godine polaze od toga da je abnormalna morfologija rezultat poremećene ćelijske biologije, koja je uzrokovana somatskim mutacijama ili ekspresijom izmenjenih gena, pa se stoga više fokusiraju na ekstenzivno genetsko profilisanje u definisanju entiteta bolesti.

Prema SZO, konačna dijagnoza AML-a se postavlja na osnovu integrisanog dijagnostičkog algoritma koji uključuje rezultate nekoliko komplementarnih metoda: citomorfološke, imunofenotipske, citogenetske i molekularno genetske analize u formi objedinjenog izveštaja [6,7].

Ishod AML je veoma heterogen i zavisi od niza faktora u vezi sa bolesnikom (godine starosti, ECOG PS (engl. *Eastern Cooperative Oncology Group performance status*), komorbiditeti) kao i u vezi sa samom bolešću (broj leukocita (Le) na dijagnozi, prethodni

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous malignant disease of the hematopoietic system characterized by clonal proliferation of undifferentiated myeloid precursors, i.e. blasts [1]. As a result of the accumulation of immature cells, normal hematopoietic cells are suppressed and damaged, which consequently manifests clinically as infection, anemia, and bleeding [2]. Occasionally, AML can have an extramedullary presentation, including central nervous system (CNS) involvement [3].

AML is a disease of older age – the average age of patients at the time of diagnosis is 69 years. It belongs to rare diseases, with an annual incidence of 4.2/100,000 inhabitants, and is more common in men (M : F = 5.1/100,000 : 3.5/100,000) [4].

Several risk factors are known to be associated with the development of AML: exposure to mutagenic environmental factors, previous chemotherapy, and other hematological diseases [2]. More recent studies have shown an association between AML and congenital inheritance within the family [5,6].

Technological advances in molecular diagnostics have led to new insights into the biology of AML. The previous World Health Organization (WHO) classification of AML, from 2016, required $\geq 20\%$ blasts as a diagnostic criterion (except in AML with recurrent cytogenetic abnormalities – AML with RCA), and included four main groups: AML with RCA, AML with myelodysplasia-related changes (AML with MRC), therapy-related AML (t-AML), and AML not otherwise specified (AML NOS) [5]. The International Consensus Classification (ICC) and the WHO classification, from 2022, improved the previous classification of AML by introducing new genetically defined entities for which the threshold value of the percentage of blasts was lowered [6,7]. Both 2022 classifications are based on the assumption that abnormal morphology stems from disrupted cell biology, which is caused by somatic mutations or altered gene expression, and therefore focus more on extensive genetic profiling in defining disease entities.

According to the WHO, the final diagnosis of AML is established based on an integrated diagnostic algorithm that includes the results of several complementary methods: cytomorphological, immunophenotypic, cytogenetic, and molecular genetic analysis in the form of a unified report [6,7].

The outcome of AML is very heterogeneous and depends on several factors related to the patient (age, Eastern Cooperative Oncology Group performance status – ECOG PS, comorbidities), as well as to the disease itself (white blood cell count (WBC) at diagnosis, previous myelodysplastic syndrome (MDS), patholog-

mijelodisplačni sindrom (MDS), patološki kariotip, molekularni markeri, prethodna citotoksična terapija) [2]. Genetske abnormalnosti predstavljaju snažan faktor rizika za nepovoljnu prognozu [1,8]. Prema preporukama Evropske mreže za leukemiju (engl. *European LeukemiaNet – ELN*), stratifikacija rizika AML-a, koja se zasniva na citogenetskim i molekularnim aberacijama, obuhvata tri grupe rizika: povoljna, intermedijarna i nepovoljna [7]. Pored toga, procena merljive rezidualne bolesti (MRB) protočnom citometrijom i PCR (engl. *polymerase chain reaction*) metodom [7] pokazala je povezanost sa pojavom relapsa bolesti i kraćim ukupnim preživljavanjem (engl. *overall survival – OS*) [9].

Na izbor terapijskog pristupa, pored ECOG PS i komorbiditeta utiče i prognostički molekularni profil AML-a [7]. Standard intenzivnog lečenja za bolesnike sa AML-om se sastoji od inicijalne indukciono terapije „7+3” (7 dana citarabin i 3 dana antraciklin) i postremisione konsolidacione terapije (protokoli bazirani na citarabinu), što je praćeno transplantacijom matičnih ćelija hematopoeze (TMČH), ukoliko je indikovana [7,10,11]. U zavisnosti od godina starosti, stopa postizanja kompletne remisije (engl. *complete remission – CR*) varira. Za bolesnike starosti ≤ 60 godina prosek CR je 60-85%, uz petogodišnje OS od oko 30% [4,10], dok je za pacijente starosti > 60 godina, stopa postizanja CR 40-60% i OS je manje od 10% [10,12].

Današnji trendovi su usmereni u pravcu personalizovanog terapijskog pristupa prema specifičnom molekularnom profilu bolesti [13]. Genomska heterogenost AML-a je dovela do razvoja terapija koje direktno ciljaju mutacije, kao što su inhibitori FLT3 (engl. *FMS-related receptor tyrosine kinase 3*) i izocitrat dehidrogenaze 1/2 (IDH1/IDH2). Ove terapije su postale deo standardnog lečenja. Pored toga, uvedeni su i drugi inovativni lekovi poput gemtuzumab ozogamicina (GO), (anti CD33 monoklonsko antitelo), i CPX-351 (liposomalna formulacija daunorubicina i citarabina) [7]. Uvođenjem terapija nižeg intenziteta, povećane su terapijske mogućnosti za sve bolesnike, posebno za starije pacijente ili za one koji ne ispunjavaju kriterijume za intenzivno lečenje. Tako je primena protokola sa BCL-2 (engl. *B cell lymphoma 2*) inhibitorom u kombinaciji sa niskim dozama citarabina ili sa hipometilirajućim agensima, pokazala unapređenje u lečenju ove grupe bolesnika [14,15].

Uprkos nedavnom obećavajućem napretku i uvođenju novih terapijskih pristupa, ishod bolesnika sa AML-om i dalje nije u potpunosti zadovoljavajući [16]. U tom smislu, u toku su brojne studije inovativnih lekova, čiji preliminarni rezultati deluju ohrabrujuće, a čiji je cilj povećanje stope izlečenja AML-a.

Cilj ovog istraživanja je da se odredi kliničko-biološki profil bolesnika sa AML-om i njegov prognostički

ikal karyotype, molecular markers, previous cytotoxic therapy) [2]. Genetic abnormalities are a strong risk factor for unfavorable prognosis [1,8]. According to the recommendations of the European Leukemia Network (ELN), the risk stratification of AML, which is based on cytogenetic and molecular aberrations, includes three risk groups: favorable, intermediate, and unfavorable [7]. In addition, assessment of measurable residual disease (MRD) by flow cytometry and the polymerase chain reaction (PCR) method [7] showed an association with the occurrence of disease relapse and shorter overall survival (OS) [9].

In addition to ECOG PS and comorbidities, the choice of therapeutic approach is influenced by the prognostic molecular profile of AML [7]. The standard of intensive treatment for patients with AML consists of initial “7+3” induction therapy (7 days of cytarabine and 3 days of anthracycline) and post-remission consolidation therapy (cytarabine-based protocols), which is followed by hematopoietic stem cell transplantation (HSCT), if indicated [7,10,11]. Depending on age, the rate of achieving complete remission (CR) varies. For patients aged ≤ 60 years, the average CR is 60-85%, with a five-year OS of about 30% [4,10], while for patients aged > 60 years, the CR rate is 40-60% and the OS is less than 10% [10,12].

Current trends are directed towards a personalized therapeutic approach based on the specific molecular profile of the disease [13]. The genomic heterogeneity of AML has led to the development of therapies that directly target mutations, such as FMS-related receptor tyrosine kinase 3 (FLT3) and isocitrate dehydrogenase 1/2 (IDH1/IDH2) inhibitors. These treatments have become part of standard treatment. In addition, other innovative drugs such as gemtuzumab ozogamicin (GO), (anti CD33 monoclonal antibody), and CPX-351 (a liposomal formulation of daunorubicin and cytarabine) have been introduced [7]. With the introduction of lower-intensity therapies, the therapeutic possibilities for all patients have increased, especially for older patients or those who are not eligible for intensive treatment. Thus, the application of a protocol with the B cell lymphoma 2 (BCL-2) inhibitor in combination with low doses of cytarabine or with hypomethylating agents has shown an improvement in the treatment of this group of patients [14,15].

Despite recent promising breakthroughs and the introduction of new therapeutic approaches, the outcome of patients with AML is still not completely satisfactory [16]. Consequently, numerous studies of innovative drugs are underway aimed at increasing the cure rate of AML. Their preliminary results seem encouraging.

uticaj na stopu OS i na preživljavanje bez znakova bolesti (engl. *disease-free survival – DFS*).

MATERIJALI I METODE

Bolesnici

Retrospektivna analiza obuhvatila je 271 bolesnika sa AML-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju Univerzitetskog Kliničkog centra Srbije, u periodu od januara 2018. do januara 2023. godine. Bolesnici sa akutnom promijelocitnom leukemijom nisu uključeni u ovo istraživanje. Dijagnoza AML-a postavljena je na osnovu integrisanog dijagnostičkog pristupa, a u skladu sa preporukama SZO iz 2016. godine [5]. Stratifikacija rizika bolesnika sa AML-om (povoljni, intermedijarni, nepovoljni) sprovedena je u skladu sa ELN preporukama [11]. U citogenetskoj analizi uzoraka koštane srži je korišćena standardna tehnika G traka [17]. Imunofenotipizacija je sprovedena metodom protodne citometrije na uzorcima koštane srži [18]. Prisustvo FLT3-ITD (engl. internal tandem duplication) i TKD (engl. tyrosine kinase domain) mutacija, kao i NPM1 (engl. nucleophosmin) mutacija je analizirano PCR metodama [19,20]. Organomegalija, limfadenopatija, hipertrofija desni i CNS infiltracija su definisane kao ekstramedularna bolest (EMB). Ispitivanje prisustva leukemijskih ćelija u likvoru sprovedeno je metodom protodne citometrije kod simptomatskih i asimptomatskih bolesnika sa karakteristikama koje su povezane sa CNS infiltracijom (laktat dehidrogenaza (LDH) ≥ 450 , Le $\geq 30 \times 10^9/l$, mijelomonocitni i/ili monocitni fenotip, ekspresija CD56, FLT3-ITD/TKD mutacije i CBF (engl. core binding factor)) u AML-u [21]. Prilikom postavljanja dijagnoze evidentirano je opšte funkcionalno stanje na osnovu ECOG PS skale [22]. Takođe je određivan i značaj postojećih komorbiditeta, na osnovu HCT-CI (engl. hematopoietic cell transplantation-specific comorbidity index) skora [23]. Kod svih bolesnika analizirani su sledeći laboratorijski parametri: leukociti – Le ($\times 10^9/l$), trombociti – Tr ($\times 10^9/l$), hemoglobin – Hb (g/l), LDH (U/l), procenat blasta u perifernoj krvi i u koštanoj srži.

Terapija

Većina bolesnika (206/271) je lečena intenzivnom terapijom (daunorubicin i citarabin po šemi „3+7”), praćeno konsolidacijom uz primenu citarabina i TMČH. Bolesnici koji nisu ispunjavali kriterijume za intenzivno lečenje (65/271) primali su neintenzivnu hemioterapiju (daunorubicin i citarabin po šemi „2+5” i „1+5”, nisko-dozni citarabin) ili suportivnu terapiju (6-merkaptopurin, hidroksiureja), u zavisnosti od opšteg funkcionalnog stanja i komorbiditetnog indeksa. Bolesnici koji su relapsirali i oni koji su bili primarno refraktarni, lečeni

This study aims to determine the clinical and biological profile of patients with AML and its prognostic impact on OS rate and disease-free survival (DFS).

MATERIALS AND METHODS

Patients

The retrospective analysis included 271 patients with AML, diagnosed and treated at the Clinic for Hematology of the University Clinical Center of Serbia (UCCS), between January 2018 and January 2023. Patients with acute promyelocytic leukemia were not included in this study. The diagnosis of AML was established based on an integrated diagnostic approach, as per the 2016 WHO recommendations [5]. Risk stratification of patients with AML (favorable, intermediate, adverse) was carried out in keeping with ELN recommendations [11]. The standard G-banding technique was used in the cytogenetic analysis of bone marrow samples [17]. Immunophenotyping was performed by flow cytometry on bone marrow samples [18]. The presence of internal tandem duplication (FLT3-ITD) and tyrosine kinase domain (TKD) mutations, as well as of nucleophosmin (NPM1) mutations was analyzed by PCR methods [19,20]. Organomegaly, lymphadenopathy, gingival hypertrophy, and CNS infiltration were defined as extramedullary disease (EMD). Examination of the presence of leukemic cells in the cerebrospinal fluid was carried out by flow cytometry in symptomatic and asymptomatic patients with characteristics associated with CNS infiltration (lactate dehydrogenase (LDH) ≥ 450 , WBC $\geq 30 \times 10^9/l$, myelomonocytic and/or monocytic phenotype, CD56 expression, FLT3-ITD/TKD mutations and core binding factor (CBF)) in AML [21]. The general functioning status was recorded based on the ECOG PS scale [22]. The significance of existing comorbidities was also determined based on the HCT-CI (hematopoietic cell transplantation-specific comorbidity index) score [23]. The following laboratory test were performed in all patients: white blood cell count - WBC ($\times 10^9/l$), platelet count - PLT ($\times 10^9/l$), hemoglobin – Hb (g/l), LDH (U/l), percentage of blasts in peripheral blood and bone marrow.

Treatment

Most of the patients (206/271) were treated with intensive therapy (daunorubicin and cytarabine 3+7 regimen), followed by consolidation with cytarabine and HSCT. Patients who were not eligible for intensive treatment (65/271) received non-intensive chemotherapy (daunorubicin and cytarabine regimens 2+5 and 1+5, low-dose cytarabine) or supportive therapy (6-mercaptopurine, hydroxyurea), depending on their general functioning status and comorbidity index.

su protokolima spašavanja (engl. *salvage*), (fludarabin, citarabin, idarubicin, po protokolu *FLAG-Ida* ili mitoksantron, etopozid, citarabin, po protoklu *MEC*).

Procena terapijskog odgovora

Procena odgovora na terapiju definisana je prema preporukama ELN [11]: CR < 5% blasta u koštanoj srži, odsustvo cirkulišućih blasta, odsustvo EMB, apsolutni broj neutrofila $\geq 1,0 \times 10^9/l$ i Tr $\geq 100 \times 10^9/l$. Relaps je definisan kao prisustvo $\geq 5\%$ blasta u koštanoj srži ili pojava blasta u perifernoj krvi, kao i pojava EMB. Rana smrt je definisana kao smrt u periodu od 28 dana od otpočinjanja indukciono hemioterapije. Refraktorna bolest (RB), OS i DFS definisani su prema predloženim preporukama [11].

Statistička analiza

Za opisivanje podataka korišćene su deskriptivne analize (aritmetička sredina, medijana, standardna devijacija, učestalost). Dužina preživljavanja je analizirana pomoću Kaplan-Majerove metode, dok je log-rank test korišćen za poređenje preživljavanja među ispitivanim grupama. Univarijantni Koksov proporcionalni regresioni model je korišćen za identifikaciju faktora rizika. Statistička značajnost je definisana na $p < 0,05$. Statistički testovi su sprovedeni u softveru za statističku analizu IBM® SPSS® Statistics V25, IBM, USA.

REZULTATI

Kliničke karakteristike

Od ukupno 271 bolesnika sa AML-om, 206 (76%) pacijenata je lečeno primenom intenzivne hemioterapije, dok je 65 (24%) lečeno neintenzivnom terapijom. Kliničke karakteristike bolesnika lečenih intenzivnom terapijom su prikazane u Tabeli 1. Studija je obuhvatila 108 muškaraca i 98 žena (M : Ž 52,4% : 47,6%), prosečne starosti $50,3 \pm 13,9$ godina.

Distribucija bolesnika prema podtipu AML-a, a u skladu sa klasifikacijom SZO iz 2016. godine, bila je: AML sa RCA je imao 51 (24,76%) pacijent, od toga je bilo 32 (15,5%) bolesnika sa NPM1, 12 (5,6%) pacijenata sa inv(16) i 7 (3,4%) bolesnika sa t(8;21); MRC AML je imalo 27 (13,1%) pacijenata; dok je t-AML bila prisutna kod 5 (2,4%) bolesnika. Najviše je bilo bolesnika sa NOS AML, 123 (59,7%) pacijenta; bilo je 7 (7%) slučajeva AML-a sa minimalnom diferencijacijom, 21 (20%) slučaj AML-a bez sazrevanja, 26 (25%) slučajeva AML-a sa sazrevanjem, 33 (32%) slučajeva akutne mijelomonocitne leukemije, 15 (15%) slučajeva akutne monoblastne i monocitne leukemije. FLT3-ITD pozitivnost je uočena u 38 (18,4%) slučajeva, dok je FLT3-TKD mutacija registrovana u 5 (2,4%) slučajeva. U našoj grupi bolesnika, naj-

Patients who relapsed and those who were primarily refractory were treated with salvage protocols (fludarabine, cytarabine, idarubicin, according to the FLAG-Ida protocol, or mitoxantrone, etoposide, cytarabine, according to the MEC protocol).

Assessment of therapeutic response

The assessment of therapeutic response was defined according to ELN guidelines [11]: CR < 5% blasts in the bone marrow, absence of circulating blasts, absence of EMD, absolute neutrophil count $\geq 1.0 \times 10^9/l$, and PLT $\geq 100 \times 10^9/l$. Relapse was defined as the presence of $\geq 5\%$ blasts in the bone marrow or the appearance of blasts in peripheral blood, as well as the presence of EMD. Early death was defined as death within 28 days of starting induction chemotherapy. Refractory disease (RD), OS, and DFS were defined according to the proposed guidelines [11].

Statistical analysis

Descriptive analysis methods (arithmetic mean, median, standard deviation, frequency). were used to describe the data. The length of survival was analyzed using the Kaplan-Meier method, while the log-rank test was used to compare survival among the studied groups. The univariate Cox proportional regression model was used to identify risk factors. Statistical significance was defined at $p < 0.05$. Statistical tests were performed with the statistical analysis software IBM® SPSS® Statistics V25, IBM, USA.

RESULTS

Clinical characteristics

Out of a total of 271 patients with AML, 206 (76%) were treated with intensive chemotherapy, while 65 (24%) were treated with non-intensive therapy. The clinical characteristics of patients treated with intensive therapy are shown in Table 1. The study included 108 men and 98 women (M : F 52.4% : 47.6%) The average age of the patients was 50.3 ± 13.9 years.

The distribution of patients according to AML subtype, and per the WHO classification from 2016, was as follows: 51 (24.76%) patients had AML with RCA, of whom there were 32 (15.5%) patients with NPM1, 12 (5.6%) patients with inv(16) and 7 (3.4%) patients with t(8;21); 27 (13.1%) patients had MRC AML; while t-AML was present in 5 (2.4%) patients. The majority were patients with AML NOS, 123 (59.7%) patients; there were 7 (7%) cases of minimally differentiated AML, 21 (20%) cases of non-maturing AML, 26 (25%) cases of maturing AML, 33 (32%) cases of acute myelomonocytic leukemia, 15 (15%) of cases of acute monoblastic and monocytic leukemia. FLT3-ITD positivity was observed

veću zastupljenost pokazali su sledeći imunofenotipski antigeni na leukemijskim ćelijama: CD13 (90,7%), CD33 (93,9%), CD34 (74,4%) i CD117 (86%). Ukupno 26,1% bolesnika je imalo CD56 pozitivnu AML.

in 38 (18.4%) cases, while FLT3-TKD mutation was registered in 5 (2.4%) cases. In our group of patients, the following immunophenotypic antigens on leukemic cells showed the highest prevalence: CD13 (90.7%), CD33 (93.9%), CD34 (74.4%), and CD117 (86%). A total of 26.1% of patients had CD56-positive AML.

Tabela 1. Kliničko-biološke karakteristike bolesnika lečenih intenzivnom hemioterapijom

Table 1. Clinical and biological characteristics of patients treated with intensive chemotherapy

Karakteristike / Characteristics	Value
Starost (godine), AS±SD / Age (years); mean ± SD	50.3 ± 13.9
Godine < 60 g naspram > 60 g; n (%) / Age < 60 y vs. > 60 y; n (%)	142 (68.9%) vs. 64 (31.1%)
EMB - limfadenopatija; n (%) / EMD – lymphadenopathy; n (%)	44 (21.6%)
EMB - hepatosplenomegalija; n (%) / EMD – hepatosplenomegaly; n (%)	61 (29.8%)
EMB - hipertrofija desni; n (%) / EMD – gingival hypertrophy; n (%)	21 (10.3%)
CNS infiltracija; n (%) / CNS infiltration n (%)	46 (22.3%)
ECOG ≥ 2 / ECOG ≥ 2	
Da; n (%) / Yes; n (%)	43 (20.9%)
Ne; n (%) / No; n (%)	163 (79.1%)
HCT-CI ≥ 3 / HCT-CI ≥ 3	
Da; n (%) / Yes; n (%)	11 (5.3%)
Ne; n (%) / No; n (%)	192 (93.2%)
BMI kg/m ² ; AS ± SD / BMI kg/m ² ; mean ± SD	25.9 ± 5.5
Le; medijana (x10 ⁹ /l), raspon / WBC; median (x10 ⁹ /l), range	6.9 (0.1-278.5)
Le > 30 x10 ⁹ /l; n (%) / WBC > 30 x10 ⁹ /l; n (%)	56 (27.2%)
Hb; AS ± SD (g/l) / Hb; mean ± SD (g/l)	95.9 ± 19.5
Tr; medijana (x10 ⁹ /l), opseg / PLT; median (x10 ⁹ /l), range	51.5 (2-396)
LDH; medijana (U/l), opseg / LDH; median (U/l), range	396 (127-12,954)
LDH ≥ 450 U/l; n (%) / LDH ≥ 450 U/l; n (%)	88 (43.3%)
Blasti u PK; % (opseg) / Blasts in PB; % (range)	19 (0-99)
Blasti u KS; % (opseg) / Blasts in BM; % (range)	57.5 (20-96)
SZO AML; (N, %) / WHO AML; n (%)	
AML sa RCA / AML with RCA	51 (24.7%)
MRC AML / MRC AML	27 (13.1%)
t-AML / t-AML	5 (2.4%)
NOS AML / AML NOS	123 (59.7%)
AML/CD56; n (%) / AML/CD56; n (%)	46 (26.1%)
FLT3-ITD ^{mut} ; n (%) / FLT3-ITD ^{mut} ; n (%)	38 (18.4%)
FLT3-TKD ^{mut} ; n (%) / FLT3-TKD ^{mut} ; n (%)	5 (2.4%)
ELN grupa rizika; n (%) / ELN risk group; n (%)	
Povoljan / Favorable	27 (13.1%)
Intermedijarni / Intermediate	128 (62.1%)
Nepovoljan / Unfavorable	51 (24.8%)

Legenda: AML – akutna mijeloidna leukemija; SD – standardna devijacija; CNS – centralni nervni sistem; AS – aritmetička sredina; ECOG – engl. Eastern Cooperative Oncology Group; ELN – engl. European Leukemia Net; EMB – ekstramedularna bolest; Hb – hemoglobin; HCT-CI – engl. hematopoietic cell transplantation-specific comorbidity index; KS – koštana srž; LDH – laktat dehidrogenaza; Le – leukociti; PK – periferna krv; BMI – engl. body mass index; SZO – Svetska zdravstvena organizacija; AML sa RCA – AML sa rekurentnim citogenetskim abnormalnostima; MRC AML – engl. AML with myelodysplasia-related changes; t-AML – engl. therapy-related AML, NOS AML – engl. AML, not otherwise specified

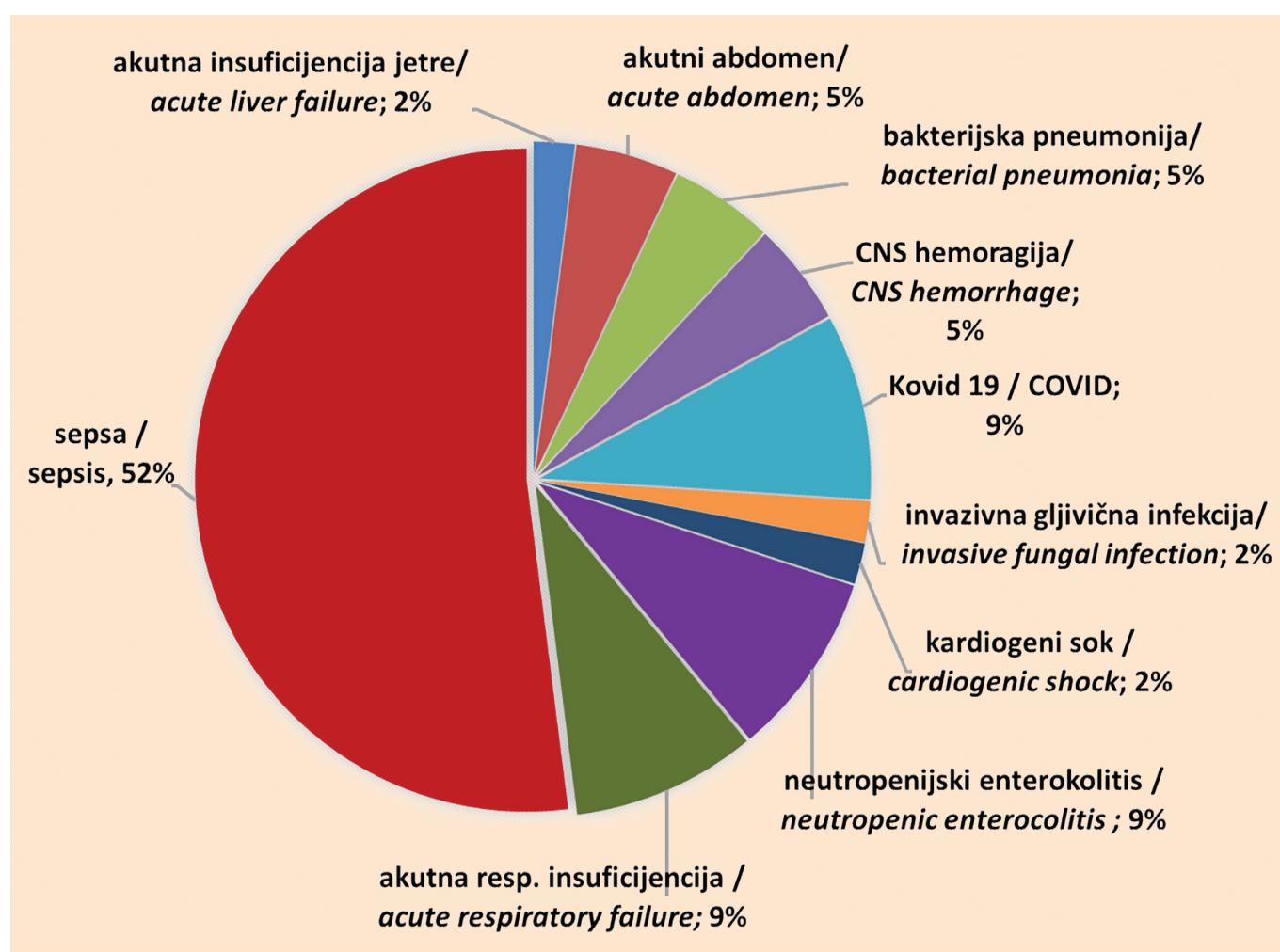
Legend: AML – acute myeloid leukemia; SD – standard deviation; CNS – central nervous system; ECOG – Eastern Cooperative Oncology Group; ELN – European Leukemia Network; EMD – extramedullary disease; Hb – hemoglobin; HCT-CI – hematopoietic cell transplantation-specific comorbidity index; BM – bone marrow; LDH – lactate dehydrogenase; WBC – white blood cell count; PB – peripheral blood; WHO – World Health Organization; BMI – body mass index; AML with RCA – AML with recurrent cytogenetic abnormalities; MRC AML – AML with myelodysplasia-related changes; AML NOS – AML not otherwise specified; t-AML – therapy-related AML

Ishod lečenja

Kompletna remisija je postignuta kod 92 od 158 (79,7%) slučajeva. Relaps bolesti je imalo 62 (67,4%) pacijenta, dok je primarno refraktornih bolesnika bilo 32 (20,3%). Rana smrt je zabeležena u 48 (23,3%) slučajeva. Glavni uzročnici rane smrti prikazani su u **Grafikonu 1**. Medijana OS je bila 12 meseci (10-13,9 meseci). Ukupno petogodišnje preživljavanje je iznosilo 19% (**Slika 1. A**). Medijana DFS je iznosila 13 meseci. Ukupno 51 od 157 (32,5%) bolesnika je lečeno transplantacijom matičnih ćelija hematopoeze. Bolesnici lečeni pomoću TMČH su imali statistički značajno duže OS (medijana 20 meseci naspram 7 meseci; $p < 0,001$) u poređenju sa bolesnicima koji nisu lečeni ovom terapijom (**Slika 1. B**).

Treatment outcome

Complete remission was achieved in 92 out of 158 (79.7%) cases. Sixty-two (67.4%) patients had disease relapse, while 32 (20.3%) were primarily refractory patients. Early death was recorded in 48 (23.3%) cases. The main causes of early death are presented in **Graph 1**. Median OS was 12 months (10-13.9 months). Overall five-year survival was 19% (**Figure 1. A**). The median DFS was 13 months. Fifty-one out of 157 (32.5%) patients were treated with hematopoietic stem cell transplantation. Patients treated with HSCT had statistically significantly longer OS (median 20 months vs. 7 months; $p < 0.001$), as compared to patients not treated with this therapy (**Figure 1. B**).



Grafikon 1. Uzroci rane smrti kod bolesnika sa AML-om lečenih intenzivnom terapijom

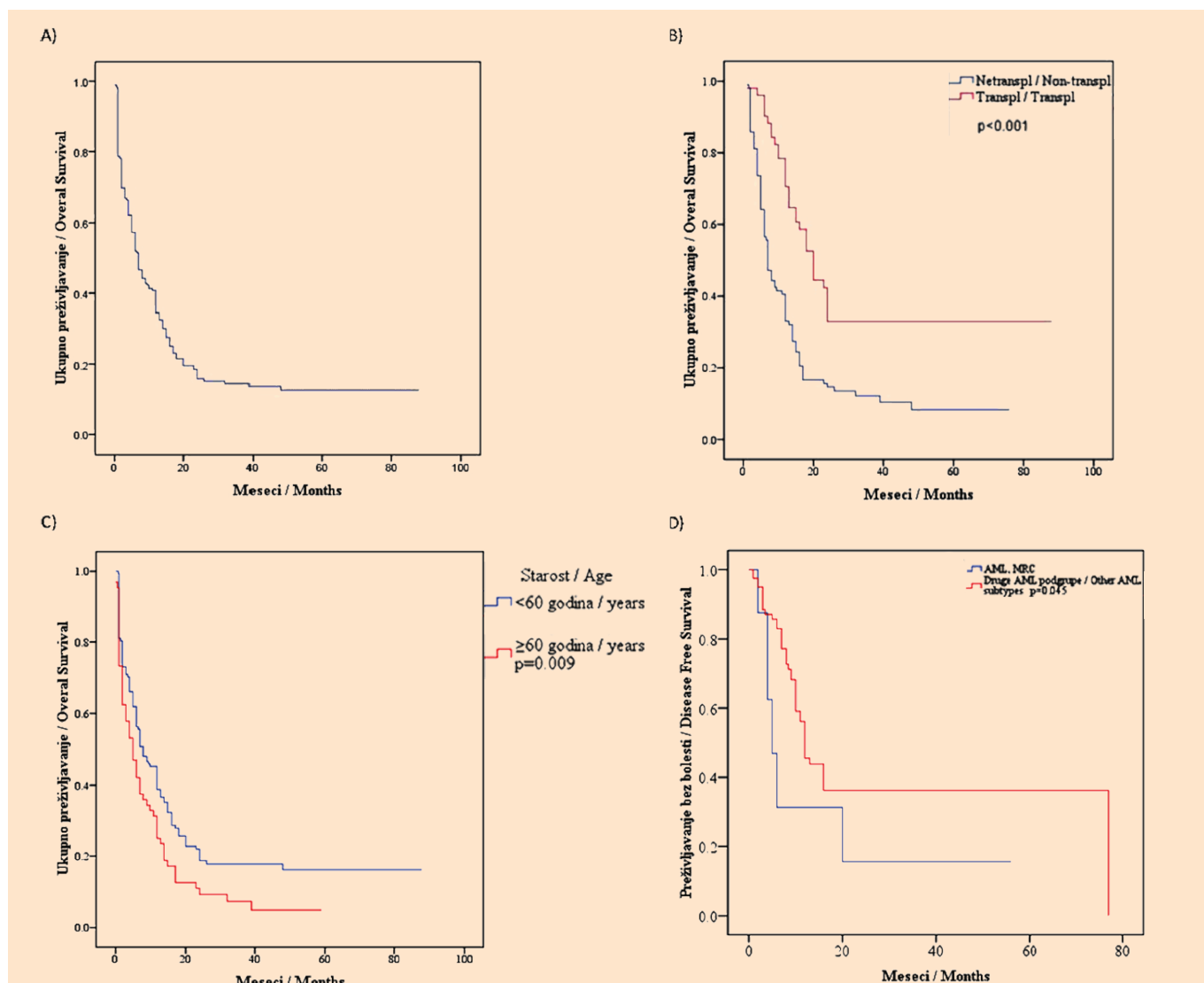
Graph 1. Causes of early death in patients with AML treated with intensive therapy

Prognostički faktori OS i DFS

Univarijantna analiza je pokazala da su sledeće ispitivane karakteristike bile značajni prediktori za kraće OS: godine ≥ 60 ($p = 0,009$), (**Slika 1. C**), $Le \geq 30 \times 10^9/l$

Prognostic factors of OS and DFS

Univariate analysis showed that the following analyzed characteristics were significant predictors of shorter OS: age ≥ 60 ($p = 0.009$), (**Figure 1. C**), $WBC \geq 30 \times 10^9/l$



Slika 1. Krive preživljavanja bolesnika sa AML-om

- A) Kriva petogodišnjeg preživljavanja bolesnika sa AML-om
- B) Kriva preživljavanja kod transplantiranih i netransplantiranih bolesnika
- C) Kriva preživljavanja kod bolesnika < 60 g naspram > 60 g
- D) Kriva preživljavanja kod bolesnika sa MRC AML-om naspram pacijenata sa drugim podtipovima AML-a

Figure 1. Survival curves of patients with AML

- A) Five-year survival curve of patients with AML
- B) Survival curve in transplanted and non-transplanted patients
- C) Survival curve in patients < 60 years vs. > 60 years
- D) Survival curve in patients with MRC AML vs. other subtypes of AML

($p = 0,031$). Ostali ispitivani prognostički faktori nisu pokazali statističku značajnost (Tabela 2). Univarijantna analiza je pokazala da je loše DFS bilo značajno povezano sa MRC AML podtipom ($p = 0,045$) (Slika 1. D).

DISKUSIJA

AML je heterogena bolest, kako po svojim citološkim, imunofenotipskim i citogenetsko-molekularnim karakteristikama, tako i po odgovoru na standardno lečenje i preživljavanju bolesnika [1,2]. Uprkos visokoj stopi CR od 60-85%, prosečna stopa petogodišnjeg OS je niska, iznosi oko 30%, i značajno se razlikuje između različitih starosnih grupa, dostižući 50% kod mlađih bolesnika,

($p = 0.031$). Other examined prognostic factors did not show statistical significance (Table 2). Univariate analysis showed that poor DFS was significantly associated with the MRC AML subtype ($p = 0.045$) (Figure 1 D).

DISCUSSION

AML is a heterogeneous disease, in terms of its cytological, immunophenotypical, cytogenetic, and molecular characteristics, but also with regards to patient response to standard treatment and patient survival [1,2]. Despite the high CR rate (60-85%), the average five-year OS rate is low, around 30%, and varies significantly among different age groups, reaching

Tabela 2. Univarijantna analiza faktora rizika za OS kod bolesnika sa AML-om lečenih intenzivnom terapijom

Karakteristike / Characteristics	Univarijantna analiza / Univariate analysis		
	p	HR	95% CI
Starost > 60 g naspram < 60 g / Age >60 y vs. < 60 y	0.009	1.015	0.064-16.165
Le ≥ 30 x10 ⁹ /l naspram Le < 30 x 10 ⁹ /l / WBC ≥ 30 x10 ⁹ /l vs. Le < 30x10 ⁹ /l	0.031	0.079	0.003-1.829
Blasti u PK ≥ 50% / Blasts in PB ≥ 50%	0.237	0.998	0.158-6.291
Blasti u KS ≥ 50% / Blasts in BM ≥ 50%	0.761	0.972	0.155-8.201
MRC AML naspram drugi AML podtipovi / MRC AML vs. other AML subtypes	0.138	0.494	0.145-1.680
Flt3-ITD ⁺ naspram Flt3-ITD ⁻ / Flt3-ITD ⁺ vs. Flt3-ITD ⁻	0.733	0.236	0.061-0.919
ECOG ≥ 2 naspram < 2 / ECOG ≥ 2 vs. < 2	0.714	0.323	0.059-1.765
CNS ⁺ naspram CNS ⁻ / CNS ⁺ vs. CNS ⁻	0.272	1.201	0.318-4.533
Nepovoljni naspram intermedijarni i povoljni rizik / Unfavorable vs. intermediate and favorable risk	0.527	14.259	1.916-106.114
LDH ≥ 450 / LDH ≥ 450	0.697	8.390	1.241-56.711
HCT-CI ≥ 3 / HCT-CI ≥ 3	0.897	1.912	0.632-5.785
AML CD56 / AML CD56	0.223	0.437	0.055-3.444

Legenda: AML – akutna mijeloidna leukemija; CNS – centralni nervni sistem; Flt3-ITD – engl. *fms-related receptor tyrosine kinase 3-internal tandem duplication*; ECOG – engl. *Eastern Cooperative Oncology Group*; Le – leukociti; PK – periferna krv; KS – koštana srž; MRC – engl. *myelodysplasia-related changes*; HCT-CI – engl. *hematopoietic cell transplantation-specific comorbidity index*; LDH – laktat dehidrogenaza

Table 2. Univariate analysis of risk factors for OS in AML patients treated with intensive therapy

Legend: AML – acute myeloid leukemia; CNS – central nervous system; Flt3-ITD – *fms-related receptor tyrosine kinase 3-internal tandem duplication*; ECOG – Eastern Cooperative Oncology Group; WBC – white blood cell count; PB – peripheral blood; BM – bone marrow; MRC myelodysplasia-related changes; HCT-CI – hematopoietic cell transplantation-specific comorbidity index; LDH – lactate dehydrogenase

Tabela 3. Univarijantna analiza faktora rizika za DFS kod bolesnika sa AML-om lečenih intenzivnom terapijom

Karakteristike / Characteristics	Univarijantna analiza / Univariate analysis		
	p	HR	95% CI
Starost > 60 g naspram < 60 g / Age >60 y vs. < 60 y	0.148	100.703	0.764-13,274.567
Le ≥ 30 x10 ⁹ /l naspram Le < 30 x10 ⁹ /l / WBC ≥ 30 x10 ⁹ /l vs. Le < 30x10 ⁹ /l	0.603	18.519	0.048-7,099.2367
Blasti u PK ≥ 50% / Blasts in PB ≥ 50%	0.638	0.145	0.007-3.093
Blasti u KS ≥ 50% / Blasts in BM ≥ 50%	0.496	60.494	0.395-9,263.056
MRC AML naspram drugi AML podtipovi / MRC AML vs. other AML subtypes	0.045	2.139	0.904-5.063
Flt3-ITD ⁺ naspram Flt3-ITD ⁻ / Flt3-ITD ⁺ vs. Flt3-ITD ⁻	0.188	1.075	0.075-15.332
ECOG ≥ 2 naspram < 2 / ECOG ≥ 2 vs. < 2	0.118	11.521	0.525-252.941
CNS ⁺ naspram CNS ⁻ / CNS ⁺ vs. CNS ⁻	0.471	1.128	0.181-7.024
Nepovoljni naspram intermedijarni i povoljni rizik / Unfavorable vs. intermediate and favorable risk	0.983	1.149	0.036-36.308
LDH ≥ 450 / LDH ≥ 450	0.873	6.929	0.231-207.864
HCT-CI ≥ 3 / HCT-CI ≥ 3	0.094	2.293	0.819-6.423
AML CD56 / AML CD56	0.911	12.887	0.26-581.361

Legenda: AML – akutna mijeloidna leukemija; CNS – centralni nervni sistem; Flt3-ITD – engl. *fms-related receptor tyrosine kinase 3-internal tandem duplication*; ECOG – engl. *Eastern Cooperative Oncology Group*; Le – leukociti; PK – periferna krv; KS – koštana srž; MRC – engl. *myelodysplasia-related changes*; HCT-CI – engl. *hematopoietic cell transplantation-specific comorbidity index*; LDH – laktat dehidrogenaza

Table 3. Univariate analysis of risk factors for DFS in AML patients treated with intensive therapy

Legend: AML – acute myeloid leukemia; CNS – central nervous system; Flt3-ITD – *fms-related receptor tyrosine kinase 3-internal tandem duplication*; ECOG – Eastern Cooperative Oncology Group; WBC – white blood cell count; PB – peripheral blood; BM – bone marrow; MRC myelodysplasia-related changes; HCT-CI – hematopoietic cell transplantation-specific comorbidity index; LDH – lactate dehydrogenase

dok je kod pacijenata starijih od 60 godina manja od 10% [4,10,24]. Brojni radovi su pokazali da prognostički faktori pri dijagnozi (citogenetsko-molekularni status, godine starosti, inicijalna leukocitoza, procenat blasta, podtip AML, ECOG PS, HCT-CI) imaju uticaj na ishod bolesti [1,8,25-30]. Takođe, u druge značajne prediktore ishoda ubrajaju se odgovor na primenjenu terapiju i rezistencija na lekove [8,31].

Naša grupa od 206 bolesnika koji su lečeni intenzivnom terapijom je bila mlađa, prosečne starosti 50,3 godina, u odnosu na literaturne podatke [4,26], te niskog ECOG PS i HCT-CI skora. Najviše je bilo bolesnika sa NOS AML-om 123 (59,7%), prema SZO klasifikaciji iz 2016. godine, a u okviru ove grupe dominirali su bolesnici sa granulocitnom diferencijacijom (80/123, odnosno 65%), a najčešći podtip je bila AML sa sazrevanjem, što odgovara literaturnim podacima [5]. U našoj grupi bolesnika, EMB pri dijagnozi je prezentovala u vidu: hepatosplenomegalije (29,8%), limfadenopatije (21,6%) i hipertrofije desni (10,3%), dok je infiltracija CNS potvrđena kod 46 (22,3%) bolesnika, što je u skladu sa nedavno objavljenim rezultatima [3].

Naši bolesnici su imali veliku stopu postizanja CR (79,7%), dok je primarnu refraktornu bolest imalo 32 (20,3%) bolesnika što odgovara literaturnim podacima [10,16]. Rana ili indukciona smrt se javila u 48 (23,3%) slučajeva. U kliničkim studijama, u poslednje dve decenije, uočava se smanjene smrtnosti povezane sa indukcionom terapijom sa 15-20% na manje od 5% [32]. Najčešći uzrok rane smrti bila je sepsa 52,6%. Infekcija je i inače opisana kao jedna od najčešćih uzroka rane smrti [33]. U našoj grupi, relaps se javio kod 62 (67,4%) bolesnika, što je više u odnosu na literaturne podatke [34,16]. Lečenje pomoću TMČH je jedina metoda kojom bolesnici mogu da se izleče od AML, što su pokazali i naši rezultati – postojala je statički značajna razlika u preživljavanju između transplantiranih i netransplantiranih bolesnika [2,7,10,11].

U skladu sa brojnim drugim studijama [25,26,31], univarijantna analiza je istakla starost bolesnika pri dijagnozi kao prediktor kliničkog ishoda. Naime, rezultati naše studije pokazuju da bolesnici stariji od 60 godina nose 50% veći rizik za kraće OS u odnosu na mlađe. Činjenica da je medijana oboljevanja od AML-a 69. godina života, ukazuje na to da najveći broj bolesnika sa AML-om spada u grupu pacijenata sa nepovoljnim rizikom, nezvano od kliničkih i genetičkih parametara, što je u skladu sa ranijim studijama [25,26]. Ovoj tvrdnji doprinosi i podatak da na svakih pet godina starosti odnos hazarda za nepovoljniji ishod lečenja raste za 22% [29]. Brojni su faktori koji bi mogli da objasne lošiju prognozu kod starijih bolesnika sa AML-om. Na prvom mestu jeste lošiji ECOG PS uslovljen brojnim komorbi-

50% in younger patients, while being less than 10% in patients older than 60 years [4,10,24]. Numerous studies have shown that prognostic factors at diagnosis (cytogenetic-molecular status, age, initial leukocytosis, percentage of blasts, AML subtype, ECOG PS, HCT-CI) have an impact on the outcome of the disease [1,8,25-30]. Also, other significant predictors of outcome include the response to applied therapy and drug resistance [8,31].

Our group comprising 206 patients treated with intensive therapy was younger, with an average age of 50.3 years, compared to literature data [4,26], with a low ECOG PS and HCT-CI score. The majority of patients had AML NOS, 123 (59.7%), according to the WHO classification from 2016, and in this group, there was a predominance of patients with granulocytic differentiation (80/123, i.e., 65%), and the most common subtype was AML with maturation, which corresponds to literature data [5]. In our group of patients, EMD at diagnosis presented as: hepatosplenomegaly (29.8%), lymphadenopathy (21.6%), and gingival hypertrophy (10.3%), while CNS infiltration was confirmed in 46 (22.3%) patients, which is in keeping with recently published results [3].

Our patients achieved a high rate of CR (79.7%), while 32 (20.3%) patients had primary refractory disease, which corresponds to literature data [10,16]. Early or induced death occurred in 48 (23.3%) cases. In clinical studies, carried out over the last two decades, mortality associated with induction therapy has been reduced from 15-20% to less than 5% [32]. The most common cause of early death was sepsis 52.6%. In fact, infection is described as one of the most common causes of early death [33]. In our group, relapse occurred in 62 (67.4%) patients, which is more as compared to literature data [34,16]. Treatment with HSCT is the only method by which patients can be cured of AML, as shown by our results – there was a statically significant difference in survival between transplanted and non-transplanted patients [2,7,10,11].

In keeping with numerous other studies [25,26,31], univariate analysis highlighted the patient's age at diagnosis as a predictor of clinical outcome. Namely, the results of our study show that patients older than 60 years have a 50% higher risk of shorter OS, compared to younger patients. The fact that the median age of AML diagnosis is 69 years indicates that the greatest number of patients with AML belong to the group of patients with an unfavorable risk, regardless of clinical and genetic parameters, which is in accordance with earlier studies [25,26]. This premise is supported by the fact that for every five years of age, the hazard ratio for a less favorable treatment outcome increases by

ditetima koji su povezani sa godinama starosti [25]. S druge strane, ranije studije su pokazale veću učestalost lošijih citogenetskih markera u starijoj populaciji u odnosu na mlađu. Naime, u studiji u kojoj su poređeni pacijenti mlađi od 56 godina u odnosu na one starije od 75 godina, nepovoljne citogenetske aberacije su bile prisutne kod 51% starijih, odnosno u 35% mlađih bolesnika sa AML-om [29]. U skladu sa time, povoljne citogenetske mutacije u grupi starijih pacijenata činile su samo 4%, dok je u grupi mlađih pacijenata 17% bilo nosilac povoljne citogenetske aberacije [29]. Veće prisustvo povoljnih aberacija kao što su *CBF AML* ili *NPM1* kod mlađih bolesnika donekle odslikava i drugačiju prirodu AML-a u različitim starosnim populacijama. Sa starošću se smanjuje procenat povoljnih citogenetskih aberacija, a raste učestalost nepovoljnih, na prvom mestu kompleksnog i monozomalnog kariotipa, koji nose nepovoljniji rizik za *CR* i *OS* [30]. Takođe, u starijoj populaciji se češće sreće tzv. *multidrug resistance* fenotip: 57% u odnosu na 33% [30].

U našoj grupi obolelih od AML-a, klasifikacija rizika po *ELN*-u nije pokazala svoj prediktivni uticaj. Razlog za to može biti veličina našeg uzorka ili mogući uzrok može biti veoma mali udeo pacijenata sa povoljnim citogenetskim aberacijama u našoj ispitivanoj grupi, odnosno 27 (13,1%). Novi lekovi poput *FLT3* inhibitora, *GO* i *CPV-351*, pokazali su svoj povoljan uticaj na ishod kod bolesnika sa AML-om [35-37]. Navedena ciljna terapija polako menja prognostičke kategorije bolesnika. Naime, uvođenje *FLT3* inhibitora u terapiju AML-a je dovelo do toga da se AML sa *FLT3* mutacijom, koja je do tada bila u najvećoj meri hemorezistentna [34,25] i spadala u grupu nepovoljnih molekularnih aberacija, svrsta u intermedijarni stepen rizika u najnovijoj *ELN* klasifikaciji [7]. Napominjemo da su u našoj studiji bolesnici lečeni standardnim hemioterapijskim protokolima, bez ciljne terapije.

Pored prethodnog prisustva MDS-a, kao i MDS-povezanih citogenetskih abnormalnosti [5], *MRC AML* je povezana sa kompleksnim i monozomalnim kariotipom, kao i genskim mutacijama povezanim sa MDS-om, kao što su: *TP53*, *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* [7]. U našoj grupi bolesnika sa *MRC AML*-om, u univarijantnoj analizi se pokazao nepovoljan uticaj na *DFS*. U studiji koja je poredila *MCR AML* bolesnike sa bolesnicima koji pripadaju podtipu *NOS AML*, prikazano je da su *MRC AML* bolesnici imali značajno nižu stopu *CR* [38]. Iako terapijske mogućnosti koje bi značajno produžile *OS* ovog podtipa AML-a, još uvek nisu istražene, terapija sa *CPX-351* pokazala je veću stopu petogodišnjeg *OS* u poređenju sa standardnom hemioterapijom [37].

22% [29]. Numerous factors could explain the poorer prognosis in elderly patients with AML, primarily poorer ECOG PS due to numerous comorbidities related to age [25]. On the other hand, previous studies have shown a higher frequency of unfavorable cytogenetic markers in the older population, as compared to the younger. Namely, in a study in which patients younger than 56 years were compared to those older than 75 years, unfavorable cytogenetic aberrations were present in 51% of older and 35% of younger patients with AML [29]. Accordingly, favorable cytogenetic mutations in the group of elderly patients accounted for only 4%, while in the group of younger patients, 17% were carriers of favorable cytogenetic aberrations [29]. The higher presence of favorable aberrations such as *CBF AML* or *NPM1* in younger patients somewhat reflects the different nature of AML in different age populations. With age, the percentage of favorable cytogenetic aberrations decreases, and the frequency of unfavorable ones increases, primarily complex and monosomal karyotypes, which carry a less favorable risk for *CR* and *OS* [30]. Also, in the older population, the so-called multidrug resistance phenotype is found more frequently, 57% versus 33% [30].

In our group of AML patients, *ELN* risk classification did not show its predictive impact. The reason for this may be the size of our sample or a possible cause may be the very small proportion of patients with favorable cytogenetic aberrations in our study group, i.e. 27 (13.1%). New drugs such as *FLT3* inhibitors, *GO* and *CPV-351*, have shown their beneficial effect on the outcome of patients with AML [35-37]. The aforementioned target therapy has been slowly changing the prognostic categories of patients. Namely, the introduction of *FLT3* inhibitors in the treatment of AML led to the fact that AML with *FLT3* mutation, which was previously mostly chemoresistant [34,25] and belonged to the group of unfavorable molecular aberrations, was classified as intermediate risk in the latest *ELN* classification [7]. It should be noted that, in our study, patients were treated with standard chemotherapy protocols, without targeted therapy.

In addition to the prior presence of MDS as well as MDS-associated cytogenetic abnormalities [5], *MRC AML* is associated with a complex and monosomal karyotype, as well as MDS-associated gene mutations such as the following: *TP53*, *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* [7]. In our group of patients with *MRC AML*, univariate analysis showed an unfavorable influence on *DFS*. In a study comparing *MCR AML* patients with patients belonging to the *AML NOS* subtype, it was shown that *MRC AML* patients had a significantly lower *CR* rate [38]. Although

ZAKLJUČAK

U našoj studiji smo potvrdili da godine starosti predstavljaju nezavistan prognostički faktor ukupnog preživljavanja kod bolesnika sa AML-om. Takođe, rezultati našeg istraživanja ukazuju da MRC AML predstavlja nepovoljan prognostički parametar za DFS. Ukupno petogodišnje preživljavanje u analiziranoj grupi je bilo loše. S tim u vezi, uvođenjem novih terapijskih pristupa kao deo standardne terapije za lečenje AML-a, kao i uključivanje bolesnika u studije sa inovativnim lekovima, može doprineti poboljšanju ishoda bolesnika sa akutnom mijeloidnom leukemijom.

SPISAK SKRAĆENICA

AML – akutna mijelodna leukemija
OS – ukupno preživljavanje (engl. *overall survival*)
DFS – preživljavanje bez znakova bolesti (engl. *disease-free survival*)
ECOG PS – engl. *Eastern Cooperative Oncology Group performance status*
ELN – Evropska mreža za leukemiju (engl. *European LeukemiaNet*)
NOS – engl. *not otherwise specified*
MRC – engl. *myelodysplasia-related changes*
CNS – centralni nervni sistem
SZO – Svetska zdravstvena organizacija
RCA – rekurentne citogenetske abnormalnosti
t-AML – engl. *therapy-related AML*
ICC – engl. *International Consensus Classification*
MDS – mijelodisplačni sindrom
MRB – merljiva rezidualna bolest
PCR – engl. *polymerase chain reaction*
TMČH – transplantacija matičnih ćelija hematopoeze
CR – kompletna remisija (engl. *complete remission*)
FLT3 – engl. *fms-related receptor tyrosine kinase 3*
FLT3-ITD – engl. *mutation an internal tandem duplication*
FLT3-TKD – engl. *tyrosine kinase domain*
NPM1 – engl. *nucleophosmin*
IDH1/IDH2 – izocitrat dehidrogenaze 1/2
GO – gemtuzumab ozogamicin
CPX-351 – liposomalna formulacija daunorubicina i citarabina
BCL-2 – engl. *B cell lymphoma 2*
EMB – ekstramedularna bolest
LDH – laktat dehidrogenaza
CBF – engl. *core binding factor*
HCT-CI – engl. *hematopoietic cell transplantation-specific comorbidity index*
RB – refraktorna bolest

Sukob interesa: Nije prijavljen.

therapeutic options that would significantly prolong OS in this AML subtype have not yet been investigated, therapy with CPX-351 has shown a higher five-year OS rate, compared to standard chemotherapy [37].

CONCLUSION

In our study, we confirmed that age is an independent prognostic factor of overall survival in patients with AML. Also, the results of our study indicate that MRC AML represents an unfavorable prognostic parameter for DFS. Overall five-year survival in the analyzed group was poor. In this regard, the introduction of new therapeutic approaches as part of standard therapy for the treatment of AML, as well as the inclusion of patients in studies with innovative drugs, can contribute to improving the outcome of patients with acute myeloid leukemia.

ABBREVIATIONS AND ACRONYMS

AML – acute myeloid leukemia
OS – overall survival
DFS – disease-free survival
ECOG PS – Eastern Cooperative Oncology Group performance status
ELN – European LeukemiaNet
NOS – not otherwise specified
MRC – myelodysplasia-related changes
CNS – central nervous system
WHO – World Health Organization
RCA – AML with recurrent cytogenetic abnormalities
t-AML – therapy-related AML
ICC – International Consensus Classification
MDS – myelodysplastic syndrome
MRD – measurable residual disease
PCR – polymerase chain reaction
HSCT – hematopoietic stem cell transplantation
CR – complete remission
FLT3 – fms-related receptor tyrosine kinase 3
FLT3-ITD – mutation an internal tandem duplication
FLT3-TKD – tyrosine kinase domain
NPM1 – nucleophosmin
IDH1/IDH2 – isocitrate dehydrogenase 1/2
GO – gemtuzumab ozogamicin
CPX-351 – liposomal daunorubicin and cytarabine
BCL-2 – B cell lymphoma 2
EMD – extramedullary disease
LDH – lactate dehydrogenase
CBF – core binding factor
HCT-CI – hematopoietic cell transplantation-specific comorbidity index
RD – refractory disease

Conflict of interest: None declared.

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UČESTALOST I ZNAČAJ RAZVOJA FEBRILNE NEUTROPENIJE KOD BOLESNIKA SA HEMATOLOŠKIM MALIGNITETIMA LEČENIH U USLOVIMA DNEVNE BOLNICE TERCIJARNE USTANOVE

ORIGINALNI RAD

ORIGINAL ARTICLE

FREQUENCY AND SIGNIFICANCE OF THE DEVELOPMENT OF FEBRILE NEUTROPENIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES TREATED IN THE DAY-CARE UNIT OF A TERTIARY INSTITUTION

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SAŽETAK

Uvod: Neutropenija uzrokovana primenom hemioterapije (HT) često se komplikuje pojavom febrilne neutropenije (FN), koja je povezana sa razvojem infekcija, redukcijom doze leka/odlaganjem hemioterapije, narušenim kvalitetom života i povećanim troškovima lečenja.

Cilj rada: Cilj rada je ispitivanje povezanosti faktora rizika za razvoj febrilne neutropenije i značaja primene granulocitnog faktora stimulacije rasta kolonija (G-CSF) kod bolesnika sa hematološkim malignitetima.

Materijali i metode: Obuhvaćeno je 90 bolesnika sa dijagnozom limfoma, multiplog mijeloma (MM) i mijelodisplaznog sindroma (MDS) lečenih u Dnevnoj bolnici Klinike za hematologiju Univerzitetskog kliničkog centra Srbije (UKCS), u periodu od januara do juna 2024.

Rezultati: Obuhvaćeno je 90 bolesnika sa sledećim dijagnozama: limfomi 70 (77,8%) pacijenata, MM 12 (13,3%) pacijenata, MDS 8 (8,9%) pacijenata, od čega 42,2% (38/90) muškog a 57,8% (52/90) ženskog pola. FN je zabeležena kod 22 (24,4%) bolesnika, dok 68 (75,6%) pacijenata nije razvilo febrilnu neutropeniju. Distribucija FN prema tipu limfoma je bila: 57,9% kod agresivnih, 31,6% kod indolentnih i 10,5% kod Hočkinovog limfoma. Uočena je povezanost febrilne neutropenije sa većom učestalošću viših kliničkih stadijuma bolesti, i za stadijum III i IV je iznosila 70%. Kod pacijenata sa FN-om je bilo značajno učestalije prisustvo velike (engl. *bulky*) tumorske mase (54,5% naspram 25%; $p = 0,010$). Pacijenti sa FN-om su imali statistički značajno veći broj primenjenih hemioterapijskih linija ($p = 0,020$), hemioterapijskih ciklusa ($p = 0,027$), ciklusa imunoterapije ($p = 0,025$), kao i veću učestalost infekcija (59,1% naspram 27,9%; $p = 0,008$), češću primenu antibiotika (95,5% naspram 33,8%; $p = 0,004$) i G-CSF-a (54,5% naspram 11,8%; $p < 0,001$), te veći broj primenjenih ampula G-CSF-a (5,0 naspram 1,0; $p < 0,001$). Dokazana je značajna razlika u višim vrednostima CRP-a kod bolesnika sa febrilnom neutropenijom (7,2% naspram 3,3%; $p = 0,012$).

Zaključak: Naša studija je pokazala da su na razvoj febrilne neutropenije uticali veći broj terapijskih linija i ciklusa imunohemioterapije, klinički stadijum bolesti, *bulky* tumorska masa i stepen agresivnosti limfoma.

Ključne reči: febrilna neutropenija, G-CSF, hematološki maligniteti, dnevna bolnica

ABSTRACT

Introduction: Chemotherapy-induced neutropenia is often complicated by the development of febrile neutropenia (FN) which is associated with infections, dose reductions/delay of chemotherapy, quality of life deterioration, and increased treatment costs.

Study aim: The study aims to research the association between risk factors for FN and the significance of applying G-CSF in patients with hematological malignancies.

Materials and methods: We evaluated 90 patients with lymphoma, multiple myeloma (MM), and myelodysplastic syndrome (MDS) treated at the Day-care Unit of the University Clinical Center of Serbia (UCCS) Clinic for Hematology, between January and June 2024.

Results: The study included 90 patients with the following diagnoses: 70 (77.8%) patients with lymphomas, 12 (13.3%) patients with MM, and 8 (8.9%) patients with MDS, of whom 42.2% (38/90) male and 57.8% (52/90) female. FN was observed in 22 (24.4%) patients, while 68 (75.6%) patients did not develop FN. The distribution of FN by lymphoma type was as follows: 57.9% in aggressive lymphomas, 31.6% in indolent lymphomas, and 10.5% in Hodgkin's lymphoma. FN was associated with a higher incidence of advanced clinical stages of disease, with 70% in stages III and IV. Patients with FN had significantly higher rates of bulky tumor mass (54.5% vs. 25%; $p = 0.010$), more lines of chemotherapy ($p = 0.020$), more cycles of chemotherapy ($p = 0.027$), more immunotherapy cycles ($p = 0.025$), as well as more infections (59.1% vs. 27.9%; $p = 0.008$), antibiotic use (95.5% vs. 33.8%; $p = 0.004$), and G-CSF administration (54.5% vs. 11.8%; $p < 0.001$), with more G-CSF ampoules used (5.0 vs. 1.0; $p < 0.001$). Higher CRP levels were also significantly associated with FN (7.2% vs. 3.3%; $p = 0.012$).

Conclusion: Our study has shown that the number of lines of therapy, the number of immunochemotherapy cycles, the clinical stage, the presence of bulky tumor mass, and the aggressiveness of the lymphoma affected FN development.

Keywords: febrile neutropenia, G-CSF, hematological malignancies, day-care unit

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UVOD

Neutropenija uzrokovana primenom hemioterapije (HT) predstavlja jedan od najčešćih neželjenih efekata lečenja i često se komplikuje febrilnom neutropenijom (FN). Neutropenija se javlja kod skoro petine hematoloških bolesnika, izuzev bolesnika kod kojih je sprovedena transplantacija matičnih ćelija hematopoeze gde se ova incidencija kreće u rasponu od 70% do 100%, za razliku od drugih onkoloških bolesnika kod kojih se kreće od 5% do 10%. Toksično dejstvo hemioterapije na hematopoetski sistem predstavlja ograničavajući faktor u adekvatnom doziranju leka. Komplikacije neutropenije uzrokovane mijelosupresivnim efektom hemioterapije igraju veliku ulogu u porastu morbiditeta i mortaliteta kod onkoloških bolesnika. Pojava febrilne neutropenije povezana je sa razvojem životno ugrožavajućih infekcija, odlaganjem hemioterapije, narušenim kvalitetom života i povećanim troškovima lečenja, što sve zajedno utiče na konačni ishod lečenja [1,2].

Upravo zbog toga je od velikog značaja identifikovanje faktora rizika koji dovode do febrilne neutropenije, u toku, kao i pre započinjanja specifičnog hematološkog lečenja. Najznačajni faktor jeste tip hemioterapije, mada do sada nije ustanovljen standardni bodovni (engl. *scoring*) sistem za poređenje agresivnosti različitih hemioterapeutika [3]. Drugi po redu najznačajniji faktor jeste osnovna bolest, iako se ovaj podatak u istraživanjima retko navodi zbog toga što neki od hematoloških maligniteta, za razliku od solidnih tumora, sami po sebi uključuju neutropeniju [4]. U literaturi se kao faktori rizika za pojavu febrilne neutropenije navode i površina tela, infiltracija koštane srži, godine starosti pri postavljanju dijagnoze, inicijalni ili broj monocita i limfocita nakon prvog ciklusa, kao i nivo hemoglobina [5–8].

U literaturi je opisana podela na faktore rizika koji su u vezi sa samim bolesnikom i faktore rizika koji su specifični za vrstu terapije. U prvu grupu spadaju prethodno podvrgavanje hemioterapijskom lečenju, inicijalna neutropenija, infiltracija koštane srži osnovnom bolešću, klinički stadijum bolesti, prisustvo komorbiditeta, lošiji ECOG performans status (engl. *Eastern Cooperative Oncology Group – ECOG performance status*), prethodno prisustvo infekcija, godine starosti, kao i tip maligniteta, uz napomenu da su bolesnici sa hematološkim malignitetima u povećanom riziku za razvoj neutropenije u odnosu na bolesnike sa solidnim tumorima, kako zbog same prirode osnovne bolesti tako i zbog intenziteta terapije. Faktori rizika specifični za vrstu terapije se mogu objasniti činjenicom da su neki lekovi mijelotoksičniji od drugih. Takođe, dokazano je da se neutropenije češće beleže u prvim ciklusima terapije.

INTRODUCTION

Chemotherapy-induced neutropenia is one of the most common side effects of treatment and is often complicated by febrile neutropenia (FN). Neutropenia occurs in almost a fifth of hematological patients, except for patients who undergo hematopoietic stem cell transplantation, among whom this incidence ranges from 70% to 100%, in contrast to other oncology patients, where it ranges from 5% to 10%. The toxic effect of chemotherapy on the hematopoietic system is a limiting factor in adequate drug dosing. Complications of neutropenia caused by the myelosuppressive effect of chemotherapy play a major role in the increase in morbidity and mortality in cancer patients. The occurrence of febrile neutropenia is associated with the development of life-threatening infections, chemotherapy treatment delay, impaired quality of life, and increased treatment costs, all of which affect the treatment outcome [1,2].

That is exactly why it is very important to identify the risk factors leading to febrile neutropenia, during and before starting specific hematological treatment. The most important factor is the type of chemotherapy, although so far, no standard scoring system has been established for comparing the aggressiveness of different chemotherapeutics [3]. The second most important factor is the underlying disease, although this information is rarely mentioned in research because some of the hematological malignancies, in contrast to solid tumors, inherently include neutropenia [4]. Body surface area, bone marrow infiltration, age at diagnosis, the initial or the number of monocytes and lymphocytes after the first cycle, as well as the hemoglobin level, are mentioned in literature as risk factors for the occurrence of febrile neutropenia [5–8].

In literature, risk factors are categorized as those associated with the patients themselves and those that are particular to the type of therapy. The first group of risk factors includes previous chemotherapy treatment, initial neutropenia, bone marrow infiltration due to the underlying disease, clinical stage of the disease, presence of comorbidities, a poorer ECOG (Eastern Cooperative Oncology Group) performance status, previous infections, age, as well as the type of malignancy, noting that patients with hematological malignancies are at increased risk of developing neutropenia, as compared to patients with solid tumors, both due to the very nature of the underlying disease and the intensity of the therapy. Risk factors particular to the type of therapy can be explained by the fact that some drugs are more myelotoxic than others. Also, it has been proven that neutropenia is more often found in the initial therapy cycles.

Stepen i razvoj neutropenije direktno utiču na rizik od nastajanja infekcije. Po težini, neutropenija se najčešće deli na četiri stepena. Stepen neutropenije gradusa 1 predstavlja ANC (engl. *absolute neutrophil count*) $\geq 1,5$ i $< 2,0 \times 10^9/l$, gradus 2 je kada je ANC $\geq 1,0$ i $< 1,5 \times 10^9/l$, gradus 3 je kada je ANC $\geq 0,5$ i $< 1,00 \times 10^9/l$, dok je gradus 4 vrednost ANC-a $< 0,5 \times 10^9/l$. FN predstavlja stanje u kojem su ANC $\leq 0,5 \times 10^9/l$ i telesna temperatura $\geq 38,3^\circ C$. Poznato je da u inflamatornom odgovoru upravo neutrofilni granulociti igraju bitnu ulogu celularnog imuniteta kao deo nespecifične zaštite organizma. Njihov broj je kompromitovan delom zbog citotoksičnog efekta terapije, a delom zbog same prirode osnovne bolesti. Upravo zbog toga, simptomi i znaci infekcije kod ovih bolesnika često budu prikriveni, a jedina manifestacija može biti povišena telesna temperatura. Preko 50% bolesnika sa febrilnom neutropenijom će ispoljiti septički sindrom, 20% – 30% ovih pacijenata će razviti težak oblik sepse, a 5% – 10% će imati septički šok. U tom slučaju, neophodna je hospitalizacija i primena intenzivne antibiotske terapije intravenskim antibioticima širokog spektra, s obzirom na stepen imunokompromitovanosti ovakvih bolesnika. Najčešće su infekcije digestivnog trakta, pluća i kože. Najčešći uzročnici su gram negativne bakterije *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, kao i aerobne, gram pozitivne koke *Staphylococcus*, *Streptococcus species* i *Enterococcus*, ali ne treba zanemariti ni često prisustvo gljivičnih infekcija [3]. Društvo za infektivne bolesti Amerike (engl. *Infectious Diseases Society of America – IDSA*) je publikovala smernice za lečenje febrilne neutropenije. Za većinu bolesnika intravenska primena antibiotika u bolničkim uslovima predstavlja standard lečenja. Antibiotsku terapiju treba birati vodeći računa o interakcijama antibiotika sa drugim lekovima i parametrima bubrežne i jetrene funkcije u zavisnosti od farmakokinetike leka [9].

Faktori stimulacije rasta kolonija (engl. *colony-stimulating factors – CSFs*) hematopoeze smanjuju trajanje i težinu stepena neutropenije i njenih komplikacija, omogućavajući brži oporavak parametara krvne slike i raniji nastavak primene započete hemioterapije [10].

CILJ RADA

Cilj rada je ispitivanje povezanosti faktora rizika za razvoj febrilne neutropenije i značaja primene granulocitnog faktora stimulacije rasta kolonija (engl. *granulocyte colony stimulating factor – G-CSF*) kod hematoloških bolesnika lečenih u Dnevnoj bolnici Klinike za hematologiju Univerzitetskog kliničkog centra Srbije.

The degree and development of neutropenia directly affect the risk of infection. Neutropenia is usually categorized into four grades, according to its severity. Grade 1 neutropenia is when the absolute neutrophil count (ANC) is ≥ 1.5 and $< 2.0 \times 10^9/l$, grade 2 is when ANC is ≥ 1.0 and $< 1.5 \times 10^9/l$, grade 3 is when ANC is ≥ 0.5 and $< 1.00 \times 10^9/l$, while grade 4 is when the ANC value is $< 0.5 \times 10^9/l$. Febrile neutropenia is a condition wherein ANC is $\leq 0.5 \times 10^9/l$ and the body temperature is $\geq 38.3^\circ C$. It is known that in inflammatory response, it is neutrophil granulocytes that play an important role in cellular immunity as part of non-specific defense of the body. Their number is compromised partly due to the cytotoxic effect of the therapy, and partly due to the very nature of the underlying disease. Precisely because of this, the symptoms and signs of infection in these patients are often hidden, and the only manifestation may be elevated body temperature. Over 50% of patients with febrile neutropenia will develop septic syndrome, 20% – 30% will develop a severe form of sepsis, and 5% – 10% will have septic shock. In that event, hospitalization and the application of intensive antibiotic therapy with broad-spectrum intravenous antibiotics are necessary, given the degree of immunocompromise of such patients. The most common are infections of the digestive tract, lungs, and skin. The most common causative agents are gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, as well as aerobic, gram-positive cocci *Staphylococcus*, *Streptococcus species*, and *Enterococcus*. However, the frequent presence of fungal infections should not be ignored either [3]. The Infectious Diseases Society of America (IDSA) has published guidelines for the treatment of febrile neutropenia. For most patients, intravenous administration of antibiotics in a hospital setting is the treatment standard. Antibiotic therapy should be chosen taking into account the interactions of antibiotics with other drugs, as well as the parameters of kidney and liver function, depending on the pharmacokinetics of the drug [9].

Colony-stimulating factors (CSFs) of hematopoiesis reduce the duration and severity of the degree of neutropenia and its complications, enabling faster recovery of blood parameters and earlier continuation of chemotherapy [10].

STUDY AIM

The study aims to examine the association between risk factors for the development of febrile neutropenia and the significance of applying granulocyte colony-stimulating factor (G-CSF) in hematological patients treated at the Day Hospital of the Hematology Clinic of the University Clinical Center of Serbia.

MATERIJALI I METODE

Istraživanje je urađeno kao retrospektivna studija. Demografske karakteristike bolesnika obuhvatile su godine starosti pri dijagnozi, pol, tip hematološkog maligniteta, klinički stadijum bolesti, ECOG performans status, infiltraciju koštane srži, prisustvo *bulky* tumorske mase, komorbiditete, prethodno prisustvo infekcije, kao i inicijalne osnovne laboratorijske parametre. Dijagnoze su postavljene prema aktuelnim vodičima za datu bolest. Uzorkom je obuhvaćeno 90 bolesnika sa dijagnozom limfoma, multiplog mijeloma (MM) i mijelodisplaznog sindroma (MDS), lečenih u Dnevnoj bolnici Klinike za hematologiju Univerzitetskog kliničkog centra Srbije, u periodu od januara do juna 2024.

Za deskripciju numeričkih podataka, u zavisnosti od normalnosti distribucije podataka, korišćeni su aritmetička sredina $\pm$ standardna devijacija ili medijana (opseg: min – max), dok su kategorijalni podaci prikazani kao učestalosti, odnosno broj ispitanika (procenat). Za testiranje značajnosti razlike između ispitivanih grupa pacijenata korišćen je t-test, Men-Vitnijev test i Hi-kvadratni test. Posmatrani klinički i laboratorijski parametri koji su se izdvojili kao statistički značajni na nivou $p < 0,01$ uključeni su u model višestruke regresione analize u cilju procene prediktora razvoja febrilne neutropenije. Kriterijum za statističku značajnost je bio $p < 0,05$ a softverski program SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) je korišćen za statističku obradu podataka. Rezultati su prezentovani tekstualno i tabelarno.

REZULTATI

Studijom je obuhvaćeno 90 bolesnika prosečne starosti $60,0 \pm 12,1$ godina. Medijana starosnog doba je iznosila 63 godine (opseg: 23 – 77). Distribucija prema dijagnozi je bila sledeća: 70 (77,8%) pacijenata sa dijagnozom limfoma, 12 (13,3%) bolesnika sa dijagnozom multiplog mijeloma i 8 (8,9%) pacijenata sa dijagnozom mijelodisplaznog sindroma. Bolesnici muškog pola su činili 42,2% (38/90) a ženskog 57,8% (52/90). Medijana praćenja iznosila je 13,5 meseci (0 – 120). Smrtni ishod je nastupio kod četiri bolesnika (4,4%).

FN je zabeležena kod 22 (24,4%) bolesnika, dok 68 (75,6%) pacijenata nije razvilo febrilnu neutropeniju. U grupi bolesnika koji su imali febrilnu neutropeniju, učestalost muškog pola je iznosila 59,1% (naspram 42,2% ženskog pola; $p = 0,065$). Učestalost dijagnoza se nije značajno razlikovala u odnosu na prisustvo febrilne neutropenije ($p = 0,525$), te je u grupi pacijenata sa febrilnom neutropenijom, 86,4% njih imalo dijagnostikovani limfom, 9,1% je imalo MM a 4,5% njih MDS. Distribucija febrilne neutropenije prema tipu limfoma je bila: 57,9% kod agresivnih, 31,6% kod indolentnih i 10,5% kod Hočkinovog limfoma.

MATERIALS AND METHODS

This is a retrospective study. Patient demographic characteristics included the following: age at diagnosis, gender, type of hematological malignancy, clinical stage of disease, the ECOG performance status, bone marrow infiltration, presence of bulky tumor mass, comorbidities, previous presence of infection, as well as initial basic laboratory parameters. Diagnoses were established according to current guidelines for the given disease. The sample included 90 patients diagnosed with lymphoma, multiple myeloma (MM), or myelodysplastic syndrome (MDS), treated at the Day-care Unit of the University Clinical Center of Serbia (UCCS) Clinic for Hematology, between January and June 2024.

For the description of numerical data, depending on the normality of the data distribution, the arithmetic mean $\pm$ standard deviation or the median (range: min-max) was used, while categorical data were presented as frequencies, the number of respondents (percentage). The t-test, the Mann-Whitney test, and the Chi-square test were used to test the significance of the difference among the examined groups of patients. Observed clinical and laboratory parameters that were statistically significant at the $p < 0.01$ level were included in the multiple regression analysis model to assess predictors of the development of febrile neutropenia. The criterion for statistical significance was $p < 0.05$ and the SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software was used for statistical data processing. The results are presented in textual and tabular form.

RESULTS

The study included 90 patients aged 60.0 ± 12.1 years. The median age was 63 years (range: 23 – 77). The distribution according to diagnosis was as follows: 70 (77.8%) patients with lymphoma, 12 (13.3%) patients with multiple myeloma, and 8 (8.9%) patients with myelodysplastic syndrome. Male patients accounted for 42.2% (38/90) while female patients accounted for 57.8% (52/90). Median follow-up was 13.5 months (0 – 120). Death occurred in four patients (4.4%).

FN was recorded in 22 (24.4%) patients, while 68 (75.6%) patients did not develop febrile neutropenia. In the group of patients with febrile neutropenia, the frequency of male patients was 59.1% (versus 42.2% female; $p = 0.065$). The frequency of diagnoses did not differ significantly in relation to the presence of febrile neutropenia ($p = 0.525$) – in the group of patients with febrile neutropenia, 86.4% were diagnosed with lymphoma, 9.1% had MM and 4.5% MDS. The distribution of febrile neutropenia according to the type of lymphoma was as follows: 57.9% in the aggressive type, 31.6% in the indolent type, and 10.5% in Hodgkin's lymphoma.

Od ukupnog broja bolesnika, bolest kategorizovanu kao klinički stadijum (KS) I imalo je 9 (10%) bolesnika, KS II je imalo 22 (24,4%) bolesnika, KS III je bio prisutan kod 28 (31,1%) pacijenata, dok je KS IV imalo 22 (24,4%) bolesnika, i nije potvrđena značajna razlika između pacijenata sa i bez febrilne neutropenije ($p = 0,191$). Uočena je povezanost febrilne neutropenije sa većom učestalošću viših kliničkih stadijuma bolesti, i za stadijume III i IV učestalost je iznosila 70,0%.

Kod pacijenata sa febrilnom neutropenijom je bilo značajno učestalije prisustvo *bulky* tumorske mase (> 7 cm) i ustanovljeno je kod 54,5% ovih bolesnika, u odnosu na pacijente bez febrilne neutropenije, kod kojih je potvrđeno u 25% slučajeva ($p = 0,010$). Infiltra-

Of the total number of patients, in 9 (10%) subjects the disease was categorized as clinical stage (CS) I, 22 (24.4%) patients had CS II, 28 (31.1%) patients had CS III, while 22 (24.4%) patients had CS IV, while no significant difference was found between patients with and without febrile neutropenia ($p = 0.191$). An association of febrile neutropenia with a higher frequency of more advanced clinical stages of the disease was observed, and for stages III and IV the frequency was 70.0%.

In patients with febrile neutropenia, the presence of a bulky tumor mass (> 7 cm) was significantly more frequent and was found in 54.5% of these patients, compared to patients without febrile neutropenia, in whom it was confirmed in 25% of cases ($p = 0.010$).

Tabela 1. Opšti podaci

Table 1. General data

Variable / Variables	n (%)
Starost / Age	
Medijana (opseg) / Median (range)	63 (23 – 77)
Pol / Gender	
M / M	38 (42.2%)
Ž / F	52 (57.8%)
Dijagnoza / Diagnosis	
Limfom / Lymphoma	70 (77.8%)/3 HL/67 NHL
Multipli mijelom / Multiple Myeloma	12 (13.3%)
Mijelodisplazni sindrom / Myelodysplastic syndrome	8 (8.9%)
Klinički stadijum / Clinical stage	
I	9 (10%)
II	22 (24.4%)
III	28 (31.1%)
IV	22 (24.4%)
Bulky TU masa > 7 cm / Bulky TU mass > 7 cm	29 (32.2%)
Infiltracija koštane srži / Bone marrow infiltration	36 (40%)
Laboratorijski parametri, medijana (opseg) / Laboratory parameters, median (range)	
Hb g/l / Hb g/l	118 (47 – 172)
MCV fl / MCV fl	92 (74 – 112)
Le $\times 10^9/l$ / WBC $\times 10^9/l$	5.5 (1.9 – 233)
Tr $\times 10^9/l$ / PLT $\times 10^9/l$	206.5 (10 – 520)
ANC $\times 10^9/l$ / ANC $\times 10^9/l$	3.2 (0.7 – 13.7)
AMC $\times 10^9/l$ / AMC $\times 10^9/l$	0.42 (0 – 12.2)
CRP mg/l / CRP mg/l	3.85 (0.1 – 116)
ECOG / ECOG	
0	60 (66.7%)
1	21 (23.3%)
2	7 (7.8%)
3	2 (2.2%)
Smrtni ishod / Fatal outcome	4 (4.4%)
Dužina praćenja u mesecima / Follow-up (months)	
Medijana (opseg) / Median (range)	13.5 (0 – 120)
FN / FN	
ima/nema / yes/no	22 (24.4%)/86 (75.6%)

Tabela 2. Sociodemografski i klinički parametri prema prisustvu febrilne neutropenije**Table 2.** Sociodemographic and clinical parameters according to the presence of febrile neutropenia

Ukupan broj (%) / Total number (%)	Febrilna neutropenija / Febrile neutropenia		p
	Ne / No 68 (75.6%)	Da / Yes 22 (24.4%)	
Pol / Gender			
Muški / Male	25 (36.8%)	13 (59.1%)	0.065
Ženski / Female	43 (63.2%)	9 (40.9%)	
Starost (godine), medijana (opseg) / Age (years), median (range)	63.0 (23 – 77)	63.0 (31 – 74)	0.408
Dijagnoza / Diagnosis			
Limfom / Lymphoma	51 (75.0%)	19 (86.4%)	0.525
MM / MM	10 (14.7%)	2 (9.1%)	
MDS / MDS	7 (10.3%)	1 (4.5%)	
Klinički stadijum / Clinical stage			
1	8 (13.1%)	1 (5.0%)	0.191
2	17 (27.9%)	5 (25.0%)	
3	23 (37.7%)	5 (25.0%)	
4	13 (21.3%)	9 (45.0%)	
Bulky TU masa > 7 cm / Bulky tumor mass > 7 cm	17 (25.0%)	12 (54.5%)	0.010
Infiltracija koštane srži / Bone marrow infiltration	26 (38.2%)	10 (45.5%)	0.548
ECOG / ECOG			
0	48 (70.6%)	12 (54.5%)	/
1	14 (20.6%)	7 (31.8%)	
2	4 (5.9%)	3 (13.6%)	
3	2 (2.9%)	0 (0.0%)	
Prethodne infekcije / Previous infections	6 (8.8%)	5 (22.7%)	0.084
Broj primenjenih hemioterapijskih linija / Number of administered lines of therapy	1 (1 – 4)	2 (1 – 6)	0.020
Broj hemioterapijskih ciklusa / Number of chemotherapy cycles	6 (1 – 25)	6 (4 – 30)	0.027
Broj ciklusa imunoterapije / Number of immunotherapy cycles	5 (0 – 18)	6 (0 – 25)	0.022
≥ 10 ciklusa hemioterapije / ≥ 10 chemotherapy cycles	12 (17.6%)	9 (40.9%)	0.025
≥ 3 hemioterapijske linije / ≥ 3 lines of chemotherapy	7 (10.3%)	8 (36.4%)	0.004
Infekcije tokom lečenja / Infections during treatment	19 (27.9%)	13 (59.1%)	0.008
Primena antibiotika / Application of antibiotics	23 (33.8%)	21 (95.5%)	0.004
Primena G-CSF-a / Application of G-CSF	8 (11.8%)	12 (54.5%)	< 0.001
Broj ampula G-CSF-a / Number of G-CSF ampoules	1 (0 – 20)	5 (1 – 28)	< 0.001
Komorbiditeti / Comorbidities	53 (77.9%)	19 (86.4%)	0.391
KOVID-19 infekcija / COVID-19 infection	7 (10.3%)	3 (13.6%)	0.665
Dužina praćenja (meseci) / Length of follow-up (months)	13.5 (0 – 120)	14 (6 – 113)	0.735
Smrtni ishod / Fatal outcome	2 (2.9%)	2 (9.1%)	0.224

Numerički podaci, u zavisnosti od normalnosti raspodele podataka, prikazani su kao aritmetička sredina ± standardna devijacija ili medijana (min-maks), a kategorijalni podaci su prikazani kao broj pacijenata i procenat, n (%); statistički značajna razlika je prikazana podebljanim slovima.

Legenda: MM – multipli mijelom; MDS – mijelodisplazni sindrom, G-CSF – engl. granulocyte colony-stimulating factor

Numerical data, depending on the normality of data distribution, are presented as the arithmetic mean ± standard deviation or as the median (min-max), and categorical data are presented as the number of patients and percentage, n (%); statistically significant difference is given in bold letters.

Legend: MM – multiple myeloma; MDS – myelodysplastic syndrome, G-CSF – granulocyte colony-stimulating factor

cija koštane srži nije pokazala značajnu razliku u odnosu na razvoj febrilne neutropenije ($p = 0,548$), kao ni u odnosu na ECOG performans status. U našem uzorku, distribucija bolesnika prema ECOG performans statusu je bila sledeća: ECOG 0 je imalo 60 (66,7%) bolesnika, ECOG 1 je ustanovljen kod 21 (23,3%) pacijenta, ECOG 2 je bio prisutan kod 7 (7,8%) pacijenata, dok je dva (2,2%) bolesnika imalo ECOG 3 (Tabela 1).

Pacijenti sa febrilnom neutropenijom su imali statistički značajno veći broj primenjenih hemioterapijskih linija ($p = 0,020$), hemioterapijskih ciklusa ($p = 0,027$) i ciklusa imunoterapije ($p = 0,025$). Pacijenti kod kojih se razvila FN su značajno učestalije imali primenjenih ≥ 3 hemioterapijskih linija (36,4% naspram 10,3%; $p = 0,004$) kao i primenjenih ≥ 10 ciklusa hemioterapije (40,9% naspram 17,6%; $p = 0,025$) ili imunoterapije (IT), (31,6% naspram 11,3%; $p = 0,042$). Takođe, kod pacijenata sa febrilnom neutropenijom je bila značajno veća učestalost infekcije tokom lečenja (59,1% naspram 27,9%; $p = 0,008$), kao i primena antibiotika (95,5% naspram 33,8%; $p = 0,004$), primena G-CSF-a (54,5% naspram 11,8%; $p < 0,001$), te broj primenjenih ampula G-CSF-a (5,0 naspram 1,0; $p < 0,001$). Posmatrani klinički parametri koji se nisu razlikovali između pacijenata sa i bez febrilne neutropenije su: komorbiditeti ($p = 0,391$), KOVID-19 infekcija ($p = 0,665$), dužina praćenja ($p = 0,735$) i smrtni ishod ($p = 0,224$), (Tabela 2).

Među pacijentima koji su razvili FN, stepen neutropenije gradusa 1 je uočen kod jednog (4,5%) bolesnika, gradus 2 je utvrđen kod četiri (18,2%) bolesnika, gradus 3 kod 10 (45,5%) pacijenata, a gradus 4 kod 7 (31,8%) bolesnika.

Bone marrow infiltration did not show a significant difference in relation to the development of febrile neutropenia ($p = 0.548$), nor in relation to the ECOG performance status. In our sample, the distribution of patients according to the ECOG performance status was as follows: ECOG 0 was present in 60 (66.7%) patients, ECOG 1 was established in 21 (23.3%) patients, ECOG 2 was present in 7 (7.8%) patients, while two (2.2%) patients had ECOG 3 (Table 1).

Patients with febrile neutropenia had a statistically significantly higher number of applied lines of chemotherapy ($p = 0.020$), chemotherapy cycles ($p = 0.027$), and immunotherapy cycles ($p = 0.025$). Patients who developed FN significantly more often had ≥ 3 lines of chemotherapy administered (36.4% vs. 10.3%; $p = 0.004$), as well as ≥ 10 chemotherapy cycles (40.9% vs. 17.6%; $p = 0.025$) or immunotherapy (IT) cycles administered, (31.6% vs. 11.3%; $p = 0.042$). Also, in patients with febrile neutropenia, there was a significantly higher frequency of infection during treatment (59.1% vs. 27.9%; $p = 0.008$), as well as a significantly higher frequency of antibiotics use (95.5% vs. 33.8%; $p = 0.004$), a significantly higher frequency of G-CSF administration (54.5% vs. 11.8%; $p < 0.001$), and a significantly higher number of administered ampoules of G-CSF (5.0 vs. 1.0; $p < 0.001$). The clinical parameters that were observed not to differ between patients with and without febrile neutropenia were as follows: comorbidities ($p = 0.391$), COVID-19 infection ($p = 0.665$), length of follow-up ($p = 0.735$), and the fatal outcome ($p = 0.224$), (Table 2).

Tabela 3. Laboratorijski parametri prema prisustvu febrilne neutropenije

Ukupan broj (%) / Total number (%)	Febrilna neutropenija / Febrile neutropenia		p
	Ne / No 68 (75.6%)	Da / Yes 22 (24.4%)	
Hb (g/l)	117.4 $\pm$ 24.3	112.9 $\pm$ 23.7	0.449
MCV (fl)	92.0 $\pm$ 7.6	89.8 $\pm$ 6.6	0.237
WBC (x10e9/l)	5.5 (2.2 – 233.0)	5.8 (1.9 – 116.0)	0.602
PLT (x10e9/l)	206.5 (10.0 – 520.0)	199.0 (51.0 – 503.0)	0.981
ANC (x10e9/l)	3.1 (0.7 – 12.4)	3.7 (0.8 – 13.7)	0.430
AMC (x10e9/l)	0.4 (0.0 – 12.2)	0.5 (0.0 – 1.1)	0.960
CRP (mg/l)	3.3 (0.1 – 116.0)	7.2 (0.1 – 116.0)	0.012

Numerički podaci, u zavisnosti od normalnosti raspodele podataka, prikazani su kao aritmetička sredina $\pm$ standardna devijacija ili medijana (min-maks) a kategorijalni podaci su predstavljeni kao broj pacijenata i procenat, n (%).

Hb – hemoglobin, MCV – engl. mean corpuscular volume, Le – leukociti, Tr – trombociti, ANC – engl. absolute neutrophil count, AMC – engl. absolute monocyte count, CRP – C reaktivni protein

Table 3. Laboratory parameters according to the presence of febrile neutropenia

Numerical data, depending on the normality of data distribution, are presented as the arithmetic mean $\pm$ standard deviation or as the median (min-max), and categorical data are presented as the number of patients and percentage, n (%).

Hb – hemoglobin, MCV – mean corpuscular volume, WBC – white blood cell count, PLT – platelet count, ANC – absolute neutrophil count, AMC – absolute monocyte count, CRP – C reactive protein

Tabela 4. Multivarijantna logistička regresija u predikciji razvoja febrilne neutropenije kod bolesnika sa hematološkim malignitetima

Nezavisne varijable / Independent variables	Multivarijantna logistička regresija / Multivariate logistic regression		
	B	p	OS (95% CI)
Bulky TU masa > 7 cm / Bulky tumor mass > 7 cm	0.588	0.419	1.8 (0.4 – 7.5)
≥ 3 hemioterapijskih linija / ≥ 3 lines of chemotherapy	0.917	0.310	2.5 (0.4 – 14.71)
Infekcije tokom lečenja / Infections during treatment	-3.547	0.055	0.03 (0.001 – 1.1)
Primena antibiotika / Application of antibiotics	4.551	< 0.001	94.7 (8.1 – 1109.7)
Primena G-CSF-a / Application of G-CSF	2.901	0.102	18.2 (0.6 – 591.1)
Broj ampula G-CSF-a / Number of G-CSF ampoules	0.154	0.028	1.2 (1.0 – 1.3)

B – koeficijent, OR – odnos šansi (engl. odds ratio), CI – interval poverenja (engl. confidence interval), podebljano – statistički značajni prediktori

Table 4. Multivariate logistic regression in predicting the development of febrile neutropenia in patients with hematological malignancies

B – coefficient, OR – odds ratio, CI – confidence interval, bold – statistically significant predictors

Između pacijenata sa i bez febrilne neutropenije upoređivani su i laboratorijski parametri i jedino su vrednosti C-reaktivnog proteina (CRP) pokazale značajnu razliku sa većim vrednostima kod bolesnika kod kojih se razvila FN (7,2% naspram 3,3%; $p = 0,012$). Ostali parametri: hemoglobin (Hb), ($p = 0,449$), MCV (engl. *mean corpuscular volume*), ($p = 0,237$), leukociti (Le), ($p = 0,602$), trombociti (Tr), ($p = 0,981$), ANC (engl. *absolute neutrophil count*), ($p = 0,430$) i AMC (engl. *absolute monocyte count*), ($p = 0,960$), nisu se značajno razlikovali u odnosu na razvoj febrilne neutropenije (Tabela 3).

Svi prediktori koji su univarijantnom analizom imali statističku značajnost na nivou od 0,01 uključeni su u model multivarijantne regresione analize sa zavisnom varijablom – razvoj febrilne neutropenije. Model sadrži šest prediktora i statistički je značajan ($p < 0,001$). Kao značajni prediktori razvoja febrilne neutropenije izdvojili su se primena antibiotika ($B = 4,551$; $p < 0,001$) i broj primenjenih ampula G-CSF-a ($B = 0,154$; $p = 0,028$), (Tabela 4).

DISKUSIJA

U našoj studiji je dokazana statistički značajna razlika u pojavi febrilne neutropenije kod bolesnika koji su prethodno primali hemioterapiju, što je u skladu sa dostupnim literaturnim podacima. Dodatak monoklonskih antitela hemioterapijskim protokolima povećava rizik od pojave febrilne neutropenije [11,12]. Dokazali smo da je pojava febrilne neutropenije bila češća i kod bolesnika kod kojih je primenjeno nekoliko terapijskih linija: ≥3 terapijske linije (36,4% naspram 10,3%; $p = 0,004$), ali i kod onih kod kojih je primenjen veći broj ciklusa istog citostatika: ≥ 10 ciklusa hemioterapije (40,9% naspram 17,6%; $p = 0,025$), uključujući i imunoterapiju (31,6% naspram 11,3%; $p = 0,042$).

Kada je reč o distribuciji febrilne neutropenije prema tipu limfoma, u rezultatima naše studije se može

Among the patients who developed FN, grade 1 neutropenia was observed in one (4.5%) patient, grade 2 was found in four (18.2%) patients, grade 3 in 10 (45.5%) patients, and grade 4 in 7 (31.8%) patients.

Laboratory parameters were also compared between patients with and without febrile neutropenia and only C-reactive protein (CRP) levels showed a significant difference, with higher values in patients who developed FN (7.2% vs. 3.3%; $p = 0.012$). Other parameters, namely: hemoglobin (Hb), ($p = 0.449$), mean corpuscular volume (MCV), ($p = 0.237$), white blood cell count (WBC), ($p = 0.602$), platelet count (PLT), ($p = 0.981$), absolute neutrophil count (ANC), ($p = 0.430$), and absolute monocyte count (AMC), ($p = 0.960$), did not differ significantly in relation to the development of febrile neutropenia (Table 3).

All predictors with statistical significance at the level of 0.01 in the univariate analysis were included in the multivariate regression analysis model with the development of febrile neutropenia as the dependent variable. The model contains six predictors and is statistically significant ($p < 0.001$). The use of antibiotics ($B = 4.551$; $p < 0.001$) and the number of administered ampoules of G-CSF ($B = 0.154$; $p = 0.028$) were distinctly significant predictors of the development of febrile neutropenia (Table 4).

DISCUSSION

Our study demonstrated a statistically significant difference in the occurrence of febrile neutropenia in patients who had previously received chemotherapy, which is in keeping with available literature data. The addition of monoclonal antibodies to chemotherapy protocols increases the risk of febrile neutropenia [11,12]. We have proven that the occurrence of febrile neutropenia was more frequent in patients who received several lines of therapy: ≥3 lines of therapy

videti najmanja učestalost kod Hočkinovog limfoma. Ovakvi rezultati se mogu objasniti činjenicom da se u našem uzorku mogu naći samo bolesnici koji su tretirani ABVD protokolom (doksorubicin, bleomicin, vinblastin i dakarbazin), te shodno tome, a u skladu sa NCCN (engl. *National Comprehensive Cancer Network*) preporukama, nije primenjivan G-CSF zbog opisane pulmološke toksičnosti bleomicina u ovakvoj kombinaciji kod bolesnika starosti < 40 godina [13]. U svom radu iz 2024. godine, Islas-Munjoz i saradnici su objavili podatke da je FN najčešća upravo kod bolesnika sa Hočkinovim limfom i kod bolesnika sa akutnom limfoblastnom leukemijom (ALL), odnosno akutnom mijeloidnom leukemijom (AML) [14]. S obzirom da je naš uzorak mali i da ne obuhvata bolesnike sa akutnim leukemijama, ove podatke ne možemo u potpunosti korelirati.

U literaturi je opisan prediktivni model razvoja neutropenije, redukcije doze leka i odlaganja primene terapije, zasnovan na hematološkim parametrima krvne slike tokom prvog ciklusa hemioterapije. Model procenjuje rizik od potrebe za redukcijom doze usled mijelosupresije i rangira bolesnike u grupe sa srednjim i visokim rizikom od nastanka neutropenije u narednim ciklusima lečenja. Shodno tome, ova podela omogućava pravovremenu primenu G-CSF-a, prevenciju febrilne neutropenije i sledstvenu adekvatnu dozu hemioterapije, bez potrebe za redukcijom. Dokazano je da kod bolesnika sa najvišim stepenom neutropenije češće dolazi do razvoja febrilne neutropenije [15,16]. U našoj studiji, treći i četvrti stepen neutropenije u grupi onih koji su razvili febrilnu neutropeniju, uočen je kod više od dve trećine bolesnika.

Dokazali smo češću pojavu febrilne neutropenije kod bolesnika sa prethodnim infekcijama, bolesnika sa uznapredovalim kliničkim stadijumom bolesti i onih koji su imali *bulky* tumorsku masu, što je opisano i u dostupnoj literaturi [6].

Interesantno je da je u našem uzorku kod bolesnika sa povoljnijim ECOG performans statusom (0 i 1) češće dolazilo do razvoja febrilne neutropenije, što nije u saglasnosti sa objavljenim studijama [17]. Ovo se može objasniti time što bolesnici lošijeg performans statusa zahtevaju lečenje u bolničkim uslovima na matičnim odeljenjima, zbog pratećih komplikacija, dok pokretni bolesnici mogu primati terapiju i u uslovima dnevne bolnice.

Teški i produženi oblici neutropenije dokazano su povezani, u mnogim istraživanjima, sa češćom pojavom infektivnog sindroma [14] što je potvrđeno i u našoj studiji: bolesnici sa febrilnom neutropenijom su imali češće infekcije (59,1% naspram 27,9%; $p = 0,008$), kao i češću primenu antibiotika (95,5% naspram 33,3%; $p < 0,001$). Literaturni podaci ukazuju da se kod hema-

(36.4% vs. 10.3%; $p = 0.004$), but also in those who received a higher number of cycles of the same cytostatic drug: ≥ 10 cycles of chemotherapy (40.9% vs. 17.6%; $p = 0.025$), including immunotherapy (31.6% vs. 11.3%; $p = 0.042$).

Regarding the distribution of febrile neutropenia according to the type of lymphoma, the results of our study show the lowest frequency in Hodgkin's lymphoma. Such results can be explained by the fact that our sample comprised only patients treated with the ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine). Because of this, and per the National Comprehensive Cancer Network (NCCN) recommendations, G-CSF was not applied due to the described pulmonary toxicity of bleomycin in this combination in patients aged < 40 years [13]. In their study from 2024, Islas-Muñoz et al. reported that FN is, indeed, most common in patients with Hodgkin's lymphoma and those with acute lymphoblastic leukemia (ALL)/acute myeloid leukemia (AML) [14]. Since our sample is small and does not include patients with acute leukemias, we cannot fully correlate these data.

The literature describes a predictive model of the development of neutropenia, drug dose reduction, and treatment delay, based on blood count hematological parameters during the first cycle of chemotherapy. The model estimates the risk of the need for dose reduction due to myelosuppression and ranks patients into groups with a medium and high risk of developing neutropenia in subsequent treatment cycles. Consequently, this patient categorization allows timely administration of G-CSF, prevention of febrile neutropenia, and the administration of an appropriate chemotherapy dose, without the need for reduction. It has been proven that patients with the highest degree of neutropenia often develop febrile neutropenia [15,16]. In our study, in the group of patients who developed febrile neutropenia, grade III and grade IV neutropenia was observed in more than two-thirds of the subjects.

We have proven the more frequent occurrence of febrile neutropenia in patients with previous infections, patients with an advanced clinical stage of the disease, and those who had a bulky tumor mass, which has also been reported in the available literature [6].

Interestingly, in our sample, patients with a more favorable ECOG performance status (0 and 1) developed febrile neutropenia more often, which is not in agreement with published studies [17]. This can be explained by the fact that patients with a poorer performance status require treatment in specialized hospital wards, due to accompanying complications, while ambulatory patients can receive therapy in day-care settings.

toloških bolesnika beleže duži periodi neutropenije, kao i neutropenije većeg stepena, te shodno tome i veći mortalitet u odnosu na bolesnike sa tumorima solidnih organa [18]. Ovi podaci naglašavaju potrebu za blagovremenim intenzivnim praćenjem i lečenjem hematoloških bolesnika sa teškim oblicima neutropenije u bolničkim uslovima. Takođe, naglašava se potreba za primenom G-CSF-a kod ovakvih bolesnika, u cilju bržeg oporavka.

Već pomenuti faktori rizika za razvoj febrilne neutropenije, kao što su uzrast, performans status, mijelosupresivni efekat terapije, odnosno izbor hemioterapeutika, stepen neutropenije, te pad vrednosti drugih parametara krvne slike, ključni su u blagovremenom prepoznavanju potrebe za primenom G-CSF-a. Neki od ovih faktora su uvršćeni u ASCO CSF preporuke (engl. *American Society of Clinical Oncology – ASCO CSF guidelines*) za profilaktičku primenu faktora stimulacije rasta kolonija [17]. U našoj studiji smo dokazali da je primena G-CSF-a bila češća kod onih pacijenata kod kojih je došlo do razvoja febrilne neutropenije (54,5% naspram 11,8%; $p < 0,001$), kao i da je kod njih primenjen veći broj ampula G-CSF-a (5,0 naspram 1,0; $p < 0,001$), što je u skladu sa do sada objavljenim podacima.

Kod četiri bolesnika je zabeležen letalni ishod zbog pratećih komorbiditeta, što je svakako nedovoljan uzorak za poređenje sa dostupnim podacima iz literature.

Ograničenja naše studije su sledeća: retrospektivni tip studije, uključivanje pacijenata samo jednog odeljenja, odnosno mali broj ispitanika, teritorijalna pripadnost određenog broja bolesnika drugim zdravstvenim centrima i samim tim nedostupnost podataka o primeni G-CSF-a u tim centrima, primena terapijskih protokola u skladu sa individualnom kliničkom praksom, kao i sekundarna primena G-CSF-a u ovoj grupi bolesnika. Ipak, prednost prikupljanja podataka u dnevnoj bolnici tercijarne ustanove je bilo prisustvo heterogene grupe bolesnika sa najvećim spektrom hematoloških dijagnoza, te će buduća istraživanja sa većim uzorkom nesumnjivo doprineti dobijanju statistički značajnijih podataka.

ZAKLJUČAK

Naša studija je pokazala da je na razvoj febrilne neutropenije uticao veći broj primenjenih terapijskih linija i ciklusa imunohemioterapije, klinički stadijum bolesti, prisustvo *bulky* tumorske mase prilikom uspostavljanja dijagnoze, kao i stepen agresivnosti tipa limfoma. Česte infekcije kod ovih bolesnika i veća potreba za primenom G-CSF-a i antibiotika naglašavaju potrebu za daljim istraživanjima u ovom polju, u cilju poboljšanja kvaliteta lečenja. Takođe, potrebno je usmeriti pažnju u pravcu preciznije identifikacije faktora rizika koji do-

Severe and prolonged forms of neutropenia have been proven to be associated, in many studies, with a more frequent occurrence of an infective syndrome [14], which was also confirmed in our study: patients with febrile neutropenia had more frequent infections (59.1% vs. 27.9%; $p = 0.008$), as well as a more frequent use of antibiotics (95.5% vs. 33.3%; $p < 0.001$). Literature data indicate that hematological patients experience longer periods of neutropenia, as well as a higher degree of neutropenia, and consequently higher mortality, compared to patients with solid-organ tumors [18]. These data emphasize the need for timely intensive monitoring and treatment of hematological patients with severe forms of neutropenia in the hospital setting. Also, the need for the application of G-CSF in such patients is emphasized, in order to achieve faster recovery in patients.

The aforementioned risk factors for the development of febrile neutropenia, such as age, performance status, the myelosuppressive effect of treatment, i.e. the choice of chemotherapeutic agents, the degree of neutropenia, and the drop in other parameters in the blood count, are key in timely recognition of the need for G-CSF administration. Some of these factors are included in the American Society of Clinical Oncology (ASCO) CSF guidelines for the prophylactic use of the colony-stimulating factor [17]. In our study, we have proven that the use of G-CSF was more frequent in those patients who developed febrile neutropenia (54.5% vs. 11.8%; $p < 0.001$) and that they were given a higher number of ampoules of G-CSF (5.0 vs. 1.0; $p < 0.001$), which is consistent with previously published data.

The fatal outcome was recorded in four patients, due to accompanying comorbidities, which is certainly not a sufficient sample for comparison with available data from literature.

The limitations of our study are as follows: retrospective type of study, the inclusion of patients from only one hospital department, i.e., a small number of respondents, the territorial affiliation of a certain number of patients to other health centers, and therefore the unavailability of data on the use of G-CSF in those centers, the application of therapeutic regimens in keeping with individual clinical practice, as well as the secondary application of G-CSF in this group of patients. However, the advantage of collecting data in a day hospital belonging to a tertiary institution was the presence of a heterogeneous group of patients with a very wide spectrum of hematological diagnoses. Therefore, future research on a larger sample will undoubtedly contribute to the obtaining of more statistically significant data.

vode do febrilne neutropenije, kako bi se sproveda pravovremena profilaksa i postigao veći uspeh u lečenju bolesnika sa hematološkim malignitetima.

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CONCLUSION

Our study showed that the development of febrile neutropenia was influenced by the number of applied lines of therapy and the number of cycles of immunochemotherapy, the clinical stage of the disease, the presence of a bulky tumor mass at the time of diagnosis, as well as the degree of aggressiveness of the specific lymphoma type. Frequently occurring infections in these patients, as well as the greater need for the use of G-CSF and antibiotics, emphasize the need for further research in this field, in order to improve the quality of treatment. Also, it is necessary to focus attention on more precise identification of risk factors that lead to febrile neutropenia, so as to implement timely prophylaxis and achieve greater success in the treatment of patients with hematological malignancies.

Conflict of interest: None declared

HEMATOLOŠKE TOKSIČNOSTI INHIBITORA CIKLIN ZAVISNIH KINAZA 4 I 6 U LEČENJU METASTATSKOG KARCINOMA DOJKE, ISKUSTVA JEDNOG CENTRA

ORIGINALNI RAD

ORIGINAL ARTICLE

HEMATOLOGICAL TOXICITIES OF CYCLIN-DEPENDENT KINASE 4 AND 6 INHIBITORS IN METASTATIC BREAST CANCER, SINGLE INSTITUTION EXPERIENCE

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SAŽETAK

Uvod: Hematološke toksikosti predstavljaju najčešće neželjeno dejstvo primene CDK4/6 inhibitora (CDK4/6i). Zbog novine ovih lekova, postoji potreba za dodatnim podacima koji bi identifikovali potencijalne predisponirajuće faktore za razvoj hematoloških toksikosti.

Cilj rada: Ova studija ima za cilj da ispita potencijalne predisponirajuće faktore za razvoj hematološke toksikosti tokom primene CDK4/6i u cilju lečenja metastatskog karcinoma dojke.

Materijal: U ovoj retrospektivnoj deskriptivnoj studiji ispitivana je primena CDK4/6i kod pacijentkinja sa metastatskim karcinomom dojke, lečenih na Odeljenju dojke IORSa u periodu od 1.1.2021. do 1.6.2024.

Rezultati: U studiju je uključeno 128 pacijentkinja; 43% je bilo na terapiji palbociclibom, a 57% ribociclibom. Medijana starosti bila je 60 godina. Medijana praćenja iznosila je 12 meseci (opseg 2–23). Neutropenija je uočena kod 82,1% pacijentkinja, a gradus 3/4 kod 43%. Redukcija doze zbog ponavljanih neutropenija gradusa 3/4 bila je potrebna kod 21,1% ispitanica. Anemije i trombocitopenije gradusa 3/4 primećene su kod 0,8% i 1,6% pacijenata. Obustava terapije zbog hematoloških toksikosti bila je neophodna kod 1,5% pacijentkinja. Nije bilo statistički značajne razlike između dva leka u pogledu incidence hematološke toksikosti ($p = 0,443$). Prethodna primena hemioterapije u metastatskoj fazi bolesti nije bila značajno povezana sa učestalošću hematološke toksikosti ($p = 0,565$). Palijativna radioterapija koštanih lezija pokazala je statistički značajnu povezanost sa potrebom za redukcijom doze CDK4/6i ($p = 0,001$, $r = 0,283$). Medijana preživljavanja bez progresije bolesti (PFS) nije dostignuta, ali nije postojao trend koji bi ukazivao na to da redukcija doze CDK4/6i utiče na PFS ($p = 0,719$).

Zaključak: Palijativna radioterapija koštanih lezija je povezana je sa češćom redukcijom doze CDK4/6i, ali redukcija doze ovih lekova nije uticala na dužinu preživljavanja.

Ključne reči: metastatski karcinom dojke, CDK4/6 inhibitori, hematološka toksikost

ABSTRACT

Introduction: Hematologic toxicity is the most common side effect of CDK4/6 inhibitors (CDK4/6i). Due to the novelty of these drugs, additional data are needed to identify potential predisposing factors for the development of hematologic toxicities.

Aim: This study aims to investigate potential predisposing factors for the development of hematological toxicity during the administration of CDK4/6i in the treatment of metastatic breast cancer.

Methods: This retrospective descriptive study investigated the application of CDK4/6i in patients with metastatic breast cancer treated at the Breast Department of IORS from 1.1.2021 to 1.6.2024.

Results: 128 patients were included in the study; 43% were treated with palbociclib and 57% with ribociclib. The median age was 60 years. Median follow-up was 12 months (range 2–23). Neutropenia was observed in 82.1% of patients and grade 3/4 in 43%. Dose reduction due to repeated grade 3/4 neutropenia was required in 21.1% of subjects. Grade 3/4 anemia and thrombocytopenia were observed in 0.8% and 1.6% of patients. Discontinuation of therapy due to hematological toxicities was necessary in 1.5% of patients. There was no statistically significant difference between the two drugs regarding the incidence of hematological toxicity ($p = 0.443$). Previous use of chemotherapy in the metastatic phase of the disease was not significantly associated with the frequency of hematological toxicity ($p = 0.565$). Palliative radiotherapy of bone lesions showed a statistically significant association with the need to reduce the dose of CDK4/6i ($p = 0.001$, $r = 0.283$). Median progression-free survival (PFS) was not reached, but there was no trend to suggest that CDK4/6i dose reduction affected PFS ($p = 0.719$).

Conclusion: Palliative radiotherapy of bone lesions is associated with more frequent dose reduction of CDK4/6i, but dose reduction of these drugs did not affect the length of survival.

Keywords: metastatic breast cancer, CDK4/6 inhibitors, hematological toxicity

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UVOD

Inhibitori ciklin-zavisnih kinaza 4 i 6 (CDK4/6) predstavljaju optimalnu terapijsku opciju u prvoj ili drugoj liniji lečenja metastatskog hormonski receptor-pozitivnog (HR+), humanog epidermalnog faktora rasta 2 (HER2) negativnog karcinoma dojke. Ovi lekovi primenjuju se zajedno sa hormonskom terapijom, u prvoj liniji sa inhibitorima aromataze, a u drugoj sa fulvestrantom [1]. Do danas tri CDK4/6 inhibitora dobila su odobrenje Američke agencije za lekove i hranu (FDA) i Evropske medicinske agencije (EMA) za primenu u ovoj indikaciji: abemaciclib, palbociclib i ribociclib. Takođe, abemaciclib je odobren od strane FDA i EMA za primenu u adjuvantnom lečenju ranog karcinoma dojke sa visokim rizikom od relapsa bolesti, dok je ribociclib u adjuvantnom pristupu pokazao značajan benefit u preživljavanju bez relapsa bolesti kod pacijentkinja u II i III stadijumu HR+/HER2- karcinoma dojke [1,2].

Osnovni princip antineoplastičnog dejstva ovih lekova zasniva se na inhibiciji CDK4/6, koja dovodi do zaustavljanja fosforilacije retinoblastom proteina (Rb) u CDK4/6-cyclin D1-Rb-p16/ink4a signalnom putu, što za posledicu ima onemogućavanje prelaska ćelija iz G1 u S fazu ćelijskog ciklusa [3]. Iako sva tri leka vrše inhibiciju CDK 4 i CDK 6, pokazano je da palbociclib i ribociclib u značajnijoj meri inhibiraju CDK 6, dok abemaciclib prevashodno deluje na CDK 4, što dovodi do postojanja razlike u profilu toksičnosti ovih lekova [4]. Zbog važne uloge CDK6 u diferencijaciji hematopoetskih ćelija, neutropenija (koja je prisutna kod 80% pacijenata) predstavlja daleko najčešće neželjeno dejstvo palbocicliba i ribocicliba [5,6]. Međutim, ova neutropenija se lako i brzo spontano oporavlja, jer nastaje zbog zaustavljanja diferencijacije hematopoetskih ćelija bez oštećenja DNK, za razliku od citotoksične neutropenije koja nastaje pod uticajem hemioterapije. S obzirom na to, terapijski ciklus ribocicliba i palbocicliba uključuje pauzu u primeni leka od 7 dana, a neutropenije grada (gr) 4 i ponavljane neutropenije gr 3 predstavljaju najčešći razlog za smanjenje doze ovih lekova [7,8]. Ovo nije slučaj kod abemacicliba, lek se primenjuje kontinuirano, a učestalost neutropenije iznosi oko 40%, a najčešće neželjeno dejstvo predstavljaju dijareje, koje su prouzrokovane inhibicijom CDK9 koja nije prisutna kod palbocicliba i ribocicliba [9]. Anemije i trombocitopenije značajno su ređe prilikom primene CDK4/6 inhibitora [7,8].

Do trenutka pisanja ovog rada, u Republici Srbiji su za lečenje metastatskog HR+/HER2- karcinoma dojke dostupni palbociclib i ribociclib, dok se abemaciclib primenjuje kao adjuvantna terapija. Cilj ove studije je da prikaže dosadašnje iskustvo našeg centra sa pal-

INTRODUCTION

Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors represent an optimal therapeutic option in the first or second line of treatment for metastatic hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-negative breast cancer. These drugs are administered in combination with hormonal therapy, in the first line with aromatase inhibitors and the second line with fulvestrant [1]. To date, three CDK4/6 inhibitors have received approval from the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in this indication: abemaciclib, palbociclib, and ribociclib. Additionally, abemaciclib has been approved by the FDA and EMA for use in the adjuvant treatment of early-stage breast cancer with a high risk of relapse, while ribociclib has demonstrated significant relapse-free survival benefits in the adjuvant setting for patients in stage II and III HR+/HER2- breast cancer [1,2].

The primary mechanism of action of these drugs is based on the inhibition of CDK4/6, which leads to the halting of retinoblastoma protein (Rb) phosphorylation in the CDK4/6-cyclin D1-Rb-p16/ink4a signaling pathway, preventing the transition of cells from the G1 to the S phase of the cell cycle [3]. Although all three drugs inhibit both CDK4 and CDK6, studies have shown that palbociclib and ribociclib inhibit CDK6 to a greater extent, while abemaciclib primarily targets CDK4, resulting in differences in the toxicity profiles of these drugs [4]. Due to the important role of CDK6 in hematopoietic cell differentiation, neutropenia (present in up to 80% of patients) is by far the most common adverse effect of palbociclib and ribociclib [5,6]. However, this neutropenia is easily and quickly reversible, resulting from halted hematopoietic cell differentiation without DNA damage, unlike cytotoxic neutropenia caused by chemotherapy. As a result, the therapeutic cycle of ribociclib and palbociclib includes a 7-day drug break, and grade 4 neutropenia and recurrent grade 3 neutropenia are the most common reasons for the dose reduction of these drugs [7,8]. This is not the case with abemaciclib, which is administered continuously, with a neutropenia rate of around 40%, while the most common adverse effect is diarrhea, caused by CDK9 inhibition, which is not seen with palbociclib and ribociclib [9]. Anemia and thrombocytopenia are significantly less frequent with the use of CDK4/6 inhibitors [7,8].

As of the time of writing this paper, palbociclib and ribociclib are available in the Republic of Serbia for the treatment of metastatic HR+/HER2- breast cancer, while abemaciclib is used as adjuvant therapy. This study aims to present the experience of our center

bociclibom i ribociclibom iz ugla hematoloških toksičnosti, kao i da se ispita potencijalne faktore rizika koji mogu dovesti do predviđanja pojave povećane učestalosti hematoloških neželjenih dejstava i potrebe za redukcijom doze ovih lekova.

MATERIJALI I METODE

Sproveli smo retrospektivnu deskriptivnu studiju, koja je uključila pacijentkinje koje su u periodu od 1.1.2021. do 1.6.2024. lečile metastatski HR+/HER- karcinom dojke palbociclibom ili ribociclibom na odeljenju dojke Instituta za onkologiju i radiologiju Srbije (IORS). Istorije bolesti pacijenatkinja pregledane su radi dobijanja podataka o godinama starosti, stadijumu bolesti, histologiji, karakteristikama relapsa, neželjenim događajima, prethodnom specifičnom onkološkom lečenju i praćenju pacijentkinja. Prikupljeni su i podaci o primeni hemioterapije u metastakoj fazi bolesti pre primene CDK4/6 inhibitora, kao i podaci o sprovođenju palijativne radioterapije (RT) koštanih lezija hematopoetski aktivnih kostiju (kičma, karlica, rebra, skapule, ramena kost ili butna kost) pre ili tokom primene CDK4/6 inhibitora. Common Terminology Criteria for Adverse Events (CTCAEv.5.0) korišćen je za gradiranje neželjenih događaja, dok je Response Evaluation Criteria in Solid Tumors (RECIST) verzija 1.1 primenjivana u cilju evaluacije bolesti. Opšte stanje pacijenata uključenih u studiju procenjavano je na osnovu Performans statusa Istočnoevropske kooperativne onkološke grupe (ECOG PS). Sve pacijentkinje su dale pisani informisani pristanak pre početka studije. Terapijski ciklus palbocicliba ili ribocicliba trajao je 28 dana, od kojih se lek primenjivao 21 dan (palbociclib u dozi 125 mg dnevno, ribociclib u dozi 600 mg dnevno), nakon čega je sledila pauza od 7 dana. U slučaju pojave neželjenih dejstava koja su zahtevala smanjenje doze leka, palbociclib je bio ordiniran u dozi 100 mg dnevno (prvi nivo dozne redukcije) ili 75 mg dnevno (drugi nivo dozne redukcije), dok je ribociclib bio ordiniran u dozama 400 mg dnevno (prvi nivo dozne redukcije) ili 200 mg dnevno (drugi nivo dozne redukcije).

Ukupno 128 pacijentkinja bilo je uključeno u ovu studiju. Većina ispitanica (95.4%) bila je dobrog opšteg stanja (ECOG PS 0 ili 1) pre početka primene CDK4/6 inhibitora. Palbociclib je primenjen kod 56 (43,8%) pacijentkinja, dok je ribociclib primenjen kod 72 (56,2%) pacijentkinje. Medijana starosti populacije bila je 60 godina (opseg 30-83). Medijana starosti u subpopulaciji koja je primenjivala palbociclib bila je 66 godina (opseg 42-83), dok je za ribociclib bila 57 godina (opseg 30-77). U većini slučajeva, CDK4/6 inhibitori primenjivani su kao prva linija terapije sa inhibitorima aromataze kod 91 (71,1%) pacijentkinje, palbociclib 37

with palbociclib and ribociclib from the perspective of hematological toxicities, as well as to examine potential risk factors that could predict the occurrence of increased hematological adverse effects and the need for dose reduction of these drugs.

MATERIALS AND METHODS

We conducted a retrospective descriptive study that included female patients treated for metastatic HR+/HER2- breast cancer with palbociclib or ribociclib from January 1, 2021, to June 1, 2024, at the Breast Department of the Institute for Oncology and Radiology of Serbia (IORS). The patients' medical histories were reviewed to obtain data on age, disease stage, histology, relapse characteristics, adverse events, previous specific oncological treatments, and patient follow-up. Data on chemotherapy use in the metastatic phase of the disease before CDK4/6 inhibitor administration, as well as the use of palliative radiotherapy (RT) for bone lesions of hematopoietically active bones (spine, pelvis, ribs, scapula, shoulder, or femur) before or during CDK4/6 inhibitor therapy, were also collected. The Common Terminology Criteria for Adverse Events (CTCAEv.5.0) was used to grade adverse events, while the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was applied to evaluate the disease. The general condition of the patients included in the study was assessed based on the Eastern Cooperative Oncology Group Performance Status (ECOG PS). All patients provided written informed consent before the study began.

The treatment cycle for palbociclib or ribociclib lasted 28 days, with the drug administered for 21 days (palbociclib at a dose of 125 mg daily, ribociclib at a dose of 600 mg daily), followed by a 7-day break. In the case of adverse events requiring dose reduction, palbociclib was administered at a dose of 100 mg daily (first dose reduction level) or 75 mg daily (second dose reduction level), while ribociclib was administered at doses of 400 mg daily (first dose reduction level) or 200 mg daily (second dose reduction level).

A total of 128 patients were included in this study. Most participants (95.4%) were in good general condition (ECOG PS 0 or 1) before starting CDK4/6 inhibitors. Palbociclib was used by 56 (43.8%) patients, while ribociclib was used by 72 (56.2%) patients. The population's median age was 60 years (range 30-83). The median age in the palbociclib subgroup was 66 years (range 42-83), while for the ribociclib subgroup, it was 57 years (range 30-77). In most cases, CDK4/6 inhibitors were administered as first-line therapy with aromatase inhibitors in 91 (71.1%) patients, with 37 (66%) receiving palbociclib and 54 (75%) receiving ribociclib. Slightly

Tabela 1. Demografske karakteristike populacije

Varijabla / Variable	n=128
Lek, n (%) / Drug, n (%)	
Palbociclib / Palbociclib	56 (43.8%)
Ribociclib / Ribociclib	72 (56.2%)
Linija terapije, n (%) / Therapy line, n (%)	
Prva linija / First line	91 (71.1%)
Druga linija / Second line	37 (28.9%)
Klinički stadijum pri dijagnozi, n (%) / Clinical stage at diagnosis, n (%)	
I	5 (3.9%)
II	39 (30.5%)
III	36 (28.1%)
IV	48 (37.5%)
Visceralne metastaze, n (%) / Visceral metastases, n (%)	
Da / Yes	67 (52.3%)
Ne / No	61 (47.7%)
RT koštanih lezija pre CDK4/6i, n (%) / Bone RT before starting CDK4/6, n (%)	
Da / Yes	51 (39.8%)
Ne / No	77 (60.2%)
Sistemska HT pre CDK4/6, n (%) i / Systemic chemotherapy before CDK4/6, n (%)	
Da / Yes	20 (15.6%)
Ne / No	108 (84.4%)
ECOG PS, n (%) / ECOG PS, n (%)	
0	48 (37.5%)
1	74 (57.9%)
2	6 (4.6%)

Legenda: RT– radiaciona terapija, HT– hemioterapija, CDK4/6i – Inhibitori ciklin zavisnih kinaza 4 i 6, ECOG PS – Performans Status Istočno Evropske Kooperativne Onkološke Grupe)

Table 1. Demographic data on the population

Legend: RT– radiotherapy, HT– chemotherapy, CDK4/6i – Cyclin Dependent Kinase 4 and 6 inhibitors), ECOG PS – Eastern Cooperative Oncology Group Performance Status)

(66%) i ribociclib 54 (75%). Nešto više od trećine pacijentkinja, 48 (37,5%), dijagnostikovano je u četvrtom kliničkom stadijumu, a visceralne metastaze bile su prisutne kod 67 (52,3%) ispitanica, 28 (50%) u palbociclib i 39 (54,2%) u ribociclib podgrupi. Kod 41 (32%) pacijentkinje sekundarni depoziti bili su prisutni samo na kostima (palbociclib 32,1%, ribociclib 31,9%). Hemioterapija je primenjena u metastatskoj fazi bolesti pre CDK4/6 inhibitora kod 20 (15,6%) pacijentkinja, a zračna terapija koštanih lezija sprovedena je kod 51 (37,5%) ispitanice.

Statistička analiza

Statistical Package for Social Sciences (SPSS) verzija 26 korišćena je za statističku analizu. Period bez progresije bolesti (PFS) definisan je kao vreme proteklo od početka terapije CDK4/6 inhibitorom do pojave progresije bolesti ili smrti zbog bilo kog uzroka. Korelacije između nominalnih varijabli ispitivane su primenom Pearsonovog koeficijenta korelacije i Fisherovog exact testa korelacije, dok su ispitivanja korelacije među ordinalnim varijablama sprovedene Man-Whitney U testom. Podaci o preživljavanju dobijeni su

over a third of the patients, 48 (37.5%), were diagnosed in the fourth clinical stage, and visceral metastases were present in 67 (52.3%) participants, 28 (50%) in the palbociclib group, and 39 (54.2%) in the ribociclib group. In 41 (32%) patients, secondary deposits were present only in the bones (palbociclib 32.1%, ribociclib 31.9%). Chemotherapy was administered in the metastatic phase of the disease before CDK4/6 inhibitors in 20 (15.6%) patients, and radiotherapy for bone lesions was performed in 51 (37.5%) patients.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 26 was used for statistical analysis. Progression-free survival (PFS) was defined as the time from the start of CDK4/6 inhibitor therapy to disease progression or death from any cause. Correlations between nominal variables were assessed using Pearson's correlation coefficient and Fisher's exact test, while correlations between ordinal variables were analyzed using the Mann-Whitney U test. Survival data were obtained using the Kaplan-Meier method. The log-rank test was used to assess the correlation between survival param-

Kaplan-Meier metodom. Log-rank test korišćen je za procenu korelacije između parametara preživljavanja. Vrednosti p manje od 0.05 smatrane su statistički značajnim.

REZULTATI

Primenom Man-Whitney U testa za nezavisan uzorak, zaključeno je da je postojala statistički značajna razlika u starosti između podgrupa palbocicliba i ribocicliba ($p < 0,001$). Nije bilo drugih statistički značajnih razlika u demografskim karakteristikama između dve podgrupe ispitanica.

Nakon medijane praćenja od 12 meseci (opseg 2-34), zabeležena su 54 slučaja progresije bolesti (42,2% pacijenatkinja). Do trenutka pisanja ovog rada još uvek nije dostignuta medijana PFS-a (procenjena aritmetička sredina: 18,029 meseci [CI 95% 15,453-20,604]).

Hematološke toksičnosti bile su veoma česte i javile su se kod 82,8% pacijentkinja. Predominantno se javljala neutropenija, koja se u bilo kom gradu tokom primene CDK4/6 inhibitora javila kod 105 (82%) ispitanica. Neutropenija gr 3 je bila prisutna u 51 (39,8%) slučaju, dok je neutropenija gr 4 bila značajno ređa i javila se kod 5 (3,9%) ispitanica. U palbociclib podgrupi, 47 (83,9%) pacijenatkinja razvilo je neutropeniju, od kojih je 24 (42,3%) imalo neutropeniju gr 3 ili 4. Slični rezultati zabeleženi su i u ribociclib podgrupi, gde se neutropenija javila kod 58 (80,6%) pacijenatkinja, a neutropenija gr 3 ili 4 kod 32 (44,5%). Nije bilo statistički značajne razlike između palbocicliba i ribocicliba kada je u pitanju incidenca neutropenije ($p = 0,857$).

eters. P -values less than 0.05 were considered statistically significant."

RESULTS

Using the Mann-Whitney U test for independent samples, it was concluded that there was a statistically significant difference in age between the palbociclib and ribociclib subgroups ($p < 0.001$). There were no other statistically significant differences in demographic characteristics between the two subgroups of patients.

After a median follow-up of 12 months (range 2–34), 54 cases of disease progression were recorded (42.2% of patients). At the time of writing this paper, the median PFS had not yet been reached (estimated mean: 18.029 months [CI 95% 15.453-20.604]).

Hematological toxicities were very common, occurring in 82.8% of patients. Neutropenia was the predominant toxicity, occurring in any grade during CDK4/6 inhibitor therapy in 105 (82%) patients. Grade 3 neutropenia was present in 51 (39.8%) cases, while grade 4 neutropenia was significantly rarer, occurring in 5 (3.9%) patients. In the palbociclib subgroup, 47 (83.9%) patients developed neutropenia, of whom 24 (42.3%) had grade 3 or 4 neutropenia. Similar results were observed in the ribociclib subgroup, where neutropenia occurred in 58 (80.6%) patients, and grade 3 or 4 neutropenia occurred in 32 (44.5%). There was no statistically significant difference between palbociclib and ribociclib in the incidence of neutropenia ($p = 0.857$).

No cases of febrile neutropenia were recorded during CDK4/6 inhibitor therapy, nor was granulocyte colony-stimulating factor (GCSF) used as a treatment

Tabela 2. Analiza podgrupa palbociclib i ribociclib

Varijabla (Variable) / Variable	Palbociclib n= 56	Ribociclib n= 72	Poređenje podgrupa palbociclib i ribociclib / Palbociclib and ribociclib subgroup comparison
Godine starosti (medijana, opseg) / Years of age, median (range)	66 (42-83)	57 (30-77)	$p < 0.001$
Visceralne metastaze, n (%) / Visceral metastases, n (%)	28 (50%)	39 (54.2%)	$p = 0.640$
Linija terapije, n (%) / Therapy line, n (%)			
Prva linija / First line	37 (66.1%)	54 (75%)	$p = 0.269$
Druga linija / Second line	19 (33.9%)	18 (25%)	
Redukcija doze, n (%) / Dose reduction, n (%)	11 (19.6%)	16 (22.2%)	$p = 0.723$
Medijana vremena do redukcije doze (meseci, opseg), n (%) / Median time to dose reduction (months, range), n (%)	8 (1-15)	3.5 (1-22)	$p = 0.544$
Neutropenija, n (%) / Neutropenia, n (%)	47 (83.9%)	58 (80.6%)	$p = 0.443$
Neutropenija gradus 3/4, n (%) / Neutropenija gradus 3/4, n (%)	24 (42.9%)	32 (44.5%)	$p = 0.857$
Radioterapija pre CDK4/6i, n (%) / Radiotherapy prior to CDK4/6i, n (%)	24 (42.9%)	27 (37.5%)	$p = 0.539$
Hemioterapija pre CDK4/6i, n (%) / Chemotherapy before CDK4/6i, n (%)	10 (17.9%)	10 (13.9%)	$p = 0.540$

Table 2. Analysis of palbociclib and ribociclib subgroup

Legenda: CDK4/6i - Inhibitori ciklin zavisnih kinaza 4 i 6

Legend: CDK4/6i - Cyclin Dependent Kinase 4 and 6 inhibitors

Nisu zabeleženi slučajevi febrilne neutropenije tokom primene CDK4/6 inhibitora, niti su faktori stimulacije rasta granulocitnih kolonija (GCSF) primenjivani kao terapija neutropenije. Neutropenije gr 4 ili ponavljane neutropenije gr 3, koje su zahtevale odlaganja terapijskog ciklusa i posledično smanjenje doze lekova, javile su se kod 27 (21,1%) pacijentkinja. U palbociclib podgrupi, 11 (19,6%) pacijentkinja je zahtevalo smanjenje doze leka, dok je u ribociclib podgrupi, taj broj iznosio je 16 (22,2%). Nije bilo statistički značajne razlike između dva leka kada je u pitanju potreba za redukcijom doze ($p = 0,723$). Medijana vremena do redukcije doze iznosila je 5 meseci (opseg 1-22).

Trombocitopenije su bile značajno ređe i zabeležene su kod 10 (7,8%) pacijentkinja, dok je po jedna (0,8%) pacijentkinja u obe podgrupe imala trombocitopeniju gr 3 ili 4. Anemija visokog gradusa zabeležena je samo u jednom slučaju terapije ribociclibom (0,8%). Transfuzije derivata krvi bile su potrebne kod dve pacijentkinje, jednom zbog postojanja trombocitopenije gr 4 i jednom zbog postojanja anemije gr 4. Potpuna obustava CDK4/6 inhibitora zbog hematološke toksičnosti bila je potrebna u samo dva (1,5%) slučaja, jednom zbog trombocitopenije gr 4 (ribociclib) i jednom zbog ponavljanih neutropenija gr 4 uprkos redukciji doze leka (palbociclib).

Prethodna primena hemioterapije u metastatskoj fazi bolesti i palijativna zračna terapija koštanih lezija ispitivane su kao potencijalni faktori rizika za razvoj hematološke toksičnosti tokom primene CDK4/6 inhibitora. Primena hemioterapije nije pokazala statistički značajnu povezanost sa incidencom hematoloških neželjenih dejstava ($p = 0,663$), niti sa potrebom za redukcijom doze lekova ($p = 0,467$). Palijativna zračna terapija koštanih lezija nije pokazala statistički značajnu povezanost sa pojavom neutropenije ($p = 0,778$), ali je bila u statistički značajnoj korelaciji sa potrebom za redukcijom doze CDK4/6 inhibitora ($p = 0,001$, $r = 0,283$). Ova korelacija postojala je i pri analizi podgrupa: palbociclib ($p = 0,029$, $r = 0,298$), ribociclib ($p = 0,019$, $r = 0,276$).

Iako medijana PFS-a još nije dostignuta, uzimajući u obzir da je medijana vremena do redukcije doze leka kraća od medijane praćenja, sprovedena je analiza preživljavanja sa ciljem da se ispita potencijalni efekat smanjenja doze CDK4/6 inhibitora usled hematološke toksičnosti na PFS. Nakon medijane praćenja od 12 meseci, nije primećena statistički značajna razlika u PFS-u kod pacijentkinja koje su bile na redukovanoj dozi CDK4/6 inhibitora zbog razvoja hematološke toksičnosti ($p = 0,179$).

Nehematološke toksičnosti visokog gradusa bile su značajno ređe od hematoloških. Razvoj hepatične tok-

for neutropenia. Grade 4 neutropenia or recurrent grade 3 neutropenia, which required delays in the therapeutic cycle and subsequent dose reductions, occurred in 27 (21.1%) patients. In the palbociclib subgroup, 11 (19.6%) patients required dose reduction, while in the ribociclib subgroup, this number was 16 (22.2%). There was no statistically significant difference between the two drugs regarding the need for dose reduction ($p = 0.723$). The median time to dose reduction was 5 months (range 1–22).

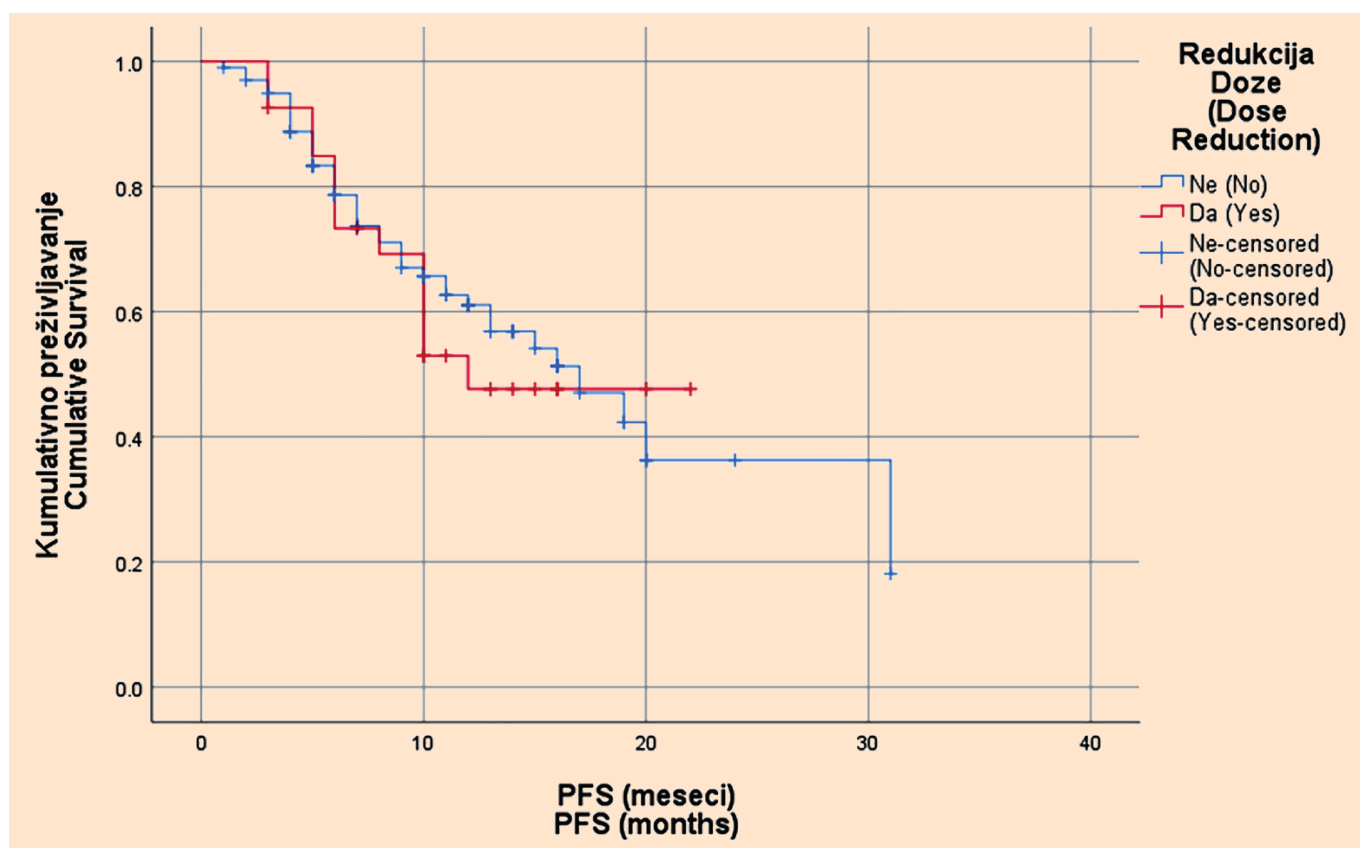
Thrombocytopenia was significantly less common, occurring in 10 (7.8%) patients, with one patient (0.8%) in each subgroup experiencing grade 3 or 4 thrombocytopenia. High-grade anemia was recorded in only one case during ribociclib therapy (0.8%). Blood transfusions were needed in two patients: one due to grade 4 thrombocytopenia and one due to grade 4 anemia. Complete discontinuation of CDK4/6 inhibitors due to hematological toxicity was necessary in only two (1.5%) cases: one due to grade 4 thrombocytopenia (ribociclib) and one due to recurrent grade 4 neutropenia despite dose reduction (palbociclib).

The prior use of chemotherapy in the metastatic phase of the disease and palliative radiation therapy for bone lesions were examined as potential risk factors for developing hematological toxicity during

Tabela 3. Hematološke toksičnosti i redukcije doze

Table 3. Hematological toxicities and dose reductions of therapy

Varijabla / Variable	n=128
Neutropenia bilo kog gradusa, n (%) / Neutropenia of any grade, n (%)	
Da / Yes	105 (82%)
Ne / No	23 (18%)
Neutropenija gradus 3 ili 4, n (%) / Neutropenia of grade 3 or 4, n (%)	
Da / Yes	56 (43.75%)
Ne / No	72 (56.25%)
Redukcija doze, n (%) / Dose reduction, n (%)	
Ne / No	101 (78.9%)
Da, Nivo 1 / Yes, level one	26 (20.3%)
Da, Nivo 2 / Yes, level two	1 (0.8%)
Palbociclib Redukcija doze, n (%) / Palbociclib Dose reduction, n (%)	n=56 (%)
Ne / No	45 (80.4%)
Da, Nivo 1 / Yes, level one	10 (18.8%)
Da, Nivo 2 / Yes, level two	1 (0.8%)
Ribociclib Redukcija doze, n (%) / Ribociclib Dose reduction, n (%)	n=72 (%)
Ne / No	56 (77.8%)
Da, Nivo 1 / Yes, level one	16 (22.2%)
Da, Nivo 2 / Yes, level two	0 (0%)



Slika 1. Kaplan-Meier kriva preživljavanja za pacijentkinje sa i bez redukcije doze CDK4/6i

Legenda: PFS- preživljavanje bez progresije bolesti, CDK4/6i - Inhibitori ciklin zavisnih kinaza 4 i 6

Picture 1. Kaplan-Meier survival curve for patients with and without dose reduction of CDK4/6i

Legend: PFS- Progression Free Survival, CDK4/6i - Cyclin Dependent Kinase 4 and 6 inhibitors

sičnosti u vidu porasta transaminaza gr 3 ili 4 zabeležen je kod 3,9% pacijentkinja, dok je smanjenje klirensa kreatinina gr 3 ili 4 zabeleženo kod 4,6% ispitanica. Produženje QTC intervala primećeno je kod 2 (1,5%) pacijentkinje koje su bile na terapiji ribociclibom. Zabeležen je i jedan slučaj alergijske reakcije na palbociclib.

DISKUSIJA

Primena palbocicliba i ribocicliba u HR+/HER2- metastatskom karcinomu dojke odobrena je na osnovu multiplih kliničkih studija koje su pokazale klinički i statistički značajan benefit u PFS-u i OS-u, u odnosu na primenu endokrine terapije bez CDK4/6 inhibitora [7-10]. Međutim, pacijenti u kliničkim studijama su precizno selektovani s aspekta godina starosti, opšteg stanja, komorbiditeta, menopauzalnog statusa, prethodnog specifičnog onkološkog lečenja, kao i mnogih drugih faktora i ne oslikavaju uvek osobine opšte populacije. U našem istraživanju, pacijentkinje koje su tretirane ribociclibom bile su statistički značajno mlađe od onih kod kojih je ordiniran palbociclib. Potencijalno objašnjenje za ovu razliku može ležati u činjenici da je u studijama, tokom primene ribocicliba, zabeležen

CDK4/6 inhibitor therapy. Chemotherapy use was not significantly associated with the incidence of hematological adverse events ($p = 0.663$) or with the need for dose reduction ($p = 0.467$). Palliative radiation therapy for bone lesions was not significantly associated with the occurrence of neutropenia ($p = 0.778$), but it was significantly correlated with the need for CDK4/6 inhibitor dose reduction ($p = 0.001$, $r = 0.283$). This correlation persisted in the subgroup analysis: palbociclib ($p = 0.029$, $r = 0.298$) and ribociclib ($p = 0.019$, $r = 0.276$).

Although the median PFS has not yet been reached, considering that the median time to dose reduction was shorter than the median follow-up time, a survival analysis was conducted to investigate the potential effect of dose reduction of CDK4/6 inhibitors due to hematological toxicity on PFS. After a median follow-up of 12 months, no statistically significant difference in PFS was observed in patients on a reduced dose of CDK4/6 inhibitors due to hematological toxicity ($p = 0.179$).

High-grade non-hematological toxicities were significantly rarer than hematological ones. The development of hepatic toxicity in the form of grade 3 or 4 transaminase elevation was recorded in 3.9% of patients, while a reduction in creatinine clearance of

značajan rizik od razvoja kardiološke toksičnosti u vidu produženja QTc intervala, kao i interakcije sa antiaritmikima i lekovima koji utiču na QTc interval, a koji se često primenjuju u starijoj populaciji [4,6,11].

Shodno zvaničnim preporukama Evropskog udruženja medikalnih onkologa (ESMO), CDK4/6 inhibitori najčešće se primenjuju kao prva linija lečenja u metastatskoj fazi bolesti [1]. Međutim, poseban entitet u lečenju metastatskog karcinoma dojke predstavlja visceralna kriza, koja je definisana kao postojanje ozbiljnog napredujućeg oštećenja funkcije vitalnog organa uslovljenog postojanjem maligne bolesti [12]. U uslovima visceralne krize zvanične preporuke savetuju primenu hemioterapije kao terapije izbora u prvoj liniji lečenja. Značajan procenat pacijentkinja koje su u našoj studiji dobijale hemioterapiju pre CDK4/6 inhibitora, nije uslovljen prethodnim postojanjem visceralne krize kod istih, već činjenicom da je hemioterapija ordinirana pre registracije CDK4/6 inhibitora u Republici Srbiji.

Hematološke toksičnosti prouzrokovane mijelosupresijom, predstavljaju najčešće neželjeno dejstvo palbocicliba i ribocicliba. Procenat pacijenata koji razviju neutropeniju tokom primene ovih lekova ne razlikuje se značajno u kliničkim studijama (74-82%) i podacima iz realne kliničke prakse (68,4%-89%) [11,13-15]. U ovom aspektu, naša populacija nije značajno odstupala u odnosu na dosadašnja ispitivanja. Takođe, nismo zabeležili značajnu razliku u učestalosti neutropenija gr 3 ili 4 u našem ispitivanju u odnosu na prethodna istraživanja [16]. Iako do sada nisu sprovedene randomizovane kliničke studije koje bi direktno poredile palbociclib i ribociclib, literaturni podaci ne ukazuju na postojanje razlike između ova dva leka iz ugla hematoloških toksičnosti, što naše ispitivanje i potvrđuje [11,13]. Posebna pažnja u studijama posvećena je i razvoju febrilne neutropenije tokom primene CDK4/6 inhibitora. Iako se neutropenije visokog gradusa javljaju kod velikog procenta pacijenata, teške infekcije i febrilna neutropenija su izuzetno retke, a u nekim istraživanjima, kao i u našem, nisu ni primećene [11,13,17,18]. Kako je neutropenija koja nastaje tokom primene CDK4/6 inhibitora uslovljena inhibicijom diferencijacije hematopoetskih ćelija, postavlja se pitanje o svrsishodnosti primene GCSF-a tokom lečenja CDK4/6 inhibitorima. Iako je GCSF primenjivan tokom kliničkih studija koje su inicijalno ispitivale ove lekove, danas mnogi autori smatraju da, s obzirom na brz oporavak neutropenije nakon pauze u primeni ovih lekova, primena GCSF-a nije opravdana [6,19]. Anemije i trombocitopenije visokog gradusa su retka neželjena dejstva ovih lekova i javljale su se kod oko 1% ispitanica u kliničkim stu-

grade 3 or 4 was noted in 4.6% of patients. QTc interval prolongation was observed in 2 (1.5%) patients receiving ribociclib. There was also one case of an allergic reaction to palbociclib.

DISCUSSION

The use of palbociclib and ribociclib in HR+/HER2- metastatic breast cancer has been approved based on multiple clinical studies that have demonstrated both clinically and statistically significant benefits in progression-free survival (PFS) and overall survival (OS) compared to endocrine therapy without CDK4/6 inhibitors [7-10]. However, patients in clinical trials were carefully selected based on age, general condition, comorbidities, menopausal status, prior specific oncological treatment, and many other factors, which do not always reflect the characteristics of the general population. In our study, patients treated with ribociclib were statistically significantly younger than those treated with palbociclib. A potential explanation for this difference could lie in the fact that during ribociclib treatment, there was a significant risk of developing cardiotoxicity, such as QTc interval prolongation, as well as interactions with antiarrhythmic drugs and other medications that affect the QTc interval, which are often used in older populations [4,6,11].

According to the official recommendations of the European Society for Medical Oncology (ESMO), CDK4/6 inhibitors are most commonly used as first-line treatment in the metastatic stage of the disease [1]. However, a distinct entity in the treatment of metastatic breast cancer is the visceral crisis, which is defined as the presence of severe progressive impairment of vital organ function due to malignant disease [12]. In cases of visceral crisis, official recommendations advise chemotherapy as the first-line treatment of choice. A significant percentage of patients in our study who received chemotherapy before CDK4/6 inhibitors did so not because of a prior visceral crisis but because chemotherapy was administered before the registration of CDK4/6 inhibitors in the Republic of Serbia.

Hematological toxicities caused by myelosuppression are the most common adverse effects of palbociclib and ribociclib. The percentage of patients who develop neutropenia during the use of these drugs does not differ significantly between clinical studies (74-82%) and real-world clinical data (68.4%-89%) [11,13-15]. In this regard, our population did not significantly differ from previous studies. Additionally, we did not observe a significant difference in the frequency of grade 3 or 4 neutropenia in our study compared to previous research [16]. Although randomized clinical trials directly comparing palbociclib and ribociclib

dijama [11,13]. Ovi rezultati su slični našim, a važno je i naglasiti da je u našem ispitivanju samo kod jedne pacijentkinje postojala potreba za nadoknadom derivata krvi tokom primene CDK4/6 inhibitora, kao i da je po primeni istih došlo do brzog oporavka anemije i trombocitopenije.

Literaturni podaci pokazuju da značajan udeo pacijenata na terapiji palbociclibom i ribociclibom tokom lečenja zahteva redukciju doze ovih lekova. Najčešći razlozi za redukciju doze su ponavljane neutropenije gr 3 koje su zahtevale odlaganje terapije, ili neutropenije gr 4. Iako se neutropenije mogu javiti u bilo kom trenutku tokom lečenja CDK4/6 inhibitorima, pokazano je da se redukcije doze najčešće sprovode u prvih 6 meseci terapije [7-11,13-17]. Naša studija saglasna je sa pomenutim podacima kada je u pitanju učestalost redukcije doze ovih lekova, a kako je medijana do redukcije doze u našoj populaciji bila 5 meseci, potvrđen je i trend redukcije doze ovih lekova u prvih 6 meseci terapije. Multipla ispitivanja bavila su se istraživanjem potencijalnog efekta smanjenja doze CDK4/6 inhibitora na preživljavanje. Do sada nema podataka koji pokazuju da opravdano smanjenje doze palbocicliba ili ribocicliba dovodi do pogoršanja parametara preživljavanja kod pacijenata [7-10,20]. Naši rezultati saglasni su sa dosadašnjim opservacijama, jer iako medijana PFS-a u našoj studiji još uvek nije dostignuta, pri analizi uticaja redukcije doze na preživljavanje nije uočena statistički značajna razlika u preživljavanju kod podgrupe pacijenata kod kojih je doza CDK4/6 inhibitora bila redukovana (Log-rank, $p = 0,179$).

Palijativna radioterapija (RT) hematopoetskih kostiju ispitivana je kao potencijalni faktor rizika za razvoj hematološke toksičnosti u našoj studiji. Antineoplastični efekat RT-a proističe iz citotoksičnog efekta zasnovanog na direktnom i indirektnom oštećenju tumorske DNK tokom G2-M faze ćelijskog ciklusa [21]. Nasuprot tome, efekat CDK4/6 inhibitora je citostatski i proističe iz zaustavljanja prelaska ćelija iz G1 u S fazu ćelijskog ciklusa. Oba mehanizma dejstva imaju kao posledicu neželjeno dejstvo u vidu razvoja neutropenije. Radioterapija može biti ordinirana sekvencijalno sa terapijom CDK4/6 inhibitorima ili tokom iste [21,22]. Većina dosadašnjih studija uključivala je obe grupe pacijenata u istraživanja, dok je naša studija ispitivala samo primenu palijativne RT kostiju pre otpočinjanja CDK4/6 inhibitora. U dosadašnjim istraživanjima, palijativna RT koštanih lezija nije pokazala statistički značajnu povezanost sa učestalošću neutropenije, ili neutropenije visokog gradusa, što su naši rezultati i potvrdili [21,22]. Međutim, u našem istraživanju postojala je statistički značajna poveza-

have not yet been conducted, literature data do not indicate a difference between these two drugs regarding hematological toxicities, which our study confirms [11,13]. Studies have given special attention to the development of febrile neutropenia during CDK4/6 inhibitor treatment. Although high-grade neutropenia occurs in a large percentage of patients, severe infections and febrile neutropenia are extremely rare, and in some studies, as well as in ours, they were not observed [11,13,17,18]. Since neutropenia caused by CDK4/6 inhibitors is due to the inhibition of hematopoietic cell differentiation, the question arises about the usefulness of granulocyte colony-stimulating factor (G-CSF) during CDK4/6 inhibitor treatment. Although G-CSF was used in clinical trials that initially investigated these drugs, many authors today believe that given the rapid recovery of neutropenia after a pause in treatment, the use of G-CSF is not justified [6,19]. High-grade anemia and thrombocytopenia are rare adverse effects of these drugs, occurring in about 1% of patients in clinical studies [11,13]. These results are similar to ours, and it is also important to note that in our study, only one patient required a blood derivative transfusion during CDK4/6 inhibitor treatment, and after its administration, there was a rapid recovery from anemia and thrombocytopenia.

Literature data show that many palbociclib and ribociclib therapy patients require dose reductions during treatment. The most common reasons for dose reduction are recurrent grade 3 neutropenia that requires treatment delays or grade 4 neutropenia. Although neutropenia can occur at any time during CDK4/6 inhibitor treatment, dose reductions most often happen within the first 6 months of therapy [7-11,13-17]. Our study is consistent with these data regarding the frequency of dose reductions, and since the median time to dose reduction in our population was 5 months, the trend of dose reductions within the first 6 months of therapy is confirmed. Multiple studies have investigated the potential effect of dose reduction on survival during CDK4/6 inhibitor treatment. Until now, no data show that justified dose reductions of palbociclib or ribociclib lead to worsened patient survival outcomes [7-10,20]. Our results align with previous observations, as although the median PFS in our study has not yet been reached, no statistically significant difference in survival was observed in the subgroup of patients who underwent CDK4/6 inhibitor dose reduction (Log-rank, $p = 0.179$).

Palliative radiotherapy (RT) to hematopoietic bones was examined as a potential risk factor for the development of hematological toxicity in our study. The antineoplastic effect of RT stems from its cytotoxic

nost palijativne RT hematopoetskih kostiju i povećane učestalosti redukcije doze CDK4/6 inhibitora usled ponavljanih hematoloških toksičnosti gradus 3 ili 4 ($p=0.001$, $r=0.283$). Ovo ispitivanje je među prvim koje je iznelo dokaze o povezanosti RT-a i potrebe za redukcijom doze CDK4/6 inhibitora [21]. Zbog različitosti metodologija sprovođenja RT-a, različitog momenta sprovođenja RT-a u odnosu na terapiju CDK4/6 inhibitorima, kao i malog broja pacijenata, poređenje studija koje su se bavile uticajem RT-a na razvoj hematoloških toksičnosti tokom primene palbocicliba i ribocicliba nije moguće. Dodatna randomizovana ispitivanja prospektivnog tipa, sa većim brojem pacijenata, uniformnijim pristupom RT metodama i dužim periodom praćenja, neophodna su kako bi se doneli definitivni zaključci o zajedničkom uticaju RT-a i CDK4/6 inhibitora na razvoj hematološke toksičnosti.

Ograničenja naše studije proizilaze iz njenog retrospektivnog dizajna, ograničenog broja pacijenata i relativnog kraćeg perioda praćenja. Takođe, nisu prikupljeni podaci o tačnoj dozi ordinirane RT. Ipak, naši rezultati uspešno oslikavaju realnu kliničku praksu primene CDK4/6 inhibitora i samim tim iznose bitne zaključke o podnošenju i toksičnostima ovih lekova.

ZAKLJUČAK

U ovoj studiji identifikovali smo postojanje statistički značajne povezanosti između palijativne RT koštanih lezija i češće potrebe za redukcijom doze CDK4/6 inhibitora. Iako su potrebe za redukcijom doze CDK4/6 inhibitora zbog hematoloških toksičnosti relativno česte, nema dokaza da redukcija doze istih utiče na dužinu preživljavanja.

Sukob interesa: Nije prijavljen.

effect, based on direct and indirect damage to tumor DNA during the G2-M phase of the cell cycle [21]. In contrast, the effect of CDK4/6 inhibitors is cytostatic, resulting from the inhibition of cell cycle transition from the G1 to the S phase. Both mechanisms of action have the undesired effect of causing neutropenia. Radiotherapy can be administered sequentially with CDK4/6 inhibitor therapy or during treatment [21,22]. Most previous studies included both groups of patients in their research, whereas our study only examined the use of palliative bone RT before initiating CDK4/6 inhibitors. In previous studies, palliative RT of bone lesions did not show a statistically significant association with the incidence of neutropenia or high-grade neutropenia, which our results also confirmed [21,22]. However, our study found a statistically significant association between palliative RT of hematopoietic bones and an increased incidence of CDK4/6 inhibitor dose reductions due to recurrent grade 3 or 4 hematological toxicities ($p=0.001$, $r=0.283$). This study is one of the first to present evidence of an association between RT and the need for CDK4/6 inhibitor dose reduction [21]. Due to differences in RT methodologies, the timing of RT in relation to CDK4/6 inhibitor therapy, and the small number of patients, comparisons between studies investigating the impact of RT on hematological toxicities during palbociclib and ribociclib treatment are not feasible. Additional prospective, randomized studies with larger patient cohorts, more uniform RT approaches, and longer follow-up periods are needed to draw definitive conclusions about the combined effects of RT and CDK4/6 inhibitors on hematological toxicity.

Our study's limitations arise from its retrospective design, the limited number of patients, and the relatively shorter follow-up period. Additionally, data on the exact dose of RT administered were not collected. Nevertheless, our results successfully reflect real-world clinical practice in the use of CDK4/6 inhibitors and thus provide important conclusions about the tolerability and toxicities of these drugs.

CONCLUSION

This study identified a statistically significant association between palliative RT of bone lesions and a more frequent need for CDK4/6 inhibitor dose reduction. Although the need to reduce the dose of CDK4/6 inhibitors due to hematological toxicities is relatively common, there is no evidence that dose reduction affects the length of survival.

Conflict of interest: None declared.

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UTICAJ INSULINSKE REZISTENCIJE NA TERAPIJSKI ODGOVOR KOD NOVODIJAGNOSTIKOVANIH BOLESNIKA SA MULTIPLIM MIJELOMOM

ORIGINALNI RAD

ORIGINAL ARTICLE

THE INFLUENCE OF INSULIN RESISTANCE ON THERAPEUTIC RESPONSE IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA

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SAŽETAK

Uvod: Prema rezultatima dosadašnjih istraživanja, ustanovljeno je da se disfunkcija IGF (engl. *insulin-like growth factor*) sistema, koja je u osnovi insulinske rezistencije sa hiperinsulinemijom, ponaša kao proliferatogeni tumorski faktor, a svoj neoplastični potencijal pokazuje i u multiplom mijelomu.

Cilj: Cilj studije je ispitivanje eventualne povezanosti insulinske rezistencije kod novodijagnostikovanih bolesnika obolelih od multiplog mijeloma (NDMM) sa postignutim terapijskim odgovorom nakon sprovedene indukciono hemioterapije.

Materijali i metode: Sprovedeno je prospektivno istraživanje koje je obuhvatilo 35 pacijenata sa NDMM-om (60% žena i 40% muškaraca), lečenih na Klinici za hematologiju Kliničkog centra Vojvodine. Vrednosti glikemije i insulinemije su određivane našte i postprandijalno a potom su izračunavane vrednosti *HOMA-IR* (našte) i *HOMA-IR* (postprandijalno). Vrednosti *HOMA-IR* > 2,2 su bile kriterijum za insulinsku rezistenciju. Analize su rađene u dva navrata – pre inicijalne hemioterapije i posle završenog lečenja. Procena terapijskog odgovora je sprovedena prema kriterijumima Srpske mijelomske grupe (SMG) i Međunarodne radne grupe za mijelom (engl. *International Myeloma Working Group – IMWG*). Statističke analize su sprovedene u SPSS programu (Verzija 22).

Rezultati: Bolesnici su bili prosečne starosti od 63,85 godina. Ukupno 86% bolesnika je povoljno odgovorilo na terapiju. Prosečna vrednost *HOMA-IR* pre terapije je bila $1,82 \pm 0,79$, a posle terapije $1,80 \pm 0,72$. Centralna vrednost medijane *HOMA-IR* pre terapije je bila 5,46 sa interkvartalnim rasponom od 1,07 – 20,57, a posle terapije je bila 5,86 sa interkvartalnim rasponom od 1,22 – 28,22. Uočena je značajna negativna korelacija *HOMA-IR* nakon primenjene terapije i postignutog terapijskog odgovora ($p = 0,040$).

Zaključak: Aktivna konkomitantna terapija insulinske rezistencije kod NDMM-a bi mogla da unapredi odgovor na primenjenu antimijelomsku terapiju.

Ključne reči: hiperinsulinemija, multipli mijelom, terapijski odgovor

ABSTRACT

Introduction: According to the results of previous research, it was found that the dysfunction of the insulin-like growth factor (IGF) system, which is the basis of insulin resistance with hyperinsulinemia, acts as a proliferative tumor factor, and its neoplastic potential is also exhibited in multiple myeloma.

Study aim: The study aims to determine whether there is an association between insulin resistance in patients newly diagnosed with multiple myeloma (NDMM) and the achieved therapeutic response after induction chemotherapy.

Materials and methods: The prospective study included 35 NDMM patients (60% women and 40% men), treated at the Clinical Center of Vojvodina Clinic for Hematology. Glycemia and insulinemia levels were determined after fasting and postprandially, upon which the values of *HOMA-IR* (after fasting) and *HOMA-IR* (postprandially) were calculated. *HOMA-IR* values > 2.2 were the criteria for insulin resistance. The analyses were performed twice – before the initial chemotherapy and after the completion of the treatment. The therapeutic response was evaluated according to the criteria of the Serbian Myeloma Group (SMG) and the International Myeloma Working Group (IMWG). Statistical analyses were performed in the SPSS program, Version 22.

Results: The average patient age was 63.85 years. In total, 86% of patients responded favorably to the therapy. The average value of *HOMA-IR* before treatment was 1.82 ± 0.79 and it was 1.80 ± 0.72 after therapy. The central median value of *HOMA-IR* before treatment was 5.46, with an interquartile range of 1.07 – 20.57, and after treatment, it was 5.86 with an interquartile range of 1.22 – 28.22. A significant negative correlation between *HOMA-IR* after applied treatment and achieved therapeutic response was observed, ($p = 0.040$).

Conclusion: Active concomitant therapy of insulin resistance in NDMM could improve the response to applied antimyeloma treatment.

Keywords: hyperinsulinemia, multiple myeloma, therapeutic response

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UVOD

Multipli mijelom (MM) je maligna bolest iz grupe monoklonskih gamopatija sa ishodištem u koštanoj srži. Odlikuje se nekontrolisanom proliferacijom tumorski izmenjenih B-limfocita – plazmocita, koji proizvode patološki izmenjene imunoglobuline (M-protein). Ustanovljeno je da MM čini 1,8% svih malignih bolesti i 10% hematoloških maligniteta. U Evropi, godišnje oboli 4,5 – 6/100.000 stanovnika [1,2]. Smatra se bolešću starijeg životnog doba, uz veću učestalost u muškoj populaciji. Iako je postignut značajan napredak u lečenju MM-a u proteklih 20 godina, ova bolest je i dalje neizlečiva, te nedovoljno predvidivog toka i ishoda [3,4]. U cilju detekcije prognostičkog i terapijskog potencijala novih biohemijskih i molekularnih pokazatelja, neophodna su dalja dopunska istraživanja.

Insulinska rezistencija predstavlja otežano ili potpuno onemogućeno preuzimanje glukoze u periferna tkiva kod obolelog u odnosu na zdravu populaciju, što dovodi do porasta serumskog nivoa insulina, uz redukciju ekspresije insulinskih receptora na perifernim tkivima te posledični razvoj promena na postreceptorskom nivou [5]. Rezultati dosadašnjih istraživanja pokazuju da je uloga hiperinsulinemije i porasta bioraspoloživosti insulinskog faktora rasta 1 (engl. *insulin-like growth factor I – IGF-1*) u razvoju malignog procesa neosporna [6]. Potencijalni mehanizmi takvog epiloga su:

- moguća oštećenja DNK uz razvoj stečenih mutacija zbog povećane produkcije slobodnih kiseoničnih radikala
- promene na postreceptorskom nivou sa hiperstimulacijom brojnih ćelijskih signalnih puteva i uticaj na ćelijski metabolizam posredovan dejstvom insulinskih faktora rasta, što direktno stimuliše i ćelijsku proliferaciju [5].

Povišen serumski nivo insulina u jetri pojačava proizvodnju *IGF1*, a smanjuje sintezu proteina koji vezuju insulinske faktore rasta (engl. *insulin-like growth factor binding proteins 1 and 2 – IGFBP1 and IGFBP2*). Stimulativni efekat na rast tumora insulin može da ostvari direktnim dejstvom ili posredstvom uticaja *IGF-1*, sa posledičnim promitogenim i antiapoptotičnim dejstvom, kao što je prikazano na Slici 1 [6]. Tom prilikom, insulin prvo reaguje sa insulinskim receptorom (INSR), koji predstavlja integralni membranski protein i ima presudnu ulogu u formiranju biološkog odgovora ćelije na stimulaciju insulinom. Hiperekspresija INSR-a, dovodi do promene i biološkog odgovora ćelije na insulin sa uticajem na proliferaciju, sazrevanje i apoptozu. Ove promene mogu značajno doprineti neoplastičnoj transformaciji ćelije [6]. Osim direktnog vezivanja insulina, za INSR se mogu vezati i *IGF-1* i *IGF-2*, sa sličnim učinkom.

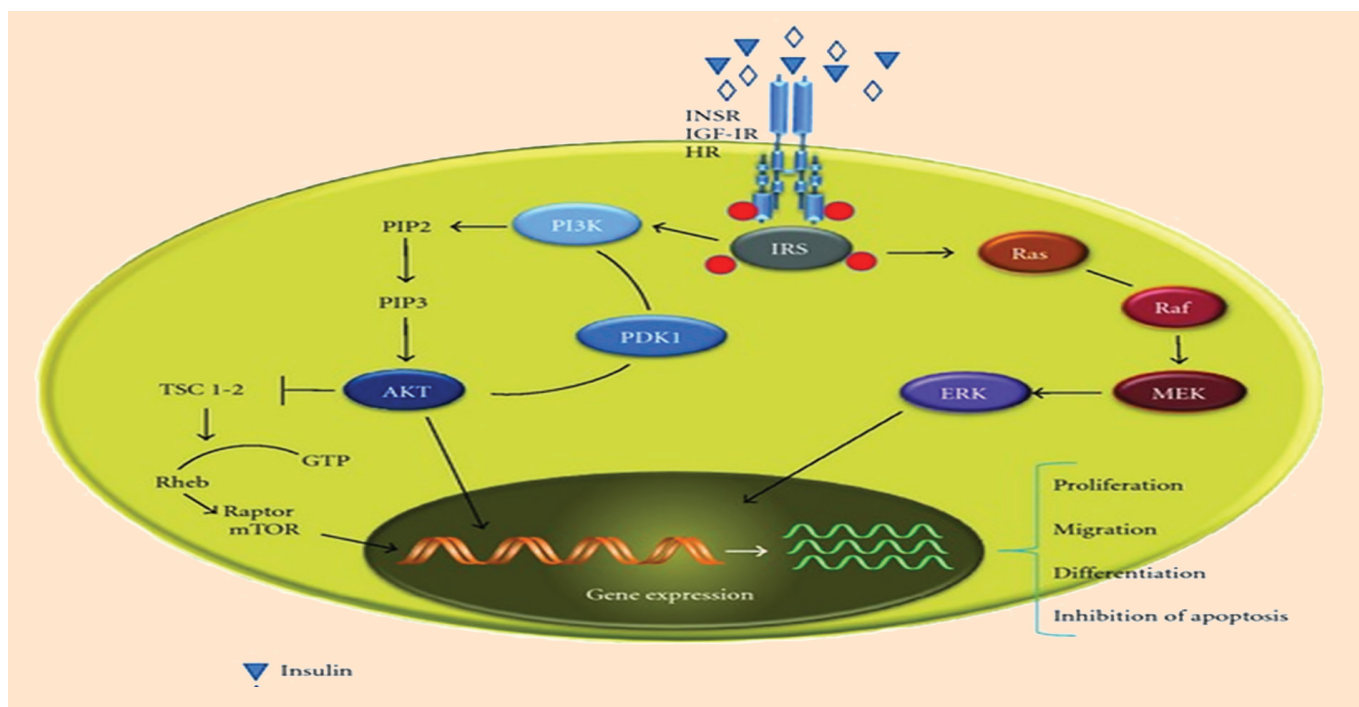
INTRODUCTION

Multiple myeloma (MM) is a malignant disease belonging to the group of monoclonal gammopathies originating in the bone marrow. It is characterized by the uncontrolled proliferation of tumorous B-lymphocytes – plasma cells, which produce pathologically altered immunoglobulins (M-protein). It was found that MM accounts for 1.8% of all malignant diseases and 10% of hematological malignancies. In Europe, 4.5 – 6/100,000 people are diagnosed annually [1,2]. Multiple myeloma is considered a disease of older age, with a higher frequency in the male population. Although significant progress has been made in the treatment of MM in the past 20 years, this disease is still incurable, and the course and outcome are insufficiently predictable [3,4]. In order to detect the prognostic and therapeutic potential of new biochemical and molecular indicators, further supplementary research is necessary.

Insulin resistance is impaired or completely incapacitated uptake of glucose into the peripheral tissues in patients affected by this condition, as compared to the healthy population, which leads to an increase in the serum level of insulin, with a reduction in the expression of insulin receptors in the peripheral tissues and the consequent development of changes at the post-receptor level [5]. The results of previous research show that the role of hyperinsulinemia and increased bioavailability of insulin-like growth factor 1 (IGF-1) in the development of the malignant process is undeniable [6]. Potential mechanisms of this are the following:

- possible DNA damage with the development of acquired mutations due to increased production of free oxygen radicals
- changes at the post-receptor level with hyperstimulation of numerous cell signaling pathways and the influence on cell metabolism mediated by the effect of insulin growth factors, which directly stimulates cell proliferation [5].

An elevated serum level of insulin in the liver increases the production of IGF1 and reduces the synthesis of insulin-like growth factor binding proteins 1 and 2 – IGFBP1 and IGFBP2. Insulin can stimulate tumor growth directly or through the influence of IGF-1, with a consequent promitogenic and antiapoptotic effect, as shown in Figure 1 [6]. In this case, insulin first reacts with the insulin receptor (INSR), which is an integral membrane protein and plays a crucial role in the formation of the cell's biological response to insulin stimulation. Hyperexpression of INSR leads to a change in the biological response of the cell to insulin with an impact on proliferation, maturation, and apoptosis. These changes can significantly contribute to the neoplastic transformation of the cell [6]. In addition to direct insu-



Slika 1. Prikaz efekta stimulacije INSR-a i pokretanja kaskade signalnih puteva koji su u osnovi biološkog odgovora ćelije na insulin (preuzeto uz dopuštenje izdavača) [6]

<https://www.ncbi.nlm.nih.gov/pmc/articles/instance/3372318/bin/EDR2012-789174.003.jpg>

Picture 1. Presentation of the effect of INSR stimulation and the initiation of the cascade of signaling pathways underlying the biological response of the cell to insulin (reproduced with permission of the publisher) [6]

<https://www.ncbi.nlm.nih.gov/pmc/articles/instance/3372318/bin/EDR2012-789174.003.jpg>

I pored brojnih istraživanja koja su do sada sprovedena, posredovanje insulinske rezistencije u karcinogenezi nije rasvetljeno na način koji bi omogućio i trasirao nove dijagnostičke i terapijske modalitete sa ciljem prevencije maligniteta, ili u slučaju već prisutne maligne bolesti, sa ciljem poboljšanja toka, odgovora na terapiju i ishoda lečenja [7].

CILJ

Cilj istraživanja je bio da se utvrdi da li postoji povezanost insulinske rezistencije kod novodijagnostikovanih bolesnika obolelih od multiplog mijeloma (NDMM) sa postignutim terapijskim odgovorom nakon sprovedene indukciono hemioterapije.

MATERIJALI I METODE

Istraživanje je sprovedeno u periodu od 2017. do 2021. godine na Klinici za hematologiju Univerzitetskog kliničkog centra Vojvodine, a nakon dobijene saglasnosti Etičkog odbora. Obuhvatilo je 35 novodijagnostikovanih bolesnika sa multiplim mijelomom, starosti 18 – 85 godina, koji do trenutka uključivanja u ovo prospektivno istraživanje nisu primili nikakvu antimijelomsku terapiju. Takođe, ispitanici nisu koristili ni lekove sa uticajem na insulinsku senzitivnost, produkciju i lučenje insulina (oralni hipoglikemici na bazi metformina, derivati sulfonilureje, tiazolidioni, SGLT-2 inhibitori,

lin binding, IGF-1 and IGF-2 can also bind to INSR, with a similar effect.

Notwithstanding the numerous studies carried out so far, the mediation of insulin resistance in carcinogenesis has not been elucidated in a way that would allow for and establish new diagnostic and therapeutic modalities to prevent malignancy, or in the case of already existing malignant disease, to improve the course, response to therapy and treatment outcomes [7].

STUDY AIM

The study aimed to determine whether there is a connection between insulin resistance in patients newly diagnosed with multiple myeloma (NDMM) and the achieved therapeutic response after induction chemotherapy.

MATERIALS AND METHODS

The research was conducted between 2017 and 2021 at the Clinic for Hematology of the University Clinical Center of Vojvodina, after obtaining the consent of the Ethics Committee. It included 35 patients newly diagnosed with multiple myeloma, aged 18 – 85 years, who had not received any anti-myeloma therapy until the time of inclusion into this prospective study. Also, the subjects did not use drugs with an impact on insulin sensitivity, insulin production, and insulin secretion

GLP1-R agonisti, insulinska terapija), kao ni hipolipide-mike sa uticajem na insulinsku senzitivnost (statini, fibrati). Dokazana druga maligna bolest je bila kriterijum za isključivanje iz studije.

U uvodnoj fazi istraživanja, nakon obavljenog razgovora, ispitanici su fizički pregledani a potom su urađene i biohemijske, dopunske hematološke i radio-loške analize predviđene protokolom Srpske mijelomske grupe (SMG) za inicijalno određivanja stadijuma NDMM-a [4].

Dopunske hematološke i biohemijske analize su obuhvatale određivanje kompletne krvne slike (KKS), parametara bubrežne funkcije (urea, kreatinin, klirens kreatinina u 24-h urinu) i aktivnosti osnovne bolesti (serumska koncentracija laktat dehidrogenaze (LDH), beta-2-mikroglobulin (β 2M), elektroforeza serumskih proteina (EFSP), serumska koncentracija imunoglobulina IgA, IgM i IgG, serumska koncentracija slobodnih lakih lanaca (SLL) κ i λ , imunofiksacija proteina u serumu i 24-h urinu), kao i standardnih inflamatornih parametara (brzina sedimentacije eritrocita (SE), C-reaktivni protein (CRP)).

U cilju procene insulinske rezistencije, merene su vrednosti glikemije i insulinemije našte i postprandijalno (2 h posle obroka), koje su dalje izračunavane prema datoj formuli: glikemija našte (mmol/l) $\times$ insulinemija našte (mIU/l) / 22,5, kako bi se odredila vrednost HOMA-IRI (engl. homeostatic model assessment-insulin resistance index) [8]. Vrednosti HOMA-IRI $> 2,2$ su bile kriterijum za dijagnozu insulinske rezistencije [9]. Određivane su vrednosti HOMA-IRIn (našte) i HOMA-IRIpp (postprandijalno, provocirano, gde su kalkulisane vrednosti glikemije i insulinemije 2 h nakon obroka).

Sve analize su rađene u dva vremena: pre započinjanja terapije i nakon završenog lečenja bolesnika. Terapijski odgovor je procenjivan prema važećim kriterijumima SMG i Međunarodne radne grupe za mijelom (engl. International Myeloma Working Group – IMWG), kao što je prikazano u **Tabeli 1**, i kvalifikovan kao: čvrsta kompletna remisija (engl. stringent complete remission – sCR), kompletna remisija (engl. complete remission – CR), veoma dobar parcijalni odgovor (engl. very good partial response – VGPR), parcijalna remisija (engl. partial remission – PR), stabilna bolest (engl. stable disease – SDi), progresija bolesti (engl. progressive disease – PD) [10].

Za svakog pacijenta su iz baze podataka prikupljeni longitudinalni podaci iz dve vremenske tačke: pre ulaska u proces hemioterapije i posle lečenja indukcionom hemioterapijom, nakon šest do sedam terapijskih ciklusa.

Same statističke analize su sprovedene u SPSS (Verzija 22) statističkom paketu, a grafički prikazi su tran-

(oral hypoglycemics based on metformin, sulfonylurea derivatives, thiazolidinediones, SGLT-2 inhibitors, GLP1-R agonists, insulin therapy), nor hypolipidemic drugs with an impact on insulin sensitivity (statins, fibrates). Another proven malignancy in a patient was an exclusion criterion.

In the initial phase of the study, after the doctor-patient interview, the subjects were physically examined, upon which biochemical, additional hematological, and radiological analyses were performed, as specified by the protocol of the Serbian Myeloma Group (SMG) for initially determining the stage of NDMM [4].

Additional hematological and biochemical analyses included the complete blood count (CBC), kidney function tests (urea, creatinine, creatinine clearance in 24-h urine collection), tests related to the activity of the underlying disease (serum level of lactate dehydrogenase (LDH), serum level of beta-2-microglobulin (β 2M), serum protein electrophoresis (SPEP), serum levels of immunoglobulins IgA, IgM, and IgG, serum levels of free light chains (FLC) κ and λ , serum immunofixation test, and 24-h urine collection), as well as standard inflammatory markers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)).

To assess insulin resistance, fasting and postprandial glycemia and insulinemia values were measured, which were further calculated according to the given formula: fasting glycemia (mmol/l) $\times$ fasting insulinemia (mIU/l) / 22.5, to calculate the value of the homeostatic model assessment-insulin resistance index (HOMA-IRI) [8]. HOMA-IRI values > 2.2 were the criterion for diagnosing insulin resistance [9]. The values of HOMA-IRIf (fasting HOMA-IRI) and HOMA-IRIpp (postprandial, provoked, where the values of glycemia and insulinemia were calculated 2 h after the meal) were calculated.

All tests were performed at two points in time: before starting the therapy and after the patient's treatment was completed. The therapeutic response was evaluated according to the valid SMG and International Myeloma Working Group (IMWG) criteria, as shown in **Table 1**, and qualified as follows: stringent complete remission (sCR), complete remission (CR), very good partial response (VGPR), partial remission (PR), stable disease (SDi), disease progression (PD) [10].

For each patient, longitudinal data were collected from the database for two points in time: before entering the chemotherapy process and after treatment with induction chemotherapy, after six to seven treatment cycles.

The statistical analyses were carried out in the SPSS (Version 22) statistical package, and the graphical representations were transposed into the Excel

Tabela 1. Klasifikacija odgovora na primenjenu terapiju, MM

sCR	✓ Odsustvo monoklonskih plazma ćelija u koštanoj srži / <i>Absence of monoclonal plasma cells in the bone marrow</i> ✓ Odsustvo M komponente u imunofiksaciji proteina u serumu i 24-h urinu / <i>Absence of the M component in serum immunofixation and in 24-h urine collection</i> Normalan SLLκ/SLLλ / <i>Normal serum FLCκ/FLCλ</i>
CR	✓ < 5% monoklonskih plazma ćelija u koštanoj srži / <i>< 5% of monoclonal plasma cells in the bone marrow</i> ✓ Odsustvo M komponente u imunofiksaciji proteina u serumu i 24-h urinu / <i>Absence of the M component in serum immunofixation and in 24-h urine collection</i> Normalan SLLκ/SLLλ / <i>Normal serum FLCκ/FLCλ</i>
VGPR	✓ imunofiksacijom detektabilna M protein u serumu i 24-h urinu / <i>Serum and urine M-protein detectable by immunofixation and in 24-h urine collection</i> ✓ Redukcija M proteina u EFSP za > 90% / <i>> 90% reduction in serum M-protein in EPSP</i> M protein < 100 mg u 24-h urinu / <i>M protein < 100 mg in 24-h urine collection</i>
PR	✓ M protein u serumu snižen za $\geq 50\%$ / <i>$\geq 50\%$ reduction of serum M-protein</i> ✓ M protein u 24-h urinu snižen za $\geq 90\%$, ili proteinurija u 24-h urinu < 200 mg / <i>M protein in 24-h urine collection reduced by $\geq 90\%$, or proteinuria in 24-h urine collection < 200 mg</i> ✓ Ukoliko je M protein u serumu i urinu nemerljiv, potrebno je da je SLLκ/λ smanjen za $\geq 50\%$ / <i>If the serum and urine M-protein are unmeasurable, it is required that serum FLCκ/λ is reduced by $\geq 50\%$</i> ✓ Redukcija infiltracije koštane srži za > 50%, ako je M protein u serumu i urinu nemerljiv i ako su laki lanci nemerljivi / <i>Reduction of bone marrow infiltration by > 50%, if the serum and urine M-protein are unmeasurable and if light chains are unmeasurable</i> ✓ Redukcija mekotkivnih infiltrata za $\geq 50\%$ / <i>Reduction of soft tissue infiltrates by $\geq 50\%$</i>
SDi	Bez kriterijuma za CR, VGPR, PR, PD / <i>Not meeting criteria for CR, VGPR, PR, or PD</i>
PD	✓ Porast M proteina u serumu (apsolutni porast $\geq 0,5$ g/dl) i/ili / <i>Increase of serum M-protein (absolute increase $\geq 0,5$ g/dl) and/or</i> ✓ Porast M proteina u urinu (apsolutni porast ≥ 200 mg/24 h) / <i>Increase of urine M-protein (absolute increase ≥ 200 mg/24 h)</i>

Legenda: sCR – čvrsta kompletna remisija (engl. *stringent complete remission*), CR – kompletna remisija (engl. *complete remission*), VGPR – veoma dobar parcijalni odgovor (engl. *very good partial response*), PR – parcijalna remisija (engl. *partial remission*), SDi – stabilna bolest (engl. *stable disease*), PD – progresija bolesti (engl. *progressive disease*)

sponovani u Excel format, radi bolje preglednosti. U cilju prikaza deskriptivnih pokazatelja i frekvencijske raspodele, korišćena je deskriptivna statistika, odnosno standardni deskriptivni statistički pokazatelji: aritmetička sredina, standardna devijacija, koeficijent zakošenosti distribucije (engl. *skewness*) i koeficijent spljoštenosti distribucije (engl. *kurtosis*), a za varijable koje se ne distribuiraju po normalnoj raspodeli prikazani su medijana i raspon, kao deskriptivni parametri. T-test za zavisne uzorke i Viloksonov test predznaka su korišćeni u cilju ukazivanja na promene parametara pre i posle indukcione hemioterapije, u zavisnosti od toga da li su analizirane normalno distribuirane varijable ili varijable koje ne prate normalnu raspodelu podataka. Međusobne relacije varijabli ispitane su putem Spirmanovog koeficijenta korelacije, s obzirom na to da postignut terapijski odgovor u ovom istraživanju predstavlja varijablu ordinalnog nivoa merenja. Statističke hipoteze su testirane na nivou statističke značajnosti (alfa nivo) od 0,05.

Table 1. Classification of response to administered therapy, MM

Legend: sCR – stringent complete remission, CR – complete remission, VGPR – very good partial response, PR – partial remission, SDi – stable disease, PD – progressive disease

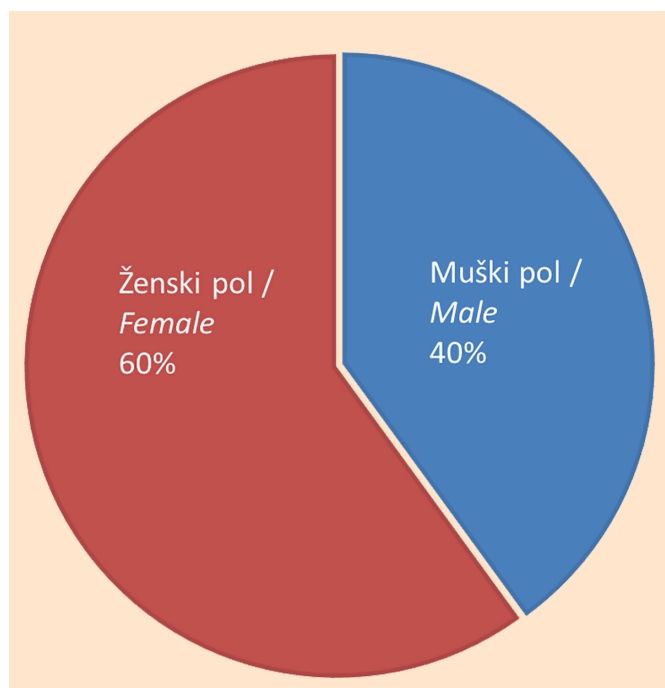
format for better visibility. In order to display descriptive indicators and frequency distribution, descriptive statistics were used, i.e. standard descriptive statistical indicators: arithmetic mean, standard deviation, skewness, and kurtosis, and for variables not distributed according to the normal distribution, median and range were presented as descriptive parameters. The t-test for dependent samples and the Wilcoxon signed-rank test were used to indicate changes in parameters before and after induction chemotherapy, depending on whether normally distributed variables or non-normally distributed variables were analyzed. Interrelationships of the variables were examined using the Spearman correlation coefficient, given that the achieved therapeutic response in this study was an ordinal variable. Statistical hypotheses were tested at a statistical significance level (alpha level) of 0.05.

RESULTS

The sex ratio of newly diagnosed patients with multiple myeloma was as follows: 60% women and 40%

REZULTATI

Kada je u pitanju polna struktura novodijagnostikovanih bolesnika obolelih od multiplog mijeloma, bilo je



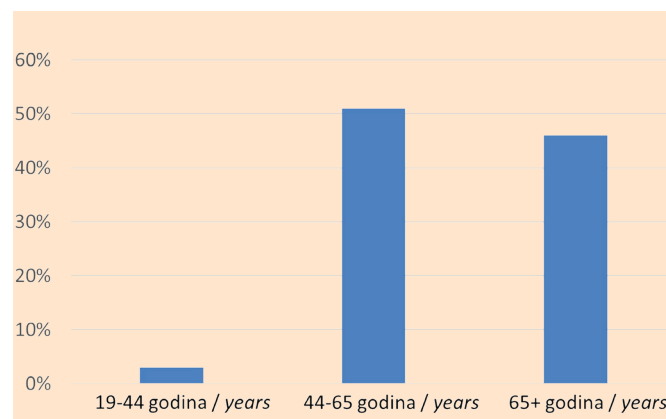
Grafikon 1. Polna struktura ispitanika u MM grupi

Graph 1. Male to female ratio in the MM group

60% žena i 40% muškaraca (**Grafikon 1**), ali ova razlika nije bila statistički značajna ($p = 0,320$). Ispitanici su bili prosečne starosti od 63,85 godina. Detektovane su statistički značajne razlike u strukturi ispitanika u odnosu na starosne grupe ($p < 0,001$). Svega 3% ispitanika je bilo starosti 19 – 41 godina, a 51% ispitanika je bilo uzrasta 45 – 65 godina (podaci prikazani u **Grafikonu 2**). Iako je čak 60% NDMM ispitanika bilo u III kliničkom stadijumu, heterogenost ispitanika u odnosu na ovu ispitivanu varijablu nije bila statistički značajna ($p = 0,200$).

Nakon sprovedene indukcionе hemioterapije je, prema **Grafikonu 3**, čak 86% ispitanika ostvarilo je odgovor na terapiju: 6% CR, 40% PR i 40% VGPR. Prosečna vrednost HOMA-IRIn je pre terapije bila $1,82 \pm 0,79$ a posle terapije $1,80 \pm 0,72$. Prosečna vrednost HOMA-IRIpp je i pre i posle primenjene terapije ispunjavala kriterijume za insulinsku rezistenciju ($\text{HOMA-IRIpp} \geq 2,2$). Centralna vrednost medijane HOMA-IRIpp je pre terapije bila 5,46 sa interkvartalnim rasponom 1,07 – 20,57, a posle terapije je bila 5,86 sa interkvartalnim rasponom 1,22 – 28,22. Nakon sprovedenog lečenja, nije detektovano statistički značajno smanjenje vrednosti, HOMA-IRIn ($p = 0,792$) i HOMA-IRIpp ($p = 0,864$). Podaci su prikazani u **Tabeli 2**.

men (**Chart 1**), however, this difference was not statistically significant ($p = 0.320$). The average age of the respondents was 63.85 years. Statistically significant differences were detected in the structure of respon-

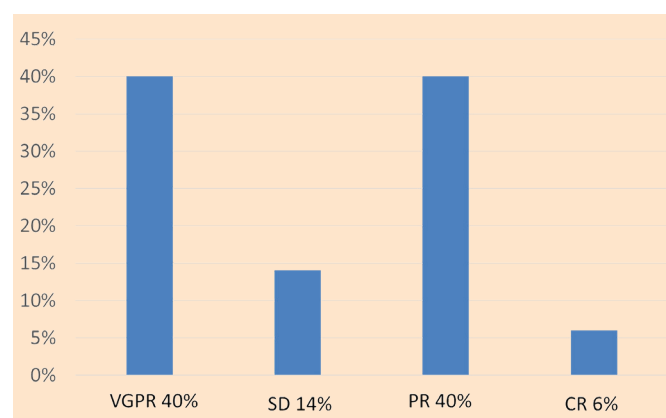


Grafikon 2. Distribucija ispitanika po starosnim intervalima u MM grupi

Graph 2. Respondent distribution by age intervals in the MM group

dents in relation to age groups ($p < 0.001$). Only 3% of respondents were aged 19 – 41 years, and 51% of respondents were aged 45 – 65 years (data presented in **Graph 2**). Although as many as 60% of NDMM subjects were in clinical stage III, the heterogeneity of the subjects regarding this variable was not statistically significant ($p = 0.200$).

After induction chemotherapy, according to **Graph 3**, as many as 86% of subjects achieved a response to treatment: 6% CR, 40% PR, and 40% VGPR. The average value of HOMA-IRIf before treatment was 1.82 ± 0.79 and after treatment, it was 1.80 ± 0.72 . The average value of HOMA-IRIpp both before and after the applied therapy met the criteria for insulin resistance



Grafikon 3. Prikaz postignutih odgovora na primenjenu terapiju u MM grupi ispitanika

Graph 3. Presentation of achieved therapeutic response in the MM respondent group

Tabela 2. Deskriptivni prikaz HOMA-IRIn i HOMA-IRIpp kod ispitivanih NDMM bolesnika

	Pre terapije / Before treatment			Posle terapije / After treatment			p
	M ± SD	Sk	Ku	M ± SD	Sk	Ku	
HOMA-IRIn / HOMA-IRIf	1.82 ± 0.79	0.70	0.35	1.80 ± 0.72	0.61	-0.2	0.792
	Mdn	Min	Max	Mdn	Min	Max	p
HOMA-IRIpp / HOMA-IRlpp	5.46	1.07	20.57	5.86	1.22	28.22	0.864

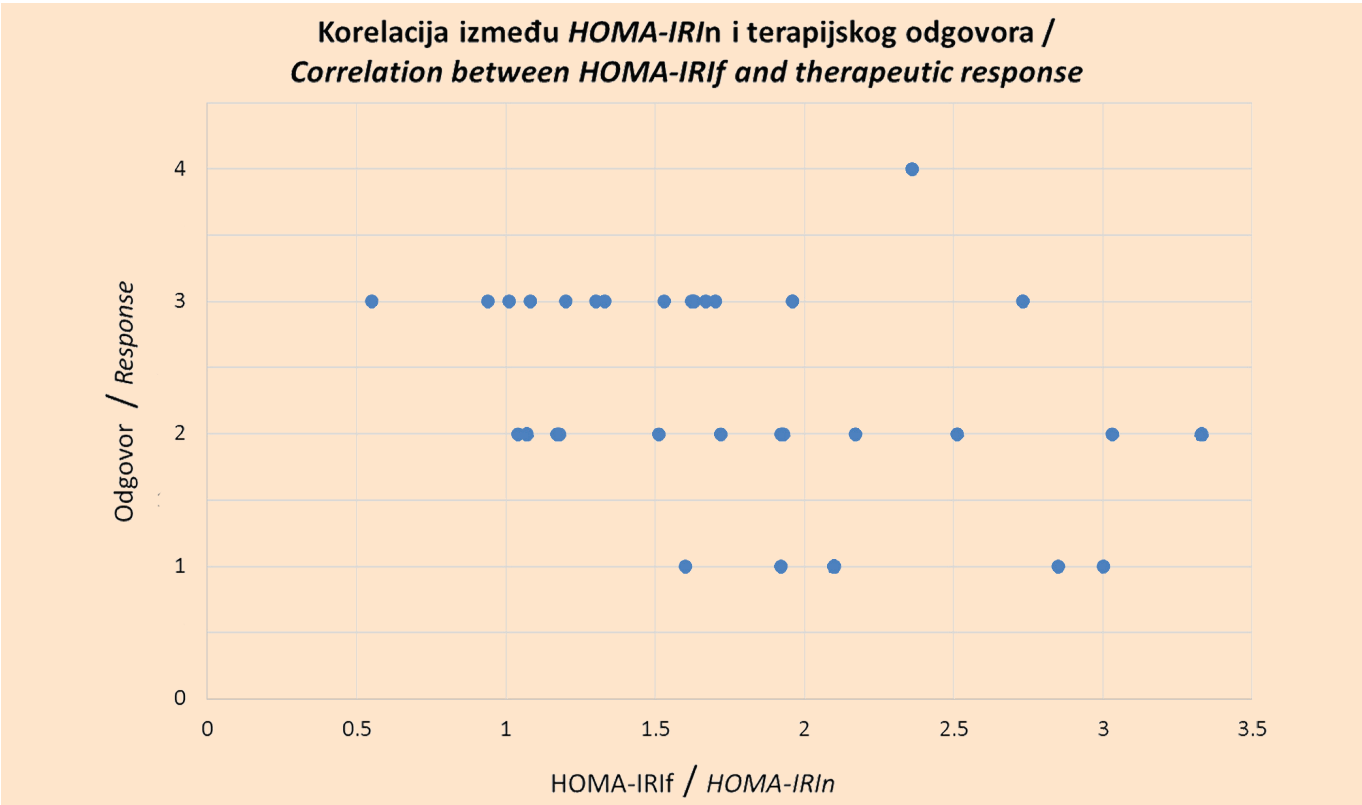
Legenda: M – aritmetička sredina (engl. *mean*), SD – standardna devijacija, Sk – koeficijent zakošenosti distribucije (engl. *skewness*), Ku – koeficijent spljoštenosti distribucije (engl. *kurtosis*), Mdn – medijana, Min – minimalna vrednost, Maks – maksimalna vrednost, p – nivo značajnosti

Table 2. Descriptive presentation of HOMA-IRIf and HOMA-IRIpp in the analyzed NDMM patients

Legend: M – mean, SD – standard deviation, Sk – skewness, Ku – kurtosis, Mdn – median, Min – minimum, Max – maximum, p – level of significance

Daljim analizama je potvrđena negativna korelacija ostvarenog terapijskog odgovora i vrednosti insulinske rezistencije našte, izmerene nakon sprovedenog lečenja. Naime, više vrednosti *HOMA-IRIn* izmerene posle sprovedenog lečenja impliciraju i lošiji terapijski odgovor ($p = 0,040$), kao što je i prikazano u **Grafikonu 4**.

($HOMA-IRIpp \geq 2.2$). The central median value of *HOMA-IRIpp* before treatment was 5.46 with an interquartile range of 1.07 - 20.57, and after treatment, it was 5.86 with an interquartile range of 1.22 - 28.22. After the treatment, no statistically significant decrease in *HOMA-IRIf* ($p = 0.792$) and *HOMA-IRIpp* ($p = 0.864$) values was detected. The data are presented in **Table 2**.



Grafikon 4. Prikaz negativne Spirmanove korelacije HOMA-IRIn i postignutog terapijskog odgovora kod NDMM

Graph 4. Presentation of the negative Spearman correlation of HOMA-IRIf and achieved therapeutic response in NDMM

DISKUSIJA

Istraživanje insulinske rezistencije kod nedijabetesne populacije NDMM bolesnika je sprovedeno sa planom evaluacije njenog eventualnog prognostičkog značaja, kao i mogućeg uticaja na tok i ishod lečenja. Iako je verifikovana inicijalno očuvana insulinska senzitivnost kod naših NDMM bolesnika, izračunate prosečne vrednosti *HOMA-IR*_{pp}, pre i nakon sprovedenog lečenja, bile su u kategoriji provocirane insulinske rezistencije. Takođe, detektovana je negativna korelacija insulinske rezistencije nakon primenjene terapije i postignutog terapijskog odgovora. Lošiji odgovor na terapiju su imali bolesnici sa višim izračunatim vrednostima *HOMA-IR*_n nakon sprovedenog lečenja. NDMM bolesnici sa ostvarenim boljim odgovorom na primenjenu terapiju su imali niži *HOMA-IR*_n. Može se reći da niže *HOMA-IR* vrednosti kod NDMM bolesnika ukazuju na veću verovatnoću remisije, kao i manju mogućnost izostanka odgovora na terapiju ili progresije pod terapijom. Ovakvi rezultati bi svakako i mogli biti očekivani, imajući u vidu do sada već detektovanu metaboličku vulnerabilnost multiplog mijeloma, čiji je glavni akter takozvani *IGF* (engl. *insulin-like growth factor*) sistem [11,12]. Ovaj sistem se sastoji iz sledećeg: dva faktora rasta – *IGF1* i *IGF2*, dva receptora – *IGF1* receptor (*IGF1R*) i *IGF2* receptor (*IGF2R*), *IGFBP*, *IGFBP* proteaze [11]. Važno je napomenuti da su *IGF1* i *IGF2* peptidi koji se strukturno u velikoj meri preklapaju sa građom proinsulina, a dosadašnjim istraživanjima je dokazan i njihov uticaj na životni vek i proliferativni indeks plazmocita, angiogenezu i aktivnost osteoklasta [13,14,15]. Visok stepen preklapanja je detektovan i u strukturi insulina i *IGF*, tako da *IGF* sistem može biti i direktno uključen u regulaciju homeostaze glukoze, vezivanjem za insulinske receptore [11,16]. Disfunkcija *IGF* sistema pogoduje razvoju insulinske rezistencije i porastu serumskog nivoa insulina dovodeći do „prezasićenosti“ insulinskih receptora *IGF1* i *IGF2* uz konsekvantne promene i na postreceptorskom nivou, stimulišući proliferaciju plazmocita i progresiju bolesti [13,17]. Samim tim se nameće zaključak da bi insulinski receptori mogli biti pogodne biološke mete za razvoj novih modaliteta u lečenju MM-a [18,19]. Drugi zaključak bi bio da se primena humanog insulina u dijabetesnoj mijelomskoj populaciji može razmatrati kao potencijalni faktor koji doprinosi progresiji MM [19,20].

Imajući u vidu i mehanizam kojim svoje dejstvo ostvaruje peroralni antidiabetesni lek, metformin, modulacijom *INSR/IGF1R* signalnog puta, može se razmatrati i antitumorski efekat ovog leka, koji je osvedočen rezultatima brojnih dosadašnjih istraživanja [14,15]. Boursi i saradnici su u svom prospektivnom istraživanju pokazali da primena metformina kod dijabetičara

Further analyses confirmed the negative correlation between the achieved therapeutic response and fasting insulin resistance values, measured after the treatment. Namely, higher values of *HOMA-IR*_f measured after treatment imply a poorer therapeutic response ($p = 0.040$), as shown in Graph 4.

DISCUSSION

The analysis of insulin resistance in the non-diabetic population of NDMM patients was conducted to evaluate its possible prognostic significance, as well as its possible influence on the course and outcome of treatment. Although initially preserved insulin sensitivity was verified in our NDMM patients, the calculated average values of *HOMA-IR*_{pp}, before and after the treatment, were in the category of provoked insulin resistance. Also, a negative correlation of insulin resistance after applied therapy and achieved therapeutic response was detected. Patients with higher calculated *HOMA-IR*_f values after treatment had a poorer response to therapy. NDMM patients with a better response to the applied therapy had a lower *HOMA-IR*_f. It can be said that lower *HOMA-IR* values in NDMM patients indicate a higher probability of remission, as well as a lower possibility of no response to therapy or progression under therapy. Such results could certainly be expected, bearing in mind the already detected metabolic vulnerability of multiple myeloma, whose main agent is the *IGF* (insulin-like growth factor) system [11,12]. This system consists of the following: two growth factors - *IGF1* and *IGF2*, two receptors - *IGF1* receptor (*IGF1R*) and *IGF2* receptor (*IGF2R*), and *IGFBP* protease [11]. It is important to note that *IGF1* and *IGF2* are peptides that structurally overlap, to a great extent, with the structure of proinsulin, and previous research has proven their influence on the lifespan and proliferative index of plasma cells, angiogenesis, and osteoclast activity [13,14,15]. A high degree of overlap was detected in the structure of insulin and *IGF*, therefore, the *IGF* system may be directly involved in glucose homeostasis regulation, by binding to insulin receptors [11,16]. Dysfunction of the *IGF* system favors the development of insulin resistance and an increase in serum insulin levels, leading to the “oversaturation” of insulin receptors with *IGF1* and *IGF2*, consequently bringing about changes at the post-receptor level, stimulating the proliferation of plasma cells and disease progression [13,17]. This leads to the conclusion that insulin receptors could be suitable biological targets for developing new modalities in the treatment of MM [18,19]. Another conclusion would be that the administration of human insulin in the diabetic myeloma population can be considered as a potential factor contributing to the progression of MM [19,20].

obolelih od monoklonske gamapatije neodređenog značaja (engl. *monoclonal gammopathy of undetermined significance* – MGUS), značajno redukuje rizik od progresije bolesti u multipli mijelom [21]. Antitumorski učinak metformina u populaciji obolelih od monoklonske gamapatije su u svom istraživanju potvrdili i Papachristu i saradnici, sa idejom o proširenju istraživanja i na nedijabetesnu populaciju [22]. Efikasnost primene metformina u nedijabetesnoj populaciji obolelih od MGUS-a, sa procenom smanjenja rizika od progresije bolesti u aktivnu formu MM, trenutno je predmet istraživanja kliničke studije NCT04850846, čiji rezultati tek treba da budu objavljeni [23].

ZAKLJUČAK

S obzirom da istraživanja sprovedena u proteklih deset godina svedoče o izraženoj metaboličkoj osetljivosti multiplog mijeloma, ne iznenađuje ni nepovoljan učinak insulinske rezistencije na ostvareni terapijski odgovor. U skladu sa tim bi i aktivan konkomitantni terapijski pristup u zbrinjavanju insulinske rezistencije i prateće hiperinsulinemije – inicijalne i/ili provocirane, u nedijabetesnoj populaciji NDMM bolesnika, bio nezanimljiv doprinos u ostvarivanju povoljnijeg odgovora na standardnu antimijelomsku terapiju.

SKRAĆENICE

β2M – beta-2-mikroglobulin
CR – kompletna remisija (engl. *complete remission*)
CRP – C-reaktivni protein
EFSP – elektroforeza serumskih proteina
GLP1-R – glukagonu slični peptid-1 receptor (engl. *glucagon-like peptide-1 receptor*)
HOMA-IRI – engl. *homeostatic model assessment-insulin resistance index*
HOMA-IRIn – *homeostatic model assessment-insulin resistance index*, našte
HOMA-IRIpp – *homeostatic model assessment-insulin resistance index*, postprandijalno
IMWG – Međunarodna radna grupa za mijelom (engl. *International Myeloma Working Group*)
INSR – insulinski receptor
Ku – koeficijent spljoštenosti distribucije (engl. *kurtosis*)
LDH – laktat dehidrogenaza
M – aritmetička sredina (engl. *mean*)
Maks – maksimalna vrednost
Mdn – medijana
MM – multipli mijelom
MGUS – monoklonska gamapatija neodređenog značaja (engl. *monoclonal gammopathy of undetermined significance*)
Min – minimalna vrednost
NDMM – novodijagnostikovani multipli mijelom
PR – parcijalna remisija (engl. *partial remission*)
PD – progresija bolesti (engl. *progressive disease*)
SD – standardna devijacija

Bearing in mind the mechanism whereby the oral antidiabetic drug metformin achieves its effect by modulating the INSR/IGF1R signaling pathway, the antitumor effect of this drug, proven by the results of numerous previous studies, should also be considered [14,15]. In their prospective study, Boursi et al. demonstrated that the application of metformin in diabetic patients with monoclonal gammopathy of undetermined significance (MGUS) significantly reduces the risk of disease progression to multiple myeloma [21]. The antitumor effect of metformin in the population of patients with monoclonal gammopathy was confirmed in their research by Papachristou et al., with the idea of extending the research to the non-diabetic population as well [22]. The effectiveness of metformin administration in the non-diabetic population of MGUS patients, with an assessment of the reduction in the risk of disease progression to active MM, is currently the subject of research in the clinical study NCT04850846, whose results have yet to be published [23].

CONCLUSION

Given that research conducted in the past ten years testifies to the pronounced metabolic sensitivity of multiple myeloma, the adverse effect of insulin resistance on the achieved therapeutic response is not surprising. Accordingly, an active concomitant therapeutic approach in treating insulin resistance and accompanying hyperinsulinemia – initial and/or provoked, in the non-diabetic population of NDMM patients, would be a significant contribution to achieving a more favorable response to standard antimyeloma therapy.

ABBREVIATIONS AND ACRONYMS

β2M – beta-2-mikroglobulin
CR – complete remission
CRP – C-reactive protein
EPSP – serum protein electrophoresis
GLP1-R – glucagon-like peptide-1 receptor
HOMA-IRI – homeostatic model assessment-insulin resistance index
HOMA-IRIf – homeostatic model assessment-insulin resistance index, after fasting
HOMA-IRIpp – homeostatic model assessment-insulin resistance index, postprandially
IMWG – International Myeloma Working Group
INSR – insulin receptor
Ku – kurtosis
LDH – lactate dehydrogenase
M – mean
Maks – maximum
Mdn – median
MM – multiple myeloma
MGUS – monoclonal gammopathy of undetermined significance

SDi – stabilna bolest (engl. *stable disease*)
Sk – koeficijent zakošenosti distribucije (engl. *skewness*)
SGLT 2 – (engl. *sodium-glucose cotransporter 2*)
SLL κ – slobodni laki lanci kapa
SLL λ – slobodni laki lanci lambda
SMG – Srpska mijelomska grupa
sCR – čvrsta kompletna remisija (engl. *stringent complete remission*)
VGPR – veoma dobar parcijalni odgovor (engl. *very good partial response*)

Sukob interesa: Nije prijavljen.

Min – minimum
NDMM – newly diagnosed multiple myeloma
PR – partial remission
PD – progressive disease
SD – standard deviation
SDi – stable disease
Sk – skewness
SGLT 2 – sodium-glucose cotransporter 2
FLC κ – kappa free light chains
FLC λ – lambda free light chains
sCR – stringent complete remission
SMG – Serbian Myeloma Group
VGPR – very good partial response

Conflict of interest: None declared.

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MIJELOPROLIFERATIVNE NEOPLAZME KOD BOLESNIKA MLAĐIH OD 40 GODINA: RETROSPEKTIVNA ANALIZA KLINIČKIH KARAKTERISTIKA

ORIGINALNI RAD

ORIGINAL ARTICLE

MYELOPROLIFERATIVE NEOPLASMS IN PATIENTS YOUNGER THAN 40 YEARS: A RETROSPECTIVE ANALYSIS OF CLINICAL CHARACTERISTICS

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SAŽETAK

Uvod: Mijeloproliferativne neoplazme (MNP) se obično dijagnosticiraju kod bolesnika starosti oko 60 godina, međutim, u kliničkoj praksi se često sreću bolesnici mlađi <40 godina.

Cilj: Procena kliničko-laboratorijskih karakteristika, učestalost tromboza i analiza terapijskog pristupa kod bolesnika sa MPN <40 godina.

Metode: Retrospektivna studija obuhvatila je 84 bolesnika, dijagnostikovanih prema SZO kriterijumima, lečenih na Klinici za hematologiju UKCS od 2000. do 2024. godine.

Rezultati: Medijana starosti iznosila je 33 godine, sa većom učestalošću žena (60,7%). Policitemija vera (PV) je nađena kod 61,9%, esencijalna trombocitemija (ET) kod 25%, a prefibrotična faza primarne mijelofibroze (prePMF) kod 13,1% ispitanika. Mutacija *JAK2V617F* je detektovana je kod 46,5% bolesnika. Mikrovaskularne simptome imalo je 27,4%, svrab 14,3%, konstitucionalne simptome 7,1%, a splenomegaliju 45,2% bolesnika. Najviši hemoglobin i hematokrit su imali bolesnici sa PV (170 g/L, 50%). Broj trombocita bio je najveći kod bolesnika sa prePMF ($1007 \times 10^9/L$), zatim kod ET ($856 \times 10^9/L$) i PV ($737,5 \times 10^9/L$). Raspodela prema stepenu fibroze koštane srži bile je sledeća: MF0 - 28,5%, MF1 - 54,8%, MF2 - 4,8%. Ukupno, 41,7% bolesnika imalo je bar jedan kardiovaskularni faktor rizika, najčešće pušenje (23,8%). Prethodne tromboze imalo je 16,7% bolesnika, a tromboze tokom praćenja (8,3%) imali su samo bolesnici sa PV. Aspirin je koristilo 86,9% bolesnika, dok su flebotomije primenjene kod skoro svih bolesnika sa PV. Citoreduktivnom terapijom lečeno je 43,9% bolesnika, 39,1% jednom TL, najčešće hidroksiurea (HU). Prema European LeukemiaNET (ELN) skoru, 82,1% bolesnika pripadalo je grupi niskog rizika. Medijana praćenja bila je 72 meseca, a dva bolesnika su preminula (2,45%).

Zaključak: Većina mlađih bolesnika sa MPN pripada ELN grupi niskog rizika. Međutim zbog razvoja tromboze (25%), prisutnih tegoba, stepena trombocitoze i splenomegalije > 18 cm, skoro 1/2 prima citoreduktivnu terapiju. To ukazuje na potrebu bolje stratifikacije, kao i upotrebu drugačijih metoda za procenu rizika bolesti, kao što je sekvencioniranje sledeće generacije (NGS).

Ključne reči: mijeloproliferativne neoplazme, tromboze, adolescenti, mlađi uzrast

ABSTRACT

Introduction: Myeloproliferative neoplasms (MPN) are typically diagnosed in patients around 60 years of age; however, in clinical practice, patients <40 years are often encountered.

Aim: Assessment of clinical-laboratory characteristics, thrombosis incidence, and therapeutic approaches in patients with MPN <40 years.

Methods: This retrospective study included 84 patients diagnosed according to WHO criteria who were treated at the Clinic of Hematology, UKCS, from 2000 to 2024.

Results: The median age was 33 years, with a higher prevalence in females (60.7%). Polycythemia vera (PV) was found in 61.9%, essential thrombocythemia (ET) in 25%, and pre-primary myelofibrosis (prePMF) in 13.1% of participants. The *JAK2V617F* mutation was detected in 46.5% of patients. Microvascular symptoms were present in 27.4%, pruritus in 14.3%, constitutional symptoms in 7.1%, and splenomegaly in 45.2% of patients. The highest hemoglobin and hematocrit levels were found in PV patients (170 g/L, 50%). The platelet count was highest in patients with prePMF ($1007 \times 10^9/L$), followed by ET ($856 \times 10^9/L$) and PV ($737.5 \times 10^9/L$). The distribution of bone marrow fibrosis was as follows: MF0 - 28.5%, MF1 - 54.8%, MF2 - 4.8%. Overall, 41.7% of patients had at least one cardiovascular risk factor, most commonly smoking (23.8%). The previous thrombosis occurred in 16.7% of patients, while thrombosis during follow-up (8.3%) was seen only in PV patients. Aspirin was used by 86.9% of patients, and phlebotomy was applied to almost all PV patients. Cytoreductive therapy was used in 43.9% of patients, with 39.1% receiving one line of treatment, most commonly hydroxyurea (HU). According to the European LeukemiaNET (ELN) score, 82.1% of patients were classified as low-risk. The median follow-up was 72 months, and two patients died (2.45%).

Conclusion: The majority of younger MPN patients belong to the low-risk ELN group. However, nearly half of the patients receive cytoreductive therapy due to the development of thrombosis (25%), the presence of symptoms, the degree of thrombocytosis, and splenomegaly > 18 cm. This indicates the need for better stratification and the use of different methods for disease risk assessment, such as next-generation sequencing (NGS).

Keywords: myeloproliferative neoplasms, thrombosis, adolescents, young age

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UVOD

Pacijenti mlađe životne dobi sa mijeloproliferativnim neoplazmama (MPN) predstavljaju posebnu subpopulaciju bolesnika čiji se broj stalno postepeno povećava [1]. Medijana starosti u trenutku postavljanja dijagnoze za klasične BCR-ABL1 negativne MPN, koje obuhvataju policitemiju veru (PV), esencijalnu trombocitemiju (ET) i primarnu mijelofibrozu (PMF), kreće se od 60-65 godina [2]. Prema podacima iz studija, pacijenti mlađi od 40 godina čine svega 10% ukupnog broja bolesnika sa MPN [3-6], dok neka druga istraživanja ukazuju da je taj udeo čak i 20% [7-9]. Od svih MPN entiteta, u populaciji mladih bolesnika, najčešća je ET sa 51%-72%, drugom mestu je PV, a najređe se javlja PMF [2,10-13]. ET se karakteriše megakariocitnom hiperplazijom i trombocitozom, bolesnici sa PV imaju značajnu eritrocitozu, dok oni sa PMF imaju fibrozu koštane srži, koja posledično kasnije dovodi do citopenija i ekstramedularne hematopoeze [14]. Kod sve tri bolesti postoji rizik od nastanka vaskularnih komplikacija, ali i akutne mijeloidne leukemije (AML), dok PV i ET mogu da se transformišu u mijelofibrozu (MF) [14].

Jedinstveno za mlade obolele od MPN je duži očekivani životni vek u odnosu na stariju populaciju, ali istovremeno, dugo trajanje bolesti pruža mogućnost za nastanak komplikacija povezanih sa bolešću i njenim lečenjem [2]. Takođe, postoje i dodatna pitanja koja se isključivo tiču ove starosne grupe, a odnose se na psihosocijalne potrebe, fertilitet i trudnoću, kao i jedinstvenu biologiju MPN [11,15]. Većina dostupnih podataka, kao i aktuelni konvencionalni prognostički modeli, dobijeni su analizom starije populacije, te se postavlja pitanje u kojoj meri precizno odražavaju jedinstvene fenotipske obrasce ili prognostičke markere ove mlađe starosne grupe.

Tako, standardni *European LeukemiaNET* (ELN) prognostički skor je baziran na godinama života (> 60) i prethodnim trombozama, i služi za određivanje načina lečenja usmerenog na smanjenje rizika od krvarenja i tromboze [16]. Stoga je njegova primena kod mladih ograničena, jer se godine života tu ne pojavljuju kao faktor, pa jedino što ove bolesnike može da svrsta u visokorizičnu grupu jesu prethodne tromboze.

Cilj ove studije bio je evaluacija kliničko-laboratorijskih karakteristika, učestalosti tromboza i analiza terapijskog pristupa kod MPN bolesnika mlađih od 40 godina.

METODE

Retrospektivno istraživanje obuhvatilo je 84 bolesnika sa dijagnozom neke od bolesti iz grupe Filadelfija negativnih MPN, dijagnostikovanih, praćenih i lečenih na Klinici za hematologiju UKCS, u periodu od 2000. do

INTRODUCTION

Younger patients with myeloproliferative neoplasms (MPN) represent a special subpopulation of patients whose number is gradually increasing [1]. The median age at diagnosis for classic BCR-ABL1-negative MPNs, which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), ranges from 60-65 years [2]. According to data from studies, patients younger than 40 make up only 10% of the total number of patients with MPN [3-6], while some other studies indicate that this proportion is even 20% [7-9]. Of all MPN entities, in the population of young patients, ET is the most common with 51%-72%, PV is second, and PMF is the least common [2,10-13]. ET is characterized by megakaryocyte hyperplasia and thrombocytosis, patients with PV have significant erythrocytosis, while those with PMF have bone marrow fibrosis, which subsequently leads to cytopenia and extramedullary hematopoiesis [14]. In all three diseases, there is a risk of vascular complications, but also of acute myeloid leukemia (AML), while PV and ET can transform into myelofibrosis (MF) [14].

Uniquely for young people with MPN is the longer life expectancy compared to the older population, but at the same time, the long duration of the disease provides the possibility for complications related to the disease and its treatment [2]. Also, there are additional questions that exclusively concern this age group and are related to psychosocial needs, fertility, and pregnancy, as well as the unique biology of MPN [11,15]. Most of the available data, as well as the current conventional prognostic models, have been obtained by analyzing the elderly population, and the question arises to what extent they accurately reflect the unique phenotypic patterns or predictive markers of this younger age group.

Thus, the standard *European LeukemiaNET* (ELN) prognostic score is based on years of life (> 60) and previous thromboses and serves to determine the treatment approach aimed at reducing the risk of bleeding and thrombosis [16]. Therefore, its application in young people is limited because age does not appear as a factor, so the only thing that can place these patients in the high-risk group is previous thromboses.

This study aimed to evaluate the clinical and laboratory characteristics, the frequency of thrombosis, and the analysis of the therapeutic approach in MPN patients under 40 years of age.

METHODS

The retrospective study included 84 patients diagnosed with one of the diseases from the Philadelphia group of negative MPNs who were diagnosed, monitored, and treated at the UKCS Hematology Clinic from

2024. godine. Dijagnoza je postavljena prema aktuelnim SZO kriterijumima [17].

Iz medicinske dokumentacije prikupljeni su sledeći podaci: 1) demografske karakteristike bolesnika; 2) prisustvo JAK2V617F mutacije; 3) simptomi; 4) prisustvo palpabilne splenomegalije; 5) parametri krvne slike; 6) vrednosti laktat-dehidrogenaze; 7) stepen fibroze kostne srži; 8) kardiovaskularni (KV) faktori rizika; 9) prisustvo i vrsta prethodnih tromboza i tromboza u toku praćenja; 10) modaliteti lečenja; 11) prognostički skorovi (ELN skor, *International Prognostic Score of Thrombosis in WHO – ET (IPSET Thrombosis)*, *International Prognostic Score for ET: Predicts Survival (IPSET survival)*, *International Prognostic Scoring System (IPSS)*); 12) dužina preživljavanja; 13) uzrok smrtnog ishoda.

Deskriptivnim statističkim parametrima, kategorijalni podaci su prikazani kao apsolutne i relativne učestalosti, a numerički podaci, s obzirom da nisu imali normalnu raspodelu, prikazani su kao medijana i opseg (Min-Max). Za testiranje razlika u učestalosti korišćen je hi-kvadrat test, a za testiranje razlika vrednosti numeričkih podataka korišćeni su Kruskal-Wallis-ov test i Mann-Whitney test. Kriterijum za statističku značajnost je bio $p < 0.05$. Podaci su obrađeni korišćenjem softverskog programa SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

REZULTATI

Studija je obuhvatila 84 bolesnika uzrasta do 40 godina sa potvrđenom dijagnozom bolesti iz grupe Filadelfija negativnih mijeloproliferativnih neoplazmi (Ph- MPN). Policitemiju veru imalo je 52 bolesnika (61,9%), esencijalnu trombocitemiju 21 bolesnik (25%), prefibrotičnu fazu primarne mijelofibroze 11 (13,1%). Nijedan bolesnik nije imao manifestnu fazu PMF. Grupu su činile dominantno žene, 51 odnosno (60,7%), dok su 33 ispitanika, (39,3%) bili muškarci (odnos $\bar{Z} : M = 1,55:1$). Medijana starosti bolesnika u trenutku postavljanja dijagnoze bila je 33 godine (od 14 do 40 godina) (Tabela 1).

Allel specifičnom PCR metodom kod 39 bolesnika (46,4%) nađena je JAK2V617F mutacija, dok je kod 24 bolesnika (28,6%) bila odsutna; kod 21 bolesnika (25%) ova analiza nije bila rađena (Tabela 1). U grupi sa PV, 31 bolesnik (59,6%) imao je JAK2V617F mutaciju, 5 bolesnika (9,6%) nije imalo, dok kod 16 bolesnika (30,7%) analiza nije bila sprovedena. Kod bolesnika sa ET, prisustvo JAK2V617F mutacije utvrđeno je kod 6 bolesnika (28,6%), dok je 10 bolesnika (47,6%) bilo negativno, a kod 5 bolesnika (23,8%) ova mutacije nije ispitivana. Svim bolesnicima sa prePMF je rađena ova analiza, pri čemu je kod 2 bolesnika (18,2%) dokazano prisustvo mutacije, dok je 9 bolesnika (81,8%) bilo negativno.

Mikrovaskularne simptome pri dijagnozi imalo je 23 bolesnika (27,4%), glavobolju 19 bolesnika (22,6%),

2000 to 2024. The diagnosis was made according to current WHO criteria [17].

The following data were collected from the medical documentation: 1) demographic characteristics of the patient; 2) presence of JAK2V617F mutation; 3) symptoms; 4) presence of palpable splenomegaly; 5) blood count parameters; 6) values of lactate dehydrogenase; 7) degree of bone marrow fibrosis; 8) cardiovascular (CVD) risk factors; 9) presence and type of previous thrombosis and thrombosis during follow-up; 10) treatment modalities; 11) prognostic scores (ELN score, *International Prognostic Score of Thrombosis in WHO – ET (IPSET Thrombosis)*, *International Prognostic Score for ET: Predicts Survival (IPSET survival)*, *International Prognostic Scoring System (IPSS)*); 12) length of survival; 13) cause of death.

Descriptive statistical parameters and categorical data are shown as absolute and relative frequencies, and numerical data, considering that they did not have a normal distribution, are shown as median and range (Min-Max). The chi-square test was used to test the differences in frequency, and the Kruskal-Wallis and Mann-Whitney tests were used to test the differences in numerical data values. The criterion for statistical significance was $p < 0.05$. Data were processed using the SPSS Statistics 22 software program (SPSS Inc., Chicago, IL, USA).

RESULTS

The study included 84 patients aged up to 40 years with a confirmed diagnosis of the disease from the Philadelphia group of negative myeloproliferative neoplasms (Ph-MPN). Polycythemia vera was present in 52 patients (61.9%), essential thrombocythemia in 21 patients (25%), and prefibrotic phase of primary myelofibrosis in 11 (13.1%). No patient had a manifest phase of PMF. The group consisted predominantly of women, 51 or so (60.7%), while 33 respondents (39.3%) were men (ratio $F : M = 1.55:1$). The median age of the patients at the time of diagnosis was 33 years (from 14 to 40 years) (Table 1).

JAK2V617F mutation was found by the Allele-specific PCR method in 39 patients (46.4%), while it was absent in 24 patients (28.6%); this analysis was not performed in 21 patients (25%) (Table 1). In the group with PV, 31 patients (59.6%) had the JAK2V617F mutation, 5 patients (9.6%) did not, while in 16 patients (30.7%), the analysis was not performed. In patients with ET, the presence of the JAK2V617F mutation was confirmed in 6 patients (28.6%), while 10 patients (47.6%) were negative, and in 5 patients (23.8%), this mutation was not examined. This analysis was performed on all patients with prePMF, whereby the presence of the mutation was proven in 2 patients (18.2%), while 9 patients (81.8%) were negative.

Tabela 1. Kliničke i laboratorijske karakteristike mladih bolesnika sa MPN

Table 1. Clinical and laboratory characteristics of young MPN patients

Varijabla / Variable	n (%)
Uzrast (godine), medijana (opseg) / Age (years), median (range)	33 (14-40)
Pol (Ž/M) / Gender (F/M)	51 (60.7%) / 33 (39.3%)
Tip MPN / MPN type	
PV	52 (61.9%)
ET	21 (25.0%)
prePMF /	11 (13.1%)
JAK2V617F mutacija / JAK2V617F mutation	
Pozitivna / Positive	39 (46.4%)
Negativna / Negative	24 (28.6%)
Nije urađena / Not done	21 (25.0%)
Simptomi / Symptoms	
Svrab / Itching	12 (14.3%)
Mikrovaskularni simptomi / Microvascular symptoms	23 (27.4%)
Glavobolja / Headache	19 (22.6%)
Konstitucionalni simptomi / Constitutional symptoms	6 (7.1%)
Palpabilna splenomegalija / Palpable splenomegaly	38 (45.2%)
Povišen LDH / High LDH, U/L	43 (51.2%)
Stepen fibroze u koštanoj srži / Bone marrow fibrosis	
MF-0 / MF-0	24 (28.5%)
MF-1 / MF-1	46 (54.8%)
MF-2 / MF-2	4 (4.8%)
Nepoznato / Unknown	10 (11.9%)
Kardiovaskularni faktori rizika / Cardiovascular risk factors	
HT / HT	8 (9.5%)
Pušenje / Smoking	20 (23.8%)
HT+ HLP / HT+ HLP	2 (2.4%)
Pušenje, HT / Smoking, HT	4 (4.8%)
Pušenje HT, HLP / Smoking, HT, HLP	1 (1.2%)
Prethodne tromboze / History of thrombosis	14 (16.7%)
Tromboze u toku praćenja / Thrombosis during follow-up	7 (8.3%)
Praćenja (meseći) / Follow-up (months)	72 (3-288)
Smrtni ishod / Death	2 (2.4%)

Numerički podaci su prikazani kao medijana (opseg Min-Max) a kategorijalni podaci kao broj pacijenata i procenat, n (%)

MPN – mijeloproliferativna neoplazma, PV – policitemija vera, ET – esencijalna trombocitemija, prePMF – prefibrotična faza primarne mijelofibroze, LDH – laktat dehidrogenaza, HT – hipertenzija, HLP – hiperlipidemija

Numerical data are presented as median (Min-Max range) and categorical data as number of patients and percentage, n (%)

MPN - myeloproliferative neoplasm, PV - polycythemia vera, ET - essential thrombocythemia, prePMF - prefibrotic phase of primary myelofibrosis, LDH - lactate dehydrogenase, HT - hypertension, HLP - hyperlipidemia

svrab 12 bolesnika (14,3%), dok su konstitucione tegobe bile prisutne kod 6 bolesnika (7,1%). Palpabilna splenomegalija nađena je kod 38 bolesnika (45,2%) (Tabela 1).

U ispitivanoj grupi bolesnika vrednosti hemoglobina ($p < 0,001$), hematokrita ($p < 0,001$), MCV ($p = 0,016$)

Microvascular symptoms at diagnosis were present in 23 patients (27.4%), headache in 19 patients (22.6%), itching in 12 patients (14.3%), and constitutional complaints were present in 6 patients (7.1%). Palpable splenomegaly was found in 38 patients (45.2%) (Table 1).

Tabela 2. Parametri krvne slike po tipovima MPN kod mladih bolesnika**Table 2.** Blood count parameters by types of MPN in young patients

Parametri / Parameters medijana (opseg) / median (range)	Tip MNP / Type MNP			p	p-vrednost između grupa / p-value between groups		
	PV	ET	prePMF		PV-ET	PV-prePMF	ET-prePMF
Hemoglobin / Hemoglobin	170.0 (140.0-316.0)	137.5 (123.0-165.0)	132.0 (98.0-161.0)	<0.001	<0.001	<0.001	0.670
Hematokrit / Hematocrit	50.0 (41.4-66.0)	41.7 (34.0-48.0)	38.8 (29.6-45.1)	<0.001	<0.001	<0.001	0.413
MCV / MCV	84.0 (68.0-100.0)	91.0 (70.0-98.0)	88.0 (65.0-92.0)	0.016	0.007	0.203	0.148
Br. Leukocita / No. Leukocyte WBC count	9.9 (5.5-22.3)	8.0 (5.4-67.0)	8.6 (7.2-15.1)	0.086	0.042	0.217	0.502
Br. Trombocita / No. Thrombocytes PLT count	737.5 (166-1122)	856.0 (553-1748)	1007.0 (461-1876)	0.003	0.018	0.003	0.381

Vrednosti su prikazane kao medijana i opseg (Min-Maks), boldirano- statistički značajna razlika ($p < 0.05$)

PV – policitemija vera, ET – esencijalna trombocitemija, prePMF – prefibrotična faza primarne mijelofibroze, MCV – prosečna zapremina eritrocita, WBC – leukociti, PLT – trombociti

Values are shown as median and range (Min-Max), bold - statistically significant difference ($p < 0.05$)

PV - polycythemia vera, ET - essential thrombocythemia, prePMF - prefibrotic phase of primary myelofibrosis, MCV – mean corpuscular volume, WBC - leukocytes, PLT - platelets Hemoglobin (g/L), Hematocrit (%), MCV (f/L), No. Leukocyte ($\times 10^9/L$), No. Thrombocytes ($\times 10^9/L$)

i trombocita ($p = 0,003$) statistički su se značajno razlikovale prema grupama ispitanika, dok vrednosti leukocita nisu pokazale značajnu razliku ($p = 0,086$). Vrednosti hemoglobina bile su značajno veće u grupi pacijenata sa PV, 170,0 g/L (opseg 140,0-316,0 g/L), u odnosu na grupu ET, 137,5 g/L (raspon 123,0-165,0 g/L), ($p < 0,001$), kao i u odnosu na grupu prePMF, 132,0 g/L (raspon 98,0-161,0 g/L), ($p < 0,001$), dok se vrednosti hemoglobina nisu razlikovale između ET i prePMF ($p = 0,670$). Vrednosti hematokrita takođe su bile značajno više u grupi pacijenata sa PV, 50,0% (raspon 41,4-66,0%), u odnosu na grupu ET, 41,7% (raspon 34,0-48,0%), ($p < 0,001$), kao i u odnosu na grupu prePMF, 38,8% (raspon 29,6-45,1%) ($p < 0,001$), dok se vrednosti hematokrita nisu razlikovale između ET i prePMF ($p = 0,413$).

Vrednosti MCV-a bile su statistički značajno veće kod pacijenata sa ET, 91,0 f/L (raspon 70,0-98,0 f/L), u odnosu na grupu sa PV, 84,0 f/L (raspon 68,0-100,0 f/L), ($p < 0,001$), dok između ispitanika ostalih grupa nije postojala značajna razlika. Vrednosti trombocita bile su značajno niže u grupi pacijenata sa PV, $737,5 \times 10^9/L$ (raspon 166,0-1122,0 $\times 10^9/L$), u odnosu na grupu ET, $856,0 \times 10^9/L$ (raspon 553,0-1748,0 $\times 10^9/L$), ($p = 0,018$), kao i u odnosu na grupu prePMF $1007,0 \times 10^9/L$ (raspon 461,0-1876,0 $\times 10^9/L$), ($p = 0,003$), dok se vrednosti trombocita nisu razlikovale između ET i prePMF ($p = 0,381$) (Tabela 2).

U celoj grupi bolesnika, vrednost laktat-dehidrogenaze (LDH) bila je povišena kod 43 (51,2%). Eritropoetin je bio snižen kod 25 bolesnika (29,8%), normalan kod

In the examined group of patients, the values of hemoglobin ($p < 0.001$), hematocrit ($p < 0.001$), MCV ($p = 0.016$), and platelets ($p = 0.003$) statistically significantly differed according to the groups of subjects, while the values of leukocytes did not show a significant difference ($p = 0.086$). Hemoglobin values were considerably higher in the group of PV patients, 170.0 g/L (range 140.0-316.0 g/L), compared to the ET group, 137.5 g/L (range 123.0- 165.0 g/L), ($p < 0.001$), as well as in relation to the prePMF group, 132.0 g/L (range 98.0-161.0 g/L), ($p < 0.001$), while the values hemoglobin did not differentiate between ET and prePMF ($p = 0.670$). Hematocrit values were also significantly higher in the PV group, 50.0% (range 41.4-66.0%), compared to the ET group, 41.7% (range 34.0-48.0%), ($p < 0.001$), as well as in relation to the prePMF group, 38.8% (range 29.6-45.1%) ($p < 0.001$), while hematocrit values did not differ between ET and prePMF ($p = 0.413$).

MCV values were statistically significantly higher in ET patients, 91.0 f/L (range 70.0-98.0 f/L), compared to the PV group, 84.0 f/L (range 68.0-100.0 f/L), ($p < 0.001$), while there was no significant difference between the subjects of the other groups. Platelet values were significantly lower in the PV group, $737.5 \times 10^9/L$ (range 166.0-1122.0 $\times 10^9/L$), compared to the ET group, $856.0 \times 10^9/L$ (range 553.0- 1748.0 $\times 10^9/L$), ($p = 0.018$), as well as in relation to the prePMF group $1007.0 \times 10^9/L$ (range 461.0-1876.0 $\times 10^9/L$), ($p = 0.003$), while platelet values did not differentiate between ET and prePMF ($p = 0.381$) (Table 2).

In the entire group of patients, the value of lactate dehydrogenase (LDH) was elevated in 43 (51.2%).

Tabela 3. Tromboze i KV faktori rizika kod mladih bolesnika sa MPN

	Dijagnoza MPN / MPN diagnosis			p
	PV	ET	prePMF	
Ukupan broj (%) / Total number (%)	52 (61.9%)	21 (25.0%)	11 (13.1%)	
Prethodne tromboze / History of thrombosis	11 (21.2%)	1 (4.8%)	2 (18.2%)	0.233
Arterijske / Arterial				
TIA /	0 (0)	1 (4.8%)	0 (0)	
AIM /	3 (5.8%)	0 (0)	0 (0)	
CVI /	2 (3.8%)	0 (0)	0 (0)	
Periferna arterijska tromboza / Peripheral arterial thrombosis	1 (1.9%)	0 (0)	0 (0)	
Venske / Venous				
DVT /	1 (1.9%)	0 (0)	0 (0)	
PE /	1 (1.9%)	0 (0)	0 (0)	
CVT /	1 (1.9%)	0 (0)	0 (0)	
PVT /	1 (1.9%)	0 (0)	2 (18.2)	
Budd-Chiari /	1 (1.9%)	0 (0)	0 (0)	
Tromboze tokom praćenja / Thrombosis during follow-up	7 (13.5%)	0 (0)	0 (0)	
Tromboza u levoj komori srca / Left ventricle thrombosis	1 (1.9%)	0 (0)	0 (0)	
AIM	2 (3.8%)	0 (0)	0 (0)	
CVI	1 (1.9%)	0 (0)	0 (0)	
Venske / Venous				
DVT	3 (5.8%)	0 (0)	0 (0)	
Broj KV faktora rizika / Number of CV risk factors				
0	27 (51.9%)	14 (66.7%)	8 (72.8%)	0.293
1	20 (38.5%)	5 (23.8%)	3 (27.3%)	
2	4 (7.7%)	2 (9.5%)	0 (0)	
3	1 (1.9%)	0 (0)	0 (0)	

Kategorijalni podaci su prikazani kao broj pacijenata i procenat n (%)

TIA – tranzitorni ishemijski atak, AIM – akutni infarkt miokarda, CVI – cerebrovaskularni insult, DVT – duboka venska tromboza, PE – plućna embolija, CVT – centralna venska tromboza, PVT – tromboza vene porte, MPN – mijeloproliferativna neoplazma, PV – policitemija vera, ET – esencijalna trombocitemija, prePMF – prefibrotična faza primarne mijelofibroze

14 bolesnika (16,7%), dok kod 45 bolesnika (53,6%) nije rađen. Step en fibroze u koštanoj srži je bio MF 0 kod 24 bolesnika (28,5%), MF 1 kod 46 bolesnika (54,8%), MF 2 kod 4 bolesnika (4,8%), a kod 10 bolesnika (11,9%) nisu bili dostupni podaci (Tabela 1).

Kardiovaskularne faktore rizika imalo je 35 bolesnika (41,7%), od čega je 8 bolesnika (9,5%) imalo hipertenziju, 20 bolesnika (23,8%) su bili pušači, udruženu pojavu hipertenzije i hiperlipidemije je imalo 2 bolesnika (2,4%), pušača koji su istovremeno imali i hipertenziju bilo je 4 (4,8%), dok su sva tri faktora rizika bila prisutna kod samo 1 bolesnika (1,2%). Raspodela prema dijagnozama u odnosu na broj kardiovaskularnih rizika bila je sledeća: 27 bolesnika sa PV (51,9%), 14 bolesnika sa ET (66,7%) i 8 bolesnika sa prePMF (72,8%)

Table 3. Thrombosis and CV risk factors in young patients with MPN

	Dijagnoza MPN / MPN diagnosis			p
	PV	ET	prePMF	
Ukupan broj (%) / Total number (%)	52 (61.9%)	21 (25.0%)	11 (13.1%)	
Prethodne tromboze / History of thrombosis	11 (21.2%)	1 (4.8%)	2 (18.2%)	0.233
Arterijske / Arterial				
TIA /	0 (0)	1 (4.8%)	0 (0)	
AIM /	3 (5.8%)	0 (0)	0 (0)	
CVI /	2 (3.8%)	0 (0)	0 (0)	
Periferna arterijska tromboza / Peripheral arterial thrombosis	1 (1.9%)	0 (0)	0 (0)	
Venske / Venous				
DVT /	1 (1.9%)	0 (0)	0 (0)	
PE /	1 (1.9%)	0 (0)	0 (0)	
CVT /	1 (1.9%)	0 (0)	0 (0)	
PVT /	1 (1.9%)	0 (0)	2 (18.2)	
Budd-Chiari /	1 (1.9%)	0 (0)	0 (0)	
Tromboze tokom praćenja / Thrombosis during follow-up	7 (13.5%)	0 (0)	0 (0)	
Tromboza u levoj komori srca / Left ventricle thrombosis	1 (1.9%)	0 (0)	0 (0)	
AIM	2 (3.8%)	0 (0)	0 (0)	
CVI	1 (1.9%)	0 (0)	0 (0)	
Venske / Venous				
DVT	3 (5.8%)	0 (0)	0 (0)	
Broj KV faktora rizika / Number of CV risk factors				
0	27 (51.9%)	14 (66.7%)	8 (72.8%)	0.293
1	20 (38.5%)	5 (23.8%)	3 (27.3%)	
2	4 (7.7%)	2 (9.5%)	0 (0)	
3	1 (1.9%)	0 (0)	0 (0)	

Categorical data are presented as the number of patients and percentage n (%)

TIA - transient ischemic attack, AIM - acute myocardial infarction, CVI - cerebrovascular insult, DVT - deep vein thrombosis, PE - pulmonary embolism, CVT - central venous thrombosis, PVT - portal vein thrombosis, MPN - myeloproliferative neoplasm, PV - polycythemia vera, ET - essential thrombocythemia, prePMF - prefibrotic phase of primary myelofibrosis

Erythropoietin was decreased in 25 patients (29.8%), normal in 14 patients (16.7%), while in 45 patients (53.6%), it was not done. The degree of fibrosis in the bone marrow was MF 0 in 24 patients (28.5%), MF 1 in 46 patients (54.8%), MF 2 in 4 patients (4.8%), and in 10 patients (11.9%) no data were available (Table 1).

Cardiovascular risk factors were present in 35 patients (41.7%), of which 8 patients (9.5%) had hypertension, 20 patients (23.8%) were smokers, 2 patients had the combined occurrence of hypertension and hyperlipidemia (2.4%), there were 4 (4.8%) smokers who also had hypertension, while all three risk factors were present in only 1 patient (1.2%). The distribution according to diagnoses in relation to the number of cardiovascular risks was as follows: 27 patients with PV

Tabela 4. Terapijski pristup i stratifikacija prema ELN skor mladih bolesnika sa MPN

	Dijagnoza MPN /MPN diagnosis			p
	PV	ET	prePMF	
Ukupan broj (%) / Total number (%)	52 (61.9%)	21 (25.0%)	11 (13.1%)	
Terapija / Therapy				
ASA / ASA	43 (82.7%)	20 (95.2%)	10 (90.9%)	0.325
VKA / VKA	1 (1.9%)	0 (0)	1 (9.1%)	
DOAK / DOAC	1 (1.9%)	0 (0)	0 (0)	
ASA+OAK / ASA+ OAC	3 (5.8%)	0 (0)	0 (0)	
Flebotomije / Phlebotomy	50 (96.2%)	3 (14.3%)	0 (0%)	<0.001
Citoreduktivna terapija / Cytoreductive treatment	24 (46.1%)	9 (42.9%)	4 (36.4%)	0.831
Standardni ELN skor / Standard ELN score				
Nizak rizik / Low risk	40 (76.9%)	20 (95.2%)	9 (81.8%)	0.181
Visok rizik / High risk	12 (23.1%)	1 (4.8%)	2 (18.2%)	
Prisutni KV faktori rizika / CV risk factors	25 (48.1%)	7 (33.0%)	3 (27.3%)	0.299

Kategorijalni podaci su prikazani kao broj pacijenata i procenat n (%), Podebljano - statistički značajna razlika učestalosti

ASA – acetilsalicilna kiselina, VKA – antagonisti vitamina K, DOAK – direktni oralni antikoagulant, OAK – oralni antikoagulant, ELN – European LeukemiaNet, KV – kardiovaskularni, PV – policitemija vera, ET – esencijalna trombocitemija, prePMF – prefibrotična faza primarne mijelofibroze

nisu imali KV faktore rizika, jedan KV faktor rizika je imalo 20 bolesnika sa PV (28,5%), 5 sa ET (23,8%) i 3 sa prePMF (27,3%), 2 KV faktora rizika su imali 4 bolesnika sa PV (7,7%), 2 sa ET (9,5%) i ni jedan bolesnik sa prePMF, dok je jedan bolesnik sa PV je imao 3 KV faktora rizika (Tabela 1, Tabela 3).

Prethodne tromboze su bile prisutne kod 14 bolesnika (16,7%), dok su tromboze u toku praćenja nastale kod 7 bolesnika (8,3%). Najviše tromboza pre dijagnoze MPN imali su bolesnici sa PV, 11 bolesnika (21,2%), dok su samo 1 bolesnik sa ET (4,8%) i 2 bolesnika sa prePMF (18,2%) imali prethodnu trombozu. Učestalost tromboza pre dijagnoze MPN se nije statistički značajno razlikovala prema grupama ispitanika ($p = 0,233$). Od 11 bolesnika sa PV, 3 (5,8%) su imala akutni infarkt miokarda, 2 (3,8%) moždani udar, dok je po jedan (1,9%) bolesnik imao perifernu arterijsku trombozu, DVT, PE, CVT, PVT, Budd Chiari. Bolesnik sa dijagnozom ET je imao tranzitorni ishemijski atak, dok su oba bolesnika sa prePMF imala PVT. U toku praćenja tromboze su isključivo nađene kod bolesnika sa PV, od čega su 3 (5,8%) bolesnika imala DVT, 2 (3,8%) bolesnika AIM, dok je po jedan (1,9%) bolesnik imao CVI ili tromb u levoj komori srca. Broj KV faktora rizika se nije značajno razlikovao između dijagnostičkih grupa pacijenata, ($p = 0,293$) (Tabela 3).

U celoj grupi bolesnika, 73 (86,9%) su bili na terapiji ASA, 2 (2,4%) na terapiji VKA, jedan (1,2%) na DOAK, a 3 bolesnika (3,6%) na kombinaciji ASA i OAK. U okviru PV,

Table 4. Therapeutic approach and stratification according to the ELN score of young MPN patients

Categorical data are presented as number of patients and percentage n (%), Bold - statistically significant difference in frequency

ASA - acetylsalicylic acid, VKA - vitamin K antagonists, DOAC – direct oral anticoagulants, OAC - oral anticoagulants, ELN - European LeukemiaNet, CV - cardiovascular, PV - polycythemia vera, ET - essential thrombocythemia, prePMF - prefibrotic phase of primary myelofibrosis

(51.9%), 14 patients with ET (66.7%) and 8 patients with prePMF (72.8%) had no CV risk factors, one CV risk factor was present in 20 patients with PV (28.5%), 5 with ET (23.8%) and 3 with prePMF (27.3%), 2 CV risk factors were present in 4 patients with PV (7.7%), 2 with ET (9.5%) and no patient with prePMF, while one patient with PV had 3 CV risk factors (Table 1, Table 3).

Previous thromboses were present in 14 patients (16.7%), while thrombosis occurred during follow-up in 7 patients (8.3%). Patients with PV had the most thrombosis before the diagnosis of MPN, 11 patients (21.2%), while only 1 patient with ET (4.8%) and 2 patients with prePMF (18.2%) had previous thrombosis. The frequency of thrombosis before the diagnosis of MPN was not statistically significantly different according to the groups of subjects ($p = 0.233$). Out of 11 patients with PV, 3 (5.8%) had an acute myocardial infarction, 2 (3.8%) had a stroke, while one (1.9%) patient each had peripheral arterial thrombosis, DVT, PE, CVT, PVT, Budd Chiari. The patient diagnosed with ET had a transient ischemic attack, while both patients with prePMF had PVT. During follow-up, thromboses were exclusively found in patients with PV, of which 3 (5.8%) patients had DVT, 2 (3.8%) patients had AMI, while one (1.9%) patient had CVI or thrombus in the left ventricle of the heart. The number of CV risk factors did not differ significantly between diagnostic groups of patients ($p = 0.293$) (Table 3).

43 (82,7%) bolesnika je lečeno ASA, po jedan (1,9%) bolesnik sa VKA i DOAK, dok su tri bolesnika lečena prethodno pomenutom kombinacijom ASA i OAK. Među bolesnicima se ET, 20 (95,2%) je lečeno ASA i nije bilo bolesnika lečenih drugim tipom antitrombozne terapije. U grupi bolesnika sa prePMF, 10 (90,9%) je bilo na terapiji ASA, dok je jedan bio na terapiji VKA. Primena ASA u terapiji nije se značajno razlikovala između posmatranih grupa pacijenata ($p = 0,325$). Ukupno, 53 bolesnika (63,1%) je lečeno flebotomijama, od čega su 50 bili bolesnici sa PV, dok je 3 bolesnika imalo ET. Lečenje flebotomijom je značajno učestalije u grupi pacijenata sa PV ($p < 0,001$). Citoreduktivnom terapijom je lečeno 37 bolesnika (43,9%), dok je raspodela prema dijagnozama bila: 24 bolesnika (46,1%) u grupi sa PV, 9 (42,9%) u grupi sa ET i 4 (36,4%) u grupi sa prePMF, bez značajne razlike u primeni citoreduktivne terapije između grupa pacijenata ($p = 0,831$). U celoj grupi bolesnika, 33 bolesnika (39,1%) je lečeno jednom terapijskom linijom, dok su 4 bolesnika (4,8%) primala dve terapijske linije. Hidroksurea (HU) je bila prva linija terapije kod 31 bolesnika (36,1%), dok je 6 bolesnika (7,2%) incijalno lečeno interferonom. U drugoj terapijskoj liniji, 3 bolesnika su dobijala HU (3,6%), dok su po dva bolesnika dobijala INF (2,4%) ili ruxolitinib (2,4%) (Tabela 4).

Od ukupnog broja bolesnika, prema standardnom ELN skor, visokom riziku je pripadalo 15 (17,9%). Raspodela u okviru dijagnoza, u odnosu na ELN skor, bila je sledeća: 12 bolesnika (23,1%) sa PV, 1 bolesnik (4,8%) sa ET i 2 bolesnika (18,2%) sa prePMF pripadali su grupi visokog rizika. Učestalost visokorizičnih pacijenata prema standardnom ELN skor nije se značajno razlikovala između dijagnoza ($p = 0,181$). Od ukupnog broja bolesnika koji su primali citoreduktivnu terapiju, njih 10 (26,7%) je bilo klasifikovano kao visokorizično. U grupi bolesnika sa ET, prema IPSET survival skor, 16 bolesnika je pripadalo grupi niskog rizika, 4 bolesnika intermedijernoj grupi, a prema IPSET thrombosis skor, 11 bolesnika je pripalo grupi niskog, a 7 bolesnika intermedijernoj grupi rizika. U grupi bolesnika sa prePMF, prema IPSS skor, 8 bolesnika je pripadalo grupi niskog, a 3 bolesnika grupi intermedijernog-1 rizika. Prethodno navedeni skorovi korišteni za bolesnike sa ET i prePMF nisu identifikovali visokorizične bolesnike u našoj kohorti (Tabela 4).

Kod dve bolesnice (2,45%) postojala je pozitivna porodična anamneza u smislu porodičnog prisustva prethodne MPN. Kod jedne bolesnice sa PV, majka je tekođe imala PV, a kod druge bolesnice sa prePMF, stric je imao ET.

Transformacija u MF dogodila se kod dve bolesnice koje su imale PV (post-PV MF), što čini 2,45% od ukupnog broja bolesnika. Vreme do fibrotične progresije je iznosilo 6 i 11,5 godina.

In the entire group of patients, 73 (86.9%) were on ASA therapy, 2 (2.4%) on VKA therapy, one (1.2%) on DOAK, and 3 patients (3.6%) on a combination of ASA and OAK. Within PV, 43 (82.7%) patients were treated with ASA, one (1.9%) patient each with VKA and DOAC, and three patients were treated with the combination mentioned above of ASA and OAK. Among the patients with ET, 20 (95.2%) were treated with ASA, and no patients were treated with another type of antithrombotic therapy. In the group of patients with prePMF, 10 (90.9%) were on ASA therapy, while one was on VKA therapy. The use of ASA in treatment did not differ significantly between the observed groups of patients ($p = 0.325$). In total, 53 patients (63.1%) were treated with phlebotomies, of which 50 were patients with PV, while 3 patients had ET. Phlebotomy treatment was significantly more frequent in the group of patients with PV ($p < 0.001$). 37 patients (43.9%) were treated with cytoreductive therapy, while the distribution according to diagnosis was: 24 patients (46.1%) in the group with PV, 9 (42.9%) in the group with ET, and 4 (36.4%) in the group with prePMF, with no significant difference in the application of cytoreductive therapy between patient groups ($p = 0.831$). In the entire group of patients, 33 patients (39.1%) were treated with one therapeutic line, while 4 patients (4.8%) received two therapeutic lines. Hydroxyurea (HU) was the first line of therapy in 31 patients (36.1%), while 6 patients (7.2%) were initially treated with interferon. In the second therapeutic line, 3 patients received HU (3.6%), while two patients each received INF (2.4%) or ruxolitinib (2.4%) (Table 4).

Of the total number of patients, according to the standard ELN score, 15 (17.9%) were at high risk. The distribution within the diagnoses, in relation to the ELN score, was as follows: 12 patients (23.1%) with PV, 1 patient (4.8%) with ET, and 2 patients (18.2%) with prePMF belonged to the group high risk. The frequency of high-risk patients according to the standard ELN score did not differ significantly between diagnoses ($p = 0.181$). Of the total number of patients who received cytoreductive therapy, 10 of them (26.7%) were classified as high-risk. In the group of patients with ET, according to the IPSET survival score, 16 patients belonged to the low-risk group, 4 patients to the intermediate group, and according to the IPSET thrombosis score, 11 patients belonged to the low-risk group, and 7 patients to the intermediate risk group. In the group of patients with prePMF, according to the IPSS score, 8 patients belonged to the group of low, and 3 patients to the group of intermediate-1 risk. The aforementioned scores used for patients with ET and prePMF did not identify high-risk patients in our cohort (Table 4).

Medijana praćenja bolesnika je iznosila 72 meseca (3-288 meseci). Dva bolesnika (2,45%) su tokom praćenja preminula, a uzrok smrtnog ishoda kod jednog su bile posttransplantacione komplikacije nakon alogene transplantacije matičnih ćelija hematopoeze, odnosno sekundarni malignitet, karcinom jetre kod drugog (Tabela 1).

DISKUSIJA

Najčešće dijagnostikovana Filadelfija negativna (Ph-) MPN kod mladih bolesnika je esencijalna trombocitemija (ET). Ovaj tip MPN najčešće se otkriva kod ispitanika ženskog pola, što objašnjava nalaz da su među mladim bolesnicima sa MPN pretežno žene (65-71%), dok je kod starijih zastupljenost ženskog pola manja i iznosi 46-58% [10,11]. U našoj kohorti, iako je češće nađena policitemija vera (PV), i dalje je dominantan ženski pol, jer žene čine skoro dve trećine ukupnog broja bolesnika.

Medijana starosti na dijagnozi u studijama sa mladim bolesnicima sa MPN kreće se između 20 i 36 godina, što je u skladu sa našim podacima gde je medijana bila 33 godine, sa rasponom od 14 do 40 godina. Ove studije su uključile bolesnike mlađe od 45 godina starosti [10,18,19].

Pacijenti mlađe životne dobi sa MPN na dijagnozi su uglavnom bez tegoba (asimptomatski), slično kao i stariji [18]. Međutim, prema podacima Sobas i saradnika, više od jedne trećine bolesnika je imalo simptome zbog hiperviskoznosti [13]. U našoj studiji, skoro trećina bolesnika imala je mikrovaskularne tegobe, koje su bile najzastupljenije. Na drugom mestu je bio svrab, a manje od 10% bolesnika je imalo konstitucione tegobe. Prema podacima iz literature, koji su nisu brojni, bolesnici sa PV češće imaju na dijagnozi konstitucione simptome, 9,2% u odnosu na 3,9% kod bolesnika sa ET [13]. Splenomegalija je nađena kod skoro polovine naših ispitanika. Prema Szuber i saradnicima, povećan promer slezine bio je prisutan kod većeg procenta mladih od 40 godina u odnosu na bolesnike starije od 60 godina [14]. Ovo je naročito značajno, uzimajući u obzir da je splenomegalija identifikovana kao faktor rizika za nastanak komplikacija i progresije bolesti, uključujući transformaciju, krvarenje i trombozu kod ove grupe bolesnika [12,13].

Kao što je i očekivano, bolesnici sa PV su na dijagnozi imali značajno više vrednosti hemoglobina i hematokrita u poređenju sa bolesnicima sa ET i prePMF ($p < 0,001$). Medijana hemoglobina kod PV bolesnika je bila 170 g/L, dok je kod bolesnika sa ET i prePMF ona iznosila 137,5 g/L i 132 g/L. Hematokrit je takođe bio najviši kod PV bolesnika, sa medijanom od 50,0%, u poređenju sa 41,7% kod ET i 38,8% kod prePMF. Tromboci-

In two patients (2.45%), there was a positive family history in terms of family presence of previous MPN. In one patient with PV, the mother also had PV, and in another patient with prePMF, the uncle had ET.

Two patients with PV (post-PV MF) were transformed to MF, accounting for 2.45% of the total number of patients. The time to fibrotic progression was 6 and 11.5 years.

Median patient follow-up was 72 months (3-288 months). Two patients (2.45%) died during follow-up, and the cause of death in one was post-transplantation complications after allogeneic hematopoietic stem cell transplantation, i.e., secondary malignancy and liver cancer in the other (Table 1).

DISCUSSION

The most frequently diagnosed Philadelphia-negative (Ph-) MPN in young patients is essential thrombocythemia (ET). This type of MPN is most often detected in female respondents, which explains the finding that among young patients with MPN, women are predominantly female (65-71%), while in the elderly, the representation of the female gender is lower and amounts to 46-58% [10,11]. In our cohort, although polycythemia vera (PV) was found more often, the female gender is still dominant, as women make up almost two-thirds of the total number of patients.

The median age at diagnosis in studies with young patients with MPN ranges between 20 and 36 years, which is consistent with our data, where the median was 33 years, with a range of 14 to 40 years. These studies included patients younger than 45 years of age [10,18,19].

Younger patients diagnosed with MPN are generally asymptomatic, similar to the elderly [18]. However, according to the data of Sobas et al., more than one-third of patients had symptoms due to hyperviscosity [13]. In our study, almost a third of patients had microvascular problems, which were the most prevalent. In second place was itching; less than 10% of patients had constitutional complaints. According to data from the literature, which are not numerous, patients with PV more often have constitutional symptoms at diagnosis, 9.2% compared to 3.9% in patients with ET [13]. Splenomegaly was found in almost half of our subjects. According to Szuber et al., an increased spleen diameter was present in a higher percentage of patients younger than 40 years compared to patients older than 60 years [14]. This is particularly significant, considering that splenomegaly has been identified as a risk factor for complications and disease progression, including transformation, bleeding, and thrombosis in this group of patients [12,13].

toza je uočena kod svih ispitivanih MPN entiteta, ali su postojale značajne razlike u broju trombocita između pacijenata sa PV i ET ($p = 0,018$), kao i između PV i pre-PMF ($p = 0,003$). Najveći broj trombocita su imali bolesnici sa prePMF (medijana $1007 \times 10^9/L$), zatim bolesnici sa ET (medijana $856 \times 10^9/L$), dok su PV bolesnici imali najmanji stepen trombocitoze (medijana $737,5 \times 10^9/L$). Prema podacima iz literature, u grupi mladih bolesnika bila je zapažena trombocitoza većeg stepena u odnosu na starije što bi se moglo objasniti povećanom incidencijom CALR mutacije u ovoj grupi, uzimajući u obzir da je ova mutacija povezana sa trombocitozom [2,11,18]. U našoj kohorti je isključivo ispitivana JAK2V617F mutacija, koja je bila pozitivna kod oko polovine bolesnika. LDH je bio povišen kod oko polovine bolesnika. Kod najvećeg broja bolesnika fibroza je odgovarala MF-1 sa više od 50%, dok su tek dva bolesnika su imali MF-2.

Prema podacima iz literature, približno 10% bolesnika sa MPN pokazuje familijarnu povezanost (klasterizaciju), što ukazuje na značaj naslednih komponenti u razvoju bolesti [14]. Učestalost familijarnih MPN je veća kod mladih bolesnika u donosu na starije bolesnike (19% vs 9%) [10]. Ovo je takođe očekivano, s obzirom i na raniju pojavu bolesti u mlađoj grupi bolesnika. U našoj kohorti, kod dva bolesnika je uočena porodična pojava MPN (2,45%), što se može objasniti relativno malom grupom ispitanika.

Vaskularne komplikacije su značajan rizik, naročito za bolesnike sa PV, a javljaju se kako pre postavljanja dijagnoze, tako i tokom daljeg prirodnog toka bolesti [11,20,21]. Dokazano je da su povezane sa dijagnozom MPN i da nekada mogu biti i prva manifestacija bolesti zbog koje se bolesnici javljaju hematologu [22,23]. U literaturi se nalazi da su prethodne tromboze bile prisutne kod 14-27% bolesnika sa PV, 6-13% bolesnika sa ET i 7-16% sa PMF [2,11,13,18,19]. Ovi podaci u velikoj meri odgovaraju zastupljenosti prethodnih tromboza u okviru različitih MPN dijagnoza i u našem istraživanju. Od svih prethodnih tromboza u našoj kohorti, najčešće su bile zastupljene tromboze splanhične cirkulacije sa 28,5% od ukupnog broja, dok je zastupljenost arterijskih i venskih tromboza bila jednaka. Drugi najčeći tip tromboze, odnosno tromboznih događaja, bio je akutni infarkt miokarda, koji se javio kod 21,4% bolesnika. Poznato je da MPN dovodi do tromboza na neuobičajenim lokalizacijama, kao što su tromboze splanhične cirkulacije, uključujući Budd-Chiarijev sindrom (BCS), tromboza vene porte (PVT) i tromboza cerebralnih vena [11,22,24]. Tromboze u toku praćena isključivo su bile prisutne kod bolesnika sa PV sa predominacijom arterijskih tromboza koje su činile skoro 2/3. Prema literaturnim podacima kod bolesnika ove uzrasne grupe češće su bile prisutne venske tromboze u toku praće-

As expected, patients with PV had significantly higher hemoglobin and hematocrit values at diagnosis than patients with ET and prePMF ($p < 0.001$). Median hemoglobin in PV patients was 170 g/L, while in patients with ET and prePMF, it was 137.5 g/L and 132 g/L. Hematocrit was also highest in PV patients, with a median of 50.0%, compared to 41.7% in ET and 38.8% in prePMF. Thrombocytosis was observed in all MPN entities examined, but there were significant differences in platelet counts between PV and ET patients ($p = 0.018$) and between PV and prePMF ($p = 0.003$). Patients with prePMF had the highest number of platelets (median $1007 \times 10^9/L$), followed by patients with ET (median $856 \times 10^9/L$), while PV patients had the lowest degree of thrombocytosis (median $737.5 \times 10^9/L$). According to data from the literature, a higher degree of thrombocytosis was observed in the group of young patients compared to the elderly, which could be explained by the increased incidence of the CALR mutation in this group, taking into account that this mutation is associated with thrombocytosis [2,11,18]. In our cohort, the JAK2V617F mutation was exclusively examined, and it was positive in about half of the patients. LDH was elevated in about half of the patients. In the most significant number of patients, fibrosis corresponded to MF-1 with more than 50%, while only two patients had MF-2.

According to data from the literature, approximately 10% of patients with MPN show a familial connection (clustering), which indicates the importance of hereditary components in the development of the disease [14]. The frequency of familial MPN is higher in younger patients than in older patients (19% vs 9%) [10]. This is also expected, considering the earlier onset of the disease in the younger group of patients. In our cohort, a familial occurrence of MPN was observed in two patients (2.45%), which the relatively small group of subjects can explain.

Vascular complications are a significant risk, especially for patients with PV, and occur both before diagnosis and during the further natural course of the disease [11,20,21]. It has been proven that they are related to the diagnosis of MPN and that sometimes, they can be the first manifestation of the disease for which the patients consult a hematologist [22,23]. In the literature, previous thrombosis was present in 14-27% of patients with PV, 6-13% of patients with ET, and 7-16% with PMF [2,11,13,18,19]. These data correspond to previous thromboses' prevalence among different MPN diagnoses and in our research. Of all the previous thromboses in our cohort, the most common was thrombosis of the splanchnic circulation, with 28.5% of the total number, while the prevalence of arterial and venous thrombosis was equal. The second most com-

nja, pa čak češće i u odnosu na starije bolesnike [10]. Autori ovu pojavu objašnjavaju većim brojem tromboza splanhične cirkulacije u toku praćenja, sa 77% od ukupnog broja venskih tromboza [12]. S druge strane, arterijske tromboze su u toku praćenja uglavnom ređe zastupljene kod mlađih bolesnika. Smatra se da je ovo u vezi sa nižom incidencom kardiovaskularnih komorbiditeta i aterosklerotskih promena u krvnim sudovima [14]. Međutim, u našoj kohorti 41,7% bolesnika je imalo KV faktore rizika, pri čemu je pušenje bilo najčešće zastupljeno, a čak 8,3% bolesnika imalo je više od jednog faktora rizika. Taj nalaz može objasniti pojavu češćih arterijskih tromboza u našoj studiji.

Prema ELN preporukama, lečenje bolesnika sa PV je bazirano na riziku od tromboze. Grupi visokog rizika pripadaju bolesnici koji su imali prethodne tromboze ili su stariji od 60 godina, dok nizak rizik podrazumeva odsustvo oba navedena faktora. Primenom ovog scoring sistema, svi bolesnici u grupi mladih pripadaju grupi niskog rizika, osim ako nisu imali prethodne tromboze [16]. Lečenje niskorizičnih bolesnika sa PV podrazumeva primenu flebotomija sa ciljem da Hct bude < 45%, kao i primenu malih doza ASA [16,25]. Pre primene antitrombotične terapije, bitno je testirati bolesnika na stečeni von Willebrandov sindrom kako bi se identifikovali bolesnici sklon krvarenju, imajući u vidu da se on može da javi čak i u odsustvu izražene trombocitoze [26]. U našoj grupi bolesnika sa PV, većina je lečena niskim dozama ASA, sa svega par bolesnika je bilo na na oralnoj antikoagulantnoj terapiji (OAK). Razlog za uvođenje OAK kod skoro svih bolesnika bile su prethodne tromboze, osim kod jednog bolesnika kod koga je ova terapija uvedena zbog atrijalne fibrilacije. Bolesnici sa visokim rizikom, prema ELN preporukama, zahtevaju pored ove dve terapije i primenu citoreduktivne terapije [16]. Dok se HU standardno koristi kod starijih bolesnika, prema ELN i NCCN (*National Comprehensive Cancer Network*) preporukama, kod mladih prvi izbor citoreduktivne terapije je rekombinovani ili pegilovani INF-alfa [16,27]. U našoj grupi bolesnika, skoro polovina bolesnika sa PV lečena je primenom citoreduktivne terapije, uglavnom HU, dok je manji broj dobijao INF-alfa. Procenat lečenih je izuzetno visok, uzimajući u obzir da je manje od četvrtine bolesnika bilo svrstano u grupu sa visokim rizikom, a drugi razlozi za uvođenje terapije su bili visoka trombocitoza, splenomegalija, trudnoća ili značajne tegobe. Slično je i sa bolesnicima sa ET, gde su svi osim jednog pripadali grupi niskog rizika, pa a ipak je 42,9% bolesnika lečeno citoreduktivnom terapijom. ELN preporuke u smislu primene citoreduktivne terapije kod bolesnika sa ET ne razlikuju se mnogo u odnosu na PV, što se ne može reći i za primenu ASA [16]. Niske doze ASA su preporučene

mon type of thrombosis, or thrombotic events, was acute myocardial infarction, which occurred in 21.4% of patients. MPN is known to lead to thromboses in unusual locations, such as thromboses of the splanchnic circulation, including Budd-Chiari syndrome (BCS), portal vein thrombosis (PVT), and cerebral vein thrombosis [11,22,24]. Thrombosis during follow-up was exclusively present in patients with PV, with a predominance of arterial thrombosis, which accounted for almost 2/3. According to literature data in patients of this age group, venous thromboses were more likely to be present during follow-up and even more often compared to older patients [10]. The authors explain this phenomenon by the higher number of thromboses of the splanchnic circulation during follow-up, with 77% of the total number of venous thromboses [12]. On the other hand, arterial thrombosis during follow-up is generally less common in younger patients. This is thought to be related to lower cardiovascular comorbidities and atherosclerotic changes in blood vessels [14]. However, in our cohort, 41.7% of patients had CV risk factors, where smoking was the most common, and even 8.3% of patients had more than one risk factor. This finding may explain the occurrence of more frequent arterial thrombosis in our study.

According to ELN recommendations, treating patients with PV is based on the risk of thrombosis. Patients who have had previous thrombosis or are older than 60 years belong to the high-risk group, while low-risk implies the absence of both mentioned factors. By applying this scoring system, all patients in the youth group belong to the low-risk group unless they have had previous thrombosis [16]. The treatment of low-risk patients with PV involves the use of phlebotomy with the aim of Hct being < 45%, as well as the use of small doses of ASA [16,25]. Before applying antiplatelet therapy, it is crucial to test the patient for acquired von Willebrand syndrome to identify patients prone to bleeding, bearing in mind that it can occur even in the absence of pronounced thrombocytosis [26]. In our group of patients with PV, most were treated with low doses of ASA, with only a few patients on oral anticoagulant therapy (OAC). The reason for the introduction of OAC in almost all patients was previous thrombosis, except for one patient in whom this therapy was introduced due to atrial fibrillation. Patients with high risk, according to ELN recommendations, require cytoreductive therapy in addition to these two therapies [16]. While HU is standardly used in elderly patients, according to ELN and NCCN (*National Comprehensive Cancer Network*) recommendations, in young people, the first choice of cytoreductive therapy is recombined or pegylated INF-alpha [16,27]. In our group of patients, al-

kod bolesnika sa JAK2 mutacijom, dok kod CALR mutacije, s obzirom na veću sklonost ka krvarenju, treba biti oprezan [14]. Ipak, u našoj studiji svi, osim jednog bolesnika su dobijali ASA, a sama CALR mutacija nije bila rađena.

Kod bolesnika sa PMF, lečenje se značajno razlikuje, i ukoliko nemaju citopeniju, prisutne visokorizične molekularne mutacije i imaju normalan kariotip, preporučuje se posmatranje, kliničke studije ili upotreba JAK inhibitora (JAKi) [14]. U suprotnom, za bolesnike visokog rizika, preporučuje se alogena transplantacija matičnih ćelija hematopoeze (TMČH) [14]. U našoj grupi bolesnika isključivo je bila dijagnostikovana prePMF, pa je terapijski pristup bio sličan onom kod ET, s obzirom da ove dve bolesti karakteriše uporediv rizik od trombotičkih komplikacija [28]. Stoga je lečenje podrazumevalo primenu aspirina, a nešto više od trećine bolesnika je bilo lečeno i citoreduktivnom terapijom što je više u odnosu na procenat bolesnika sa trombozom (18.2%). Jedna bolesnica, sa progresijom u post-PV MF nakon 11,5 g od dijagnoze, transplantirana je, ali je preminula zbog nastanka posttransplantacionih komplikacija. Većina bolesnika u našoj grupi lečena je jednom linijom citoreduktivne terapije, a svega 4,8% bolesnika je zahtevalo drugu terapijsku liniju, i to HU, INF ili ruxolitinib.

Trenutno, ne postoje prognostički skorovi koji su specifično dizajnirani ili validirani za procenu mladih MPN bolesnika. U našoj kohorti za stratifikaciju rizika, kako je ranije pomenuto korišćen je standardni ELN skor, ali i IPSET srurvival i IPSET thrombosis za bolesnike sa ET i IPSS skor kod bolesnika sa prePMF [15,29,30]. Prema IPSET survival i IPSET thrombosis skorovima najveći broj bolesnika je pridao grupi niskog rizika, a ni jedan bolesnik nije pripadao grupi visokog rizika. Slično je i kod prePMF, gdje je IPSS identifikovao bolesnike uglavnom kao niskorizične.

Najčešći uzrok smrtnog ishoda u ovoj grupi bolesnika se pripisuje osnovnoj bolesti, transformaciji ili komplikacijama koje nastaju nakon TMČH [10]. Literaturni podaci ukazuju na to da, iako ne postoji razlika u učestalosti transformacije u MF ili AML između mladih i starijih bolesnika sa MPN, medijana dužine perioda do transformacije je značajno duža kod mladih (< 45 g) u odnosu na starije (> 65 g) bolesnike (19-20 odnosno 7-8 godina) zbog veće dužne praćenja [11]. Samo kod dve bolesnice u našoj kohorti došlo je do progresije u post-PV MF, što se može objasniti kratkom medijanom praćenja koja je iznosila oko 3 godine. Podaci iz literature ukazuju da je > 45% transformacija zabeleženo nakon > 10 godina od postavljanja dijagnoze, pa stoga dužina praćenja predstavlja bitan faktor [13]. Smrtni ishod je nastupio kod dva bolesnika, i to, kako je ranije

most half of the patients with PV were treated with cytoreductive therapy, mainly HU, while a smaller number received INF-alpha. The percentage of those treated is exceptionally high, taking into account that less than a quarter of patients were classified as high-risk, and other reasons for the introduction of therapy were high thrombocytosis, splenomegaly, pregnancy, or significant complaints. It is similar to patients with ET, where all but one belonged to the low-risk group, and yet 42.9% of patients were treated with cytoreductive therapy. ELN recommendations regarding the application of cytoreductive therapy in patients with ET do not differ much compared to PV, which cannot be said for the application of ASA [16]. Low doses of ASA are recommended in patients with JAK2 mutation, while caution should be exercised in patients with CALR mutation, given the greater tendency to bleeding [14]. However, in our study, only one patient received ASA, and the CALR mutation was not performed.

In patients with PMF, treatment varies significantly, and if they do not have cytopenia, high-risk molecular mutations are present and have a normal karyotype; observation, clinical studies, or the use of JAK inhibitors (JAKi) are recommended [14]. Otherwise, for high-risk patients, allogeneic hematopoietic stem cell transplantation (HSCT) is recommended [14]. In our group of patients, only prePMF was diagnosed, so the therapeutic approach was similar to that of ET, considering that these two diseases are characterized by a comparable risk of thrombotic complications [28]. Therefore, the treatment involved the use of aspirin, and slightly more than a third of patients were treated with cytoreductive therapy, which is higher than the percentage of patients with thrombosis (18.2%). One patient, with progression to post-PV MF after 11.5 years from diagnosis, was transplanted but died due to post-transplantation complications. Most patients in our group were treated with one line of cytoreductive therapy, and only 4.8% of patients required a second line of treatment, namely HU, INF, or ruxolitinib.

Currently, no prognostic scores are specifically designed or validated for evaluating young MPN patients. In our risk stratification cohort, as previously mentioned, the standard ELN score was used for IPSET survival and IPSET thrombosis for patients with ET and IPSS score for patients with prePMF [15,29,30]. According to the IPSET survival and IPSET thrombosis scores, most patients belonged to the low-risk group, and not a single patient belonged to the high-risk group. It is similar to prePMF, where the IPSS identified patients primarily as low-risk.

The most common cause of death in this group of patients is attributed to the underlying disease, trans-

navedeno, kod bolesnice sa post PV PMF, usled komplikacija TMČH, a kod drugog bolesnika razlog zbog sekundarnog maligneta.

ZAKLJUČAK

Adolescenti i mlade odrasle osobe čine posebnu subpopulaciju unutar MPN, pokazujući jedinstvene karakteristike po kojima se razlikuju od starijih bolesnika. Kao rezultat dugog trajanja bolesti u ovoj starosnoj grupi, postoji veći rizik od trombotičkih događaja, posebno tromboza splanhične cirkulacije, ali i veći rizik od transformacije u agresivniju formu bolesti zbog dužine praćenja. Naša studija pokazuje da većina mladih bolesnika sa MPN, prema standardnom ELN skor, pripada grupi niskog rizika. Međutim, značajan broj njih razvija ozbiljne komplikacije, poput tromboza (25%), simptoma, trombocitoze i splenomegalije (> 18 cm), a kao rezultat, skoro polovina bolesnika se leči citoreduktivnom terapijom.

Ovi podaci ukazuju na potrebu za daljim istraživanjima kako bi se razvili prognostički modeli specifični za mlade MPN bolesnike, čime bi se omogućila preciznija stratifikacija rizika i optimalan terapijski pristup. Prisutnost familijarne pojave bolesti ukazuje na potrebu za sveobuhvatnim genetičkim testiranjem. Upotreba NGS (next-generation sequencing) u ispitivanju epigenetskih mutacija kod mladih bolesnika može značajno doprineti razumevanju genetskih promena koje utiču na tok bolesti i odgovor na terapiju.

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formation, or complications that occur after TMJ [10]. Literature data indicate that, although there is no difference in the frequency of transformation to MF or AML between younger and older patients with MPN, the median length of the period to transformation is significantly longer in younger (< 45 y) compared to older (> 65 y) patients. patients (19-20 or 7-8 years old) due to greater follow-up [11]. Only two patients in our cohort had progression to post-PV MF, which can be explained by the short median follow-up of about 3 years. Data from the literature indicate that > 45% of transformations were recorded after > 10 years from diagnosis, and therefore, the length of follow-up is an essential factor [13]. Death occurred in two patients, as previously stated, in a patient with post-PV PMF due to complications of TMJ and in the second patient due to secondary malignancy.

CONCLUSION

Adolescents and young adults form a particular subpopulation within MPN, showing unique characteristics distinguishing them from older patients. As a result of the long duration of the disease in this age group, there is a higher risk of thrombotic events, especially thrombosis of the splanchnic circulation, but also a higher risk of transformation into a more aggressive form of the disease due to the length of follow-up. Our study shows that most young patients with MPN, according to the standard ELN score, belong to the low-risk group. However, a significant number of them develop serious complications, such as thrombosis (25%), symptoms, thrombocytosis, and splenomegaly (> 18 cm), and as a result, almost half of the patients are treated with cytoreductive therapy.

These data indicate the need for further research to develop prognostic models specific to young MPN patients, enabling more precise risk stratification and an optimal therapeutic approach. The presence of a familial occurrence of the disease indicates the need for comprehensive genetic testing. The use of NGS (next-generation sequencing) in examining epigenetic mutations in young patients can significantly contribute to understanding genetic changes that affect the course of the disease and the response to therapy.

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ABVD PROTOKOL NIJE ADEKVATAN ZA SVE PACIJENTE SA UZNAPREDOVALIM KLASIČNIM HOČKINOVIM LIMFOMOM: PETOGODIŠNJE ISKUSTVO JEDNOG CENTRA IZ SVAKODNEVNE KLINIČKE PRAKSE

ORIGINALNI RAD

ORIGINAL ARTICLE

ABVD DOES NOT FIT ALL ADVANCED-STAGE CLASSICAL HODGKIN LYMPHOMA PATIENTS: REAL-WORLD FIVE-YEAR SINGLE-CENTER EXPERIENCE

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SAŽETAK

Uvod/Cilj: Uznappedovali klasični Hočkinov limfom (UkHL) predstavlja terapijski izazov zbog značajnog procenta neuspeha prve terapijske linije. Cilj rada je da se opiše lečenje bolesnika sa UkHL-om u svakodnevnoj kliničkoj praksi ABVD protokolom.

Metode: U ovoj retrospektivnoj studiji su ispitivani kliničko-laboratorijski parametri, lečenje i ishod bolesnika kod kojih je UkHL dijagnostikovao u periodu od 2016. do kraja 2020. godine.

Rezultati: Kohortu je činilo 49 bolesnika sa UkHL-om čija je medijana praćenja bila 47 meseci (opseg: 1 – 79). Najvažnije kliničko-laboratorijske karakteristike su sumirane u Tabeli 1.

Svi bolesnici su inicijalno lečeni ABVD protokolom. Ukupni odgovor je iznosio 72,3% (kompletni odgovor = 61,7%; parcijalni odgovor = 10,6%), dok je ostalih 27,7% pacijenata ispoljilo refraktornost. Još 10,6% bolesnika je relapsiralo naknadno. Od ispitivanih parametara (Tabela 1) samo je povišena sedimentacija eritrocita ($SE \geq 50$ mm u prvom satu) uticala na kraće preživljavanje bez progresije (PBP), (medijana PBP = 19 meseci naspram nedostignuta kod bolesnika sa $SE < 50$ mm u prvom satu; $p = 0,039$), dok je prisustvo velike tumorske mase (engl. *bulky tumor mass/bulky disease*) bilo povezano sa kraćim ukupnim preživljavanjem (UP), ($p = 0,044$). Takođe, primarno refraktorni bolesnici su imali značajno kraće UP (medijana UP = 54 meseca naspram nedostignuta kod bolesnika sa postignutom remisijom; $p = 0,004$). Medijana PBP-a i UP-a nije dostignuta; četvorogodišnje PBP i UP iznosili su 61%, odnosno 89%. Pacijenti koji su lečeni autolognom transplantacijom (AT) u relapsiranoj/refraktornoj (R/R) bolesti imali su duže PBP ($p = 0,02$), ali ne i UP. Brentuksimab vedotin (BV) je uspešno primenjen kod četiri od 14 bolesnika, kod troje u konsolidaciji nakon autologne transplantacije.

Zaključak: Značajan broj bolesnika sa UkHL-om ne može da bude izlečen ABVD protokolom, već je neophodno za njih obezbediti intenzivnije lečenje ili inovativne terapije.

Ključne reči: uznappedovali Hočkinov limfom, ABVD, preživljavanje, autologna transplantacija

ABSTRACT

Introduction/Aim: Advanced-stage classical Hodgkin lymphoma (AScHL) is a therapeutic challenge due to chemoresistance. This study aims to present real-world data on the application of the ABVD regimen (doxorubicin, bleomycin, vinblastine, dacarbazine) in patients with AScHL.

Methods: This retrospective study examines the clinical and laboratory parameters, as well as the treatment and outcome of patients diagnosed with AScHL, in the period between 2016 and 2020.

Results: The cohort consisted of 49 patients with AScHL. Median follow-up was 47 months (range: 1 – 79). The most important clinical and laboratory characteristics are summarized in Table 1.

All patients were initially treated with ABVD. The overall response rate was 72.3% (complete response = 61.7%; partial response = 10.6%), while 27.7% of patients exhibited refractoriness. Additionally, 10.6% relapsed at a later stage. Of the investigated parameters (Table 1), only an elevated erythrocyte sedimentation rate (ESR) ≥ 50 mm in the first hour was associated with shorter progression-free survival (PFS), (median PFS = 19 months vs. not reached (NR), in patients with ESR < 50 mm in the first hour; $p = 0.039$), while the presence of bulky disease was associated with shorter overall survival (OS), ($p = 0.044$). Also, refractory patients had significantly shorter OS (median OS = 54 months vs. NR in patients who achieved remission; $p = 0.004$). The median PFS and OS were not achieved; four-year PFS and OS were 61% and 89%, respectively. Patients treated with autologous transplantation (AT) in relapsed/refractory disease had a longer PFS ($p = 0.02$), but not a longer OS. Brentuximab vedotin (BV) was successfully used in 4/14 patients, of whom three patients received it as consolidation treatment after AT.

Conclusion: A significant number of patients with AScHL cannot be cured with ABVD, thus more intensive treatment or innovative therapies are warranted.

Keywords: advanced-stage Hodgkin lymphoma, ABVD, survival, autologous transplantation

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UVOD

Klasični Hočkinov limfom (kHL) čini približno 95% svih slučajeva Hočkinovog limfoma. Preostalih 5% su pacijenti sa histološkim podtipom Hočkinovog limfoma sa nodularnom limfocitnom predominacijom, koji zahteva značajno drugačije lečenje i ima drugačiju prognozu. U okviru najnovije klasifikacije limfoidnih maligniteta, Hočkinov limfom sa nodularnom limfocitnom predominacijom je reklasifikovan kao nodularni limfocitno-predominantni B-ćelijski limfom [1,2]. Klasični HL pokazuje bimodalnu starosnu distribuciju i dijagnostiku se nakon ekscizije biopsije limfnih čvorova i histopatološke i imunohistohemijske potvrde CD30-pozitivnih Rid-Šternbergovih i srodnih tumorskih ćelija u okruženju koje obiluje inflamatornim ćelijama [3,4]. Stadijum bolesti i stratifikacija rizika kod pacijenata sa kHL-om oslanjaju se na En Arbor klasifikaciju i prisustvo faktora rizika, različito definisanih u različitim studijskim grupama [5].

Kombinovana hemioterapija (HT) sa ili bez radioterapije (RT) je decenijama bila osnova lečenja kHL-a [6]. Pacijenti se mogu izlečiti u oko 90% slučajeva u ranoj fazi, dok se pacijenti sa uznapredovalim stadijumom klasičnog Hočkinovog limfoma (UkHL) suočavaju sa 25% – 30% rizika od hemorezistencije ili relapsa [3,7]. Modalitet kombinovanog lečenja (HT + RT) nudi bolju dugoročnu kontrolu bolesti nego sama hemioterapija u ranoj fazi bolesti ali po cenu značajno češće kasne toksičnosti, uključujući srčanu i tiroidnu disfunkciju i solidne tumore. Pojava drugih kasnih neželjenih događaja kao što su neplodnost, plućna bolest, akutna leukemija i mijelodisplastični sindrom više zavisi od intenziteta hemioterapije [8–10].

U pogledu bezbednosti i efikasnosti, istorijski postoje dva glavna konkurentna pristupa lečenju UkHL-a: eskalirani BEACOPP protokol (BEACOPPesc), (bleomicin, etopozid, doksorubicin, ciklofosamid, vinkristin, prokarbazin i prednizon) Nemačke studijske grupe za Hočkinov limfom (engl. *German Hodgkin Study Group* – GHSG), koji obezbeđuje bolju kontrolu bolesti ali po cenu veće stope i akutne i kasne toksičnosti, i ABVD (doksorubicin, bleomicin, vinblastin i dakarbazin) protokol – čvrsto utemeljena kombinovana hemioterapija koja se primenjuje širom sveta, sa manje neželjenih efekata, ali ujedno sa slabijom dugoročnom kontrolom bolesti. Nekoliko kliničkih ispitivanja zasnovanih na lečenju koje je vođeno pozitronskom emisijom tomografijom/kompjuterskom tomografijom (PET/CT) bilo je usmereno na smanjenje intenziteta terapije kod privremeno PET negativnih pacijenata sa UkHL-om i, posledično, na smanjenje toksičnosti, uz očuvanu efikasnost lečenja [11–13]. Novi oblici lečenja, kao što je kombinacija brentuksimab vedotina (BV) i tradicional-

INTRODUCTION

Classical Hodgkin lymphoma (cHL) accounts for approximately 95% of all HL cases. The remaining 5% are patients with nodular lymphocyte-predominant Hodgkin lymphoma, a histological subtype with significantly different treatment and prognosis. Within the most recent classification of lymphoid malignancies, nodular lymphocyte-predominant HL has been reclassified as nodular lymphocyte-predominant B-cell lymphoma [1,2]. Classical HL exhibits bimodal age distribution and is diagnosed after excisional lymph node biopsy and histopathological and immunohistochemical confirmation of CD30-positive Reed-Sternberg and related tumor cells in an abundant inflammatory background [3,4]. Disease staging and risk stratification of cHL patients rely on the Ann Arbor classification and the presence of risk factors, defined differently across different study groups [5].

Combined chemotherapy (ChT) with or without radiotherapy (RT) has been the mainstay of treating cHL for decades [6]. Patients can be cured in around 90% of early-stage cases, while patients with advanced-stage classical Hodgkin lymphoma (AScHL) face a 25% – 30% risk of chemoresistance or relapse [3,7]. Combined modality treatment (ChT + RT) offers better long-term disease control than ChT alone in early-stage disease, however, at the cost of significantly more frequent late toxicities, including cardiac and thyroid dysfunction and solid tumors. The occurrence of other late events such as infertility, pulmonary disease, acute leukemia, and myelodysplastic syndrome rather depends on ChT intensity [8–10].

In terms of safety and efficacy, historically there have been two main competing approaches to treating AScHL: the German Hodgkin Study Group (GHSG) BEACOPP escalated regimen (BEACOPPesc) (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone), producing better disease control at the cost of higher rates of both acute and late toxicities, and the ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) regimen, a well-established combined ChT applied world-wide, with fewer adverse effects, but also inferior long-term disease control. Several clinical trials based on positron emission tomography/computed tomography (PET/CT) guided treatment were aimed at reducing treatment intensity in interim PET-negative AScHL patients and, consequently, reducing toxicity with preserved treatment efficacy [11–13]. New forms of treatment, such as brentuximab vedotin (BV) combined with traditional ChT have improved progression-free and overall survival, at the same time achieving reduced toxicity [14,15].

ne hemioterapije, poboljšali su preživljavanje bez progresije bolesti kao i ukupno preživljavanje, istovremeno postižući smanjenje toksičnosti [14,15].

Osnova lečenja pacijenata sa relapsiranim/refraktornim (R/R) kHL-om je hemioterapija koja ne ispoljava unakrsnu rezistenciju praćena visokodoznom hemioterapijom (engl. *high-dose chemotherapy – HDC*) i autolognom transplantacijom matičnih ćelija hematopoeze (ATMČH) kod hemiosenzitivnih pacijenata. Ovaj pristup dovodi do dugotrajne kontrole bolesti u približno 50% slučajeva [16]. Preživljavanje je dodatno poboljšano uvođenjem novih terapija, kao što su BV i inhibitori imunskih kontrolnih tačaka (anti-PD1 antitela). Pokazalo se da je mesto novih terapija u lečenju pacijenata sa relapsiranim/refraktornim (R/R) Hočkinovim limfomom u premošćavanju do ATMČ i održavanju nakon ATMČ, kao i u kombinaciji sa hemioterapijom spašavanja (engl. *salvage chemotherapy – salvage ChT*) pre ATMČ [7]. Monoterapija kod hemiorezistentnih pacijenata i pacijenata koji ne ispunjavaju kriterijume za ATMČ nije pokazala uspešnost u obezbeđivanju dugoročne kontrole bolesti [17,18].

Postoji nekoliko studija koje opisuju iskustva iz svakodnevne kliničke prakse u primeni ABVD protokola kod pacijenata sa UkHL-om [19–22]. Neophodno je prikupiti iskustva iz svakodnevne prakse izvan kliničkih ispitivanja, odnosno kontrolisanih okolnosti, kako bi se dobila šira slika ishoda lečenja kod pacijenata sa UkHL-om.

Ova studija ima za cilj da ispita ishode pacijenata sa UkHL-om koji su lečeni ABVD protokolom u našem centru u periodu od pet godina, te da identifikuje faktore rizika za neuspeh lečenja i proceni ishode pacijenata kod kojih lečenje ABVD protokolom nije bilo uspešno.

MATERIJALI I METODE

Pacijenti i ishodi lečenja

Sproveli smo retrospektivnu unicentričnu studiju kako bismo analizirali ishode 49 pacijenata sa dijagnozom UkHL-a u skladu sa GHSG-om [5]. Ovim pacijentima je dijagnostikovao kHL tokom petogodišnjeg perioda između januara 2016. i decembra 2020. godine. Pacijenti su lečeni su na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije, u Beogradu. Dijagnoza je postavljena nakon biopsije uvećanih limfnih čvorova i histopatološkog pregleda tumorskog tkiva od strane iskusnog hematopatologa. Pacijenti su podvrgnuti standardnim hematološkim pregledima bez inicijalnog PET/CT pregleda, dodeljen im je klinički stadijum prema En Arbor klasifikaciji i svrstani su u odgovarajuću terapijsku grupu (engl. *advanced stage – AS*) prema GHSG stratifikaciji rizika [5]. Klinički i laboratorijski parametri pri postavljanju dijagnoze su prikupljeni iz bolničke dokumentacije pacijenata i korelirani sa ishodom lečenja i vremenom preživljavanja. Terapijski od-

The cornerstone of treating relapsed/refractory (R/R) cHL patients is non-cross-resistant ChT followed by high-dose ChT (HDC) and autologous stem cell transplantation (ASCT) in chemosensitive patients. This approach leads to long-term disease control in approximately 50% of cases [16]. Survival is further improved by introducing novel therapies, such as BV and immune checkpoint inhibitors (anti-PD1 antibodies). Bridging to and maintenance after ASCT, as well as combination with salvage ChT prior to ASCT seem to be the most appropriate ways to use these novel therapies in the relapsed/refractory (R/R) setting [7]. Monotherapy in chemoresistant and ASCT-ineligible patients failed to provide long-term disease control [17,18].

There are several studies describing real-world experience with ABVD in patients with ASCHL [19–22]. It is necessary to gather experience from daily practice outside trials, i.e., controlled circumstances, in order to get a broader overview of treatment outcomes in ASCHL patients.

This study aims to examine the outcomes of ASCHL patients treated with ABVD at our center over a period of five years, identify risk factors for treatment failure, and assess the outcomes of patients who had experienced ABVD treatment failure.

MATERIALS AND METHODS

Patients and treatment outcomes

We conducted a single-center retrospective study to analyze the outcomes of 49 patients diagnosed with ASCHL as per the GHSG [5]. These patients were diagnosed with cHL during the five years between January 2016 and December 2020 and were treated at the Clinic for Hematology of the University Clinical Center of Serbia, in Belgrade. The diagnosis was established following the biopsy of enlarged lymph nodes and histopathological examination of tumor tissue by an experienced hematopathologist. The patients underwent standard hematological work-up with no initial PET/CT examination. They were assigned an Ann Arbor clinical stage and the appropriate treatment group (advanced stage – AS) according to the GHSG risk stratification [5]. Clinical and laboratory parameters at diagnosis were collected from patient hospital records and correlated with the treatment outcome and survival time. Treatment response and disease relapse were assessed according to the Lugano Criteria [23]. All patients were initially treated with the ABVD regimen which consisted of doxorubicin (25 mg/m²), bleomycin 10 mg/m², (vinblastine 6 mg/m²), and dacarbazine (375 mg/m²). Progression-free survival was calculated as the number of months from treatment initiation to disease relapse

govor i relaps bolesti su procenjeni prema Lugano kriterijumima [23]. Svi pacijenti su inicijalno lečeni ABVD protokolom koji se sastojao od doksorubicina (25 mg/m²), bleomicina (10 mg/m²), vinblastina (6 mg/m²) i dakarbazina (375 mg/m²). Preživljavanje bez progresije je izračunato kao broj meseci od početka lečenja do relapsa bolesti ili smrti usled bilo kojeg uzroka, šta god sa prvo dogodi. Ukupno preživljavanje je definisano kao vreme u mesecima od dijagnoze do smrti ili do datuma cenzorisanja na poslednjoj kontroli. Ispitali smo da li su ključni demografski i klinički parametri povezani sa terapijskim odgovorom, preživljavanjem bez progresije i ukupnim preživljavanjem.

Studija je sprovedena prema Helsinškoj deklaraciji iz 1964. godine i odobrena od strane Etičkog komiteta Medicinskog fakulteta Univerziteta u Beogradu, Beograd, Srbija.

Statistička analiza

Kategoričke varijable su predstavljene kao apsolutni brojevi i frekvencije, dok su kontinuirane varijable predstavljene kao medijana sa opsegom, srednje vrednosti sa standardnom devijacijom (SD) i devedesetpetoprocenatni interval poverenja (engl. 95% *confidence interval* – 95% *CI*). Distribucija podataka je procenjena pomoću Šapiro-Vilk testa. Povezanost između kategoričkih varijabli analizirana je Fišerovim egzaktnim testom i Hi-kvadratnim testom. Vreme preživljavanja pacijenata je analizirano prema Kaplan-Majerovom modelu. Razlike u distribuciji preživljavanja među grupama pacijenata na osnovu različitih karakteristika pacijenata definisane su pomoću log-rank testa.

Za statističku analizu korišćen je softver SPSS 21.0 (IBM). Sve analize su izvršene na osnovu dvostranih p vrednosti, a značajnost je definisana kao $p < 0,05$.

REZULTATI

Opis kohorte

Medijana praćenja je bila 47 meseci (opseg: 1 – 79). Medijana starosti pacijenata u trenutku uspostavljanja dijagnoze je bila 34 godine (opseg: 18 – 74). Muškarci su bili zastupljeni u kohorti 1,6 puta više od žena. Učestalosti histoloških podtipova su bile sledeće: nodularna skleroza 73,8%, mešovita celularnost 9,5%, limfocitna deplecija 2,4%, limfocitima bogat 7,1%, kombinovana nodularna skleroza/mešovita celularnost 7,1%, dok je sedam pacijenata bilo neklasifikovano. Pacijenti su klasifikovani na osnovu klasifikacije Evropske organizacije za istraživanje i lečenje raka (engl. *European Organisation for Research and Treatment of Cancer* – EORTC) i Asocijacije za istraživanje limfoma (engl. *Lymphoma Study Association* – LYSA). Sedam (14,3%) pacijenata je

or death from any cause, whatever occurred first. Overall survival was defined as the time in months from diagnosis to death or to the date of censoring at the latest follow-up. We examined whether key demographic and clinical parameters were associated with treatment response, progression-free survival, and overall survival.

The study was conducted per the 1964 Helsinki Declaration and approved by the Ethics Committee of the University of Belgrade School of Medicine, Belgrade, Serbia.

Statistical Analysis

Categorical variables are presented as absolute numbers and frequencies, while continuous variables are presented using medians with ranges, means with standard deviation (SD), and a 95% confidence interval (95% CI). The data distribution was assessed using the Shapiro-Wilk test. The association between categorical variables was analyzed using the Fisher exact test and the Chi-square test. Survival times were analyzed according to the Kaplan-Meier estimator. Survival distribution differences among patient groups based on various patient characteristics were defined using the log-rank test.

SPSS 21.0 software (IBM) was used for statistical analysis. All analyses were performed based on two-tailed p values and significance was defined as $p < 0.05$.

RESULTS

Cohort description

The median follow-up was 47 months (range: 1 – 79). The median age when the diagnosis was established was 34 years (range: 18 – 74). Men were represented in the cohort 1.6 times more than women. The frequencies of histological subtypes were as follows: nodular sclerosis 73.8%, mixed cellularity 9.5%, lymphocyte depletion 2.4%, lymphocyte rich 7.1%, combined nodular sclerosis/mixed cellularity 7.1%, while seven patients were unclassified. The patients were classified based on the European Organisation for Research and Treatment of Cancer (EORTC) and Lymphoma Study Association (LYSA) classification. Seven (14.3%) patients were classified as being in the intermediate stage, while 42 (85.7%) patients were classified as being in the advanced stage (AS). According to the Ann Arbor staging system, seven (14.3%) patients were classified as being in stage II, 26 (53%) patients as being in stage III, and 16 (32.7%) patients were classified as being in stage IV. Thirty-eight (77.6%) patients presented with B symptoms, 58% exhibited a bulky mass ≥ 7 cm, while 30% of the patients had a bulky mass ≥ 10 cm. More than

Tabela 1. Kliničke karakteristike pacijenata sa UkHL-om

Karakteristike / Characteristics	Values
Starost (godine), medijana (opseg) / Age (years), median (range)	34 (18 – 74)
Pol, n (%) / Gender, n (%)	
Muški / Male	30 (61.2%)
Ženski / Female	19 (38.8%)
EORTC/LYSA stadijum, n (%) / EORTC/LYSA stage, n (%)	
Intermedijarni / Intermediate	7 (14.3%)
Uznappedovali / Advanced	42 (85.7%)
En Arbor stadijum, n (%) / Ann Arbor stage, n (%)	
II	7 (14.3%)
III	26 (53%)
IV	16 (32.7%)
B simptomi, n (%) / B symptoms, n (%)	
A	11 (22.4%)
B	38 (77.6%)
Velika tumorska masa ≥ 7 cm, n (%) / Bulky disease ≥ 7 cm, n (%)	25 (58%)
Velika tumorska masa ≥ 10 cm, n (%) / Bulky disease ≥ 10 cm, n (%)	12 (30%)
Ekstranodalna bolest, n (%) / Extranodal disease, n (%)	15 (34.8)
IPS, n (%) / IPS, n (%)	
0 – 2	19 (54.3%)
3 – 7	16 (45.7%)
Hb (g/l), srednja vrednost $\pm$ SD / Hb (g/l), mean $\pm$ SD	118.73 $\pm$ 22.90
WBC ($\times 10^9/l$), medijana (opseg) / WBC ($\times 10^9/l$), median (range)	9.7 (1.6 – 25.0)
Ly ($\times 10^9/l$), medijana (opseg) / Ly ($\times 10^9/l$), median (range)	1.12 (0.14 – 4.18)
PLT ($\times 10^9/l$), srednja vrednost $\pm$ SD / PLT ($\times 10^9/l$), mean $\pm$ SD	348.250 $\pm$ 125.662
ESR (mm u prvom satu), medijana (opseg) / ESR (mm in the 1 st hour), median (range)	59 (14 – 120)
CRP (mg/l), medijana (opseg) / CRP (mg/l), median (range)	56.35 (5.6 – 361.7)

UkHL – uznapredovali klasični Hočkinov limfom (engl. *advanced stage classical Hodgkin lymphoma* – *AsCHL*); EORTC/LYSA – Evropska organizacija za istraživanje i lečenje raka, engl. *European Organisation for Research and Treatment of Cancer* – EORTC/Asocijacija za istraživanje limfoma, engl. *Lymphoma Study Association* – LYSA; IPS – međunarodni prognostički skor; engl. *International Prognostic Score*; Hb – hemoglobin; SD – standardna devijacija; WBC – bela krvna zrnca, engl. *white blood cells*; Ly – limfociti, engl. *lymphocytes*; PLT – trombociti, engl. *platelets*; ESR – sedimentacija eritrocita, engl. *erythrocyte sedimentation rate*; CRP – C-reaktivni protein

klasifikovano u grupu onih u intermedijarnom stadijumu, dok je 42 (85,7%) pacijenta klasifikovano u grupu onih koji su u uznapredovalom stadijumu (engl. *advanced stage* – AS) bolesti. Prema En Arbor klasifikaciji, sedam (14,3%) pacijenata je bilo u II stadijumu, 26 (53%) pacijenata u III stadijumu, a 16 (32,7%) pacijenata je bilo u IV stadijumu bolesti. Trideset osmoro (77,6%) pacijenata je imalo B simptome, 58% je pokazalo prisustvo velike tumorske mase (engl. *bulky tumor mass/bulky disease*) dimenzija ≥ 7 cm, dok je 30% pacijenata imalo veliku tumorsku masu dimenzija ≥ 10 cm. Više od 90% slučajeva velike tumorske mase je bilo locirano u medijastinumu. Petnaest (34,8%) pacijenata je imalo

Table 1. Clinical characteristics of ASCHL patients

ASCHL – *advanced stage classical Hodgkin lymphoma*; EORTC/LYSA – *European Organisation for Research and Treatment of Cancer/Lymphoma Study Association*; IPS – *International Prognostic Score*; Hb – *hemoglobin*; SD – *standard deviation*; WBC – *white blood cells*; Ly – *lymphocytes*; PLT – *platelets*; ESR – *erythrocyte sedimentation rate*; CRP – *C-reactive protein*

90% of cases of a bulky mass were mediastinal. Fifteen (34.8%) patients presented with extranodal disease (END), of whom 1/3 had two extranodal localizations. Fifteen out of 42 patients (35.7%) exhibited spleen involvement. A low International Prognostic Score (IPS) was calculated in 19/35 (54.3%) patients, and a high IPS was found in 16/35 (45.7%) patients. The basic clinical and laboratory parameters are summarized in Table 1.

First-line treatment and outcomes

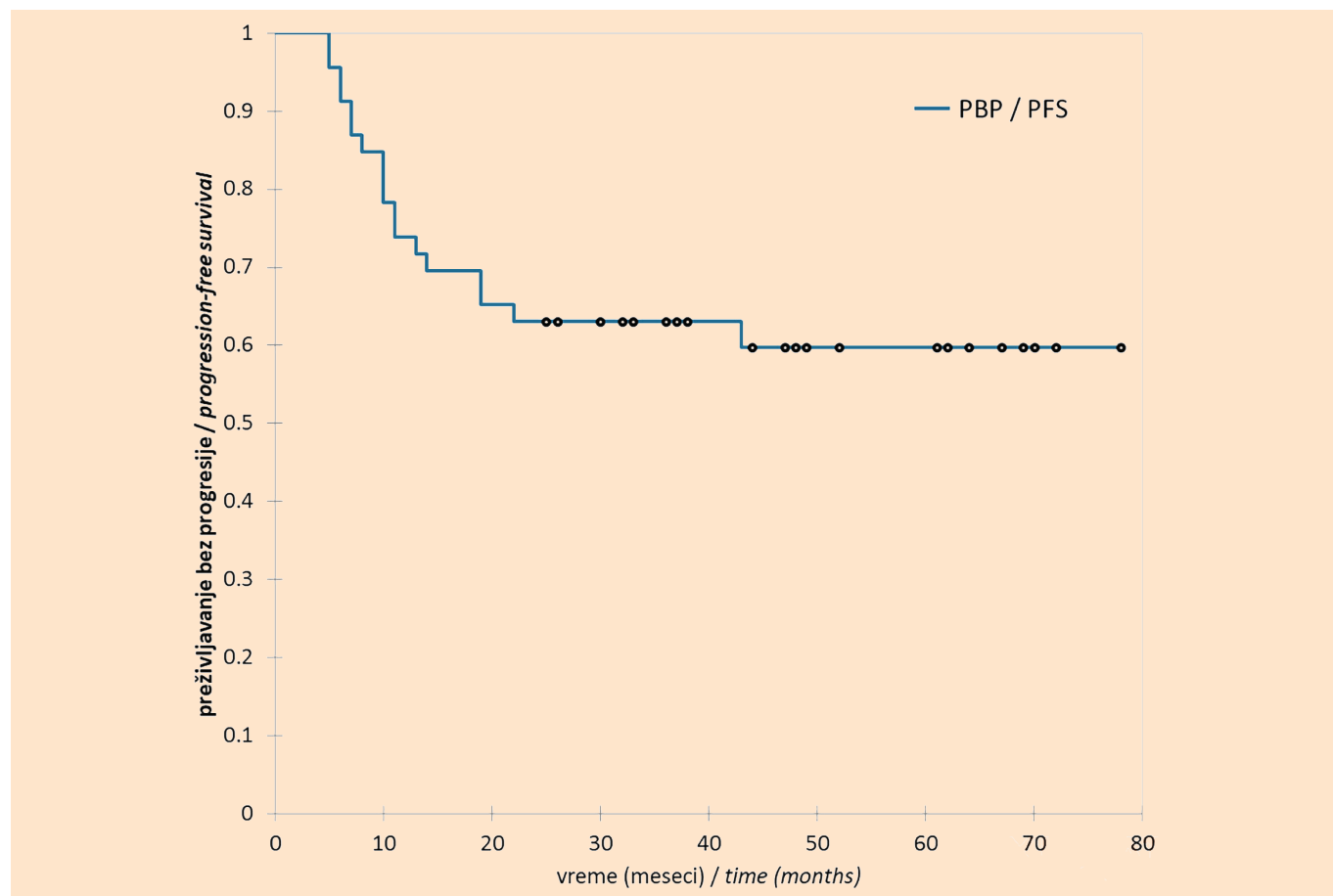
All patients received upfront ABVD treatment. Interim PET/CT was done in 12 patients with modification of further treatment intensity in six patients: three pa-

ekstranodalnu bolest (engl. *extranodal disease* – END), od kojih je 1/3 imala dve ekstranodalne lokalizacije. Petnaest od 42 (35,7%) pacijenata je pokazalo zahvaćenost slezine. Nizak međunarodni prognostički skor (engl. *International Prognostic Score* – IPS) izračunat je kod 19/35 (54,3%) pacijenata, dok je visok IPS utvrđen kod 16/35 (45,7%) pacijenata. Sažeti prikaz osnovnih kliničkih i laboratorijskih parametara dat je u **Tabeli 1**.

Prva terapijska linija i ishodi lečenja

Svi pacijenti su primili ABVD indukcionu terapiju. Kod 12 pacijenata je urađena/učinjena rana procena PET/CT pregledom sa modifikacijom daljeg intenziteta lečenja kod šest pacijenata: tri pacijenta su prešla na BEACOPPesc, dok je kod tri pacijenta bleomicin izostavljen i lečenje je nastavljeno AVD-om. Sveukupno gledano, medijana broja ciklusa primenjenih u prvoj terapijskoj liniji iznosila je šest ciklusa hemoterapije (opseg: 3 – 8). Deset od 45 (22%) pacijenata je podvrgnuto adjuvantnoj terapiji zračenjem. Ukupni odgovor je potvrđen u 72,3% slučajeva (potpuni odgovor (engl. *complete response* – CR) 61,7%; delimični odgovor (engl. *partial response* – PR) 10,6%), dok je preostalih 27,7%, odno-

tients switched to BEACOPPesc, while in three patients bleomycin was discontinued and treatment was continued with AVD. Overall, patients received a median of six cycles of chemotherapy (range: 3 – 8) as first-line treatment. Ten out of 45 (22%) patients underwent adjuvant radiation therapy. Overall response was confirmed in 72.3% of the cases (complete response (CR) 61.7%; partial response (PR) 10.6%), while the remaining 27.7%, i.e., 13 patients had primary refractory disease. Additionally, 5 (10.6%) patients relapsed after a median of 19 months (range: 8 – 33). No statistically significant differences in response to first-line treatment regarding gender, age (cut-off value 50 years), Ann Arbor and EORTC/LYSA stage, presence of B symptoms, presence of END, bulky disease (≥ 7 cm), erythrocyte sedimentation rate (ESR) ≥ 50 mm in the first hour, risk group according to IPS, and administered RT were observed. Median PFS (**Figure 1**) and OS (**Figure 2**) for the whole cohort were not reached, while the mean PFS and OS were 51.991 months (95% CI 42.290 – 61.692) and 71.017 months (95% CI 64.558 – 77.475), respectively. On the other hand, the four-year PFS was 61%, and the four-year OS was 89%. Patients with ESR

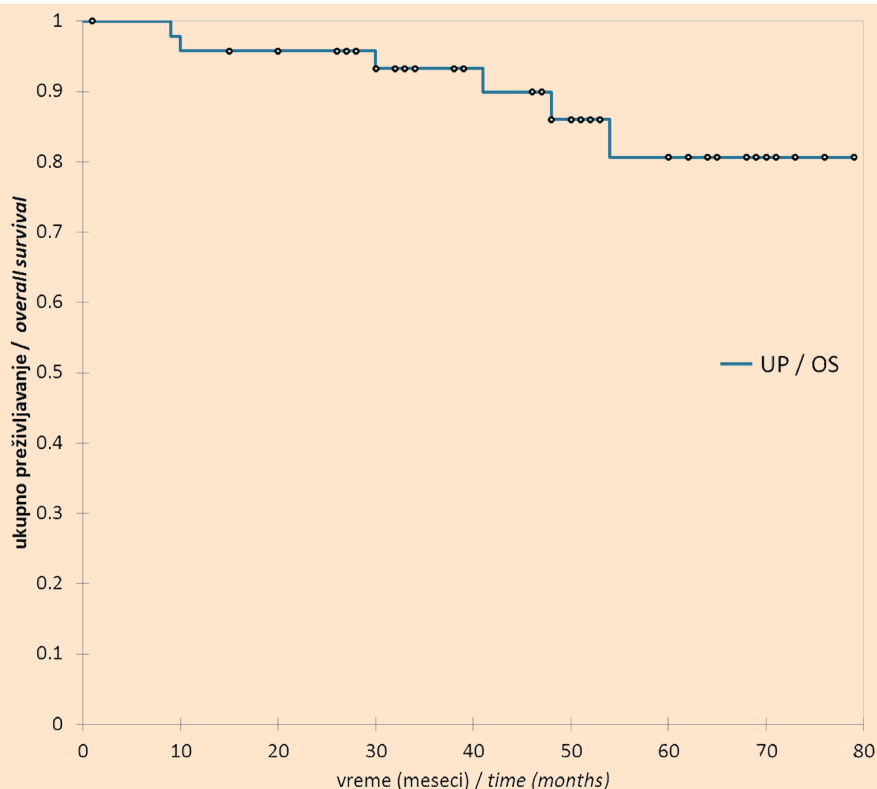


Slika 1. Preživljavanje bez progresije u celoj kohorti

Medijana PBP-a nije dostignuta; srednja vrednost = 51,991 mesec; 95% CI 42,290 – 61,692 meseca; četvorogodišnje PBP iznosilo je 61%

Figure 1. Progression-free survival for the whole cohort

Median PFS was not reached; mean = 51.991 months; 95% CI 42.290 – 61.692 months; four-year PFS was 61%.

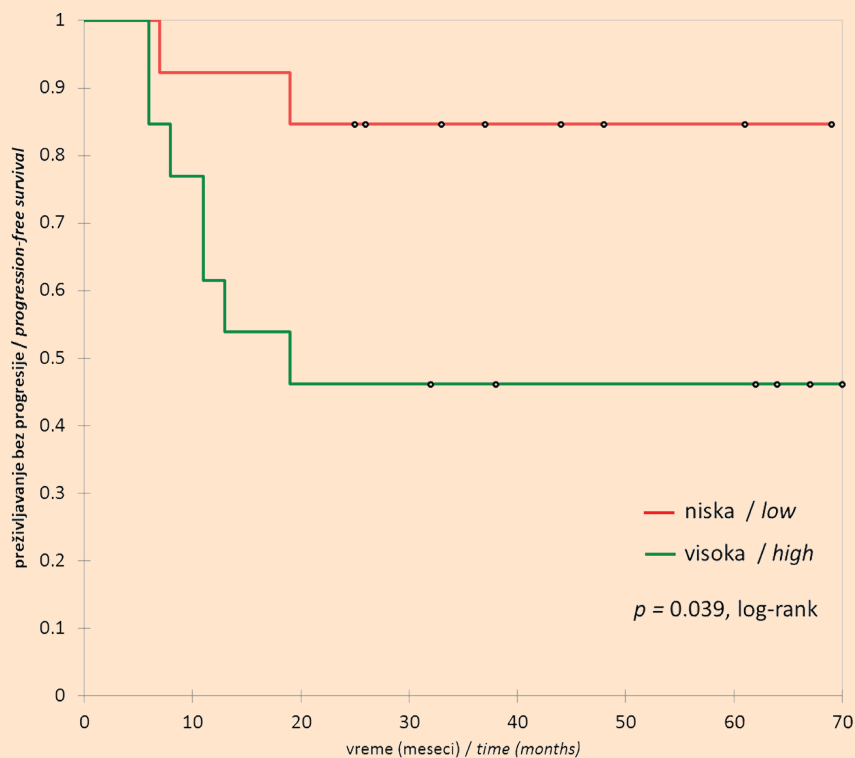


Slika 2. Ukupno preživljavanje u celoj kohorti

Medijana UP-a nije dostignuta; srednja vrednost = 71,017 meseci; 95% CI 64,558 – 77,475 meseci; četvorogodišnje UP je bilo 89%

Figure 2. Overall survival for the whole cohort

Median OS was not reached; mean = 71.017 months; 95% CI 64.558 – 77.475 months; four-year OS was 89%

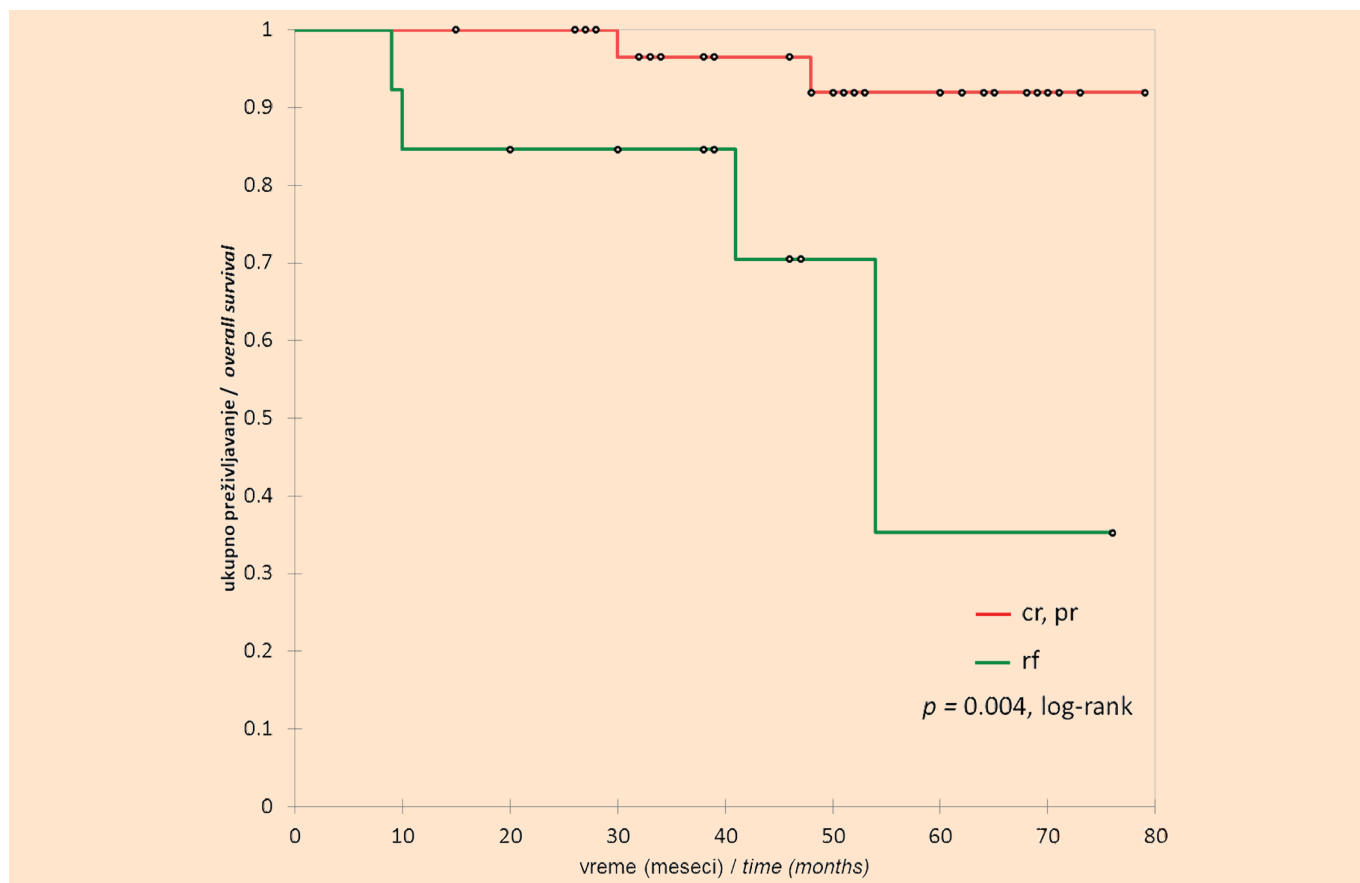


Slika 3. Sedimentacija eritrocita i preživljavanje bez progresije

Niska ($ESR < 50$ mm u prvom satu): medijana nije dostignuta; srednja vrednost = 60,385 meseci; 95% CI 44,747 – 76,022 meseca
Visoka ($ESR \geq 50$ mm u prvom satu): medijana = 19 meseci; 95% CI nije utvrđen; $p = 0,039$, log-rank test

Figure 3. Erythrocyte sedimentation rate and progression-free survival

Low ($ESR < 50$ mm in the 1st hour): median not reached; mean = 60.385 months; 95% CI 44.747 – 76.022 months
High ($ESR \geq 50$ mm in the 1st hour): median = 19 months; 95% CI not determined; $p = 0.039$, log-rank test



Slika 4. Odgovor na prvu terapijsku liniju i ukupno preživljavanje

Pacijenti koji su dostigli cr/pr: medijana nije dostignuta; srednja vrednost = 75,885 meseci; 95% CI 69,987 – 81,783 meseca

Pacijenti sa refraktornom bolešću (rf): medijana = 54 meseca; 95% CI 35,150 – 72,850 meseci; $p = 0,004$, log-rank test

cr – kompletni odgovor (engl. *complete response*); pr – parcijalni odgovor (engl. *partial response*); rf – refraktorna bolest

Figure 4. Response to first-line treatment and overall survival

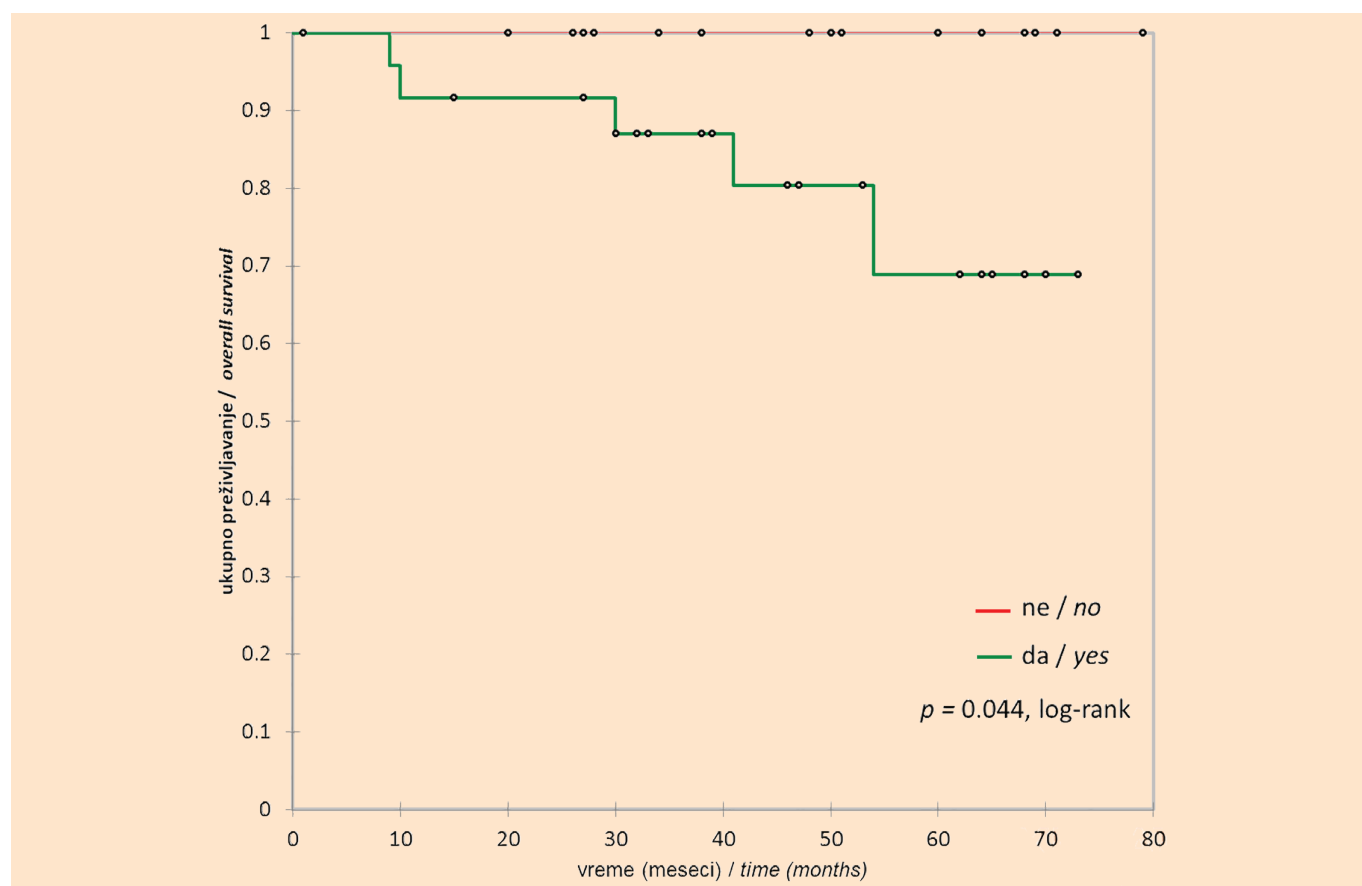
Patients who achieved cr/pr: median not reached; mean = 75.885 months; 95% CI 69.987 – 81.783 months

Patients with refractory disease (rf): median = 54 months; 95% CI 35.150 – 72.850 months; $p = 0.004$, log-rank test

cr – complete response; pr – partial response; rf – refractory disease

sno 13 pacijenata, imalo primarno refraktornu bolest. Nadalje, pet (10,6%) pacijenata je imalo relaps nakon medijane od 19 meseci (opseg: 8 – 33). Nema statistički značajnih razlika u odgovoru na prvu terapijsku liniju u odnosu na pol, starost (granična vrednost 50 godina), En Arbor i EORTC/LYSA stadijum, prisustvo B simptoma, prisustvo ektranodalne bolesti, prisustvo velike tumorske mase (≥ 7 cm), sedimentaciju eritrocita (engl. *erythrocyte sedimentation rate* – ESR) ≥ 50 mm u prvom satu, rizičnu grupu prema IPS-u i primenjenu radioterapiju. Medijane PBP-a (Slika 1) i UP-a (Slika 2) za celu kohortu nisu dostignute, dok su srednje vrednosti PBP-a i UP-a bile 51,991 mesec (95% CI 42,290 – 61,692), odnosno 71,017 meseci (95% CI 64,558 – 77,475). Sa druge strane, četvorogodišnje PBP je bilo 61%, a četvorogodišnje UP je bilo 89%. Pacijenti sa ESR ≥ 50 mm u prvom satu su imali kraće PBP nakon ABVD, u poređenju sa pacijentima sa ESR < 50 mm u prvom satu (medijana PBP = 19 meseci naspram nedostignuta; $p = 0,039$, log-rank test), (Slika 3). Pol, starost (granična vrednost 50 godina), EORTC/LYSA stadijum (intermedijarni na-

≥ 50 mm in the first hour had shorter PFS after ABVD, as compared to the patients with ESR < 50 mm in the first hour (median PFS = 19 months vs. not reached, respectively; $p = 0.039$, log-rank test) (Figure 3). Gender, age (cut-off value 50 years), EORTC/LYSA stage (intermediate vs. advanced), presence of B symptoms, presence of END, bulky disease, IPS (0 – 2 vs. 3 – 7), and administered RT were not significant regarding PFS. Patients who were refractory to first-line treatment had significantly shorter OS, as compared to the patients who achieved CR/PR (median OS = 54 months vs. not reached; $p = 0.004$, log-rank test), (Figure 4). Additionally, patients who presented with a large tumor mass (bulky mass ≥ 7 cm) experienced significantly decreased OS, as compared to the patients without a bulky mass ($p = 0.044$, log-rank test), (Figure 5). OS did not differ significantly in relation to gender, age (cut-off value 50 years), EORTC/LYSA stage, presence of B symptoms, END at presentation, ESR, IPS (0 – 2 vs. 3 – 7), and RT within first-line treatment.



Slika 5. Prisustvo velike tumorske mase i ukupno preživljavanje

Pacijenti bez prisustva velikih tumorskih masa (Ne): srednja vrednost i medijana nisu utvrđene (bez događaja)

Pacijenti sa velikom tumorskom masom (≥ 7 cm), (Da): medijana nije dostignuta; srednja vrednost = 61,412 meseca; 95% CI 51,435 – 71,389 meseci (značajno kraće UP); $p = 0,044$, log-rank test

Figure 5. Bulky disease and overall survival

Patients without bulky disease (No): mean and median not determined (no events)
Patients with bulky mass ≥ 7 cm (Yes): median not reached; mean = 61.412 months; 95% CI 51.435 – 71.389 months (significantly shorter OS); $p = 0.044$, log-rank test

spram uznapređovalog), prisustvo B simptoma, prisustvo ektranodalne bolesti, prisustvo velike tumorske mase, *IPS* (0 – 2 naspram 3 – 7) i primenjena RT nisu bili značajni za PBP. Pacijenti koji su bili refraktorni na prvu terapijsku liniju su imali značajno kraće UP u poređenju sa pacijentima koji su dostigli CR/PR (medijana UP = 54 meseca naspram nedostignuta; $p = 0,004$, log-rank test), (Slika 4). Pored toga, pacijenti koji su imali veliku tumorsku masu (≥ 7 cm) su imali značajno smanjeno UP, u poređenju sa pacijentima bez velike tumorske mase ($p = 0,044$, log-rank test), (Slika 5). UP se nije značajno razlikovalo u odnosu na pol, starost (granična vrednost 50 godina), *EORTC/LYSA* stadijum, prisustvo B simptoma, prisustvo ektranodalne bolesti, *ESR*, *IPS* (0 – 2 naspram 3 – 7) i RT u okviru prve terapijske linije.

Ishod kod pacijenata sa relapsiranom/refraktornom bolešću

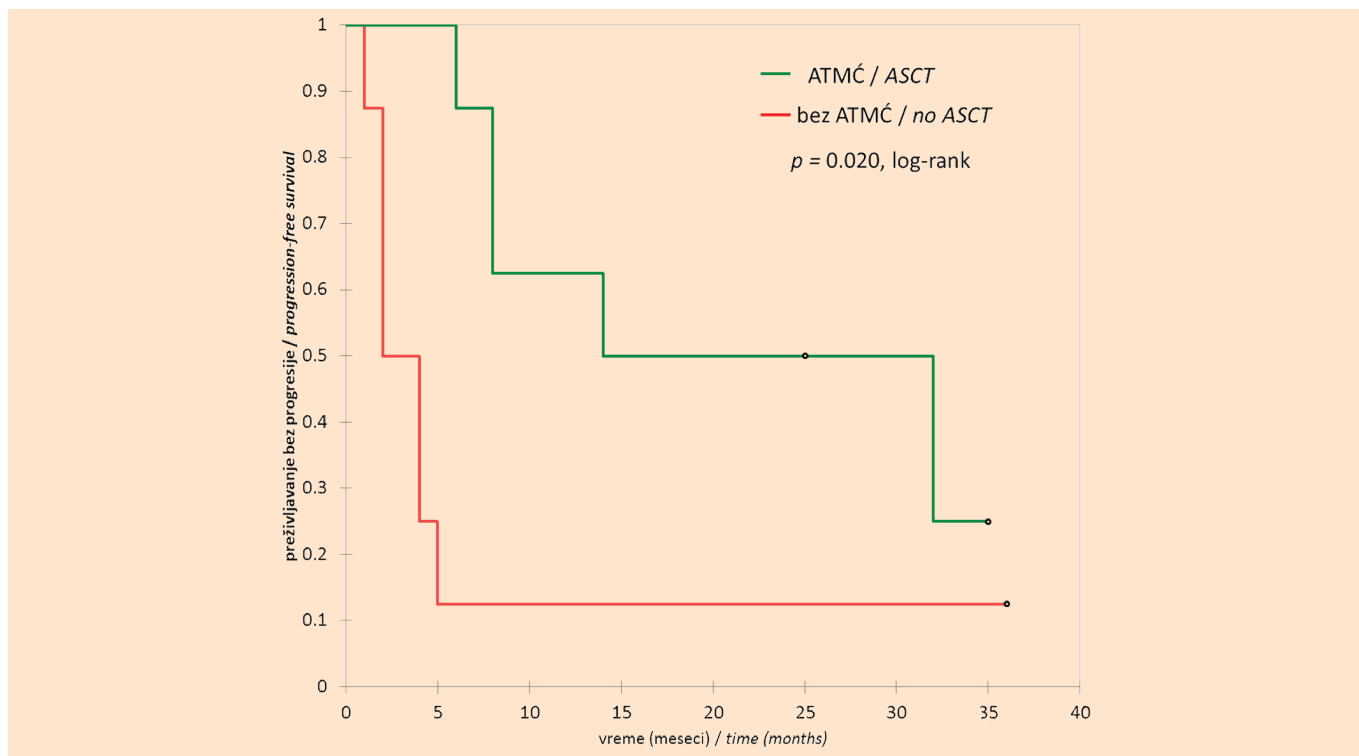
Među 18 bolesnika sa relapsiranom/refraktornom bolešću (od kojih je 13 pacijenata imalo primarni refraktorni HL, a pet je relapsiralo), 17 pacijenata je lečeno

The outcome of relapsed/refractory patients

Among the 18 R/R patients (of whom 13 patients had primary refractory HL and five relapsed), 17 were treated with salvage ChT $\pm$ ASCT following HDC. Patients treated with HDC + ASCT after salvage ChT had a significantly longer PFS than those without ASCT ($p = 0.020$, log-rank test), (Figure 6), however, with no confirmed significant advantage in OS. Fourteen patients with R/R disease received BV in monotherapy, of whom three were consolidated after ASCT (all in sustained CR after BV), while 11 patients were treated with BV following a median of three lines of therapy (range: 2 – 4). Of the eleven patients, only one patient achieved CR, one was still on BV at the latest follow-up, while the other nine patients were refractory. The median number of administered BV cycles was 12.

DISCUSSION

Currently, there are various recommendations for the upfront treatment of AsCHL [5,24,25]. In keeping with the results of the recent major clinical trials and sub-



Slika 6. Autologna transplantacija matičnih ćelija hematopoeze i PBP kod R/R uznapredovalog Hoćkinovog limfoma

ATMČH: medijana = 14 meseci; 95% CI 8,174 – 36,174 meseci

Bez ATMČ: medijana = 2 meseca; 95% CI 0,337 – 3,663 meseci

Pacijenti koji su primili HDC + ATMČ nakon hemioterapije spašavanja imali su značajno duže PBP od pacijenata bez ATMČ ($p = 0,020$, log-rank test).

Figure 6. Autologous stem cell transplantation and PFS in R/R advanced Hodgkin lymphoma

ASCT: median = 14 months; 95% CI 8.174 – 36.174 months

No ASCT: median = 2 months; 95% CI 0.337 – 3.663 months

Patients who received HDC + ASCT after salvage ChT experienced significantly longer PFS than those without ASCT ($p = 0.020$, log-rank test).

hemioterapijom spašavanja $\pm$ ATMČ nakon visokodozne hemioterapije (HDC). Pacijenti lečeni pomoću HDC + ATMČ nakon hemioterapije spašavanja su imali značajno duže PBP od onih kod kojih nije primenjena ATMČ ($p = 0,020$, log-rank test), (Slika 6), međutim, bez potvrđene značajne prednosti za UP. Četrnaest pacijenata sa R/R bolešću je primilo BV u monoterapiji, od kojih je tri konsolidovano nakon ATMČ (svi sa dugotrajnim CR-om nakon BV), dok je 11 pacijenata lečeno BV-om nakon medijane od tri terapijske linije (opseg: 2 – 4). Od tih 11 pacijenata, samo jedan pacijent je postigao CR, jedan je još uvek bio na BV-u na poslednjoj kontroli, dok je ostalih devet pacijenata bilo refrakturno. Medijana primenjenih ciklusa BV-a bila je 12.

DISKUSIJA

Trenutno postoje različite preporuke za indukciono lečenje UkHL-a [5,24,25]. U skladu sa rezultatima nedavnih velikih kliničkih ispitivanja i sledstveno ažuriranim relevantnim smernicama za lečenje, kliničari mogu da biraju između konvencionalnih hemioterapijskih opcija (ABVD ili BEACOPPesc) i kombinacije BV i hemioterapije [15,26]. Ključni cilj lečenja je postizanje maksimalne terapijske koristi uz izbegavanje akutnih i kasnih

sekvencijalno relevantne terapijske opcije, kliničari mogu da biraju između konvencionalnih ChT opcija (ABVD or BEACOPPesc) and a combination of BV and ChT [15,26]. The key objective of the treatment is to get maximal therapeutic benefits while avoiding acute and late side effects that could jeopardize the patients' quality of life and survival.

Lower rates of remission in our study than in other real-world studies can be explained by the fact that a significant number of patients from the cohort were diagnosed in 2019 and 2020 and were treated during the coronavirus disease of 2019 (COVID-19) pandemic with probable treatment delays and subsequent negative impact on outcomes [20,22,27]. However, this is only a speculation as we did not collect information about COVID-19 and treatment delay from the records of the patients in our cohort. Additionally, although most refractory patients were eventually treated at our institution with completely accessible medical records, a number of patients who achieved adequate disease control were subsequently followed up by hematologists in their local area, which removed them from the reach of our institution, making such patients not fully eligible for this study.

After a four-year follow-up of the patients in our cohort, PFS was 61%, and OS was 89%. It is noteworthy

neželjenih efekata koji bi mogli da ugroze kvalitet života i preživljavanje pacijenata.

Niže stope remisije u našoj studiji nego u drugim studijama iz svakodnevne kliničke prakse mogu se objasniti činjenicom da je značajan broj pacijenata iz ove kohorte dijagnostikovao 2019. i 2020. godine. Ovi pacijenti su lečeni tokom pandemije koronavirusne bolesti 2019 (COVID-19), te je kod njih verovatno došlo do odlaganja lečenja i posledičnog negativnog uticaja na ishode [20,22,27]. Međutim, ovo je samo naša pretpostavka jer nismo prikupili informacije o COVID-19 oboljenju i kašnjenju u lečenju iz dokumentacije pacijenata u našoj kohorti. Osim toga, iako je većina refraktornih pacijenata na kraju lečena u našoj ustanovi sa potpuno dostupnom medicinskom dokumentacijom, jedan broj pacijenata koji je postigao adekvatnu kontrolu bolesti, nakon toga je praćen od strane hematologa na njihovoj teritoriji stanovanja, što ih je učinilo nedostupnim za praćenje od strane naše ustanove, a samim tim oni nisu u potpunosti ispunili kriterijume za ovu studiju.

Nakon četvorogodišnjeg praćenja pacijenata u našoj kohorti, PBP je bilo 61% dok je UP bilo 89%. Važno je napomenuti da je UP bilo duže u našoj studiji nego u prethodnoj velikoj kohortnoj studiji iz naše ustanove, koju su sprovedi Anđelić i saradnici (četvorogodišnje UP od 89% naspram petogodišnjeg UP od 76%), najverovatnije zbog dostupnosti novih terapijskih modaliteta za lečenje pacijenata sa R/R kHL-om u Srbiji poslednjih godina [22]. S druge strane, ukupno preživljavanje u našoj studiji je bilo uporedivo sa ukupnim preživljavanjem u većini prospektivnih kliničkih ispitivanja koja su se bavila ABVD protokolom u UKHL-u [28–32].

Kada su u pitanju faktori rizika, u našoj kohorti je utvrđeno da je samo povišena sedimentacija eritrocita povezana sa PBP-om, dok su pacijenti sa velikom tumorskom masom (≥ 7 cm) i pacijenti koji su bili primarno refraktorni na prvu terapijsku liniju pokazali značajno kraće UP. U retrospektivnoj studiji koju su sprovedi Avigdor i saradnici, a koja opisuje ishod za 221 pacijenta sa UKHL-om lečenog ABVD protokolom u prvoj terapijskoj liniji, starosti 60+ godina, visok IPS i pozitivan rani PET/CT bili su povezani sa kraćim PBP-om [27]. U drugoj studiji iz svakodnevne kliničke prakse, koju su sprovedi Anđelić i saradnici u našoj bolnici, viši IPS i postojanje više od jedne ektranodalne lokalizacije bili su povezani sa lošijim PBP-om, dok su visoki IPS, ESR > 50 mm/h, > 1 ektranodalna lokalizacija i prisustvo velike tumorske mase bili u korelaciji sa značajno kraćim UP-om [22]. U našoj studiji, samo su povišena sedimentacija, prisustvo velike tumorske mase i primarna refraktornost pokazali prognostički značaj u prvoj terapijskoj liniji, najverovatnije zbog malog broja ispitanika, za razliku od dve gore pomenute studije koje su uključivale

thy that OS was longer in our study than in a previous large cohort study from our institution by Andjelic et al., (four-year OS 89% vs. five-year OS 76%), most probably because of the availability of novel treatment options for R/R CHL patients in Serbia in recent years [22]. On the other hand, OS in our study was comparable to OS in most of the prospective clinical trials dealing with ABVD in ASCHL [28–32].

Regarding the risk factors, in our cohort, only elevated ESR was found to be associated with PFS, while patients with bulky disease ≥ 7 cm and patients that were primarily refractory to the first treatment line exhibited significantly shorter OS. In a retrospective study conducted by Avigdor et al., which reports on the outcome of 221 ASCHL patients treated with ABVD as first-line therapy and aged 60+ years, a high IPS and positive early PET/CT were associated with a shorter PFS [27]. In another real-world study by Andjelic et al., conducted at our hospital, a higher IPS and more than one extranodal localization were associated with poorer PFS, while high IPS, ESR > 50 mm/h, > 1 extranodal site, and bulky disease correlated with significantly shorter OS [22]. In our study, only elevated ESR, bulky disease, and primary refractoriness showed prognostic significance in first-line treatment, most probably due to the small study sample, as opposed to the other two abovementioned studies which included 221 (Avigdor et al.) and 314 (Andjelic et al.) ASCHL patients treated with ABVD, respectively [22,27].

Up to 30% of patients in clinical trials are refractory to combined first-line ChT, while one retrospective study from the United States, which included 167 patients with ASCHL, confirmed that 55% of patients were treated with second-line therapy, which makes this number significantly higher in the real-world setting [21]. Our data show that 27.7% of patients were initially refractory to ABVD while an additional 10% relapsed after a median period of 19 months, implying that a certain number of AS patients treated with ABVD will eventually need further treatment. Autologous stem cell transplantation following salvage ChT and HDC remains the standard of care in the R/R setting with a potential for 50% of R/R patients to be cured [16]. The addition of novel therapeutic options, such as BV or checkpoint inhibitors (nivolumab or pembrolizumab) to salvage protocols prior to ASCT, dramatically improves posttransplant survival [7]. Our study confirmed the significance of ASCT in consolidation after salvage ChT in R/R ASCHL.

As mentioned above, novel therapies, including BV and anti-PD1 antibodies, have significantly changed the landscape of the contemporary treatment of CHL. Brentuximab vedotin was initially approved in 2011

221 (Avigdor i sar.) i 314 (Anđelić i sar.) pacijenata sa UkHL-om lečenih ABVD protokolom [22,27].

Do 30% pacijenata u kliničkim ispitivanjima je refraktorno na kombinovanu hemioterapiju u prvoj terapijskoj liniji, dok je u jednoj retrospektivnoj studiji iz Sjedinjenih Američkih Država, koja je obuhvatila 167 pacijenata sa UkHL-om, potvrđeno da je 55% pacijenata lečeno u drugoj terapijskoj liniji, što čini ovaj broj je znatno većim u svakodnevnoj kliničkoj praksi [21]. Naši podaci pokazuju da je 27,7% pacijenata u početku bilo refraktorno na ABVD, dok je dodatnih 10% relapsiralo nakon medijane od 19 meseci, što ukazuje na to da će određenom broju pacijenata sa UkHL-om koji su lečeni ABVD protokolom na kraju biti potrebna terapija spašavanja. Visokodозна hemioterapija sa autolognom transplantacijom matičnih ćelija nakon hemioterapije spašavanja ostaje standard lečenja kod R/R pacijenata sa potencijalom da 50% R/R bolesnika bude izlečeno [16]. Dodavanje novih terapijskih opcija, kao što su BV ili inhibitori imunskih kontrolnih tačaka (nivolumab ili pembrolizumab) protokolima spašavanja pre ATMČ, drastično poboljšava preživljavanje nakon transplantacije [7]. Naša studija je potvrdila značaj ATMČ u konsolidaciji nakon hemioterapije spašavanja kod R/R UkHL-a.

Kao što je već navedeno, nove terapije, uključujući BV i anti-PD1 antitela, značajno su promenile situaciju u savremenom lečenju kHL-a. Brentuksimab vedotin je prvobitno odobren 2011. godine od strane američke Uprave za hranu i lekove (engl. *Food and Drug Administration*), za pacijente sa relapsom nakon ATMČ ili za pacijente posle ≥ 2 linije lečenja koji ne ispunjavaju uslove za ATMČ. Ključno ispitivanje AETHERA i iskustvo iz svakodnevne kliničke prakse su potvrdili korist od post-ATMČ konsolidacije sa BV-om za pacijente visokog rizika, dok njegova upotreba kao monoterapija u kontekstu terapije spašavanja kod pacijenata koji su imali relaps nakon ATMČ ili nisu ispunjavali kriterijume za ATMČ, nije pokazala da ostvaruje dovoljnu dugoročnu kontrolu bolesti [17,18,33-35]. Rezultati naše studije po pitanju primene BV-a su u skladu sa ovim nalazima, međutim, mali uzorak (14 pacijenata u našoj kohorti je primilo BV) onemogućio je adekvatnu statističku analizu koja bi donela relevantne zaključke.

Naša studija ima nekoliko ograničenja:

1. Kohorta je premala da bi se napravila poređenja koja bi imala dovoljnu statističku snagu.
2. Ne postoje bezbednosni podaci o toksičnosti ABVD protokola tokom i posle terapije i njenom uticaju na ishod.
3. Nema podataka o KOVID-19 statusu onih pacijenata iz kohorte koji su lečeni tokom pandemije bolesti KOVID-19, te njegovom mogućem uticaju na odlaganje lečenja i posledičnim implikacijama na ishod.

by the US Food and Drug Administration, for patients relapsed after ASCT or for patients after ≥ 2 lines of treatment who are not eligible for ASCT. The pivotal AETHERA trial and real-world experience confirmed the benefit of post-ASCT consolidation with BV for high-risk patients, while its use as monotherapy in the salvage setting in patients who had relapsed after ASCT or were not eligible for ASCT did not show sufficient long-term disease control [17,18,33-35]. The results of our study concerning BV are in line with these findings, however, the small sample (14 patients in our cohort received BV) made it impossible to perform an adequate statistical analysis that would have yielded relevant conclusions.

There are several limitations to our study:

1. The cohort is too small to make comparisons that would have enough statistical power.
2. There are no safety data about the toxicity of ABVD during and after treatment and its impact on the outcome.
3. There are no data about the COVID-19 status of the patients from the cohort treated during the COVID-19 pandemic, its possible impact on delaying treatment, and consequent implications for the outcome.

CONCLUSION

In our cohort, patients with higher erythrocyte sedimentation levels had a shorter progression-free survival. Additionally, the presence of a bulky mass and primary refractory disease were associated with shorter overall survival. Patients with relapsed or refractory classical Hodgkin lymphoma showed a significantly better prognosis when they underwent consolidation therapy with autologous stem cell transplantation. Monotherapy with brentuximab vedotin was not beneficial outside the post-transplant consolidation setting. A significant number of advanced-stage classical Hodgkin lymphoma patients can not be cured with ABVD and need more intensive and/or novel therapeutic options in first-line treatment.

ABBREVIATIONS AND ACRONYMS

ABVD – doxorubicin, bleomycin, vinblastine, dacarbazine
 AS – advanced-stage
 ASCT – autologous stem cell transplantation
 AVD – doxorubicin, vinblastine, dacarbazine
 BEACOPPesc – bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone, escalated
 BV – brentuximab vedotin
 cHL – classical Hodgkin lymphoma
 ChT – chemotherapy

ZAKLJUČAK

U našoj kohorti, pacijenti sa višim vrednostima sedimentacije eritrocita imali su kraće preživljavanje bez progresije bolesti. Pored toga, prisustvo velike tumorske mase i primarna refraktorna bolest su bili povezani sa kraćim ukupnim preživljavanjem. Pacijenti sa relapsom ili refraktornim klasičnim Hočkinovim limfomom pokazali su značajno bolju prognozu kada su bili podvrgnuti konsolidacionoj terapiji sa autolognom transplantacijom matičnih ćelija. Monoterapija sa brentuksimab vedotinom nije bila uspešna van konsolidacije nakon transplantacije. Značajan broj pacijenata sa uznapredovalim klasičnim Hočkinovim limfomom ne može biti izlečen ABVD protokolom i iziskuje intenzivnije i/ili nove opcije lečenja u prvoj terapijskoj liniji.

SPISAK SKRAĆENICA

ABVD – doksorubicin, bleomicin, vinblastin i dakarbazin
AS – uznapredovali stadijum bolesti; engl. *advanced-stage*
ATMČ – autologna transplantacija matičnih ćelija (engl. *autologous stem cell transplantation – ASCT*)
AVD – doksorubicin, vinblastin i dakarbazin
BEACOPPesc – eskalirani bleomicin, etopozid, doksorubicin, ciklofosamid, vinkristin, prokarbazin i prednizon
BV – brentuksimab vedotin
kHL – klasični Hočkinov limfom (engl. *classical Hodgkin lymphoma – cHL*)
HT – hemioterapija
KOVID-19 – koronavirusna bolest 2019 (engl. *coronavirus disease of 2019 – COVID-19*)
CR – potpuni odgovor; engl. *complete response*
END – ekstranodalna bolest; engl. *extranodal disease*
EORTC/LYSA – Evropska organizacija za istraživanje i lečenje raka; engl. *European Organisation for Research and Treatment of Cancer – EORTC/Asocijacija za istraživanje limfoma*; engl. *Lymphoma Study Association – LYSA*
ESR – sedimentacija eritrocita; engl. *erythrocyte sedimentation rate*
GHSG – Nemačka studijska grupa za Hočkinov limfom; engl. *German Hodgkin Study Group*
HDC – visokodozna hemioterapija; engl. *high-dose chemotherapy*
IPS – međunarodni prognostički skor; engl. *International Prognostic Score*
UP – ukupno preživljavanje (engl. *overall survival – OS*)
PET/CT – pozitronska emisiona tomografija/kompjuterizovana tomografija; engl. *positron emission tomography/computed tomography*
PBP – preživljavanje bez progresije (engl. *progression-free survival – PFS*)
PR – parcijalni odgovor; engl. *partial response*
R/R – relapsiran-a/refraktoran-na (engl. *relapsed/refractory*)
RT – radioterapija
SD – standardna devijacija

Sukob interesa: Nije prijavljen.

COVID-19 – coronavirus disease of 2019

CR – complete response

END – extranodal disease

EORTC/LYSA – European Organisation for Research and Treatment of Cancer/Lymphoma Study Association

ESR – erythrocyte sedimentation rate

GHSG – German Hodgkin Study Group

HDC – high-dose chemotherapy

IPS – International Prognostic Score

OS – overall survival

PET/CT – positron emission tomography/computed tomography

PFS – progression-free survival

PR – partial response

R/R – relapsed/refractory

RT – radiotherapy

SD – standard deviation

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ANEMIA AND HEART FAILURE

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SAŽETAK

Prisustvo anemije kod bolesnika sa srčanom slabošću predstavlja nezavisni loš prognostički faktor. Etiologija anemije je multifaktorijska, a u njoj učestvuju kako priroda same hronične srčane slabosti, tako i starije životno doba ovih bolesnika i učestali komorbiditeti. Apsolutni ili funkcionalni deficit gvožđa, čak i u odsustvu anemije, kod bolesnika sa srčanom slabošću značajno utiče na opadanje kvaliteta života, te na povećanje učestalosti hospitalizacije i mortaliteta. Kompleksna etiologija i patofiziologija anemije otežavaju pravilno tumačenje laboratorijskih parametara metabolizma gvožđa, a takođe iziskuju i specifičan terapijski pristup.

Ključne reči: anemija, srčana slabost, etiologija, lečenje

ABSTRACT

The presence of anemia in patients with heart failure is a significant independent adverse prognostic factor. The etiology of anemia is multifactorial and the nature of heart failure itself, advanced age, and frequent comorbidities contribute to its development. Notably, absolute or functional iron deficiency, even in the absence of anemia, significantly diminishes the quality of life, increases hospitalization frequency, and raises mortality rates in patients with heart failure. The intricate etiology and pathophysiology of anemia present a challenge for the accurate interpretation of laboratory parameters of iron metabolism and necessitate a tailored therapeutic approach.

Keywords: anemia, heart failure, etiology, treatment

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UVOD

Anemija i dalje predstavlja veliki globalni zdravstveni problem, iako je poslednjih godina primećen značajan opadajući trend, nastao zahvaljujući preventivnim merama. Na osnovu analize sprovedene 2019. godine u 204 zemlje širom sveta, procenjeno je da 1,8 milijardi ljudi ima anemiju, sa starosno prilagođenom prevalencijom od 23% [1]. Najnoviji epidemiološki podaci pokazuju da oko 64 miliona ljudi u svetu boluje od srčane slabosti. Dok se incidencija srčane slabosti u razvijenim zemljama stabilizuje ili blago opada, njena prevalencija raste zbog uspešnijeg lečenja ishemijske bolesti srca, koja je glavni uzrok srčane slabosti, kao i zbog novih terapijskih pristupa u lečenju same srčane slabosti, te ukupnog starenja svetske populacije [2].

Prema važećim kriterijumima Svetske zdravstvene organizacije (SZO), anemija se kod odraslih osoba definiše kao pad nivoa hemoglobina (Hb) ispod 120 g/l kod žena, odnosno ispod 130 g/l kod muškaraca. U zavisnosti od stepena pada nivoa hemoglobina u krvi, anemija se klasifikuje na sledeći način: blaga ($Hb \geq 110$ g/l), srednje teška (80 g/l – 109 g/l) i teška ($Hb < 80$ g/l) [3]. Srčana slabost (engl. *heart failure* – HF) je sindrom nastao kao rezultat strukturnih i/ili funkcionalnih oštećenja srca koji dovode do povećanja intrakardijalnog pritiska i/ili smanjenja minutnog volumena srca u mirovanju ili tokom fizičkog napora. Srčana slabost se može klasifikovati u sledeće tri grupe: 1) *HF_{rEF}* – srčana slabost sa smanjenom ejectionom frakcijom leve komore (engl. *HF with reduced left ventricular ejection fraction*), ($LVEF \leq 40\%$); 2) *HF_{mrEF}* – srčana slabost sa umereno smanjenom LVEF (engl. *HF with moderately reduced LVEF*), ($LVEF = 41\% - 49\%$); i 3) *HF_{pEF}* – srčana slabost sa očuvanom LVEF (engl. *HF with preserved LVEF*), ($LVEF \geq 50\%$) [4,5]. Najnoviji epidemiološki podaci pokazuju da oko 64 miliona ljudi širom sveta boluje od srčane slabosti.

Epidemiološka istraživanja su otkrila da je prevalencija anemije kod obolelih od srčane slabosti oko 30% kod ambulantnih pacijenata, a čak 50% kod hospitalizovanih pacijenata, što je mnogo više nego u opštoj populaciji [6]. Utvrđeno je da se incidencija anemije povećava sa stadijumom, odnosno funkcionalnom klasom srčane slabosti, u rasponu od 9%, kod *New York Heart Association* klase I (engl. *NYHA Class I*), do 79%, kod *NYHA* klase IV [7]. Štaviše, incidencija anemije progresivno raste sa trajanjem srčane slabosti. Rezultati studije *Studies of Left Ventricular Dysfunction* (SOLVD) pokazali su da je 9% pacijenata sa normalnim nivoom hemoglobina u vreme dijagnoze srčane slabosti razvilo anemiju u prvoj godini praćenja [8], dok je u studiji *Carvedilol and Metoprolol European Trial* (COMET) prevalencija anemije bila iznad 25%, nakon petogodišnjeg praćenja [9].

INTRODUCTION

Anemia remains a major global health problem, although a significant downward trend has been observed in recent years, thanks to preventive measures. Based on the analysis conducted in 2019, in 204 countries worldwide, it has been estimated that 1.8 billion people have anemia, with an age-adjusted prevalence of 23% [1]. The latest epidemiological data show that around 64 million people in the world suffer from heart failure (HF). While the incidence of HF in developed countries is stabilizing or slightly declining, its prevalence is increasing due to the improved treatment of ischemic heart disease, which is the main cause of HF, as well as due to new therapeutic approaches to HF treatment and the overall aging of the global population [2].

According to the current World Health Organization (WHO) criteria, anemia in adults is defined as a drop in hemoglobin (Hb) levels below 120 g/l in women, i.e., below 130 g/l in men, and, depending on the degree of Hb reduction, it is graded in the following manner: mild ($Hb \geq 110$ g/l), moderate (80 g/l – 109 g/l), and severe ($Hb < 80$ g/l) [3]. Heart failure (HF) is a syndrome resulting from structural and/or functional heart impairments leading to an increase in intracardiac pressures and/or a decrease in cardiac output at rest or during exertion. HF can be classified into the following three groups: 1) HF with reduced left ventricular ejection fraction (*HF_{rEF}*), ($LVEF \leq 40\%$); 2) HF with moderately reduced LVEF (*HF_{mrEF}*), ($LVEF = 41\% - 49\%$); and 3) HF with preserved LVEF (*HF_{pEF}*), ($LVEF \geq 50\%$) [4,5]. The latest epidemiological data show that approximately 64 million people worldwide suffer from HF.

Epidemiological studies have revealed that the prevalence of anemia in patients with HF is around 30% in outpatients and as high as 50% in hospitalized patients, which is much higher than in the general population [6]. It has been found that the incidence of anemia increases with the HF stage, i.e., HF functional class, ranging from 9%, in *New York Heart Association* (NYHA) Class I, to 79%, in NYHA Class IV [7]. Furthermore, the incidence of anemia progressively increases with the duration of HF. The results of the *Studies of Left Ventricular Dysfunction* (SOLVD) showed that 9% of patients with normal Hb levels at the time of HF diagnosis developed anemia within the first year of follow-up [8], while in the *Carvedilol and Metoprolol European Trial* (COMET), the prevalence of anemia was above 25%, after five-year follow-up [9].

In the recommendations of the European Society of Cardiology (ESC) for the treatment of HF, iron deficiency (ID), irrespective of the presence of anemia, is particularly recognized as an adverse risk factor in

U preporukama Evropskog kardiološkog društva (engl. *European Society of Cardiology – ESC*) za lečenje srčane slabosti, deficit gvožđa (engl. *iron deficiency – ID*) je, bez obzira na to da li je prisutna i anemija, posebno prepoznat kao nepovoljan faktor rizika kod *HFmrEF* i *HFrEF*, koji značajno otežava tok bolesti. Zbog toga je neophodno proveriti prisustvo anemije i deficita gvožđa kod svih bolesnika sa srčanom slabošću [4,5]. Studija sprovedena u 48 medicinskih centara u Francuskoj (studijaska grupa *CARENFER*), koja je obuhvatila 1.475 pacijenata sa dekompenzovanom srčanom slabošću i hroničnom srčanom slabošću, a čija je prosečna starost bila 78 godina, pokazala je ukupnu prevalenciju deficita gvožđa od 49,5%. Međutim, kada su bolesnici stratifikovani prema težini srčane slabosti – dekompenzovana srčana slabost (60,1% ispitanika) i hronična srčana slabost, i zatim podeljeni u tri kategorije prema preporukama *ESC*-a, prevalencija deficita gvožđa kod pacijenata sa dekompenzovanom srčanom slabošću je iznosila 58,1%, u poređenju sa prevalencijom od 39% kod onih sa hroničnom srčanom slabošću. Među pacijentima sa hroničnom srčanom slabošću, prevalencija deficita gvožđa je bila najveća u *HFpEF* grupi (*HFpEF* 57,3%; *HFmrEF* 47,4%; *HFrEF* 44,3%) [10]. Novija istraživanja ukazuju na to da anemija značajno doprinosi smanjenju tolerancije fizičkog napora kod osoba sa *HFpEF* [11], ali i da sam deficit gvožđa značajno povećava rizik od mortaliteta kod pacijenata sa *HFpEF* [12].

ETIOLOGIJA I PATOFIZIOLOGIJA ANEMIJE KOD SRČANE SLABOSTI

Anemija kod srčane slabosti ima složenu i multifaktorsku etiologiju, u kojoj sama srčana slabost i prateća stanja igraju značajnu ulogu. Preko 85% pacijenata sa srčanom slabošću pati od dve ili više hroničnih bolesti, a prosečan broj kardiovaskularnih i nekardiovaskularnih komorbiditeta po hospitalizovanom pacijentu sa srčanom slabošću iznosi oko četiri. Pored anemije, uobičajeni nekardiovaskularni komorbiditeti srčane slabosti uključuju i gojaznost, dijabetes, bubrežnu insuficijenciju, neravnotežu elektrolita, poremećaj funkcije štitaste žlezde, hroničnu opstruktivnu bolest pluća, apneju u snu, i maligne bolesti [13,14]. Pacijenti sa srčanom slabošću su po pravilu starije osobe koje često ispoljavaju karakteristike anemije povezane sa starošću, što može biti u vezi sa nepravilnom ishranom (siromaštvo, neuhranjenost) i hroničnim zapaljenjem [15]. Anemija kod srčane slabosti je obično blaga do srednje teška, a njen primarni uzrok je često deficit gvožđa, bilo da je apsolutni ili funkcionalni. Čak i bez anemije, deficit gvožđa može značajno da smanji funkcionalnost i kvalitet života [1]. Procenjuje se da 47% – 68% pacijenata sa hroničnom srčanom slabošću ima apsolutni ili funkcionalni deficit gvožđa [16].

HFmrEF and *HFrEF* that significantly complicates the course of the disease. This is why it is necessary to carry out screening for the presence of anemia and ID in all patients with HF [4,5]. A study conducted in 48 medical centers in France (*CARENFER* study group), which included 1,475 patients with decompensated HF and chronic HF, whose average age was 78 years, showed a total prevalence of ID of 49.5%. However, when the patients were stratified according to the severity of HF – decompensated HF (60.1% of respondents) and chronic HF, and then divided into three categories according to *ESC* recommendations, the prevalence of ID among patients with decompensated HF was 58.1%, as compared to a prevalence of 39% in those with chronic HF. Among the latter, the prevalence of ID was the highest in the *HFpEF* group (*HFpEF* 57.3%; *HFmrEF* 47.4%; *HFrEF* 44.3%) [10]. New research indicates that anemia significantly contributes to reduced exercise tolerance in people with *HFpEF* [11], but also that ID itself markedly increases the risk of mortality in patients with *HFpEF* [12].

ETIOLOGY AND PATHOPHYSIOLOGY OF ANEMIA IN HEART FAILURE

The etiology of anemia in heart failure is complex and multifactorial, wherein HF itself and associated conditions play a significant role. Over 85% of patients with HF suffer from two or more chronic diseases, and the average number of cardiovascular and non-cardiovascular comorbidities per hospitalized patient with HF is approximately four. In addition to anemia, common non-cardiovascular comorbidities include obesity, diabetes, renal failure, electrolyte imbalance, thyroid gland dysfunction, chronic obstructive pulmonary disease, sleep apnea, and malignant diseases [13,14]. Patients with HF are typically older and often exhibit characteristics of anemia related to old age, which can be linked to improper nutrition (poverty, malnutrition) and chronic inflammation [15]. Anemia in HF is usually mild to moderate, and its primary cause is often iron deficiency, whether absolute or functional. Even without anemia, ID can significantly reduce functionality and quality of life [1]. It is estimated that 47% – 68% of patients with chronic HF have absolute or functional ID [16].

Iron is an integral component of the metalloprotein hemoglobin, essential for transporting oxygen to all cells in the body. Due to its chemical properties, which allow it to accept and release electrons easily, changing from a divalent (ferrous) to a trivalent (ferric) form, iron is also an integral part of enzymes and enzyme activators involved in crucial body processes. Iron controls the expression of aconitase, a key enzyme in the Krebs

Gvožđe je sastavni deo metaloproteina hemoglobina, koji je neophodan za transport kiseonika do svih ćelija u telu. Zbog svojih hemijskih osobina, koje mu omogućavaju da lako prima i otpušta elektrone, prelazeći iz dvovalentnog (fero) u trovalentni (feri) oblik, gvožđe je takođe sastavni deo enzima i aktivatora enzima uključenih u ključne procese u telu. Gvožđe kontroliše ekspresiju akonitaze, ključnog enzima u Krebsovom ciklusu (ciklus limunske kiseline), koji olakšava prenos elektrona u redoks reakcijama. Pošto srčani mišić obiluje mitohondrijama, neophodne su odgovarajuće koncentracije gvožđa za pravilan tok redoks reakcija i za sprečavanje stvaranja slobodnih radikala koji utiču na remodelovanje i apoptozu kod srčane slabosti [17].

Uzroci apsolutnog deficita gvožđa (engl. *iron deficiency – ID*) i sideropenijske anemije (engl. *iron deficiency anemia – IDA*) su raznovrsni, od nepravilne ishrane (npr. vegetarijanska ishrana; veganska ishrana; ishrana zasnovana uglavnom na mleku i mlečnim proizvodima; ishrana zasnovana na hrani bogatoj fitatima – žitarice, soja, mahunarke; prekomerno konzumiranje pića koja sadrže tanine – kafa, čaj, crno vino), preko malapsorpcije u dvanaestopalačnom crevu (npr. autoimuni atrofični gastritis, celijakija, necelijačna glutenska enteropatija i drugi sindromi malapsorpcije, barijatrijska hirurgija, zapaljenke bolesti creva); povećane potrebe za gvožđem (npr. tokom postoperativnog oporavka; tokom primene eritropoetina – EPO), urođenog nedostatka transportera gvožđa (npr. hem oksigenaza, dvovalentni metalni transporter 1 – DMT1), do povećanog gubitka krvi (npr. krvarenje iz urogenitalnog i digestivnog trakta), što je najčešći uzrok anemije u razvijenim zemljama [18,19].

Funkcionalni deficit gvožđa je nedostatak gvožđa u cirkulaciji koje ostaje zarobljeno u makrofagima, što se javlja u stanjima hronične upale. Prevalencija funkcionalnog deficita gvožđa kod pacijenata sa srčanom slabošću, koja je takođe hronično proinformatorno stanje, iznosi oko 18% [20]. Povećana proizvodnja proinformatornih citokina, kao što su interleukin-6 (IL-6), IL-1 β , IL-2, IL-8, IL-33, interferon- γ (IFN- γ), galektin-3, faktor nekroze tumora-1 (engl. *tumor necrosis factor-1 – TNF-1*), pentaksin-3, te smanjena proizvodnja zaštitnih citokina, kao što je IL-10, dokazane su kod srčane slabosti, dovodeći do sledećih pojava: a) hipoferemije izazvane povećanom proizvodnjom hepcidina, što izaziva degradaciju ferroportina, izvoznika intracelularnog gvožđa, zbog čega gvožđe ostaje zarobljeno u makrofagima, enterocitima i hepatocitima i nije dostupno prekursorima eritropoeze u koštanoj srži; b) direktnog suzbijanja eritropoeze; c) umerenog skraćivanja životnog veka eritrocita usled njihove pojačane razgradnje; g) smanjene proizvodnje EPO-a [21–24]. Hronična bubrežna insuficijencija takođe doprinosi smanjenoj proizvodnji EPO-a,

cycle (citric acid cycle) that facilitates electron transfer in redox reactions. Since the heart muscle is abundant with mitochondria, appropriate iron concentrations are necessary for the proper course of redox reactions and for preventing the formation of free radicals that affect remodeling and apoptosis in HF [17].

The causes of absolute iron deficiency (ID) and iron deficiency anemia (IDA) are diverse, ranging from improper nutrition (e.g., vegetarian diet; vegan diet; diet based mainly on milk and dairy products; diet based on foods rich in phytates – cereals, soy, legumes; excessive intake of beverages with tannins – coffee, tea, red wine), malabsorption in the duodenum (e.g., autoimmune atrophic gastritis, celiac disease, non-celiac gluten enteropathy and other malabsorption syndromes, bariatric surgery, inflammatory bowel diseases – IBD), increased iron needs (e.g., during postoperative recovery; during the administration of erythropoietin – EPO), congenital deficiency of iron transporters (e.g., heme oxygenase, divalent metal transporter 1 – DMT1), to increased blood loss (e.g., bleeding from the urogenital and digestive tract), which is the most common cause of anemia in developed countries [18,19].

Functional ID is a deficiency of circulating iron that remains trapped in macrophages, which occurs in states of chronic inflammation. The prevalence of functional ID in patients with HF, which is also a chronic proinflammatory condition, is about 18% [20]. Increased production of proinflammatory cytokines, such as interleukin-6 (IL-6), IL-1 β , IL-2, IL-8, IL-33, interferon- γ (IFN- γ), galectin-3, tumor necrosis factor-1 (TNF-1), pentaxin-3, and reduced production of protective cytokines, such as IL-10, have been proven in HF, leading to the following: a) hypoferremia caused by the increased secretion of hepcidin, which causes the degradation of ferroportin, the exporter of intracellular iron, which is why iron remains captured in macrophages, enterocytes, and hepatocytes and is not available to the precursors of erythropoiesis in the bone marrow; b) direct suppression of erythropoiesis; c) moderate shortening of the lifespan of erythrocytes due to their increased destruction; d) reduced production of EPO [21–24]. Chronic kidney failure also contributes to reduced EPO production, and almost 45% of patients with terminal renal failure develop HF, i.e., cardiorenal syndrome type 4 [25,26].

It's important to note that various medications can often cause ID/IDA. Certain drugs like antihistamines (H2 blockers), antacids, proton pump inhibitors (PPIs), some antibiotics (penicillin, ciprofloxacin, tetracyclines), cholestyramine, and anticonvulsants reduce iron absorption. On the other hand, nonsteroidal anti-rheumatic drugs, antiplatelet drugs, and anticoag-

a kod skoro 45% pacijenata sa terminalnom bubrežnom insuficijencijom dolazi do razvoja srčane slabosti, odnosno kardioresalnog sindroma tipa 4 [25,26].

Važno je naglasiti da različiti lekovi često mogu izazvati deficit gvožđa/sideropenijsku anemiju. Određeni lekovi, kao što su antihistaminici (H2 blokatori), antacidi, inhibitori protonske pumpe (engl. *proton pump inhibitors* – PPIs), neki antibiotici (penicilin, ciprofloksacin, tetraciklini),olestiramin i antikonvulzanti, smanjuju apsorpciju gvožđa. S druge strane, nesteroidni antireumatski lekovi, antiagregacioni lekovi i antikoagulansi dovode do krvarenja [18]. Štaviše, ACE inhibitori (engl. *angiotensin-converting enzyme inhibitors* – ACE inhibitors) i AT1-antagonisti (blokatori angiotenzina) deluju preko renin-angiotenzin-aldosteron sistema (RAAS), umanjujući na taj način efekte remodelovanja i simpatičke efekte, a svojim uticajem na angiotenzin II suzbijaju sintezu EPO-a i proliferaciju eritroidnih progenitora u koštanoj srži [20,27]. Uz to, ACE inhibitori dovode do povećane proizvodnje N-acetil-seril-aspartil-lizil-prolina, što utiče na smanjenje sekrecije EPO-a, a ovo dovodi do smanjenja eritrocitopoeze i pada koncentracije hemoglobina za 5 g/l [27,28].

Maligna oboljenja su, uz kardiovaskularne bolesti, glavni uzrok morbiditeta i mortaliteta, kao i vodeći uzrok prerane smrti u razvijenim zemljama. Antineoplastična terapija (konvencionalni citostatici, imunoterapija i ciljana terapija, terapija zračenjem) može dovesti do ranih i kasnih kardiovaskularnih komplikacija, najčešće do srčane slabosti [29,30], ali i do pojave ili pogoršanja anemije [31]. Sa druge strane, primećeno je da upotreba SGLT2 inhibitora (engl. *sodium-glucose cotransporter 2 inhibitors*), koja značajno smanjuje mortalitet kod pacijenata sa kardioresalnim sindromom, ima povoljan efekat i na anemiju. Iako tačan mehanizam nije poznat, veruje se da SGLT inhibitori deluju na homeostazu gvožđa, smanjuju lučenje proinflamatornih citokina i indukuju lučenje EPO-a [27,32,33].

Pored gore opisanih uzroka, pad nivoa hemoglobina kod pacijenata sa kongestivnom srčanom slabošću može se pripisati hipervolemiji, koja dovodi do hemodilucije, a koju karakteriše povećanje zapremine plazme uz normalnu masu eritrocita. Do 46% pacijenata sa srčanom slabošću ima hemodiluciju [34].

UTICAJ ANEMIJE NA KVALITET ŽIVOTA I MORTALITET PACIJENATA SA SRČANOM INSUFICIJENCIJOM

Čak i kad nema srčane slabosti, teška anemija (pad nivoa hemoglobina na 40 g/l – 50 g/l) dovodi do zadržavanja natrijuma i vode, smanjene bubrežne perfuzije, smanjene glomerularne filtracije, kao i neurohormonske aktivacije. Ovaj kardioresalni odgovor proizilazi iz

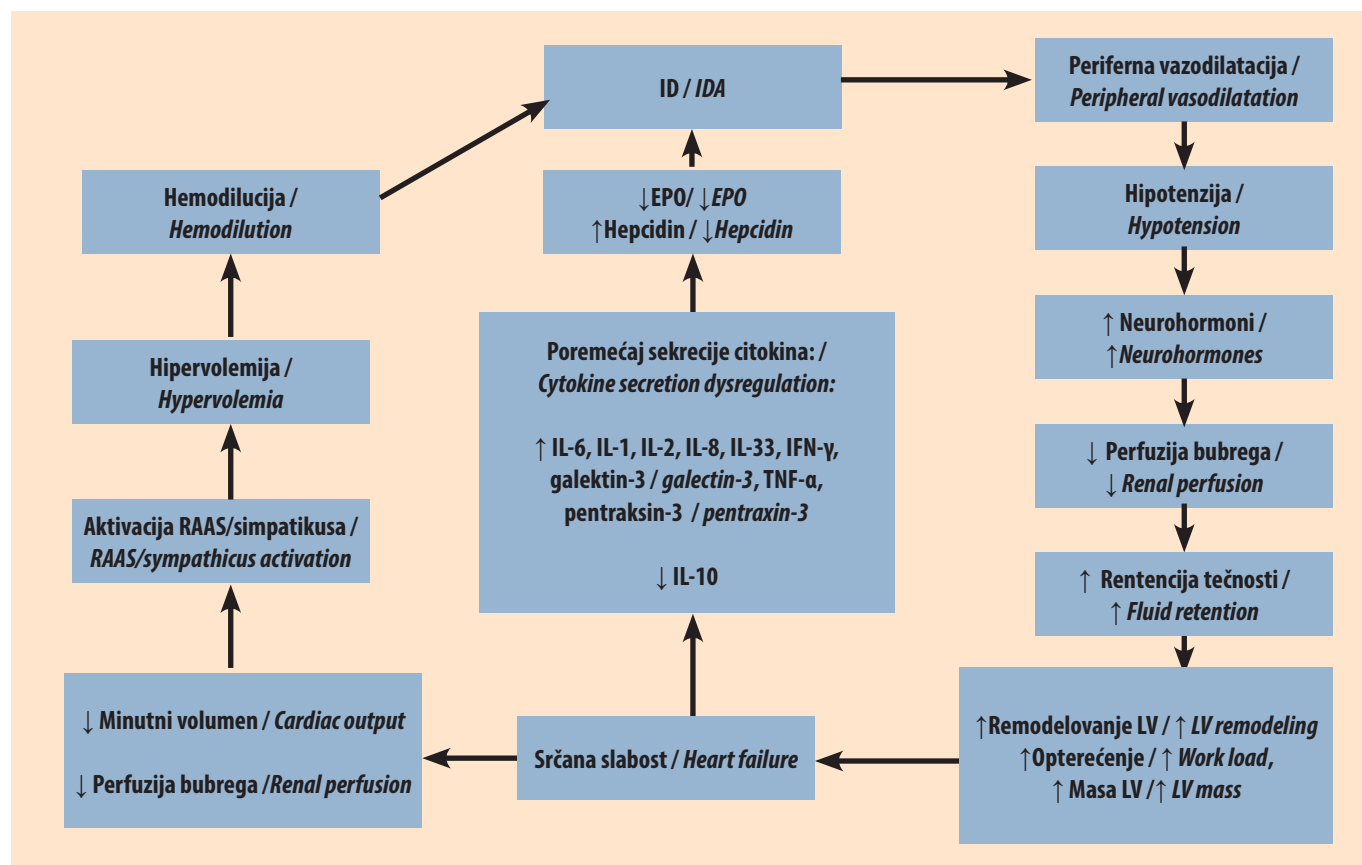
ulants lead to bleeding [18]. Furthermore, angiotensin-converting enzyme inhibitors (ACE inhibitors) and AT1-antagonists (angiotensin blockers) act via the renin-angiotensin-aldosterone system (RAAS), thereby curtailing remodeling and sympathetic effects, and, by affecting angiotensin II, they suppress the synthesis of EPO and the proliferation of erythroid progenitors in the bone marrow [20,27]. Additionally, ACE inhibitors lead to an increase in N-acetyl-seryl-aspartyl-lysyl-proline production, which affects the reduction of EPO secretion, leading to decreased erythrocytopoiesis and a drop in Hb concentration by 5 g/l [27,28].

Malignant diseases, along with cardiovascular diseases, are the main cause of morbidity and mortality, as well as the leading cause of premature death in developed countries. Antineoplastic therapy (conventional cytostatics; immunotherapy and targeted therapy; radiation therapy) can lead to early and late cardiovascular complications, most often HF [29,30], as well as the onset or worsening of anemia [31]. On the other hand, the use of sodium-glucose cotransporter (SGLT2) inhibitors, which has significantly reduced mortality in patients with cardioresnal syndrome, has also been observed to have a beneficial effect on anemia. Although the exact mechanism is not known, it is believed that SGLT inhibitors act on iron homeostasis, reduce the secretion of pro-inflammatory cytokines, and induce the secretion of EPO [27,32,33].

In addition to the causes described above, a decline in Hb levels in patients with congestive HF can be attributed to hypervolemia, leading to hemodilution, which is characterized by an increase in plasma volume with a normal erythrocyte mass. Up to 46% of patients with HF have hemodilution [34].

THE IMPACT OF ANEMIA ON THE QUALITY OF LIFE AND MORTALITY OF PATIENTS WITH HEART FAILURE

Even in the absence of HF, severe anemia (a drop in Hb to 40 g/l – 50 g/l) leads to sodium and water retention, decreased renal perfusion, decreased glomerular filtration, and neurohormonal activation. This cardioresnal response stems from the effect of anemia on blood viscosity, oxygen pressure in the microvasculature, and nitric oxide availability. Mild anemia also contributes to neurohormonal activation (catecholamines; natriuretic peptides) and exacerbation of HF [35]. Anemia is an independent factor of poor prognosis in HF with left ventricular dysfunction, and its incidence increases with the severity of HF. According to the study: Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE), the annual mortality risk in anemic HF patients is 28%, compared to 16%



Legenda: ID – deficit gvožđa (engl. *iron deficiency*); IDA – sideropenijska anemija (engl. *iron deficiency anemia*); RAAS – renin-angiotenzin-aldosteron sistem; IL – interleukin; IFN – interferon; TNF – tumor nekrotični faktor; LV – leva komora (engl. *left ventricle*)

Legend: ID – iron deficiency; IDA – iron deficiency anemia; RAAS – renin-angiotensin-aldosterone system; IL – interleukin; TNF – tumor necrosis factor; LV – left ventricle

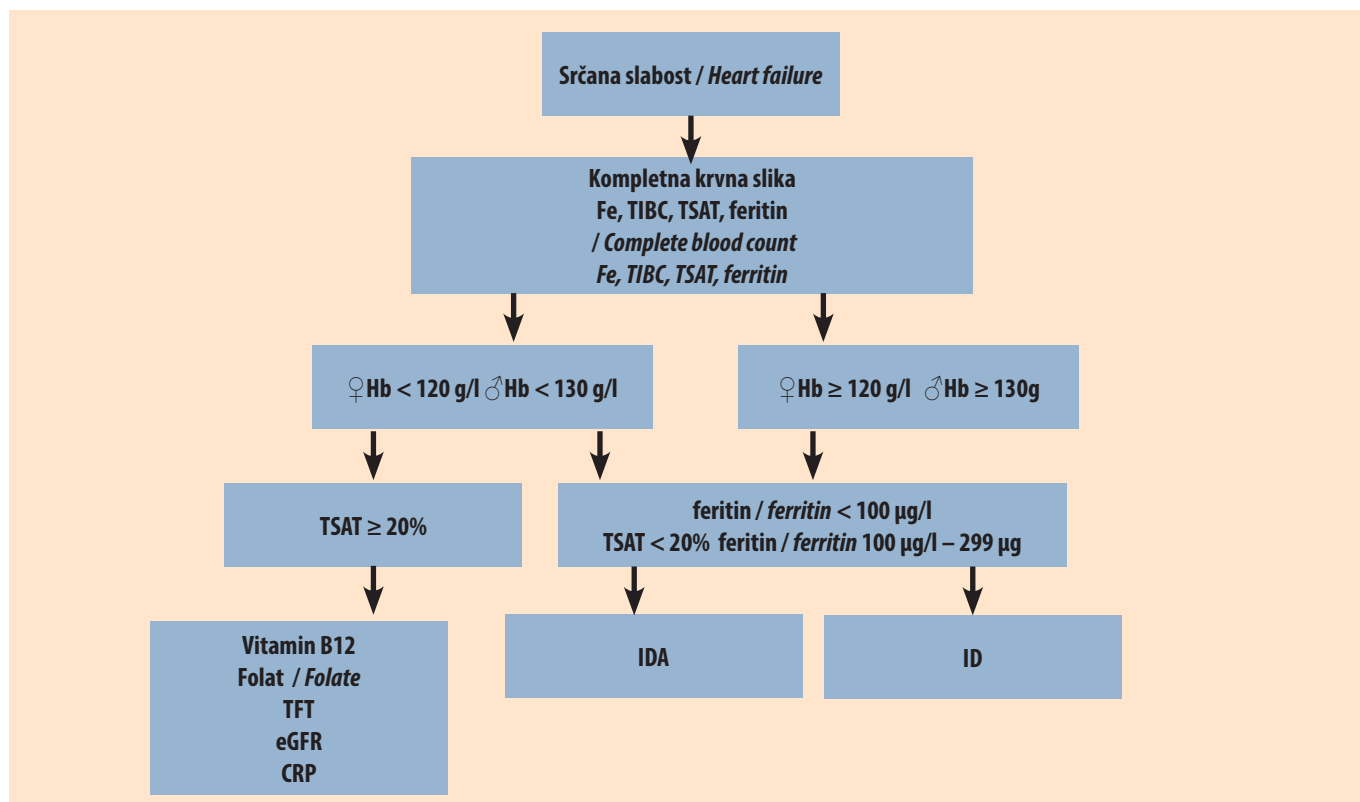
Slika 1. Patofiziološki mehanizmi međusobnog uticaja anemije i srčane slabosti

Figure 1. Pathophysiological mechanisms of the mutual effects of anemia and heart failure

uticaja anemije na viskozitet krvi, pritisak kiseonika u mikrovaskulaturi i dostupnost azot-oksida. Blaga anemija takođe doprinosi neurohormonskoj aktivaciji (kateholamini; natriuretski peptidi) i pogoršanju srčane slabosti [35]. Anemija je nezavisan faktor loše prognoze kod srčane slabosti sa disfunkcijom leve komore, a njena incidencija raste sa težinom srčane slabosti. Prema studiji: *Randomized Eranercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE)*, godišnji rizik od smrtnosti kod anemičnih pacijenata sa srčanom slabošću je 28%, u poređenju sa 16% kod pacijenata sa srčanom slabošću bez anemije. Porast nivoa hemoglobina za 10 g/l smanjuje rizik od mortaliteta za 15,8%, a rizik od hospitalizacije za 14,2% [7]. Nasuprot tome, svako smanjenje hematokrita za 1% nosi povećanje rizika od smrtnosti od 11%, kao što je pokazano u studiji: *Prospective Randomized Amlodipine Survival Evaluation (PRAISE)* [36]. Rizik od ponovne hospitalizacije je takođe veći, a povećava se za 3,3% pri svakom padu nivoa hemoglobina od 10 g/l, nakon otpusta iz bolnice [20]. Studija: *Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS)* je pokazala da je korekcija anemije i povećanje nivoa hemoglobina za

in HF patients without anemia. An increase in Hb by 10 g/l reduces the risk of mortality by 15.8% and the risk of hospitalization by 14.2% [7]. Conversely, every decrease in hematocrit by 1% carries an 11% increase in mortality risk, as shown in the study: *Prospective Randomized Amlodipine Survival Evaluation (PRAISE)* [36]. The risk of rehospitalization is also higher, increasing by 3.3% for every drop in the Hb level by 10 g/l, upon discharge from hospital [20]. The study: *Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS)* showed that correction of anemia and an increase in Hb by 10 g/l was associated with a reduction in left ventricular mass by 4.1 g/m², over a period of 24 weeks [37]. Anemic patients with HF have a reduced exercise tolerance and poorer quality of life, and the costs of treating these patients are up to 25% higher [38].

In the absence of anemia, ID alone is also an independent factor of poor prognosis in HF. Doppler echocardiographic monitoring has shown that iron substitution in HF patients with ID significantly improves cardiac function [38]. Additionally, the six-year survival rate is significantly lower in non-anemic patients with HFpEF who have ID [12].



Legenda: Fe – gvožđe; TIBC – ukupni kapacitet vezivanja gvožđa (engl. *total iron binding capacity*); TSAT – saturacija transferina (engl. *transferrin saturation*); Hb – hemoglobin; TFT – testovi funkcije tireoidne žlezde; eGFR – procenjena brzina glomerulske filtracije (engl. *estimated glomerular filtration rate*); CRP – C reaktivni protein; IDA – sideropenijska anemija (engl. *iron deficiency anemia*); ID – deficit gvožđa (engl. *iron deficiency*)

Legend: Fe – iron; TIBC – total iron binding capacity; TSAT – transferrin saturation; Hb – hemoglobin; TFT – thyroid function tests; eGFR – estimated glomerular filtration rate; CRP – C reactive protein; IDA – iron deficiency anemia; ID – iron deficiency

Slika 2. Pojednostavljeni algoritam dijagnostike deficita gvožđa kod bolesnika sa srčanom slabošću

Figure 2. Simplified algorithm of iron deficiency diagnostics in patients with heart failure

10 g/l povezana sa smanjenjem mase leve komore za 4,1 g/m², tokom perioda od 24 nedelje [37]. Anemični bolesnici sa srčanom slabošću imaju smanjenu toleranciju fizičkog napora i lošiji kvalitet života, a troškovi lečenja ovih bolesnika su i do 25% veći [38].

U odsustvu anemije, sam deficit gvožđa je takođe nezavisan faktor loše prognoze kod srčane slabosti. Praćenje Dopler ehokardiografijom je pokazalo da supstitucija gvožđa kod pacijenata sa srčanom slabošću koji imaju deficit gvožđa značajno poboljšava srčanu funkciju [38]. Pored toga, šestogodišnja stopa preživljavanja je značajno niža kod neanemičnih pacijenata sa HFpEF koji imaju deficit gvožđa [12].

LABORATORIJSKE KARAKTERISTIKE ANEMIJE KOD SRČANE SLABOSTI I DIJAGNOSTIČKI ALGORITAM

Standardni laboratorijski pokazatelji sideropenijske anemije – mikrocitni eritrociti (MCV < 80 fl); hipofermija (↓Fe); povećan ukupni kapacitet vezivanja gvožđa (engl. *total iron binding capacity* – TIBC); smanjena saturacija transferina (engl. *transferrin saturation* – TSAT), (TSAT < 20%); kao i smanjen feritin (< 30 mg/l), obično

LABORATORY CHARACTERISTICS OF ANEMIA IN HEART FAILURE AND THE DIAGNOSTIC ALGORITHM

The standard laboratory indicators of IDA – microcytic erythrocytes (MCV < 80 fl), hypoferrremia (↓Fe), increased total iron binding capacity (TIBC), decreased transferrin saturation (TSAT < 20%), and reduced ferritin (< 30 µg/l) are usually absent in patients with HF, due to the above-described pathophysiological mechanisms of anemia in HF. In HF and other chronic inflammatory conditions, due to the fact that ferritin is a reactant of the acute phase, its values of up to 100 µg/l speak in favor of absolute ID, if all other laboratory characteristics of ID/IDA are present. In patients with HF whose blood ferritin level is 100 ng/ml – 299 ng/ml, ID is considered to exist, if TSAT is < 20%. These cut-off values were taken from nephrology, as they are acceptable and specific for diagnosing and treating ID [39]. When all the obtained laboratory findings are inconclusive, additional tests, such as reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR), and the sTfR-transferrin index, can be performed

nisu prisutni kod pacijenata sa srčanom slabošću zbog gore opisanih patofizioloških mehanizama anemije kod srčane slabosti. Kod srčane slabosti i drugih hroničnih inflamatornih stanja, zbog činjenice da je feritin reaktant akutne faze, njegove vrednosti do 100 mg/l govore u prilog apsolutnog deficita gvožđa, ukoliko su prisutne sve druge laboratorijske karakteristike deficita gvožđa/sideropenijske anemije. Kod pacijenata sa srčanom slabošću čiji je nivo feritina u krvi 100 ng/ml – 299 ng/ml, smatra se da postoji deficit gvožđa ukoliko je TSAT < 20%. Ove granične vrednosti su uzete iz nefrologije, pošto su prihvatljive i specifične za dijagnostikovanje i lečenje deficita gvožđa [39]. Kada se na osnovu svih dobijenih laboratorijskih nalaza ne može doneti zaključak o vrsti anemije, u specijalizovanim laboratorijama se mogu uraditi ispitivanja dodatnih parametara, kao što su sadržaj retikulocitnog hemoglobina (engl. *reticulocyte hemoglobin content* – CHr), solubilni transferrinski receptor (engl. *soluble transferrin receptor* – sTfR) i sTfR-transferinski indeks (engl. *sTfR-transferrin index*). Pored testova gvožđa, neophodno je testirati nivoe C-reaktivnog proteina (CRP), vitamina B12, folata, tiroidnih hormona i renalnih parametara [38], kao i ispitati znakove manifestnog odnosno okultnog krvarenja [40].

Kako normalan nivo hemoglobina ne isključuje postojanje deficita gvožđa, prema aktuelnim preporukama, kod svih bolesnika sa srčanom slabošću treba sprovesti laboratorijsko testiranje pokazatelja metabolizma gvožđa [4,5,14]. Pojednostavljeni algoritam laboratorijske dijagnostike za ispitivanje deficita gvožđa/sideropenijske anemije predstavljen je na Slici 2.

LEČENJE ANEMIJE KOD SRČANE SLABOSTI

Prema preporukama Svetske zdravstvene organizacije, nadoknadu gvožđa treba započeti pri nivoima feritina ispod 30 mg/l, pošto se simptomi kod zdravih osoba javljaju već u fazi pojave deficita gvožđa [3]. Zbog povoljne cene i lakoće primene, u lečenju deficita gvožđa/sideropenijske anemije, lekovi prvog izbora su standardni oralni preparati (divalentnog i trovalentnog) gvožđa [3,19]. Međutim oko 30% – 70% pacijenata ne podnosi dobro terapiju standardnim oralnim preparatima gvožđa. Unos hrane i istovremena upotreba drugih lekova, kao što su H2-blokatori i inhibitori protonske pumpe koji menjaju pH duodenuma, mogu umanjiti apsorpciju Fe³⁺ u digestivnom traktu, što dovodi do simptoma kao što su mučnina, povraćanje, poremećaj varenja i zatvor [41]. Štaviše, standardni oralni preparati gvožđa imaju ograničenu efikasnost kod srčane slabosti zbog edema crevne sluzokože i pojačanog lučenja hepcidina. Sveukupno gledano, stepen crevne apsorpcije standardnih oralnih preparata gvožđa smanjen je na svega 5% [42]. U studijama IRON-HF [43]

in specialized laboratories. Along with iron tests, it is essential to test the levels of C-reactive protein (CRP), vitamin B12, folate, thyroid hormones, and renal parameters [38], as well as to test for signs of manifest or occult bleeding [40].

As a normal Hb level does not rule out iron deficiency, according to current recommendations, all patients with HF should undergo iron metabolism laboratory tests [4,5,14]. A simplified algorithm of ID/IDA laboratory diagnostics is presented in Figure 2.

TREATMENT OF ANEMIA IN HEART FAILURE

According to WHO recommendations, iron replacement should be started at ferritin levels below 30 µg/l, as symptoms in healthy persons already appear in the ID phase [3]. Due to their low cost and ease of administration, in the treatment of ID/IDA the drugs of first choice are standard (divalent and trivalent) oral iron supplements [3,19]. However, in about 30% – 70% of patients, standard orally administered iron supplements are not well tolerated. Food intake and concomitant use of other medications, such as H₂-blockers and proton pump inhibitors that change duodenal pH, can decrease Fe³⁺ absorption in the digestive tract, leading to symptoms such as nausea, vomiting, indigestion, and constipation [41]. Furthermore, standard oral iron supplements have limited efficacy in HF due to edema of the intestinal mucosa and high hepcidin secretion. Overall, the degree of intestinal absorption of standard oral iron supplements is reduced to only 5% [42]. The ineffectiveness of standard oral iron supplements in HF was demonstrated in the IRON-HF [43] and IRONOUT-HF [44] studies.

A pilot study conducted on 50 patients with symptomatic HFrEF indicated that oral sucrosomial iron (SI) can overcome the hepcidin-ferroportin barrier and lead to a slight increase in ferritin, as well as to the improvement in exercise tolerance and quality of life, after six months of therapy [45]. SI is an oral supplement wherein ferric-pyrophosphate is coated with a matrix containing phospholipids and sucrose (tricalcium phosphate and starch), which is absorbed from the digestive tract in a hepcidin-independent way – paracellularly and transcellularly via enterocytes and M cells, throughout the entire small intestine [46]. The release of iron from SI during digestion is less than 5%, unlike all other oral preparations (75% – 85%), which significantly reduces the possibility of toxic effects of iron on the mucosa of the digestive tract, leading to improved intestinal tolerance and high bioavailability [47,48]. However, the increase in ferritin, although significantly higher during SI supplementation, as compared to standard oral iron supplementation, is markedly infe-

i *IRONOUT-HF* [44], pokazana je neefikasnost standardnih oralnih preparata gvožđa kod srčane slabosti.

Pilot studija sprovedena na 50 pacijenata sa simptomatskom *HFrEF* je pokazala da sukrozomalno gvožđe (engl. *sucrosomal iron* – *SI*), koje se unosi oralnim putem, može da prevaziđe hepcidin-feroportin barijeru i da dovede do blagog povećanja feritina, kao i do poboljšanja tolerancije fizičkog napora i unapređenja kvaliteta života, nakon šest meseci terapije [45]. *SI* je oralni preparat u kojem je feri-pirofosfat obložen matriksom koji sadrži fosfolipide i estar sukroze (trikalcijum fosfat i skrob), a koji se apsorbira iz digestivnog trakta na način koji je nezavisan od hepcidina – paracelularno i transcelularno putem enterocita i ćelija, u celom tankom crevu [46]. Oslobođanje gvožđa iz sukrozomalnog gvožđa tokom varenja je manje od 5%, za razliku od svih ostalih oralnih preparata (75% – 85%), čime se značajno smanjuje mogućnost toksičnog dejstva gvožđa na sluzokožu digestivnog trakta, što dovodi do poboljšanja crevne tolerancije i do visoke bioraspoloživosti [47,48]. Međutim, povećanje nivoa feritina, iako značajno veće pri suplementaciji sukrozomalnim gvožđem, u poređenju sa standardnim oralnim preparatima gvožđa, značajno je inferiornije u odnosu na intravenoznu (iv) primenu gvožđa, koja ima stopostotnu bioraspoloživost. Neophodne su studije na mnogo većem broju pacijenata, kao i duže praćenje pacijenata, kako bi se utvrdilo mesto sukrozomalnog gvožđa u lečenju deficita gvožđa/sideropenijske anemije kod pacijenata sa srčanom slabošću [49].

Prema evropskim i američkim preporukama, intarvensko davanje gvožđa je zamenska terapija prvog izbora u lečenju deficita gvožđa/sideropenijske anemije kod pacijenata sa srčanom slabošću [4,5,14]. Rezultati dve velike međunarodne studije: *FAIR-HF*, sprovedene na 459 pacijenata sa srčanom slabošću *NYHA* klase II i III [50], kao i *CONFIRM-HF*, sprovedene na 304 ambulantna pacijenta sa simptomatskom slabošću i $EF \leq 45\%$ [51], pokazali su značajno smanjenje simptoma, bolju toleranciju fizičkog napora i poboljšanje kvaliteta života, kod pacijenata koji su primali gvožđe karboksimaltozu, tokom dvadesetčetvoronedeljnog odnosno jednogodišnjeg praćenja. Rezultati randomizovane studije *IRONMAN*, sprovedene na 1.137 pacijenata u 70 bolnica u Velikoj Britaniji, pokazali su da je lečenje gvožđe derizomaltozom značajno smanjilo broj hospitalizacija i mortalitet od kardiovaskularnih događaja [52]. Za razliku od pacijenata sa hroničnom srčanom slabošću, intravenozno davanje gvožđa pacijentima sa akutnom srčanom slabošću, nakon stabilizacije epizode srčane insuficijencije, ne utiče na preživljavanje pacijenata, što pokazuje studija *AFFIRM-AHF*, koja je obuhvatila 1.107 pacijenata sa akutnom srčanom slabošću [53]. Intravenozna terapija gvožđem ima veće

prior to intravenous (I.V.) iron administration, which has 100% bioavailability. Studies on a much larger number of patients and longer follow-ups are necessary to determine the place of SI in ID/IDA treatment in HF [49].

According to European and American guidelines, I.V. iron is the replacement therapy of choice for ID/IDA in HF [4,5,14]. The results of two large international studies: *FAIR-HF*, conducted on 459 patients with HF *NYHA* class II and III [50], as well as *CONFIRM-HF*, conducted on 304 outpatients with symptomatic HF and $EF \leq 45\%$ [51], showed a significant reduction of symptoms, better exercise tolerance, and improvement in quality of life, in patients who received ferric carboxymaltose during 24-week or one-year follow-up. The results of the randomized *IRONMAN* study, conducted on 1,137 patients in 70 hospitals in Great Britain, showed that treatment with ferric derisomaltose significantly reduced the number of hospitalizations and mortality from cardiovascular events [52]. Unlike patients with chronic HF, the administration of I.V. iron in patients with acute HF, after the stabilization of the heart failure episode, does not affect patient survival, as shown by the *AFFIRM-AHF* study, which included 1,107 patients with acute HF [53]. I.V. iron has higher direct costs (use of hospital resources) and indirect costs (absence from work) than oral therapy. However, a drop in the total number of hospitalizations due to the exacerbation of HF or complications in patients with HF who had received I.V. iron indicates its long-term economic cost-effectiveness [54]. More recent I.V. iron preparations have a significantly lower rate of reported side effects than earlier preparations, such as ferridextran. Based on the results of individual randomized clinical trials, as well as a meta-analysis of published results that showed a decrease in the risk of hospitalization [55,56], in the ESC guidelines, I.V. iron is recommended in patients with symptomatic *HFmrEF* as well as in patients with *HFrEF* and ID, to reduce HF symptoms and improve quality of life (recommendation class I, level of evidence A), while ferric carboxymaltose and ferric derisomaltose are listed as recommended I.V. replacement therapy for the treatment of ID in patients with symptomatic *HFmrEF* and *HFrEF*, to reduce the risk of hospitalization (recommendation class IIa, level of evidence A) [5]. The American guidelines do not single out specific I.V. forms [14].

In patients with coexisting HF and renal failure, the administration of EPO can be effective. It is important to note that correcting anemia until the Hb level reaches the normal level (130 g/l) does not reduce cardiovascular mortality. In fact, normalization of Hb significantly increases the risk of thromboembolic complications [57], which is why it is not recommend-

direktne troškove (korišćenje bolničkih resursa) i indirektne troškove (odsustvo sa posla) od oralne terapije. Međutim, smanjenje ukupnog broja hospitalizacija usled pogoršanja srčane slabosti ili komplikacija, kod pacijenata sa srčanom slabošću koji su primili gvožđe intravenozno, ukazuje na njegovu dugoročnu ekonomsku isplativost [54]. Noviji iv preparati gvožđa imaju značajno nižu stopu prijavljenih neželjenih efekata od ranijih preparata, kao što je feridekstran. Na osnovu rezultata pojedinačnih randomizovanih kliničkih studija, kao i meta-analize objavljenih rezultata koji su pokazali smanjenje rizika od hospitalizacije [55,56], u ESC smernicama, intravenozno gvožđe se preporučuje kod pacijenata sa simptomatskom *HFmrEF* i pacijenata sa *HfrEF* i deficitom gvožđa, u cilju ublažavanja simptoma srčane slabosti i unapređenja kvaliteta života (preporuka klase I, nivo dokaza A), dok se gvožđe karboksimaltoza i gvožđe derizomaltoza navode kao preporučena intravenozna supstituciona terapija za lečenje deficita gvožđa kod pacijenata sa simptomatskom *HFmrEF* i *HfrEF*, radi smanjenja rizika od hospitalizacije (preporuka klase IIa, nivo dokaza A) [5]. Američke smernice ne izdvajaju konkretne intravenozne oblike terapije [14].

Kod pacijenata sa istovremenom srčanom slabošću i bubrežnom insuficijencijom, primena EPO-a može biti delotvorna. Važno je napomenuti da korekcija anemije do postizanja normalnog nivoa hemoglobina (130 g/l) ne smanjuje kardiovaskularni mortalitet. Štaviše, normalizacija hemoglobina značajno povećava rizik od tromboembolijskih komplikacija [57], zbog čega se ne preporučuje [5,14]. Pošto je anemija kod srčane slabosti obično blaga, transfuzije eritrocita su retko indikovane. Meta-analiza objavljenih studija o preživljavanju pacijenata sa akutnim koronarnim sindromom i anemijom, pokazala je da je preživljavanje bilo značajno bolje kada su transfuzije davane pri nivoima hemoglobina ispod 100 g/l, u poređenju sa restriktivnom strategijom transfuzije, pri nivoima hemoglobina ispod 80 g/l [58]. Međutim, za anemične pacijente sa srčanom slabošću ne postoje definitivne preporuke niti randomizovane studije i meta-analize koje procenjuju njihovo preživljavanje i njihove ishode u odnosu na restriktivni odnosno liberalni pristup transfuzijskoj podršci. U nedostatku zvaničnih smernica, pojedini autori predlažu da se razmotri liberalan pristup transfuzijama eritrocita (Hb = 80 g/l – 90 g/l) za kritično bolesne pacijente sa srčanom slabošću [59].

ZAKLJUČAK

Visoka prevalencija deficita gvožđa/sideropenijske anemije kod srčane slabosti zahteva blagovremenu dijagnozu i lečenje kod svih pacijenata, kako bi se unapredio njihov kvalitet života i poboljšalo preživljavanje.

Sukob interesa: Nije prijavljen.

ed [5,14]. Since anemia in HF is usually mild, RBC transfusions are seldom indicated. A meta-analysis of published studies on the survival of patients with acute coronary syndrome and anemia showed that survival was significantly better when transfusions were given at Hb levels below 100 g/l, as compared to a restrictive transfusion strategy, with Hb levels below 80 g/l [58]. However, for anemic HF patients, there are no definitive recommendations nor randomized studies and meta-analyses evaluating their survival and outcomes, in relation to the restrictive or liberal approach to transfusion support. In the absence of official guidelines, individual authors suggest considering a liberal approach regarding erythrocyte transfusions (Hb = 80 g/l – 90 g/l) for critically ill HF patients [59].

CONCLUSION

The high prevalence of ID/IDA in HF requires timely diagnosis and treatment for all patients, to improve their quality of life and survival.

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DRUG-INDUCED THROMBOCYTOPENIA

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SAŽETAK

Lekovi predstavljaju čest uzrok trombocitopenije, pogotovo kod hospitalizovanih bolesnika. Učestalost ove neželjene reakcije na lekove kreće se oko 10/1 000 000 stanovnika godišnje i prema mehanizmu nastanka može se klasifikovati kao imunska i neimunska. Lekovi poput citostatika, linezolida, ganciklovira, valaciclovira, Aspirina i vankomicina dovode do neimunskog oblika trombocitopenije, tako što deluju proapoptotički na trombocite ili direktno citotoksično na megakariocite i trombocite. S druge strane, imunski oblik uzrokovan je antitelima specifičnim za lek koja se, u prisustvu leka ili njegovog metabolita, vezuju za trombocitne antigene dovodeći do ubrzane razgradnje trombocita. Osim ovog klasičnog oblika lekom uzrokovane imune trombocitopenije (LITP), koji se odlikuje akutnom trombocitopenijom teškog stepena (nadir broja trombocita $< 20 \times 10^9/L$) i krvarenjem, prepoznati su i posebni oblici: heparinom uzrokovana trombocitopenija (HIT), trombocitopenija uzrokovana blokatorima kontrolnih tačaka i vakcinom indukovana trombozna trombocitopenija (VITT). HIT je najčešća LITP u kojoj je nadir broja trombocita najčešće oko $60 \times 10^9/L$, a kliničkom slikom dominiraju tromboze, i to venske i, ređe, arterijske. S druge strane, VITT se odlikuje nastankom tromboze i trombocitopenije 4 do 30 dana od primene adenovirusnih vektorskih vakcina a smrtnost u ovom obliku LITP se kreće između 25% i 60%.

Ključne reči: lek, trombocitopenija, heparin, patofiziološki mehanizmi, algoritmi lečenja

ABSTRACT

Drugs could cause thrombocytopenia, mostly in hospitalized patients. The incidence of this adverse reaction to medicines is around 10/1,000,000 inhabitants/year. Depending on the pathophysiological mechanism, drug-induced thrombocytopenia can be classified into immune and non-immune. Drugs such as cytostatics, linezolid, ganciclovir, valacyclovir, aspirin, and vancomycin can induce a non-immune form of thrombocytopenia. They achieve this by exerting direct cytotoxic effects on megakaryocytes and platelets, or through proapoptotic mechanisms that affect platelets. On the other hand, the immune form is caused by drug-specific antibodies, which, in the presence of the drug or its metabolite, bind to platelet antigens, leading to accelerated destruction of platelets. Apart from this classic form of drug-induced immune thrombocytopenia (DITP), which is characterized by the acute onset of severe thrombocytopenia (nadir platelet counts $< 20 \times 10^9/L$) and bleeding, special forms such as heparin-induced thrombocytopenia (HIT), thrombocytopenia caused by the use of immune checkpoint inhibitors, and vaccine-induced thrombotic thrombocytopenia (VITT) are identified. HIT is the most common DITP in which nadir platelet count is usually around $60 \times 10^9/L$ and the clinical presentation is dominated by thrombosis (venous and less often arterial). Conversely, VITT is characterized by the onset of thrombosis and thrombocytopenia between 4 and 30 days after the administration of adenoviral vector vaccines. The mortality in this form of LITP ranges between 25% and 60%.

Keywords: drug, thrombocytopenia, heparin, pathophysiological mechanisms, treatment algorithms

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UVOD

Lekovi predstavljaju čest uzrok trombocitopenije, pogotovo kod hospitalizovanih bolesnika. Nastanak trombocitopenije do sada je opisan tokom primene više od 300 lekova, čiji se spisak nalazi na: "Platelets on the web" (<https://www.ouhsc.edu/platelets/ditp.html>) [1]. Učestalost lekovima uzrokovane trombocitopenije se procenjuje na 10/1.000.000 stanovnika godišnje i značajno je češći kod odraslih u poređenju sa decom [1]. Smatra se da u jedinicama intenzivne nege čak 25% bolesnika ima neki od oblika lekovima uzrokovane trombocitopenije [2]. Prema mehanizmu nastanka, lekovima uzrokovane trombocitopenije se mogu klasifikovati na imunske i neimunske [3-5].

NEIMUNSKLE LEKOVIMA UZROKOVANE TROMBOCITOPENIJE

Lekovi poput citostatika, linezolida, ganciklovira, valaciklovira, Aspirina i vankomicina mogu dovesti do trombocitopenije, bilo proapoptotičkim mehanizmima na trombocite ili direktnim citotoksičnim dejstvom na megakariocite i trombocite [1,3,4]. Trombocitopenija u toku primene linezolida registruje se u slučaju primene leka duže od dve nedelje i u slučaju njegovih visokih koncentracija u krvi; posledica je dozno zavisne reverzibilne mijelosupresije u toku koje je trombocitopenija najviše pogođena [6]. Sa druge strane, citostatiki oksaliplatin i irinotekan, osim neimunskim, mogu izazvati trombocitopeniju i imunskim mehanizmom [7,8].

IMUNSKLE LEKOVIMA UZROKOVANE TROMBOCITOPENIJE

Lekovima uzrokovana imunska trombocitopenija (LITP) posredovana je antitelima klase IgG (ređe IgA/M) specifičnim za lek, koja se u prisustvu leka ili njegovih metabolita nekovalentno vezuju za trombocitne antigene putem svog Fab regiona, dovodeći do ubrzane razgradnje trombocita aktivacijom komplementa i/ili fagocitozom. Pritom, glikoproteinski (GP) kompleksi, kao što su GPIIb/IIIa i GPIb/IX, najčešći su trombocitni antigeni u klasičnom obliku LITP (Shema 1A). U zavisnosti od mehanizma vezivanja razlikuje se šest tipova antitela (Tabela 1) [4].

Osim heparina koji najčešće dovodi do LITP, po učestalosti se kao uzročnici ističu: acetaminofen, abciksimab, amjodaron, ampicilin, amlodipin, kotrimoksazol, karbamazepin, hlorpropamid, drospironon, digitalis, gentamicin, eptifibatid, danazol, moksonidin, etambutol, diklofenak, tirofiban, haloperidol, efalizumab, irinotekan, ibuprofen, piperacilin, preparati zlata, fenitoin, triamteren/hidrohlortiazid, naproksen, hidrohlortiazid, oksaliplatin, interferon alfa, metildopa,

INTRODUCTION

Medications are a frequent cause of thrombocytopenia, particularly among hospitalized patients. Thrombocytopenia has been associated with the use of over 300 different medications, a list of which is available at "Platelets on the web" (<https://www.ouhsc.edu/platelets/ditp.html>) [1]. The incidence of drug-induced thrombocytopenia is estimated at 10 cases per 1,000,000 people per year and is significantly higher in adults than in children [1]. It is believed that in intensive care units as many as 25% of patients have some form of drug-induced thrombocytopenia [2]. Drug-induced thrombocytopenia can be classified into immune and non-immune types based on its underlying mechanism [3-5].

NON-IMMUNE DRUG-INDUCED THROMBOCYTOPENIA

Drugs like cytostatics, linezolid, ganciclovir, valacyclovir, aspirin, and vancomycin can cause thrombocytopenia either through pro-apoptotic mechanisms affecting platelets or by direct cytotoxic effects on megakaryocytes and platelets [1,3,4]. Thrombocytopenia associated with linezolid typically occurs when the drug is administered for more than two weeks or when its blood concentrations are high. This condition results from dose-dependent, reversible myelosuppression, with thrombocytopoiesis being the most affected [6]. Conversely, cytostatics like oxaliplatin and irinotecan can cause thrombocytopenia not only through non-immune mechanisms but also via immune-mediated pathways [7,8].

IMMUNE DRUG-INDUCED THROMBOCYTOPENIA

Drug-induced immune thrombocytopenia (DITP) is mediated by drug-specific IgG antibodies (rarely IgA or IgM), which non-covalently bind to platelet antigens via their Fab region in the presence of the drug or its metabolites. This binding accelerates platelet destruction through complement activation and/or phagocytosis. Additionally, glycoprotein (GP) complexes like GPIIb/IIIa and GPIb/IX are the most common platelet antigens involved in the classic form of drug-induced immune thrombocytopenia (DITP) (Figure 1A). Depending on the binding mechanism, there are six types of antibodies (Table 1) [4].

Besides heparin, which is the most frequent cause of DITP, other common causative agents include acetaminophen, abciximab, amiodarone, ampicillin, amlodipine, co-trimoxazole, carbamazepine, chlorpropamide, drospironone, digitalis, gentamicin, eptifibatide,

Tabela 1. Lekom uzrokovana imunološka trombocitopenija-mehanizmi (prilaženo iz Arnold DM, Cuker A. [4])

Mehanizam / Mechanism	Primer leka / Example of drugs	Učestalost / Incidence
Kininski tip / Quinine-type	Kinin, kinidin, sulfonamidi, nesteroidni antiinflamatorni lekovi / Quinine, sulfonamide antibiotics, non-steroidal anti-inflammatory drugs	26 slučajeva / 1 000 000 korisnika kinina nedeljno / 26 cases per million quinine users per week
Hapten-zavisni / Hapten-dependent	Lek se kovalentno vezuje za membranski protein i podstiče lek-specifično vezivanje pomoću LZAt / Drug links covalently to membrane protein and induces drug-specific binding by DDAb	Penicilin, neki cefalosporinski antibiotik / Penicillin, some cephalosporin antibiotics
Fibanski tip / Fiban-type	Lek reaguje sa GPIIb/IIIa i indukuje nove epitope za LZAt / Drug reacts with GPIIb/IIIa and induces neoepitope for the DDAb	Tirofiban, Eptifibatid / Tirofiban, Eptifibatide
Lek-specifični / Drug-specific	LZAt prepoznaje mišju komponentu himernog Fab fragmenta specifičnog za GPIIIa / DDAb recognize murine component of chimeric Fab fragment specific for GPIIIa	Abciximab / Abciximab
Autoantitelo / Autoantibody	Lek indukuje stvaranja antitela, koje potom reaguje sa autolognim trombocitima u odsustvu leka / Drug induces antibody that reacts with autologous platelets in absence of drug	Soli zlata, prokainamid / Gold salts, procainamide
Imunski kompleksi / Immune complexes	Antitela sa ciljanim antigenima stvaraju imunske komplekse / Antibodies form immune complexes with their target antigens	Heparin, komponente adenovirusne vektorske vakcine protiv COVID-19 / Heparin, a component of adenoviral vector-based vaccine against COVID-19

Legenda: LZAt - antitela zavisna od prisustva leka; LMWH - niskomolekularni heparin (engl. low-molecular-weight heparin); UFH - nefrakcionisani heparin (engl. unfractionated heparin)

Table 1. Drug-induced immune thrombocytopenia—mechanisms (adapted from Arnold DM, Cuker A. [4])

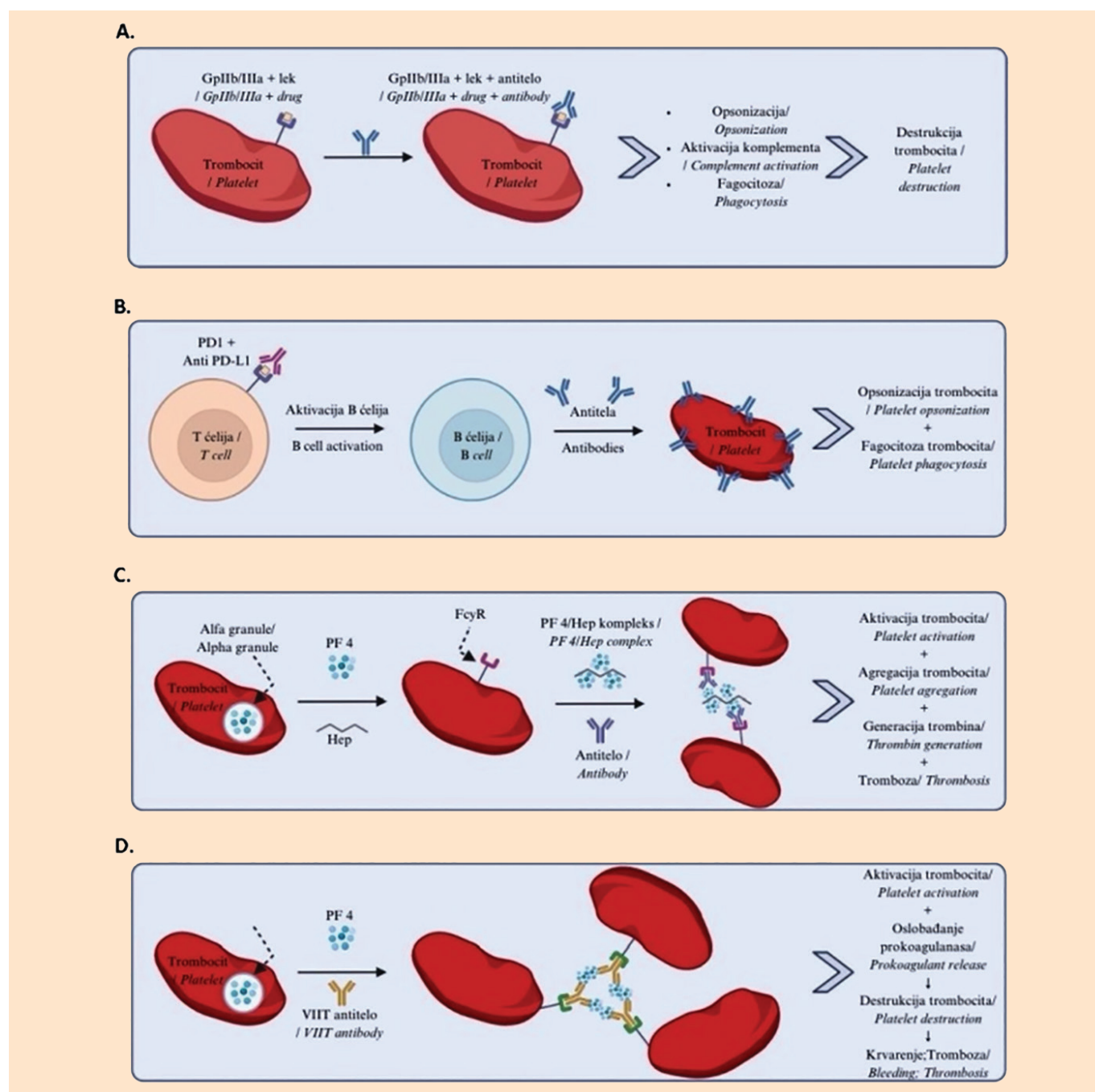
Legend: DDAb - drug dependant antibodies; LMWH - Low-molecular-weight heparin; UFH - unfractionated heparin

nalidiksinska kiselina, kinin, kinidin, rifampin, ranitidin, sulfisoksazol, simvastatin, valproična kiselina i vankomicin [4,9]. Osim lekova, neka hrana i pića kao i biljni preparati, mogu takođe prouzrokovati LITP [10].

Vreme proteklo od uvođenja leka do nastanka trombocitopenije najčešće je jedan do tri dana, ukoliko je lek prethodno korišćen, ili 7-15 dana ako lek nije prethodno primenjivan [1]. Izuzetak su lekovi iz grupe antagonista GPIIb/IIIa (abciximab, tirofiban, eptifibatid) koji dovode do trombocitopenije najčešće nekoliko sati ili čak minuta po započinjanju terapije [4]. Međutim, LITP može da nastane i posle obustave leka ili da perzistira duže vreme posle obustave leka, a primeri su terapija zlatom, alemtuzumabom i lekovima iz grupe blokatora kontrolnih tačaka (engl. immune check-point inhibitori) [4]. Naime, u kliničkoj studiji faze 2 u kojoj su ispitivani efikasnost i bezbednost alemtuzumaba u lečenju relapsno-remitentnog oblika multiple skleroze, kod 6/216 pacijenta registrovana je trombocitopenija koja je nastala 1-15 meseci od primene poslednje doze leka a uspešno je lečena standardnom terapijom za primarnu imunološku trombocitopeniju (ITP) [11]. Od interesa je napomenuti da su opisani i slučajevi LITP

danazol, moxonidine, ethambutol, diclofenac, tirofiban, haloperidol, efalizumab, irinotecan, ibuprofen, piperacillin, gold preparations, phenytoin, triamterene/hydrochlorothiazide, naproxen, hydrochlorothiazide, oxaliplatin, interferon alfa, methyldopa, nalidixic acid, quinine, quinidine, rifampin, ranitidine, sulfisoxazole, simvastatin, valproic acid, and vancomycin [4,9]. Along with medications, certain foods, beverages, and herbal preparations can also cause DITP [10].

The onset of thrombocytopenia typically occurs within one to three days after starting the drug if it has been used before, or within 7 to 15 days if the drug is being administered for the first time [1]. Exceptions include GPIIb/IIIa antagonists (such as abciximab, tirofiban, and eptifibatide), which can cause thrombocytopenia within hours or even minutes after starting therapy [4]. However, DITP can occur even after discontinuing the drug or persist for an extended period. Examples include therapy with gold, alemtuzumab, and immune checkpoint inhibitors [4]. In a Phase 2 clinical study evaluating the efficacy and safety of alemtuzumab for treating relapsing-remitting multiple sclerosis, thrombocytopenia was observed in 6 out of 216 pa-



Shema 1. Shematski prikaz patofiziologije A. Lekovima uzrokovane tromboticopenije (LITP) – klasične; B. Tromboticopenije indukovane lekovima blokatorima kontrolnih tačaka; C. Heparinom-indukovane tromboticopenije (HIT); D. Vakcinom indukovane trombozne tromboticopenije (VITT). (Prilagođeno iz Marini et al. [3])

Figure 1. Schematic representation of the pathophysiology of A. Drug-induced thrombocytopenia (DITP) - classic; B. Immune-checkpoint inhibitor (ICI) induced thrombocytopenia; C. Heparin-induced thrombocytopenia (HIT); D. Vaccine-induced immune thrombotic thrombocytopenia (VITT). (Adapted from Marini et al. [3])

koji su nastali posle dužeg vremena od uvođenja leka – pet meseci od uvođenja dabigatrana kod pacijentkinje sa atrijskom fibrilacijom [12] ili čak 10 godina od uvođenja interferona beta-1a pacijentkinji sa dijagnozom multiple skleroze [13]. Sa druge strane, učestalost LITP u sklopu lečenja blokatorima kontrolnih tačaka kreće se od 0,2-2,8% i veoma retko je teškog stepena i sa fatalnim ishodom. Najveći broj opisanih slučajeva uzro-

tients. This condition emerged 1 to 15 months after the last dose of the drug and was effectively managed with standard treatment for primary immune thrombocytopenia (ITP) [11]. It is of interest to note that cases of drug-induced immune thrombocytopenia (DITP) have also been reported long after the drug was introduced—such as five months after starting dabigatran in a patient with atrial fibrillation [12], or even ten

kovan je nivolumabom i pembrolizumabom. Smatra se da je posledica oporavka T limfocita u sklopu imunološke terapije sa posledičnim stvaranjem autoantitela na trombocitne antigene, kao u ITP [14] (Shema 1B).

KLINIČKA SLIKA LEKOM UZROKOVANE IMUNSKJE TROMBOCITOPENIJE

Trombocitopenija u sklopu LITP nastaje akutno, može biti teškog stepena (nadir broja trombocita $< 20 \times 10^9/L$), uz životno ugrožavajuće krvarenje [1,3,4]. Naime, pokazano je da čak 67% bolesnika krvari, da njih 9% ima masivno krvarenje, a da čak 0,8% egzitira [15]. Međutim, kod heparinom uzrokovane trombocitopenije (HIT), nadir broja trombocita je oko $60 \times 10^9/L$, retko se registruje trombocitopenija $< 20 \times 10^9/L$, a umesto krvarenja kliničkom slikom dominiraju tromboze [16]. Životno ugrožavajuće tromboze se takođe javljaju i u vakcinom indukovanoj tromboznoj trombocitopeniji (VITT). U LITP mogu se javiti i febrilnost, hipotenzija, sinkopa, mučnina i povraćanje [3,4].

PROCENA VEROVATNOĆE NASTANKA LEKOM UZROKOVANE TROMBOCITOPENIJE

Algoritam za procenu verovatnoće nastanka LITP je prikazan Shemom 2. S obzirom na to da u rutinskoj praksi nisu dostupni specifični dijagnostički testovi ili biomarkeri za LITP, prvi korak u lečenju LITP je posumnjati na lek kao uzrok trombocitopenije da bi potom lek koji je sumnjiv odmah bio obustavljen i zamenjen lekom drugačije hemijske strukture. Ukoliko bolesnik koristi veći broj lekova koji su istovremeno uvedeni, savetuje se korekcija celokupne terapije [4]. S obzirom da se oporavak broja trombocita očekuje u proseku za 7 dana (opseg: 1-15), a prisutan je hemoragijski sindrom, preporučuju se primena kortikosteroida, intravenskih imunoglobulina i terapijske izmene plazme, a samo u slučaju intenzivnog krvarenja transfuzija trombocita. Međutim, tek po isteku 4-5 poluživota uzročnika LITP ili njegovog metabolita može se očekivati porast broja trombocita. Takođe, transfuzija trombocita biće neefikasna dokle god su prisutni bilo lek bilo njegovi metaboliti u plazmi pacijenta [4]. S druge strane, ukoliko su blokatori kontrolnih tačaka uzrokovali LITP, modifikacija doze ovih lekova u zavisnosti od broja trombocita regulisana je preporukama Američkog udruženja onkologa [17,18]. U diferencijalnoj dijagnozi LITP treba razmatrati ITP, trombocitopeniju u sklopu sepse, diseminovanu intravaskularnu koagulaciju (DIK), trombotičnu trombocitopenijsku purpuru (TTP), i posttransfuzionu purpuru [4]. Neophodno je pacijenta posavetovati da doživotno izbegava osumnjičeni lek.

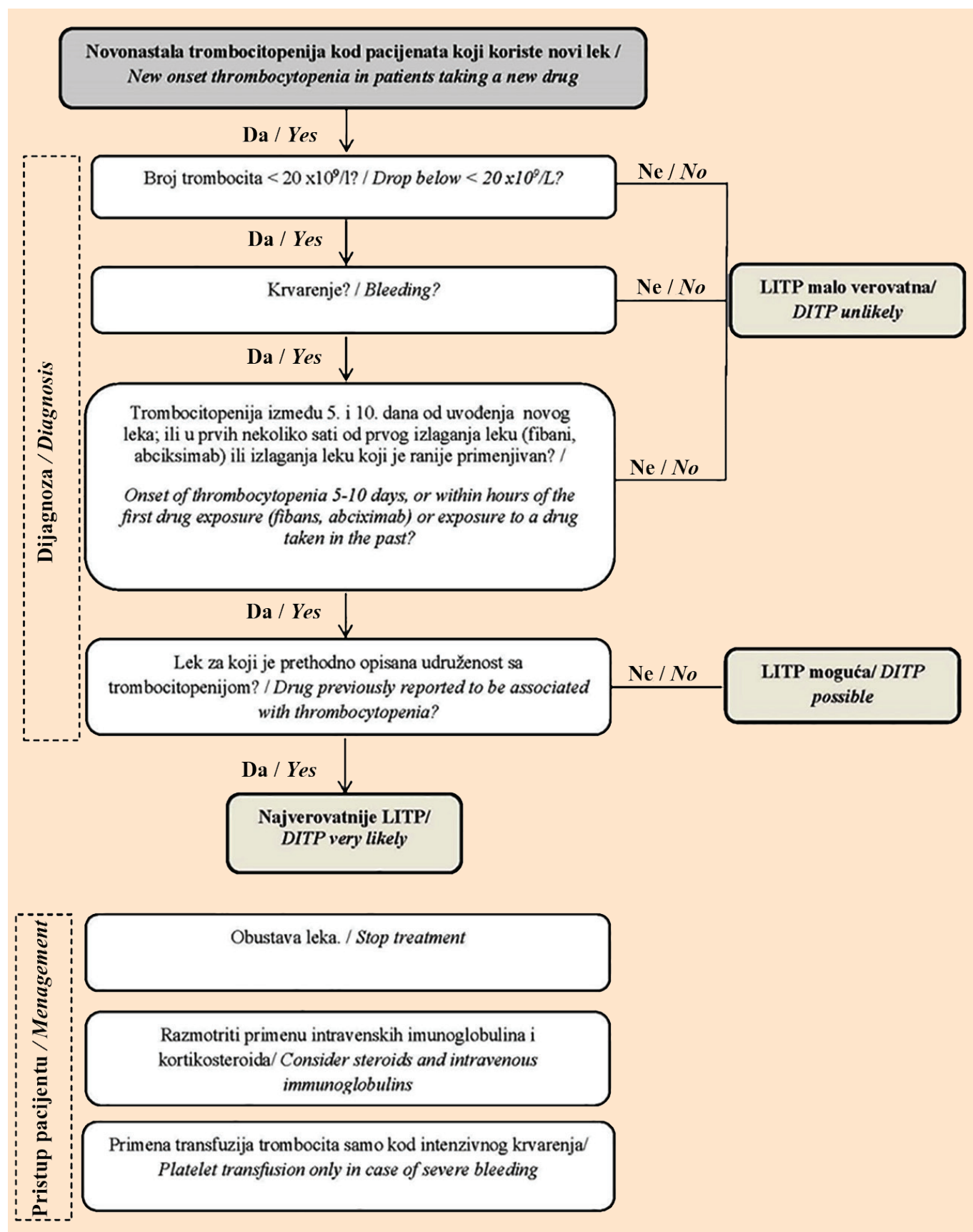
years after beginning interferon beta-1a treatment in a patient with multiple sclerosis [13]. Conversely, the incidence of DITP associated with checkpoint blockers ranges from 0.2% to 2.8%. Severe or fatal cases are extremely rare. Most of the reported cases were associated by nivolumab and pembrolizumab. It is believed that this condition arises from the recovery of T lymphocytes during immunotherapy, which leads to the formation of autoantibodies against platelet antigens, similar to what occurs in immune thrombocytopenia (ITP) [14] (Figure 1B).

CLINICAL PRESENTATION OF DRUG-INDUCED THROMBOCYTOPENIA

In drug-induced immune thrombocytopenia (DITP), thrombocytopenia typically presents acutely and can be severe, with nadir platelet counts dropping below $20 \times 10^9/L$ and potentially leading to life-threatening bleeding [1,3,4]. Studies have shown that 67% of patients with DITP experience bleeding, with 9% of these cases being classified as massive bleeding. Additionally, 0.8% of patients may experience fatal outcomes [15]. In heparin-induced thrombocytopenia (HIT), the lowest nadir platelet count typically reaches around $60 \times 10^9/L$, with counts below $20 \times 10^9/L$ being rare. Instead of bleeding, HIT is primarily characterized by thromboses [16]. Life-threatening thromboses also occur in vaccine-induced thrombotic thrombocytopenia (VITT). In DITP, symptoms such as fever, hypotension, syncope, nausea, and vomiting may also occur [3,4].

EVALUATION OF THE LIKELIHOOD OF DRUG-INDUCED THROMBOCYTOPENIA

Figure 2 illustrates the algorithm for estimating the likelihood of DITP. Since specific diagnostic tests or biomarkers for drug-induced thrombocytopenia (DITP) are not routinely available, the initial step in managing DITP is to identify a suspected drug as the cause. The suspected drug should be promptly discontinued and replaced with an alternative medication of a different chemical structure. If the patient is taking multiple medications introduced simultaneously, it is advisable to adjust the entire therapy regimen [4]. Since platelet count recovery typically occurs within an average of 7 days (range: 1-15 days), and if hemorrhagic symptoms are present, the use of corticosteroids, intravenous immunoglobulins, and therapeutic plasma exchange is recommended. Platelet transfusion should be considered only in cases of severe bleeding. However, an increase in platelet count is typically expected only after the causative agent of DITP or its metabolite has been cleared from the body, which usually occurs after 4-5 half-lives of the drug. Platelet transfusion will be inef-



Shema 2. Pristup dijagnozi i lečenju pacijenta sa novonastalom trombocitopenijom kod kojih se sumnja na lekovima uzrokovanu trombocitopeniju (LITP) (prilagođeno iz Arnold DM, Cuker A. [4])

Figure 2. Diagnostic approach and management of a patient with new-onset thrombocytopenia in whom drug-induced immune thrombocytopenia (DITP) is suspected (adapted from Arnold DM, Cuker A. [4])

HEPARINOM UZROKOVALA TROMBOCITOPENIJA

HIT je stečeni protrombotični poremećaj izazvan primenom heparina, nefrakcionisanog ili niskomolekularnog. Učestalost HIT-a posle primene niskomolekularnog heparina je 10 puta ređa nego posle upotrebe nefrakcionisanog oblika [16]. Patofiziološki mehanizam HIT-a se zasniva na sintezi antitela, najčešće klase IgG, na imunogeni kompleks sastavljen od trombocitnog faktora 4 (PF4) i heparina. Vezivanje ovog trojnog kompleksa za trombocite dovodi do ubrzanog klirensa trombocita i nastanka trombocitopenije sa jedne strane, i aktivacije i agregacije trombocita i generacije trombina sa druge strane. Istovremena aktivacija endotela i monocita, sa posledičnom ekspresijom tkivnog faktora na njihovoj površini, dodatno potencira stvaranje trombina (Shema 1C). HIT nastaje 5-10 dana od uvođenja heparina a izuzetno ranije kod pacijenata lečenih heparinom u prethodna tri meseca. Retko, HIT može da se ispolji i po obustavi heparina [16,19]. Kod 5-10% pacijenata sa HIT-om koji ispolje DIK može nastati teži stepen trombocitopenije $< 20 \times 10^9/L$ [16]. Osim smanjenja broja trombocita za 50% od najvećeg registrovanog broja trombocita pre uvođenja heparina, u 20-50% slučajeva nastaju venske, a ređe i arterijske tromboze u narednih 30 dana. Pored toga, HIT se može ispoljiti i bolnim kožnim eritematoznim i nekrotičnim promenama, naročito na mestu injekcija, intrakardijalnim trombima i mezenterijalnim infarktima. Opisuju se i pojava febrilnosti, eritema lica i prolazne amnezije nastale 30 minuta posle primene intravenskog bolusa heparina [16]. Obustava heparina kod bolesnika sa HIT-om je nedovoljna mera te se mora primeniti alternativna antikoagulantna terapija. Pritom, težak stepen trombocitopenije nije kontraindikacija za primenu ove antikoagulantne terapije [16].

PRISTUP PACIJENTU SA SUMNJOM NA HIT

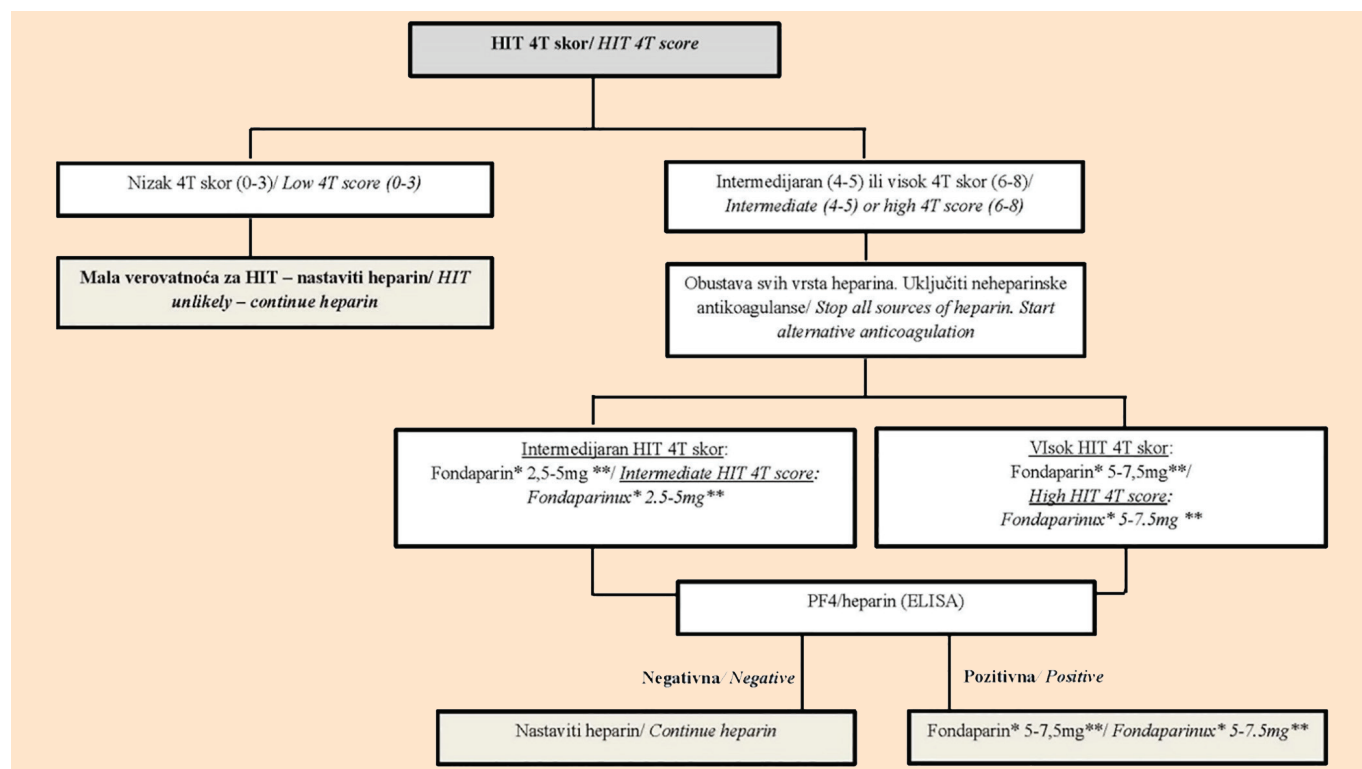
Ukoliko se registruje trombocitopenija kod bolesnika koji je na terapiji heparinom izračunavanjem 4Ts skora procenjuje se verovatnoća za nastanak HIT-a (Tabela 2) [16,19].

Ovaj bodovni sistem ima umereno pozitivnu prediktivnu i visoko negativnu prediktivnu vrednost. S druge strane, laboratorijska potvrda HIT-a bazira se na dokazu antitela uperenih na kompleks PF4-heparin. U rutinskoj upotrebi je nekoliko enzimskih imunosorbentnih (ELISA) testova koji imaju visoku negativnu prediktivnu vrednost što ih čini korisnim u isključivanju HIT-a. Međutim, usled njihove niske specifičnosti pozitivan test nema veliku vrednost u potvrdi kliničke sumnje na HIT. Pristup pacijentu sa sumnjom na HIT prikazan je Shemom 3.

ffective as long as the drug or its metabolites remain present in the patient's plasma [4]. On the other hand, if checkpoint blockers are the cause of DITP, dose adjustments based on platelet counts should follow the guidelines provided by the American Society of Clinical Oncology [17,18]. In the differential diagnosis of DITP, it is important to consider immune thrombocytopenia (ITP), thrombocytopenia due to sepsis, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and post-transfusion purpura [4]. The patient should be advised to avoid the suspected drug for life.

HEPARIN-INDUCED THROMBOCYTOPENIA

HIT is an acquired prothrombotic disorder triggered by the use of heparin, whether unfractionated or low molecular weight. The incidence of HIT with low molecular weight heparin is about ten times lower compared to unfractionated heparin [16]. The pathophysiological mechanism of HIT involves the formation of antibodies, typically of the IgG class, against an immunogenic complex composed of platelet factor 4 (PF4) and heparin. The binding of this ternary complex to platelets results in their accelerated clearance, leading to thrombocytopenia. Simultaneously, it triggers platelet activation and aggregation, as well as thrombin generation. Simultaneous activation of endothelial cells and monocytes, along with the expression of tissue factor on their surfaces, further enhances thrombin generation (Figure 1C). HIT typically occurs 5-10 days after starting heparin therapy, but it can develop much earlier in patients who have received heparin within the previous three months. Rarely, HIT can also occur after stopping heparin [16,19]. In 5-10% of patients with HIT who develop disseminated intravascular coagulation (DIC), thrombocytopenia may be more severe, with platelet counts dropping below $20 \times 10^9/L$ [16]. In addition to a 50% reduction in the highest recorded platelet count before starting heparin, 20-50% of patients may develop venous, and less frequently arterial, thrombosis within the following 30 days. HIT can also present with painful erythematous and necrotic skin changes, particularly at injection sites, as well as intracardiac thrombi and mesenteric infarcts. Symptoms such as fever, facial erythema, and transient amnesia can occur within 30 minutes after administering an intravenous bolus of heparin [16]. Merely suspending heparin in patients with HIT is inadequate; alternative anticoagulant therapy must be implemented. At the same time, severe thrombocytopenia is not a contraindication for using alternative anticoagulant therapy in HIT [16].



Shema 3. Protokol postupanja kod sumnje na heparinom uzrokovanu trombocitopeniju (HIT) (prilagođeno iz Crowther M, Pishko A. [16])

*fondaparinux je za sada jedini dostupan neheparinski antikoagulant u Republici Srbiji

**u zavisnosti od telesne mase

Figure 3. SHIT protocol (adapted from Crowther M, Pishko A. [16])

*fondaparinux is the only non-heparin anticoagulants in Serbia currently

** depending on body weight

Naime, ukoliko je 4Ts skor ≤ 3 , verovatnoća HIT-a je mala, zbog čega se ne savetuje obustava heparina, ali ukoliko je skor intermedijaran ili visok (≥ 4), neophodna je obustava svih vrsta heparina, što podrazumeva i propiranje venskih linija i upotrebu ekstrakorporalnih membranskih oksigenatora, a potom uvođenje neheparinskih antikoagulanasa (NHA). Takođe je neophodno određivanje titra antiheparinskih antitela. Ukoliko su antiheparinska antitela odsutna treba obustaviti NHA i nastaviti primenu heparina, a u slučaju pozitivnog testa, lečenje se nastavlja sa NHA [16,19]. Izbor NHA zavisi od karakteristika i dostupnosti pojedinih NHA sa jedne strane, i karakteristika pacijenta sa druge [16].

Varfarin i ostali oralni antikoagulasi ne koriste se u lečenju pacijenata sa aktivnim HIT jer u HIT-u sa dubokom venskom trombozom, pogotovo sa vrednošću INR > 4 , smanjenim nivoom proteina C i povišenim trombin-antitrombin kompleksima može nastati gangrena ekstremiteta. Međutim, ovi lekovi se mogu primeniti kod klinički stabilnih pacijenata čije je stvaranje trombina kontrolisano sa NHA i koji su normalizovali broj trombocita [16]. Ukoliko se kod pacijenta sa HIT-om razvila tromboza, antikoagulantnu terapiju treba sprovoditi tokom najmanje tri meseca. S druge strane, ukoliko je HIT kod pacijenta protekao bez tromboze,

APPROACH TO A PATIENT WITH SUSPECTED HIT

If a patient on heparin therapy develops thrombocytopenia, the likelihood of HIT is assessed using the 4Ts score (Table 2) [16,19].

This scoring system has a moderate positive predictive value and a high negative predictive value. On the other hand, laboratory confirmation of HIT relies on detecting antibodies specific to the PF4-heparin complex. Several enzyme-linked immunosorbent assay (ELISA) tests are routinely used and have a high negative predictive value, making them effective for ruling out HIT. However, due to their low specificity, a positive test result has limited value in confirming the clinical suspicion of HIT. The approach to a patient with suspected HIT is outlined in Figure 3.

If the 4Ts score is ≤ 3 , the probability of HIT is considered low, so discontinuing heparin is not necessary. However, if the score is intermediate or high (≥ 4), all forms of heparin should be stopped, which includes removing venous lines and discontinuing the use of extracorporeal membrane oxygenators. In such cases, non-heparin anticoagulants (NHAs) should be introduced. It is also important to measure the titer of

Tabela 2. 4Ts bodovni sistem za procenu rizika od nastanka heparinom indukovane trombocitopenije (HIT) (prilagođeno iz Crowther M, Pishko A. [16])

Parametar / Category	Broj poena / Points
Trombocitopenija / Thrombocytopenia	
Broj Tr snižen za > 50% od inicijalne vrednosti i minimalan broj Tr > 20x10 ⁹ /L / Platelet count fall > 50% and platelet nadir > 20x10 ⁹ /L	2
Broj Tr snižen za 30-50% od inicijalne vrednosti ili minimalan broj Tr 10-19x10 ⁹ /L / Platelet count fall 30-50% or platelet nadir 10-19x10 ⁹ /L	1
Broj Tr snižen < 30% od inicijalne vrednosti ili minimalni broj Tr < 10 ⁹ /L / Platelet count fall < 30% or platelet nadir < 10 ⁹ /L	0
Vreme proteklo od uvođenja heparina do pojave trombocitopenije / Timing of platelet count fall	
Između 5. i 10. dana terapije ili ≤ 1 dana u slučaju primene heparina tokom prethodnih 30 dana / Clear onset between days 5 - 10 or falls ≤ 1 day in case of prior heparin exposure during the last 30 days	2
> 10. dana ili ≤ 1 dana u slučaju primene heparina tokom prethodnih 30-100 dana / Onset > 10 day or falls ≤ 1 day in case of prior heparin exposure within prior 30-100 days	1
≤ 4 dana od početka terapije ili > 14 dana po izlaganju / Platelet count falls < 4 days or > 14 days after exposure	0
Tromboze i ostale komplikacije / Thrombosis or other sequelae	
Nova potvrđena tromboza i: nekroza kože, akutna sistemska reakcija posle intravenskog bolusa nefrakcionisanog heparina, progresivna ili rekurentna tromboza, eritem kože, bez nekroze / New thrombosis (confirmed); necrotizing and non-necrotizing skin lesions; acute systemic reaction post-intravenous UFH bolus; progressive or recurrent thrombosis	2
Sumnja na trombozu (nedokazana) / Suspected thrombosis (not proven)	1
Nijedno od navedenog / None of the above	0
Trombocitopenija, drugi uzroci / Other causes of thrombocytopenia	
Nema / None apparent	2
Mogući drugi uzroci / Possible	1
Dokazani drugi uzroci / Definite	0

Legenda: Tr- trombociti

lečenje se sprovodi tokom četiri nedelje [16,19]. Pacijent kome je jednom dijagnostikovano HIT više se ne sme izlagati heparinu, osim pod kontrolom specijalizovanog tima [20].

VAKINOM INDUKOVANA TROMBOZNA TROMBOCITOPENIJA

VITT se odlikuje nastankom tromboze i trombocitopenije 4 do 30 dana od primene adenovirusne vektorske vakcine. Naime, učestalost VITT je 2.5-38/milion primenjenih prvih doza ChadOx1 nCov19 i 1.9/ milion drugih doza [21]. S druge strane, saopštena je učestalost VITT od 3.5/milion primenjenih doza Ad26.CoV2.S [22]. Smatra se da je ključni događaj u nastanku VITT stvaranje antitela klase IgG na PF4 usled formiranja neoantigena na PF4 prilikom njegovog vezivanja za komponentu vakcine. Naime, anti-PF4 antitelo vezuje se za PF4 na površini trombocita što prouzrokuje aktivaciju

Table 2. 4Ts score for heparin-induced thrombocytopenia (HIT) (adapted from Crowther M, Pishko A. [16])

Parametar / Category	Broj poena / Points
Trombocitopenija / Thrombocytopenia	
Broj Tr snižen za > 50% od inicijalne vrednosti i minimalan broj Tr > 20x10 ⁹ /L / Platelet count fall > 50% and platelet nadir > 20x10 ⁹ /L	2
Broj Tr snižen za 30-50% od inicijalne vrednosti ili minimalan broj Tr 10-19x10 ⁹ /L / Platelet count fall 30-50% or platelet nadir 10-19x10 ⁹ /L	1
Broj Tr snižen < 30% od inicijalne vrednosti ili minimalni broj Tr < 10 ⁹ /L / Platelet count fall < 30% or platelet nadir < 10 ⁹ /L	0
Vreme proteklo od uvođenja heparina do pojave trombocitopenije / Timing of platelet count fall	
Između 5. i 10. dana terapije ili ≤ 1 dana u slučaju primene heparina tokom prethodnih 30 dana / Clear onset between days 5 - 10 or falls ≤ 1 day in case of prior heparin exposure during the last 30 days	2
> 10. dana ili ≤ 1 dana u slučaju primene heparina tokom prethodnih 30-100 dana / Onset > 10 day or falls ≤ 1 day in case of prior heparin exposure within prior 30-100 days	1
≤ 4 dana od početka terapije ili > 14 dana po izlaganju / Platelet count falls < 4 days or > 14 days after exposure	0
Tromboze i ostale komplikacije / Thrombosis or other sequelae	
Nova potvrđena tromboza i: nekroza kože, akutna sistemska reakcija posle intravenskog bolusa nefrakcionisanog heparina, progresivna ili rekurentna tromboza, eritem kože, bez nekroze / New thrombosis (confirmed); necrotizing and non-necrotizing skin lesions; acute systemic reaction post-intravenous UFH bolus; progressive or recurrent thrombosis	2
Sumnja na trombozu (nedokazana) / Suspected thrombosis (not proven)	1
Nijedno od navedenog / None of the above	0
Trombocitopenija, drugi uzroci / Other causes of thrombocytopenia	
Nema / None apparent	2
Mogući drugi uzroci / Possible	1
Dokazani drugi uzroci / Definite	0

Legend: UFH - unfractionated heparin

anti-heparin antibodies. If anti-heparin antibodies are absent, NHAs should be discontinued, and heparin administration can be resumed. If the test is positive, continue treatment with NHA [16,19]. The choice of NHA depends on both the specific characteristics and availability of each NHA and the individual patient's clinical condition [16].

Warfarin and other oral anticoagulants are not used in the treatment of patients with active HIT. In cases of HIT with deep vein thrombosis, particularly with an INR value > 4, there is a risk of limb gangrene due to reduced levels of protein C and elevated thrombin-antithrombin complexes. However, these drugs can be used in clinically stable patients whose thrombin generation is controlled with NHA and whose platelet count has normalized [16]. If a patient with HIT develops thrombosis, anticoagulant therapy should be continued for at least three months. Conversely, if the

trombocita uz oslobađanje prokoagulantnih supstanci (Shema 1D). Oko dve nedelje posle vakcinacije, javljaju se krvarenje usled teškog stepena trombocitopenije i tromboza, najčešće u cerebralnim venskim sinusima, kojoj prethodi intenzivna glavobolja. Dokazano je da anti-PF4 antitela perzistiraju u organizmu obolelih od VITT čak 12 do 15 meseci od vakcinacije [23]. Kod 30% pacijenata javlja se intrakranijalno krvarenje koje je često fatalno. Smrtnost u VITT se kreće od 20- 60% [24-25]. Po svom kliničkom ispoljavanju VITT veoma liči na HIT, a jedina razlika je što pacijent nije prethodno bio izložen heparinu. U lečenju VITT koriste se neheparinski antikoagulansi, intravenski imunoglobulini i kortikosteroidi ukoliko je broj trombocita $< 50 \times 10^9/L$, dok transfuzije trombocita treba izbegavati zbog rizika od tromboze.

Sukob interesa: Nije prijavljen.

patient's HIT resolves without thrombosis, treatment should be continued for four weeks [16,19]. A patient who has been diagnosed with HIT should avoid re-exposure to heparin, unless under the supervision of a specialized medical team [20].

VACCINE-INDUCED THROMBOTIC THROMBOCYTOPENIA

VITT is characterized by the onset of thrombosis and thrombocytopenia occurring 4 to 30 days after administration of an adenoviral vector vaccine. The frequency of VITT is approximately 2.5 to 38 cases per million doses administered for the first dose of the ChAdOx1 nCoV-19 vaccine and about 1.9 cases per million for the second dose [21]. Conversely, the frequency of VITT with the Ad26.CoV2.S vaccine has been reported as 3.5 cases per million doses administered [22]. The development of VITT is believed to be primarily triggered by the generation of IgG antibodies against platelet factor 4 (PF4). This occurs due to the formation of a neoantigen on PF4 when it binds to the vaccine component. Namely, the anti-PF4 antibodies bind to PF4 on the surface of platelets, leading to platelet activation and the release of procoagulant substances (Figure 1D). Approximately two weeks after vaccination, bleeding may occur due to severe thrombocytopenia and thrombosis, commonly affecting the cerebral venous sinuses and often preceded by intense headaches. It has been proven that anti-PF4 antibodies persist in the body of VITT patients even 12 to 15 months after vaccination [23]. Intracranial bleeding occurs in about 30% of patients, and it is often fatal. Mortality in VITT ranges from 20-60% [24-25]. Clinically, VITT closely resembles HIT, the only difference being that the patient has not previously been exposed to heparin. In the treatment of VITT, non-heparin anticoagulants, intravenous immunoglobulins, and corticosteroids are used if the platelet count is $< 50 \times 10^9/L$. Platelet transfusions should be avoided due to the risk of exacerbating thrombosis.

Conflict of interest: None declared.

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KRVARENJE UDRUŽENO SA PRIMENOM DIREKTNIH ORALNIH ANTIKOAGULANSA

HEMORRHAGIC COMPLICATIONS ASSOCIATED WITH THE USE OF DIRECT ORAL ANTICOAGULANTS

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SAŽETAK

Ključ lečenja i prevencije venskog tromboembolizma (VTE) predstavlja primena antikoagulantne terapije. Dugi niz godina, kamen temeljac je predstavljala primena antagonista vitamina K (VKA) koja je bila povezana sa mnogobrojnim preprekama i komplikacijama. Uvođenjem nove generacije direktnih oralnih antikoagulantnih lekova (DOAK) neke od prepreka, kao što su brzina postizanja terapijskog efekta, fiksna doza, inhibicija manjeg broja koagulacionih faktora, manji broj interakcija sa lekovima su prevaziđene, uz zadržavanje adekvatnog bezbedonosnog profila. Stoga su se DOAK vrlo brzo našli u preporukama za lečenje i prevenciju VTE, kao i za prevenciju ishemijskih komplikacija kod pacijenata sa atrijskom fibrilacijom bez mehaničke valvule. Ipak, primenom antikoagulantne terapije, narušava se homeostaza, te lečenjem ili sprečavanjem VTE sa jedne strane, povećavamo rizik od krvarenja sa druge. Brojne studije, kako randomizovane kliničke, tako i retrospektivne su analizirale rizik od krvarenja nakon primene DOAK u poređenju sa VKA, kao i između DOAK međusobno. Nedvosmisleno je pokazano da je rizik od intrakranijalne hemoragije manji sa DOAK u poređenju sa VKA, dok je većina studija pokazala da je i rizik od major krvarenja isti ili čak i manji sa DOAK. Upravo veća bezbednost, efikasnost, kao i jednostavna primena DOAK su dovela do toga da se sve češće primenjuju u svakodnevnoj kliničkoj praksi, te se čini da će VKA postepeno postati deo istorije, odnosno da će se koristiti samo u određenoj, jasno definisanoj populaciji.

Ključne reči: krvarenje, venski tromboembolizam, antikoagulansi

ABSTRACT

The milestone of treating and preventing venous thromboembolism (VTE) is the application of anticoagulants. For many years the cornerstone was the use of vitamin K antagonists (VKAs), but it was associated with numerous obstacles and complications. With the introduction of a new generation of direct oral anticoagulants (DOAC), some of the difficulties, such as delayed onset/offset of the action, individual dose modifications, inhibition of several coagulation factors, need for frequent monitoring of prothrombin time, multiple drug interactions, have been overcome, while maintaining an adequate safety profile. Therefore, DOACs have rapidly replaced VKAs as a standard of care in the treatment and prevention of VTE, as well as in the prevention of ischemic complications in patients with non-valvular atrial fibrillation. However, the expected consequence of the use of anticoagulant drugs is increased bleeding risk. Several randomized and retrospective studies have analyzed the risk of bleeding associated with the use of DOACs compared to VKAs and between DOACs. It has been clearly shown that intracranial hemorrhage risk is decreased with DOAC compared to VKA, while most studies have shown that the risk of major bleeding is the same or even lower with DOAC. Considering DOAC's efficacy, excellent safety, and simple application compared with VKAs, it does not surprise their increasingly frequent application in everyday clinical practice. Will VKAs gradually become a part of history, or will their use be limited to a specific, clearly defined population? The time has to show.

Keywords: bleeding risk, venous thromboembolism, anticoagulants

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UVOD

Antagonisti vitamina K (VKA) su kamen temeljac u prevenciji i lečenju venske tromboembolije (VTE) više od pola veka. Međutim, njihova upotreba takođe ima brojne poteškoće, kao što su odloženi početak/prestanak delovanja, individualna varijabilnost doze, brojne interakcije lekova i hranljivih materija i potreba za čestim praćenjem terapijskog opsega [1]. Sve navedeno je dovelo do stvaranja novih lekova, direktnih oralnih antikoagulanasa (DOAK), koji blokiranjem faktora IIa (dabigatran) ili faktora Xa (rivaroksaban, edoksaban, apiksaban) postižu željeni antikoagulantni efekat [2]. DOAK, kao klasa lekova, efikasni su i bezbedni, barem kao VKA, sa fiksним rasporedom doza, manjim brojem interakcija lek-lek i bez potrebe za praćenjem [2]. Navedene prednosti dovele su do toga da su DOAK u velikoj meri zamenili VKA, pa se sada sve više koriste u prevenciji i lečenju VTE, kao i u prevenciji ishemijskog moždanog udara kod pacijenata sa ne-valvularnom atrijalnom fibrilacijom (NVAf) [2,3]. Međutim, VKA su i dalje poželjna opcija kod pacijenata sa valvularnom AF i antifosfolipidnim sindromom [4]. Prirodna posledica antikoagulantnog dejstva i VKA i DOAK je krvarenje, koje može biti teško i ponekad opasno po život. Lečenje krvarenja povezanih sa DOAK može biti izazovno iz nekoliko razloga: rutinski testovi koagulacije ne pokazuju antikoagulacionu aktivnost, reverzibilni agensi često nisu dostupni, a u nekim slučajevima mogu biti čak i protrombotični [5]. Sa kliničke tačke gledišta, ključno je utvrditi koliko često se može očekivati krvarenje povezano sa upotrebom DOAK i koji su prediktivni faktori za ovu komplikaciju. Na osnovu toga bi se mogle napraviti dalje strategije i planovi lečenja.

RIZIK OD KRVARENJA KOD PACIJENATA SA AKUTNOM VTE

Nekoliko randomizovanih kontrolisanih studija (RCT) uporedilo je efikasnost i bezbednost različitih DOAK sa VKA ili heparinom niske molekularne težine (LMVH) kod pacijenata sa akutnom VTE [6]. U okviru pomenutih studija praćena je incidencija major i non-major krvarenja (Tabela 1). Major krvarenje je definisano kao klinički teško krvarenje udruženo sa smanjenjem nivoa hemoglobina za > 20 g/L, potrebom za transfuzijom ≥ 2 jedinice koncentrovanih eritrocita, krvarenje u "kritične" lokalizacije (npr. intrakranijalna, retroperitonealna) ili fatalno krvarenje. Sva druga krvarenja od kliničkog značaja okarakterisana su kao non-major krvarenja [7]. U RE-COVER studiji, koja je upoređivala bezbednost dabigatrana i varfarina, rizik od major krvarenja bio je sličan u obe grane (HR 0,82; 95% CI, 0,45-1,48, $p = 0,38$), dok je rizik od bilo kakvog krvare-

INTRODUCTION

Vitamin K antagonists (VKAs) have been a cornerstone in preventing and treating venous thromboembolism (VTE) for over half a century. However, their use also has numerous difficulties, such as a delayed onset/offset of action, individual dosage variability, numerous drug and nutrient interactions, and the need for frequent monitoring of the therapeutic range [1]. All of the above led to the generation of new medicines, direct oral anticoagulants (DOACs), which, by blocking Factor IIa (dabigatran) or Factor Xa (rivaroxaban, edoxaban, apixaban), achieve the desired anticoagulant effect [2]. DOACs, as a class of drugs, are effective and safe, at least as VKAs, with fixed-dose schedules, fewer drug-drug interactions, and no need for monitoring [2]. The mentioned benefits have led to the fact that DOACs have largely replaced VKAs, so they are now increasingly used in the prevention and treatment of VTE, as well as in the prevention of ischemic stroke in patients with non-valvular atrial fibrillation (NVAf) [2,3]. However, VKAs are still a preferable option in patients with valvular AF and antiphospholipid syndrome [4]. The natural consequence of the anticoagulant effect of both VKA and DOAC is bleeding, which can be severe and sometimes life-threatening. Management of DOACs associated with bleeding can be challenging for several reasons: routine coagulation tests do not demonstrate anticoagulation activity, reversible agents are often not available, and in some cases, they can even be prothrombotic [5]. From a clinical point of view, it is crucial to establish how frequently bleeding associated with DOAC use could be expected and what the predictive factors are for this complication. According to this, further strategies and treatment plans could be made.

BLEEDING RISK IN PATIENTS WITH ACUTE VTE

Several randomized controlled trials (RCT) have compared the efficacy and safety of different DOACs with VKA or low molecular weight heparin (LMWH) in patients with acute VTE [6]. Within the mentioned studies, the incidence of major and non-major bleeding was monitored (Table 1). Bleeding was defined as major if it was clinically overt and if it was associated with a fall in the hemoglobin level of at least 20 g per liter, resulted in the need for transfusion of 2 or more units of red cells, involved a critical site (e.g., intracranial, retroperitoneal), or was fatal. All other hemorrhages of clinical importance were characterized as non-major bleedings [7]. In the RE-COVER study, which compared the safety of dabigatran versus warfarin, the risk of major bleeding was similar in both arms (HR 0.82; 95% CI, 0.45-1.48, $p = 0.38$), while the

nja bio niže u grani tretiranoj dabigatranom (HR 0,71, 95% CI, 0,59-0,85, $p < 0,001$) [8]. Dve studije, EINSTEIN-PE i EINSTEIN-DVT, i njihova objedinjena analiza, upoređivale su bezbednost rivaroksabana u poređenju sa enoksabanom ili varfarinom. Pokazalo se da je rizik od major krvarenja u grani lečenoj sa rivaroksabanom značajno manji u odnosu na standardnu terapiju (HR 0,54, 95% CI, 0,37-0,79, $p = 0,002$), a apsolutni rizik od krvarenja smanjen je za 0,8% u korist rivaroksabana [9-11]. Podaci iz studije AMPLIFY pokazali su superiornost apiksabana u odnosu na varfarin kada su major krvarenja u pitanju (HR 0,31, 95% CI, 0,17-0,55, $p < 0,001$), dok je Hokusai-VTE studija pokazala da je incidencija major krvarenja slična između konvencionalne terapije i edoksabana (HR 0,84, 95% CI, 0,59-1,21, $p = 0,35$) [12,13]. Sve gore pomenute studije su pokazale da je učestalost intrakranijalnog krvarenja kod pacijenata lečenih sa DOAK, bez obzira na specifičnu vrstu korišćenog DOAK-a, niža od one sa varfarinom. Što se tiče gastrointestinalnog krvarenja, učestalost je bila nešto veća sa dabigatranom, manja sa apiksabanom, a ista sa rivaroksabanom i edoksabanom [8-13].

Iako rezultati navedenih randomizovanih studija govore u prilog DOAK, podaci iz RWE (*Real World Evidence*) studija su drugačiji. Tri velike RWE studije iz Sjedinjenih Američkih Država (SAD) koje su upoređivale apiksaban i varfarin, pokazale su da je učestalost major krvarenja manja kod pacijenata lečenih apiksabanom (HR 0,67-0,76). Štaviše, učestalost non-major klinički značajnog krvarenja je takođe bila niža sa apiksabanom (HR 0,77) tokom perioda praćenja od 6 meseci [14-16]. Studija sprovedena u Francuskoj na 10 440 pacijenata sa VTE lečenih sa apiksabanom i 36 922 pacijenta lečena rivaroksabanom, pokazala je da je rizik od major krvarenja manji pod apiksabanom u poređenju sa varfarinom (10 775 pacijenata), dok je sličan kod pacijenata lečenih rivaroksabanom i varfarinom tokom šestomesečnog praćenja [17]. Do sličnog zaključka kada je rivaroksaban u pitanju, došli su i autori XALIA studije u kojoj je rizik od major krvarenja pod rivaroksabanom i standardnom terapijom (enoksaban/varfarin) gotovo identičan [18]. GARFIELD-VTE opservaciona studija nije pokazala statistički značajnu razliku u pojavi bilo major ili bilo kog drugog krvarenja kod pacijenata sa VTE lečenih DOAK i VKA [19].

Rezultati i RCT i RWE studija su pokazali da većina DOAK ima identičan ili manji rizik od krvarenja kod pacijenata sa akutnom VTE u poređenju sa standardnom terapijom. Ipak, pitanje je kolika je učestalost krvarenja ukoliko se porede različiti lekovi iz DOAK grupe. Poredeći međusobno rizik od krvarenja kod pacijenata na rivaroksabanu i apiksabanu, Jin i saradnici su pokazali da je učestalost ne-intrakranijalnog krvarenja manja u

risk of any bleeding was lower in dabigatran treated arm (HR 0.71, 95% CI, 0.59-0.85, $p < 0.001$) [8]. Two studies, EINSTEIN-PE and EINSTEIN-DVT, and their pooled analysis compared the safety of rivaroxaban to enoxaban or warfarin. It was shown that the risk of major bleeding in the rivaroxaban arm was significantly lower compared to standard therapy (HR 0.54, 95% CI, 0.37-0.79, $p = 0.002$), and the absolute risk of bleeding was reduced by 0.8% in favor of rivaroxaban [9-11]. Data from the AMPLIFY study showed apixaban superiority compared to warfarin for major bleeding (HR 0.31, 95% CI, 0.17-0.55, $p < 0.001$), while the Hokusai-VTE study showed that the incidence of major bleeding was similar between edoxaban and conventional therapy (HR 0.84, 95% CI, 0.59-1.21, $p = 0.35$) [12,13]. All of the studies mentioned above demonstrated that the frequency of intracranial hemorrhage in DOAC-treated patients, despite the type of DOAC used, is lower than that of warfarin. Regarding gastrointestinal bleeding, it was slightly more familiar with dabigatran, less common with apixaban, and the same with rivaroxaban and edoxaban [8-13].

Although the results of the RCTs speak in favor of DOAC, data from RWE (Real World Evidence) studies are different. Three large RWE studies from the United States of America (USA) comparing apixaban and warfarin showed that the frequency of major bleeding was lower in patients treated with apixaban (HR 0.67-0.76). Furthermore, the frequency of non-major clinically significant bleeding was also lower with apixaban (HR 0.77) during the follow-up period of 6 months [14-16]. A study in France on 10,440 patients with VTE treated with apixaban and 36,922 patients treated with rivaroxaban reported that the risk of major bleeding was lower with apixaban than with warfarin (10,775 patients), while it was similar in patients treated with rivaroxaban and warfarin during a 6-month follow-up [17]. Regarding rivaroxaban, the authors of the XALIA study came to a similar conclusion, confirming that the risk of major bleeding was almost identical between rivaroxaban and standard therapy (enoxaban/warfarin) [18]. The GARFIELD-VTE observational study showed no statistically significant difference in the occurrence of either major or any other bleeding in patients with VTE treated with DOAC and VKA [19].

Results from both RCT and RWE studies have demonstrated that most DOACs have an identical or lower risk of bleeding in patients with acute VTE compared with standard therapy. Yet, it remains a question: are the bleeding risks associated with the use of different DOAC drugs comparable? Evaluating the risk of bleeding in patients on rivaroxaban and apixaban,

Tabela 1. Poređenje rizika od krvarenja između varfarina i DOAK kod pacijenata sa akutnim VTE, rezultati odabranih studija**Table 1.** Results of selected studies comparing the risk of bleeding between DOACs and warfarin in acute VTE setting

Studija / Study	Vrsta krvarenja / Type of bleeding	Lek / Drug	Rezultati / Results
AMPLIFY (12) / AMPLIFY (12)	Bilo koje krvarenje / Any bleeding	apiksaban / apixaban	HR 0.31, 95% CI, 0.17-0.55, $p < 0.001$
Hokusai-VTE (13) / Hokusai-VTE (13)	Bilo koje krvarenje / Any bleeding	edoksaban / edoxaban	HR 0.84, 95% CI, 0.59-1.21, $p = 0.35$
RE-COVER (8) / RE-COVER (8)	Major krvarenje / Major bleeding	dabigatran / dabigatran	HR 0.82; 95% CI, 0.45-1.48, $p = 0.38$
RE-COVER (8) / RE-COVER (8)	Bilo koje krvarenje / Any bleeding	dabigatran / dabigatran	HR 0.71, 95% CI, 0.59-0.85, $p < 0.001$
EINSTEIN-PE and EINSTEIN-DVT* (9,10,11) / EINSTEIN-PE and EINSTEIN-DVT* (9,10,11)	Major krvarenje / Major bleeding	rivaroksaban / rivaroxaban	HR 0.54, 95% CI, 0.37-0.79, $p = 0.002$
RWE studije SAD (14,15,16) / RWE studies USA (14,15,16)	Major krvarenje / Major bleeding	apiksaban / apixaban	HR 0.67-0.76, $p < 0.05$
RWE studije SAD (14,15,16) / RWE studies USA (14,15,16)	Bilo koje krvarenje / Any bleeding	apiksaban / apixaban	HR 0.77, $p < 0.05$
RWE studija Francuska (17) / RWE study France (17)	Major krvarenje / Major bleeding	apiksaban / apixaban	Niži / lower
RWE studija Francuska (17) / RWE study France (17)	Bilo koje krvarenje / Any bleeding	rivaroksaban / rivaroxaban	sličan rizik / similar risk
XALIA* (18) / XALIA* (18)	Major krvarenje / Major bleeding	rivaroksaban / rivaroxaban	Sličan rizik / similar risk

* u poređenju sa varfarinom i edoksabanom
RWE - Real World Evidence (Dokazi iz kliničke prakse)

* compared to warfarin and edoxaban
RWE - Real World Evidence

grani lečenju apiksabanom [20]. Novija meta-analiza potvrdila je manju učestalost major krvarenja sa apiksabanom u poređenju sa rivaroksabanom, dok nisu uočene razlike između apiksabana i dabigatrana ili dabigatrana i rivaroksabana [21].

RIZIK OD KRVARENJA KOD PACIJENATA SA EKSTENDIRANOM PRIMENOM DOAK NAKON VTE

Lečenje VTE se zasniva na primeni antikoagulantne terapije, a prema važećim smernicama, primarno lečenje obično traje 3-6 meseci. Kod pacijenata sa VTE izazvanim prolaznim faktorima rizika (hirurškim ili nehirurškim) terapija se završava u primarnoj fazi [22]. S druge strane, poseban oprez je potreban kod pacijenata sa hroničnim faktorima rizika ili kod onih koji su imali neprovociranu VTE, te vodiči u ovoj grupi predlažu eksteniranu, sekundarnu terapiju i primenu antikoagulanasa sa ciljem prevencije rekurentnih VTE

Jin et al. showed that the incidence of non-intracranial bleeding was lower in the apixaban-treated arm [20]. A more recent meta-analysis confirmed a lower frequency of major bleeding with apixaban compared to rivaroxaban, while they found no differences between apixaban and dabigatran or dabigatran and rivaroxaban [21].

BLEEDING RISK IN PATIENTS WITH EXTENDED VTE TREATMENT

The treatment of VTE is based on the use of anticoagulant therapy, and according to the current guidelines, primary treatment usually lasts 3-6 months. In patients with VTE provoked by transient risk factors (surgical or non-surgical), therapy is completed in the primary phase [22]. On the other hand, particular concerns are needed in patients with unprovoked VTE or those exposed to chronic risk factors. The guidelines in this group suggest extended secondary therapy and the

[22]. Studija EPSTEIN- Extended, koja je analizirala bezbednost i efikasnost ekstenzije primene rivaroksabana u poređenju sa placebo tokom 6-12 meseci, pokazala je da je rizik od ponovnog VTE smanjen za 82% u grani koja je primala rivaroksaban u kojoj je zabeleženo 0,7% major krvarenja, odnosno 5,4% klinički relevantnih non-major krvarenja [9]. Apiksaban, bilo u terapijskoj dozi (5 mg) ili u dozi za tromboprolaksu (2,5 mg), značajno je smanjio rizik od ponovnih VTE događaja u poređenju sa placebo, bez povećanja rizika od velikog krvarenja (0,5% u placebo grupi, 0,2% u apiksaban u grupi od 2,5 mg i 0,1% u grupi sa 5 mg apiksabana) [23]. Meta-analiza koja je uključila 16 RTC i 22 000 pacijenata pokazala je da je upotreba bilo kog oralnog antikoagulansa (VKA ili DOAK) značajno redukovala rizik od retromboza u poređenju sa placebo ili Aspirinom, dok je veća incidencija major krvarenja zabeležena samo u grani lečenoj VKA u poređenju sa Aspirinom/placebo [24]. Novija meta-analiza (14 RTC i 13 kohortnih studija), upoređujući produženu upotrebu DOAK ili VKA tokom još tri meseca kod pacijenata sa prvom neprovociranom trombozom, pokazala je da dugoročni rizik od major krvarenja u obe ispitivane grupe nije zanemarljiv [25].

Na osnovu ovih zapažanja, biće potrebne dodatne analize i podaci iz svakodnevne kliničke prakse da bi se doneo definitivni zaključak o bezbednosti dugoročne primene DOAK.

RIZIK OD KRVARENJA KOD PACIJENATA SA NEVALVULARNOM AF

Direktni oralni antikoagulansi se koriste za prevenciju ishemijskog moždanog udara i sistemske embolizacije kod pacijenata sa NVA [2,3]. Nekoliko RTC (ROCKET AF, RE-LY, ENGAGE AF-TIMI 48, ARISTOTLE) upoređivalo je rizik od krvarenja između DOAK i standardne terapije VKA (Tabela 2).

Rizik od major krvarenja bio je manji kod pacijenata lečenih dabigatranom u dozi od 110 mg (HR 0,80), apiksabanom (HR 0,69) i niskom/visokom dozom edoksabana (HR 0,47/0,80) u poređenju sa varfarinom. Sa druge strane, rizik je bio sličan u granama lečenim primenom dabigatrana u dozi od 150 mg i rivaroksabana u poređenju sa varfarinom. Rizik od intrakranijalnog krvarenja je značajno smanjen upotrebom DOAK, dok je rizik od gastrointestinalnog krvarenja bio veći sa rivaroksabanom, dabigatranom 150 mg i visokim dozama edoksabana u poređenju sa varfarinom [26-29]. Meta-analiza na 94 656 pacijenata pokazala je da apiksaban 5 mg/12h, dabigatran 110 mg/12h, edoksaban 30 mg/dan i edoksaban 60 mg/dan dovode do značajno nižeg rizika od major krvarenja u poređenju sa varfarinom. Rizik od major krvarenja bio je veći sa dabiga-

use of anticoagulants to prevent recurrent VTE [22]. The EPSTEIN-Extended study, which analyzed the safety and efficacy of extended use of rivaroxaban compared to placebo for 6-12 months, showed that the risk of recurrent VTE was reduced by 82% in the rivaroxaban arm, with 0.7% incidence of major bleeding, and 5.4% of clinically relevant non-major bleeding [9]. Apixaban, either in the therapeutic dose (5mg) or in the thromboprophylaxis dose (2.5 mg), significantly reduced the risk of recurrent VTE events compared to placebo, without increasing the risk of major bleeding (0.5% in the placebo group, 0.2% in the apixaban 2.5mg group and 0.1% in the apixaban 5mg group) [23]. A meta-analysis that included 16 RTCs and 22,000 patients showed that the use of any oral anticoagulant (VKAs or DOACs) significantly reduced the risk of new thromboembolism compared to placebo or Aspirin. The higher incidence of major bleeding was noted only in the VKA arm compared to Aspirin/placebo [24]. A more recent meta-analysis (14 RTC and 13 cohort studies), comparing the extended use of DOACs or VKAs for another three months in patients with first unprovoked thrombosis, described that the long-term risks and outcomes of anticoagulant-related major bleeding were significant with both VKAs and DOACs [25].

Based on these observations, additional analyses and data from RWE will be needed to reach a definitive conclusion on the safety of long-term use of DOACs.

BLEEDING RISK IN PATIENTS WITH NON-VALVULAR AF

Direct oral anticoagulants are used to prevent ischemic stroke and systemic embolization in patients with NVA [2,3]. Several RTCs (ROCKET AF, RE-LY, ENGAGE AF-TIMI 48, ARISTOTLE) compared bleeding risks between DOACs and standard VKA therapy (Table 2).

The risk of major bleeding was lower in patients treated with dabigatran 110 mg (HR 0.80), apixaban (HR 0.69), and low/high dose edoxaban (HR 0.47/0.80) compared to warfarin. In comparison, the risk was similar in the dabigatran 150 mg and rivaroxaban arm compared to warfarin. The risk of intracranial hemorrhage was significantly decreased by using DOACs than VKAs. The risk of gastrointestinal bleeding was higher with rivaroxaban, dabigatran 150mg, and high-dose edoxaban compared to warfarin [26-29]. A meta-analysis of 94,656 patients showed that apixaban 5 mg/12h, dabigatran 110 mg/12h, edoxaban 30 mg/day, and edoxaban 60 mg/day lead to a significantly lower risk of major bleeding compared to warfarin. The risk of major bleeding was higher with dabigatran 150 mg/12h than with apixaban 5 mg/12h, with rivaroxaban 20 mg/12h compared to

Tabela 2. Različiti sistemi bodovanja za procenu verovatnoće krvarenja kod pacijenata sa AF

	HAS-BLED	OBRI	ATRIA	ORBIT	ABC	NBP	HEMORR2HAGES
Hipertenzija / Hypertension	✓		✓			✓	✓
Abnormalna funkcija bubrega/jetne / Abnormal renal/liver function	✓						✓
Moždani udar / Stroke	✓	✓	✓				✓
Istorija krvarenja ili predispozicija / Bleeding history or predisposition	✓			✓	✓	✓	✓
Krvarenje iz GI trakta / GI tract bleeding		✓					
Labilan iNR / Labile iNR	✓	✓					
Starost / Age	✓	✓	✓	✓	✓		✓
Lekovi (NSAID)/alkohol / Drugs (NSAID)/Alcohol	✓						✓
Ozbiljni komorbiditet / Serious comorbidity		✓					
Pol / Sex			✓			✓	
Dijabetes Melitus / Diabetes Mellitus			✓				
Hronična srčana insuficijencija / Chronic Heart Failure			✓				
Proteinurija / Proteinuria			✓				
eGFR			✓	✓		✓	
Anemija / Anaemia				✓	✓	✓	✓
Antiplatelet th / Antiplatelet th				✓		✓	✓
GDF-15					✓		
cTn-hs					✓		
Trombocitopenija / Thrombocytopenia						✓	✓
Rizik od pada / Fall risk						✓	✓
Serumski holesterol / Serum cholesterol						✓	
Istorija maligniteta / Malignancy history							✓
Genetski faktori / Genetic factors							✓

GI – gastrointestinalni, iNR – internacionalni normalizovani odnos, NSAID – nesteroidni antiinflamatorni lekovi, eGFR – procenjena glomerulska filtracija, CDF-15 – Faktor diferencijacije rasta 15, cTn-hs – visokosenzitivni troponin

Table 2. Risk assessment models to evaluate bleeding probability in patients with AF

GI – gastrointestinal, iNR - International normalized ratio, NSAID - Non-steroidal anti-inflammatory drugs, eGFR - Estimated glomerular filtration rate, CDF-15 - Growth differentiation factor 15, cTn-hs - High-sensitivity cardiac troponin

tranom 150 mg/12 h nego sa apiksabanom 5 mg/12 h, sa rivaroksabanom 20 mg/12 h u poređenju sa apiksabanom 5 mg/12 h i sa rivaroksabanom 20 mg/12h u poređenju sa edoksabanom 60 mg/dan. Ipak, rizik od intrakranijalnog krvarenja je bio nizak, sa skoro svim DOAK u svim dozama [30].

Novija meta-analiza, koja je uključivala veliki broj retrospektivnih kohortnih studija i skoro pola miliona pacijenata iz Evrope sa NVAF, upoređujući bezbednost DOAK u odnosu na VKA, zaključila je da ne postoji veći rizik od major krvarenja sa DOAK u odnosu na VKA (HR 0,94, 95% CI, 0,87–1,02). Nasuprot tome, uočene su razlike između različitih DOAK. Tako su, na primer, pacijenti na rivaroksabanu imali nešto veću učestalost krvarenja od VKA, dok je učestalost kod dabigatrana i apiksabana u poređenju sa VKA bila niža [31]. Pored toga, rezultati drugih studija potvrdili su da rizik od

apixaban 5 mg/12h, and with rivaroxaban 20 mg/12h compared to edoxaban 60 mg/day. However, the risk of intracranial bleeding was low, with almost all DOACs at all doses [30].

A more recent meta-analysis, which included a large number of retrospective cohort studies and almost half a million patients from Europe with NVAF, comparing the safety of DOACs versus VKAs concluded that there is no greater risk of major bleeding with DOACs versus VKAs (HR 0.94, 95% CI, 0.87–1.02). Conversely, the differences were observed between different DOACs. Thus, for example, patients on rivaroxaban had a slightly higher bleeding frequency than VKAs, while the frequency with dabigatran and apixaban compared to VKAs was lower [31]. Additionally, the results of other studies confirmed that the risk of bleeding and effectiveness vary within different DOACs. The

krvarenja i efikasnost variraju unutar različitih DOAK. Nemačka studija je primetila da je edoksaban efikasniji u smanjenju rizika od ishemijskog moždanog udara i sistemske embolizacije u poređenju sa drugim DOAK i VKA, uz manji rizik od krvarenja u poređenju sa rivaroksabanom i VKA, odnosno isti kao apiksaban [32]. Upoređujući DOAK, nekoliko studija je naglasilo da je apiksaban bezbedniji kada se uzme u obzir rizik od major krvarenja i gastrointestinalnog/intrakranijalnog krvarenja u poređenju sa rivaroksabanom, pa čak i dabigatranom, dok je njegova efikasnost u poređenju sa drugim DOAK još uvek predmet rasprave [33,34].

PREDIKTIVNI FAKTORI ZA RAZVOJ KRVARENJA

Između 2-4% pacijenata sa AF na terapiji VKA godišnje razvije krvarenje, a od toga njih 0,61% intrakranijalno krvarenje. U slučaju VTE, veliko krvarenje se javlja kod 7,22/100 pacijenata godišnje, sa stopom mortaliteta od 9% [35]. Imajući u vidu navedene rizike, jasno je da je neophodno identifikovati i određene prediktivne faktore za razvoj hemoragijskih komplikacija.

Prethodno krvarenje u anamnezi je najvažniji faktor rizika povezan sa major krvarenjem kod pacijenata na terapiji antikoagulansima. Pored navedenog, drugi relevantni faktori rizika su starija životna dob, bolesti jetre ili bubrega, maligniteti, trombocitopenija, istovremena primena antiagregacione terapije i prekomerna antikoagulacija usled neadekvatno visokih doza DOAK ili loše kontrolisanog INR (International Normalized Ratio). Ne treba zanemariti ni postojanje minimalnih lezija sluzokože u gastrointestinalnom ili urinarnom traktu [6].

Sa namerom da se identifikuju pacijenti sa povećanim rizikom od krvarenja, danas postoje brojni sistemi bodovanja. Među najčešće korišćenim i potvrđenim kod pacijenata sa AF je HAS-BLED („Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile INR, Elderly ≥ 65 , Drugs (NSAID)/alcohol”), koji stratifikuje pacijente u tri grupe rizika (nizak, umeren i visok). Pacijenti sa visokim rizikom od krvarenja prema HAS-BLED skor ≥ 3 imaju godišnju stopu major krvarenja $> 3,74\%$ [36]. Takođe, postoje i drugi sistemi bodovanja za pacijente sa AF: OBRI, ATRIA, ORBIT, ABC, NBP, HEMORR₂HAGES [6]. Slično, kod pacijenata sa VTE na oralnoj antikoagulantnoj terapiji, postoje različiti sistemi bodovanja (RIETE, *Registro Informatizado de Enfermedad TromboEmbólica*; ACCP, *American College of Chest Physician*; VTE-BLEED) koji kombinuju starost, stepen anemije, prethodna krvarenja, funkciju jetre/bubrega, trombocitopeniju, konkomitantnu terapiju, druge komorbiditete, postojanje kancera da bi se pacijent stratifikovao u odgovarajuću grupu rizika (Tabela 3) [6].

German study observed that edoxaban is more effective in reducing the risk of ischemic stroke and systemic embolization compared to other DOACs and VKAs, carrying a lower risk of bleeding compared to rivaroxaban and VKAs, and the same as apixaban [32]. Comparing DOACs, several studies have emphasized that apixaban is safer when considering the risk of both major bleeding and gastrointestinal/intracranial bleeding compared to rivaroxaban and even dabigatran, while its efficacy compared to other DOACs is still a subject of debate [33,34].

PREDICTORS OF BLEEDING

Anticoagulant therapy has an annual rate of bleeding between 2-4% among patients receiving VKA, and the annual risk of intracranial hemorrhage is 0.61%. In the case of VTE, major bleeding occurs in 7.22/100 patients/year, with a mortality rate of 9% [35]. Therefore, it is crucial to identify certain predictive factors for hemorrhagic complications and to stratify patients into risk groups.

History of previous bleeding is the most important risk factor associated with major bleeding in patients on anticoagulant therapy. In addition to the above, other relevant risk factors are older age, liver or kidney diseases, malignancies, thrombocytopenia, concomitant use of antiplatelet therapy, and excessive anticoagulation due to inadequately high doses of DOACs or poorly controlled INR (International Normalized Ratio). The existence of minimal mucosal lesions in the gastrointestinal or urinary tract should not be ignored either [6].

Intending to identify those patients at increased bleeding risk, there are numerous scoring systems available today. Among the most used and validated in patients with AF is HAS-BLED (“Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile INR, Elderly ≥ 65 , Drugs (NSAID)/alcohol”), which stratifies patients into three risk groups (low, moderate and high). Patients with a high risk of bleeding according to HAS-BLED score ≥ 3 have an annual rate of significant bleeding $> 3.74\%$ [36]. Also, other scoring systems exist for patients with AF: OBRI, ATRIA, ORBIT, ABC, NBP, HEMORR₂HAGES [6]. Similarly, in patients with VTE on oral anticoagulant therapy, there are different scoring systems (RIETE, *Registro Informatizado de Enfermedad TromboEmbólica*; ACCP, *American College of Chest Physician*; VTE-BLEED) combining age, severity of anemia, previous bleeding, liver/kidney function, platelet count, concomitant therapy, other comorbidities, existence of cancer to stratify the patient into the appropriate risk group (Table 3) [6].

Tabela 3. Različiti sistemi bodovanja za procenu verovatnoće krvarenja kod pacijenata sa VTE

Faktor rizika / Risk factor	RIETE	ACCP	VTE-BLEED
Abnormalna funkcija bubrega / Abnormal renal function	✓	✓	✓
Istorija krvarenja ili predispozicija / Bleeding history or predisposition	✓	✓	✓
Starost / Age	✓	✓	✓
Anemija / Anaemia	✓	✓	✓
Abnormalna funkcija jetre / Abnormal liver function		✓	
Istorija moždanog udara/TIA / History of stroke/TIA		✓	
Lekovi (NSAID)/Alkohol / Drugs (NSAID)/Alcohol		✓	
Ozbiljni komorbiditet / Serious comorbidity		✓	
Dijabetes Melitus / Diabetes Mellitus		✓	
Česti padovi / Frequent falls		✓	
Nedavna operacija / Recent surgery		✓	
Trombocitopenija / Thrombocytopenia		✓	
Antitrombotična terapija / Antiplatelet therapy		✓	
Loša kontrola antikoagulanasa / Poor anticoagulant control		✓	
Metastatski karcinom / Metastatic cancer		✓	
Aktivni malignitet / Active malignancy		✓	✓
Pacijent sa hipertenzijom / Male patient with hypertension			✓
Istorija maligniteta / Malignancy history	✓		
Klinički očigledna plućna embolija / Clinically overt pulmonary embolism	✓		

NSAID – nesteroidni antiinflamatorni lekovi; TIA – Transzitorni ishemijski napad; VTE – venska tromboembolija

Table 3. Risk assessment models to evaluate bleeding probability in patients with VTE

NSAID – Non-steroidal anti-inflammatory drugs; TIA – Transient ischemic attack; VTE – Venous thromboembolism

Na osnovu svega navedenog, najvažnija je klinička procena, adekvatna evaluacija svih faktora rizika za krvarenje kod datog pacijenta, te na osnovu toga proceniti rizik i korist od antikoagulantne terapije i mogućnosti krvarenja usled korišćenja iste.

NASTAVAK ANTIKOAGULANTNE TERAPIJE NAKON KRVARENJA

Ponovno započinjanje antikoagulantne terapije nakon povlačenja krvarenja je izazovna klinička odluka, koju je potrebno doneti individualno za svakog pacijenta, a često uz pomoć multidisciplinarnog tima sačinjenog od specijaliste za hemostazu, kardiologa, gastroenterologa, neurologe i specijaliste intenzivne nege [37]. S jedne strane, prerano ponovno uvođenje može povećati rizik od ponovnog krvarenja, dok sa druge strane, kasniji nastavak antikoagulantne terapije povećava rizik od tromboembolijskih događaja i smrti [38]. Podaci u vezi sa ponovnim uvođenjem DOAK kod pacijenata koji su doživeli krvarenje su oskudni. Kohortna studija Tapaskara i saradnika pokazala je da je ponovno uvođenje varfarina povezano sa povećanim rizikom od rekurentnog gastrointestinalnog krvarenja (HR, 2,12;

Therefore, careful evaluation of individual patient bleeding risk factors and clinical judgment remains the best approach to minimizing the personal risk of bleeding during anticoagulant treatment.

RESUMING ANTICOAGULATION THERAPY AFTER BLEEDING

Restarting anticoagulation therapy after the resolution of bleeding is a challenging clinical decision. It should be made individually for every patient, usually by a multidisciplinary team, including hemostasis specialists, cardiologists, gastroenterologists, neurologists, and intensive care specialists [37]. On the one hand, early restart can increase the risk of rebleeding, while on the other hand, later continuation of anticoagulation therapy increases patients' risk of thromboembolic events and mortality [38]. Data regarding restarting DOACs in patients who experienced bleeding are scarce. A cohort study by Tapaskar et al. showed that the restart of warfarin was associated with an increased risk of recurrent gastrointestinal bleeding (HR, 2.12; 95% CI, 1.43–3.14; $p < 0.001$), while DOAC resumption was not associated with recurrent bleed-

95% CI, 1,43–3,14; $p < 0,001$), dok nastavak DOAK nije bio povezan sa ponovnim krvarenjem (HR, 1,43; 95% CI, 0,81–2,52; $p = 0,22$). Ipak, ista studija je pokazala da je rivaroksaban jedini DOAK povezan sa rekurentnim gastrointestinalnim krvarenjem (HR, 2,73; 95% CI, 1,43–5,20; $p = 0,002$) [39]. Kada je reč o intrakranijalnom krvarenju, Poli i saradnici su pokazala da pacijenti koji uzimaju varfarin imaju veći rizik od rekurentnog intrakranijalnog krvarenja u poređenju sa pacijentima na terapiji DOAK (OR 1,9; 95% CI 0,7–6,7). Međutim, u DOAK kohorti je bila primećena veća učestalost rekurentnog ne-cerebralnog krvarenja [40]. Na drugom kraju spektra, nastavak primene antikoagulanasa je bio povezan sa manjim brojem tromboembolijskih događaja i smanjenim mortalitetom [41,42]. Nakon otklanjanja krvarenja, određivanje pravog vremena za ponovno uvođenje DOAK je najizazovnije pitanje. Sengupta et al. pokazali su da ponovno uvođenje DOAK u roku od 30 dana nakon krvarenja nije povezano sa tromboembolijom u roku od 90 dana (HR, 0,98; 95% CI, 0,37–2,21) ili rekurentnim gastrointestinalnim krvarenjem (HR, 1,44; 95% CI 0,72–436). Prema BSG/ESGE smernicama, ponovno uvođenje DOAK što je pre moguće do sedmog dana nakon njihovog prekida može biti razumno u većini slučajeva [44]. Međutim, treba napomenuti da je ovaj zaključak donet na osnovu podataka sa varfarinom, a podaci iz randomizovanih kontrolisanih studija nedostaju.

ZAKLJUČAK

U poređenju sa VKA, DOAK su bar jednako efikasni u lečenju i prevenciji VTE kao i u prevenciji ishemijskog insulta i sistemskih embolizacija kod pacijenata sa AF, a sve to uz potvrđeno značajno manji rizik od intrakranijalnog krvarenja. Takođe, većina studija je pokazala da je rizik od major krvarenja manji kada se koriste DOAK, dok je rizik od gastrointestinalnog krvarenja varijabilan i zavisi od vrste DOAKa.

Razvijeni su brojni sistemi bodovanja koji pomažu objektivizaciji, jasnoj stratifikaciji i identifikaciji pacijenata sa većim rizikom od krvarenja. Međutim, individualno profilisanje rizika od krvarenja i izbor odgovarajućih DOAK-a na osnovu komorbiditeta, starosti, prethodnog krvarenja, opšteg stanja i interakcija lekova mogu dodatno smanjiti rizik od hemoragijskih komplikacija. Takođe, intenzivnije i češće praćenje bilo bi važno kod pacijenata sa visokim rizikom od krvarenja. Na osnovu navedenog, postavlja se pitanje da li će DOAK zameniti VKA kao prihvatljiviji oblik prevencije i lečenja VTE u većini kliničkih indikacija posle više od jednog veka.

Sukob interesa: Nije prijavljen.

ing (HR, 1.43; 95% CI, 0.81–2.52; $p = 0.22$). However, the same study showed that rivaroxaban was the only individual DOAC associated with recurrent gastrointestinal bleeding (HR, 2.73; 95% CI, 1.43–5.20; $p = 0.002$) [39]. When the word is about intracranial hemorrhage, Poli et al. showed that patients taking warfarin were at higher risk of intracranial hemorrhage recurrence than patients taking DOACs (OR 1.9; 95% CI 0.7–6.7). However, non-cerebral major re-bleeding was more common in the DOAC cohort [40]. On the other end of the spectrum, resuming anticoagulants was associated with fewer thromboembolic events and decreased mortality [41,42]. After resolving the bleeding, determining the right timing for DOAC restart is the most challenging question. Sengupta et al. showed that reintroducing DOACs within 30 days after bleeding was not associated with thromboembolism within 90 days (HR, 0.98; 95% CI, 0.37–2.21) or recurrent gastrointestinal bleeding (HR, 1.44; 95% CI 0.72–2.68) [43]. According to BSG/ESGE guidelines, restarting DOACs as soon as possible by day seven after their interruption could be reasonable in most cases [44]. However, it should be noted that this conclusion was made in parallel with warfarin, and data from randomized controlled trials are lacking.

CONCLUSION

Compared to VKA, DOACs are at least as effective in treating and preventing VTE as in preventing ischemic insult and systemic embolization in patients with AF, with a confirmed significantly lower risk of intracranial bleeding. Also, most studies showed that the risk of major bleeding is lower when using DOACs, while the risk of gastrointestinal bleeding is variable and depends on the type of DOACs.

Numerous scoring systems have been developed to help objectify, clearly stratify, and identify patients at higher risk of bleeding. However, individual bleeding risk profiling and selection of the appropriate DOACs based on the patient's comorbidities, age, previous bleeding, general condition, and drug interactions can further reduce the risk of hemorrhagic complications. Also, more intensive and frequent monitoring would be important in patients at high risk of bleeding. Based on the above, the question arises whether DOACs will replace VKAs as a more acceptable form of VTE prevention and treatment in most clinical indications after over a century.

Conflict of interest: None declared.

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TREATMENT OF PRIMARY MYELOFIBROSIS, WHERE WE STAND TODAY?

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SAŽETAK

Uvod: Primarna mijelofibroza (PMF) je klonsko oboljenje hematopoeze koje odlikuju konstitutivne tegobe, uvećanje slezine, anemija i često trombocitopenija.

Cilj rada: prikazati uporedne podatke o sprovedenim studijama i lekovima koji su nam dostupni ali i novim terapijskim opcijama.

Metode: analiza publikovanih kliničkih studija i relevantnih radova.

Rezultati: u ovom trenutku zlatni standard lečenja bolesnika sa PMF je još uvek ruxolitinib, prvi predstavnik JAK inhibitora (JAKi). Postiže smanjenje volumena slezine (SVS $\geq$ 35%) kod 41,9% bolesnika u odnosu na placebo i smanjuje tegobe >50% (TSS<50%) kod 49,5% lečenih. Nije idealan lek, jer se tokom primene javlja anemija, trombocitopenija i sklonost ka infekcijama, pa određeni broj bolesnika mora da prekine lečenje. Fedratinib je drugi JAKi, koji ima dobre rezultate kao druga linija nakon neuspeha ruxolitiniba. Primenjen u prvoj liniji, fedratinib postiže SVS $\geq$ 35% od 47% uz TSS<50 od 40%.

Kod bolesnika sa transfuziono zavisnom anemijom, dobre rezultate je postigao momelotinib, koji nije bio inferioran u odnosu na ruxolitinib (SVS $\geq$ 35% je sličan, 26.5% u odnosu na 29% za lečene ruxolitinibom). Primena momelotiniba je smanjila transfuzionu zavisnost za 17% u odnosu na ruxolitinib (66% prema 49%). Kod bolesnika sa trombocitopenijom (Tr 50-100x10⁹/L), pakritinib predstavlja najnoviju terapijsku opciju; postiže smanjenje slezine u odnosu na modifikovane doze ruxolitiniba bez daljeg pogoršanja trombocitopenije.

Kombinacije sa ruxolitinibom (navitoklaks, pelabresib) imaju za cilj da značajno poboljšaju odgovor, prvenstveno smanjenjem slezine.

Zaključak: lečenje PMF je i dalje izazov. Primena ruxolitiniba omogućava dobru kontrolu bolesti kod skoro polovine bolesnika, a novi lekovi treba da omoguće lečenje refrakternih bolesnika ili da poboljšaju odgovor.

Ključne reči: primarna mijelofibroza, JAK inhibitori, ruxolitinib, fedratinib, momelotinib, pakritinib

ABSTRACT

Introduction: Primary myelofibrosis (PMF) is a clonal hematopoietic neoplastic disease characterized by constitutive complaints, splenomegaly, anemia and very often thrombocytopenia.

Aim: To analyze and compare data from clinical trials, focusing on both current and emerging therapeutics.

Methods: The analysis of published clinical trials and relevant papers.

Results: Currently, the gold standard for treating primary myelofibrosis (PMF) is ruxolitinib, the first-in-class JAK inhibitor (JAKi). It has achieved a $\geq$ 35% reduction in spleen volume (SVR) in 41.9% of patients compared to placebo and has led to a >50% improvement in symptoms, as measured by the Total Symptom Score (TSS), in 49.5% of treated individuals. Ruxolitinib is not an ideal drug, as some patients experience worsening anemia, thrombocytopenia, and an increased susceptibility to various infections. Some patients are required to discontinue the treatment or reduce the dosage. Another approved JAK inhibitor, fedratinib, has shown promising results as a second-line treatment following ruxolitinib failure. As a first-line therapy, fedratinib achieved a spleen volume reduction (SVR) of $\geq$ 35% in 47% of patients, with a >50% improvement in TSS in 40% of cases.

In transfusion-dependent patients, momelotinib has demonstrated good results, showing non-inferiority to ruxolitinib, with similar spleen volume reduction (SVR $\geq$ 35%) rates of 26.5% compared to 29% with ruxolitinib. Momelotinib treatment reduced transfusion independence by 17% compared to ruxolitinib (66% vs 49%). In thrombocytopenic patients (Plt 50-100 x10⁹/L), pacritinib is the newest therapy; it reduces spleen volume compared to adjusted ruxolitinib dosing without worsening thrombocytopenia.

Drugs combined with ruxolitinib (e.g. navitoklaks, pelabresib) have the purpose to improve the outcome, especially in spleen reduction.

Conclusion: Treating PMF remains a challenge. While ruxolitinib provides effective disease control in nearly half of patients, new therapies are needed to enhance outcomes both overall and in those with refractory disease.

Keywords: primary myelofibrosis, JAK inhibitors, ruxolitinib, fedratinib, momelotinib, pacritinib

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UVOD

Primarna mijelofibroza (PMF) je klonsko oboljenje matične ćelije hematopoeze koje odlikuje specifičan niz genetskih i patofizioloških poremećaja koji dovodi do neadekvatnog sazrevanja matičnih ćelija megakariocitne i granulocitne loze u kostnoj srži [1,2].

U nastanku svih Filadelfija negativnih mijeloproliferativnih neoplazmi učesvuju mutacije koje se javljaju već na nivou matičnih ćelija hematopoeze i ćelija progenitora. Smatra se da postoje dve vrste mutacija, tzv *driver* ili mutacije pokretači bolesti i „epigenetske“, odnosno dodatne mutacije. Mutacije pokretači bolesti pogađaju 3 gena bitna za nastanak i održavanje ćelijskog fenotipa PMF i to su mutacija *JAK2* V617 (*JAK2*), mutacija *Calreticulin* gena (*CALR* exon 9 indel) i mutacija *c-MPL* W515K/L (*MPL*) gena [3]. Ove mutacije se javljaju u značajnom procentu kod bolesnika sa PMF (*JAK2* mutacija je pozitivna u 50-60%, *CALR* kod oko 25% bolesnika, dok je po oko 10% *MPL* pozitivno ili potpuno negativno na ove tri bitne mutacije-trostruko negativne) [3]. Dodatne mutacije su odgovorne za molekularne interakcije i za progresiju bolesti u pravcu akutne leukemije i određuju brzinu i agresivnost pogoršanja bolesti. Te mutacije obuhvataju gene koji regulišu epigenetsku regulaciju (*ASXL1*, *TET2*), delovanje *splicing* procesa na nivou RNK (*SRSF2*, *U2AF1*), mehanizme remodelacije hromatina (*DNMT3A*, *EZH2*) kao i regulaciju tumor supresornih mehanizama i apoptoze (na prvom mestu *p53* gen, ali i *BCL-X_L*, retko, *BCL-2*) [4,5].

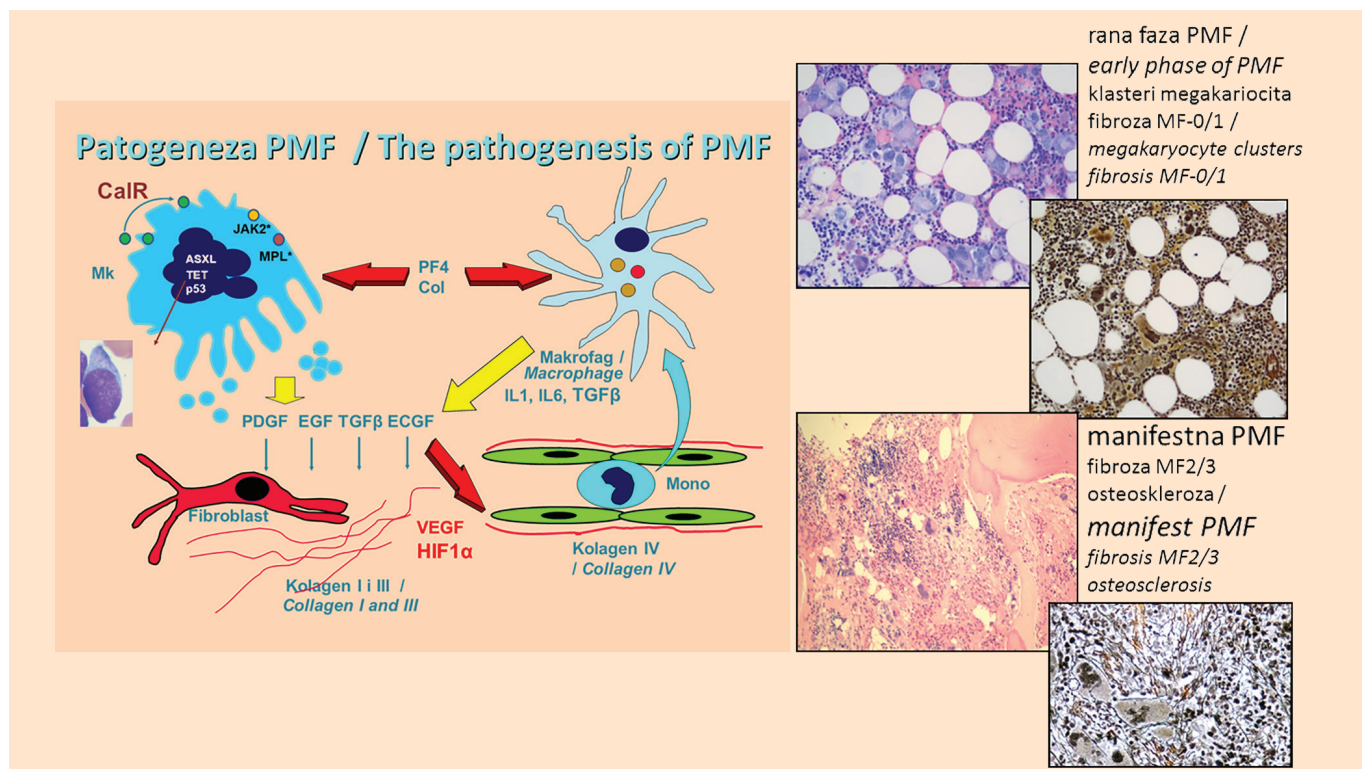
U etiopatogenezi PMF genetski izmenjene matične i progenitorne ćelije reaguju na, često genetski izmenjene, ćelije strome koštane srži, neadekvatnim mehanizmima regulacije i nastankom kontinuiranog zapaljenskog odgovora (hiperinflamacije) [6,7]. Ovakav odgovor dovodi do reakcije strome i aktivacije fibroblasta u smislu stvaranja profibrogenih i proangiogenih citokina (Shema 1) kao što su interleukin-1 β (IL-1 β), β -fibroblastni faktor rasta (β -FGF „fibroblastic growth factor“), transformišući faktor rasta beta (TGF- β , *transforming growth factor*- β), faktor rasta poreklom iz trombocita (PDGF, *platelet derived growth factor*), vaskularni endotelijalni faktor rasta (VEGF, *vascular endothelial growth factor*) [1,6,7]. Poremećaj ove regulacije dovodi do disregulacije i drugih puteva kao što je AKT/mTOR [7,8] ili do disregulacije puteva oksidativnog stresa [8], što za posledicu ima nekontrolisanu proliferaciju ne samo megakariocitnih već i granulocitnih progenitora kao i neoangiogenezu [9-11]. Proliferacija veziva u početku se ogleda kroz retikulinsku fibrozu koštane srži da bi u kasnijim stadijumima bolesti retikulin polimerizovao u kolagen a time i omogućio formiranje novih koštanih gredica

INTRODUCTION

Primary myelofibrosis (PMF) is a clonal disorder of hematopoietic stem cells, marked by specific genetic mutations and pathophysiological changes that disrupt the normal maturation of megakaryocyte and granulocyte lineage cells in the bone marrow [1,2].

All Philadelphia-negative myeloproliferative neoplasms arise from mutations that originate at the level of hematopoietic stem cells and progenitor cells. There are thought to be two types of mutations: the so-called “driver” or disease-initiating mutations, and “epigenetic” or additional mutations. Disease-causing mutations in primary myelofibrosis (PMF) primarily involve three key genes that are crucial for the development and maintenance of the disease phenotype: the *JAK2* V617F mutation, the *calreticulin* (*CALR*) gene mutation (exon 9 indel), and the *c-MPL* W515K/L mutation [3]. These mutations are found in a significant percentage of patients with PMF: the *JAK2* mutation is present in 50-60% of cases, the *CALR* mutation in about 25%, and the *MPL* mutation in approximately 10%. A subset of patients may be triple-negative, lacking all three of these key mutations [3]. Additional mutations play a crucial role in driving molecular interactions and contribute to the progression of the disease towards acute leukemia. These mutations also influence the rate and aggressiveness of disease progression. They involve genes that regulate various critical processes, including epigenetic modification (*ASXL1*, *TET2*), RNA splicing (*SRSF2*, *U2AF1*), chromatin remodeling (*DNMT3A*, *EZH2*), and the regulation of tumor suppressor mechanisms and apoptosis (primarily the *p53* gene, as well as *BCL-X_L* and, less commonly, *BCL-2*) [4,5].

In the etiopathogenesis of PMF, genetically altered stem and progenitor cells interact with often mutated stromal cells in the bone marrow, leading to dysregulated mechanisms and the development of a persistent inflammatory response, or hyperinflammation [6,7]. This response triggers stromal reactions and activates fibroblasts, leading to the production of profibrogenic and proangiogenic cytokines (Scheme 1), including interleukin-1 β (IL-1 β), β -fibroblast growth factor (β -FGF), transforming growth factor-beta (TGF- β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) [1,6,7]. Disruption of this regulation leads to the dysregulation of pathways such as AKT/mTOR [7,8] and oxidative stress pathways [8], resulting in uncontrolled proliferation of both megakaryocyte and granulocyte progenitors, as well as neoangiogenesis [9-11]. The proliferation of connective tissue initially manifests as reticulin fibrosis in the bone marrow. In the later stages of the disease, reticulin fibers polymerize into collagen, leading to the formation of new bone structures (osteogenesis) [2,6]. This progression results in the irreversible



Shema 1. Osnovni prikaz patofizioloških događaja u nastanku primarne mijelofibroze.

IL-1: interleukin-1; β-FGF: β-fibroblastni faktor rasta; TGF-β transformišući faktor rasta; PDGF: faktor rasta poreklom iz trombocita; VEGF: vaskularni endotelijalni faktor rasta; PF4: trombocitni faktor 4; Col kolagen; EGF: epidermalni faktor rasta; NO*: azot monoksid i nitrozo-oksidativni radikali; HIF1α: hipoksija inducibilni faktor 1α. JAK2, CALR, MPL, ASXL, TET i p53 geni od značaja za patogenezu PMF.

Scheme 1. Key pathophysiological events in the development of primary myelofibrosis.

IL-1: interleukin-1; β-FGF: β-fibroblastic growth factor; TGF-β: transforming growth factor-β; PDGF: platelet-derived growth factor; VEGF: vascular endothelial growth factor; PF4: platelet factor 4; Col: collagen; EGF: epidermal growth factor; NO*: nitrogen monoxide and nitroso-oxidative radicals; HIF1α: hypoxia-inducible factor 1-alpha. JAK2, CALR, MPL, ASXL, TET and p53 driving and bystander genes crucial for the pathogenesis of PMF.

(osteogeneza) [2,6], čime PMF ulazi u ireverzibilnu fazu insuficijencije koštane srži. U toku te faze tranzicije, matične ćelije i progenitori migriraju u slezinu i jetru gde nastaje mijeloidna metaplazija pa se u kasnim stadijumima bolesti hematopoeza odvija u ekstramedularnim zonama (slezina i jetra) uz sve znake insuficijencije koštane srži [1].

Otkriće JAK2 V617F mutacije 2005. godine u četiri nezavisne laboratorije u svetu (JAK2 gen je smešten na hromozomu 9), kao i otkriće mutacije c-MPL (receptora za trombopoetin) i kasnije mutacije na CALR genu (takođe na 9. hromozomu) [1,5] omogućilo je bolje sagledavanje potencijalnih terapijskih meta za razvoj terapije PMF koja je do tada bila više palijativna i simptomatsko-supotivna. Jedina terapijska opcija koja je tada, a i sada, mogla da ponudi mogućnost izlečenja je transplantacija matičnih ćelija hematopoeze, ali je ona rezervisana za manji broj mlađih bolesnika kod kojih postoji HLA podudarni davalac [4].

Sa druge strane, u PMF je opisan veći broj citogenetskih aberacija koje sa svoje strane dovode do genetskih poremećaja i kao takve uz nepovoljne molekularne mutacije utiču na brzinu pogoršanja bolesti kao i na nastanak leukemijske transformacije [12].

phase of bone marrow insufficiency in PMF. During this transitional phase, stem cells and progenitors migrate to the spleen and liver, where myeloid metaplasia occurs. Consequently, in the late stages of the disease, hematopoiesis shifts to these extramedullary sites, resulting in signs of bone marrow insufficiency [1].

The discovery of the JAK2 V617F mutation in 2005 by four independent laboratories (the JAK2 gene is located on chromosome 9), along with the identification of mutations in the c-MPL gene (thrombopoietin receptor) and later the CALR gene (also located on chromosome 9) [1,5], significantly advanced our understanding of potential therapeutic targets for PMF. Prior to these discoveries, treatment options were primarily palliative and focused on symptom management. The only therapeutic option that offers a potential cure is hematopoietic stem cell transplantation. However, this treatment is typically reserved for a limited number of younger patients who have an HLA-matched donor [4].

Conversely, numerous cytogenetic aberrations have been identified in PMF, leading to genetic disorders that, in conjunction with unfavorable molecular mutations, influence the rate of disease progression and the likelihood of leukemic transformation [12].

U kliničkoj praksi, bolesnici sa PMF se danas dele na bolesnike sa ranom fazom PMF, što je često čak i hipercelularna, prefibrotička, faza PMF (stepen fibroze MF-0 i MF-1) i bolesnike sa manifestnom, overt, PMF kod kojih postoje sve odlike bolesti kao i fibroza MF-2 i MF-3 stepena. U ranoj fazi bolesti najizraženiji klinički problemi su vezani za postojanje trombocitoze i rizik od trombotskih događaja [13,14], dok su u manifestnoj fazi odlike bolesti pojava konstitutivnih sistemskih simptoma (inflamatorni efekti), uvećanje slezine pa i jetre, često pojava anemije i trombocitopenije, što čini terapijske zahvate komplikovanijim [1].

Posebna situacija je kliničko razlikovanje esencijalne trombocitemije i rane faze primarne trombocitoze jer u drugom slučaju je vrlo bitno pažljivo pratiti bolesnika u kasnijem toku bolesti da bi se blagovremeno uvela specifična terapija i/ili planirala transplantacija matičnih ćelija [15].

Postavlja se pitanje kada treba lečiti bolesnike sa PMF [1,3,4,14]. To zavisi od faze bolesti, kliničkih manifestacija kao i bioloških pokazatelja. U kliničkoj praksi se leči trombocitoza primenom hidroksiuree ili (PEG)-interferona kod mlađih bolesnika. Primena interferona je pokazala i antiproliferativni efekat kao i delovanje na smanjenje fibroznih i inflamatornih procesa. Anemija se leči primenom transfuzije eritrocita, kao i primenom eritropoetina i sintetskih androgena sa promenljivim uspehom, a pokušani su i imunomodulatorni lekovi kao što su steroidi i talidomid. Najveći problem je lečenje uvećanja slezine, jer klasični citostatici kao hidroksiurea, busulfan, alkeran i pipobroman nose rizike za pogoršanje citopenije, naročito trombocitopenije, i time onemogućavaju lečenje.

U proceni bolesnika pri izboru terapije sagledavaju se brojni parametri, pa su poslednjih godina razvijeni specifični prognostički modeli od kojih najbolju kliničku primenu imaju DIPSS+ skor (pored kliničkih koristi i negativne citogenetske parametre) (https://qxmd.com/calculate/calculator_315/dipss-plus-score-for-prognosis-in-myelofibrosis) [16] kao i kompozitni modeli koji uključuju i citogenetiku i molekularne markere kao MIPSS70 (<http://www.mipss70score.it/>) [17] ili GIPSS skor (<https://globalrph.com/medcalcs/prognostic-scoring-for-myelofibrosis/>) [2,4]. Ovi skorovi omogućavaju da se kod bolesnika proceni dalji rizik bolesti, na prvom mestu rizik od leukemijske transformacije i smrti bolesnika od progresije i da se proceni pravi trenutak za uvođenje druge specifične terapije ili da se planira transplantacija matičnih ćelija ako je izvodljivo. Svi skorovi razvrstavaju bolesnike u tri ili, češće, četiri kategorije koje se značajno razlikuju prema preživljavanju (za DIPSS+ nizak/low rizik 15 godina, umereni-1/intermediate-1 rizik 6,6 godina, dok su bole-

In clinical practice, PMF patients are currently categorized into two groups: those in the early phase, which often includes a hypercellular, prefibrotic phase (fibrosis stages MF-0 and MF-1), and those with manifest, overt PMF, where all disease features are present along with advanced fibrosis (stages MF-2 and MF-3). In the early stage of the disease, the most significant clinical issues are thrombocytosis and the associated risk of thrombotic events [13,14]. As the disease progresses to the manifest stage, patients may experience systemic symptoms (such as inflammation), splenomegaly, hepatomegaly, and complications like anemia and thrombocytopenia, which complicate therapeutic interventions [1].

A critical aspect of clinical practice is distinguishing between essential thrombocythemia and the early phase of primary myelofibrosis. In the latter case, careful monitoring is essential throughout the disease's progression to timely initiate specific therapy and/or plan for stem cell transplantation if necessary [15].

When should patients with PMF be treated [1,3,4,14]? It depends on the stage of the disease, clinical manifestations, and biological indicators. In clinical practice, thrombocytosis is typically managed with hydroxyurea or PEG-interferon, particularly in younger patients. Interferon has demonstrated both antiproliferative effects and benefits in reducing fibrotic and inflammatory processes. Anemia is managed with red blood cell transfusions, erythropoietin, and synthetic androgens, although success varies. Additionally, immunomodulatory drugs such as steroids and thalidomide have been attempted. The treatment of splenomegaly poses significant challenges because traditional cytostatics such as hydroxyurea, busulfan, alkeran, and pipobroman can exacerbate cytopenias, particularly thrombocytopenia, thereby complicating treatment options.

Several parameters are considered when selecting therapy for patients, leading to the development of specific prognostic models. Among these, the DIPSS+ score is widely used for clinical assessment, incorporating clinical benefits and negative cytogenetic parameters (https://qxmd.com/calculate/calculator_315/dipss-plus-score-for-prognosis-in-myelofibrosis) [16]. Additionally, composite models that integrate both cytogenetic and molecular markers, such as MIPSS70 (<http://www.mipss70score.it/>) [17] and the GIPSS score (<https://globalrph.com/medcalcs/prognostic-scoring-for-myelofibrosis/>) are also utilized [2,4]. These scores help assess the patient's risk of disease progression, particularly the likelihood of leukemic transformation and mortality. They also guide decisions on the timing of additional specific therapies or the potential need for stem cell transplantation if appropriate. All scores categorize patients into three or, more often, four distinct risk groups, each associated with varying survival

snici sa umerenim-2/intermediate-2 živeli 2,9 godina i sa visokim/high rizikom svega 1.3 god) [16].

Nakon otkrića JAK2 mutacije, došlo je do relativno brzog razvoja većeg broja lekova koji deluju na JAK/STAT signalni put koji su označeni kao JAK inhibitori (JAKi). Sve registracione studije ovih lekova su pošle od bolesnika sa uznapredovalim fazama bolesti (manifestna PMF sa intermedijskim-2 i visokim rizikom, uz manifestnu mijelofibrozbu, najčešće MF-2 i MF-3 patološkog gradusa). Tek kasnije su se izdvojili preparati specifičnijeg terapijskog profila koji imaju drugačije delovanje i mogu se primenjivati kod bolesnika sa izraženom anemijom (momelotinib) ili trombocitopenijom (pakritinib).

Danas su u Americi i Evropi registrovani sledeći JAK inhibitori:

	inhibitor gena:	odobrenje u SAD	odobrenje u Evropi
ruksolitinib	JAK1 i JAK2	2011	2012
fedratinib	JAK2, FLT3, Ret, JAK3	2019	2020
momelotinib	JAK1, JAK2, ACVR1	2023	2024
pakritinib	JAK2, Flt-3, ACVR1	2022	Ne

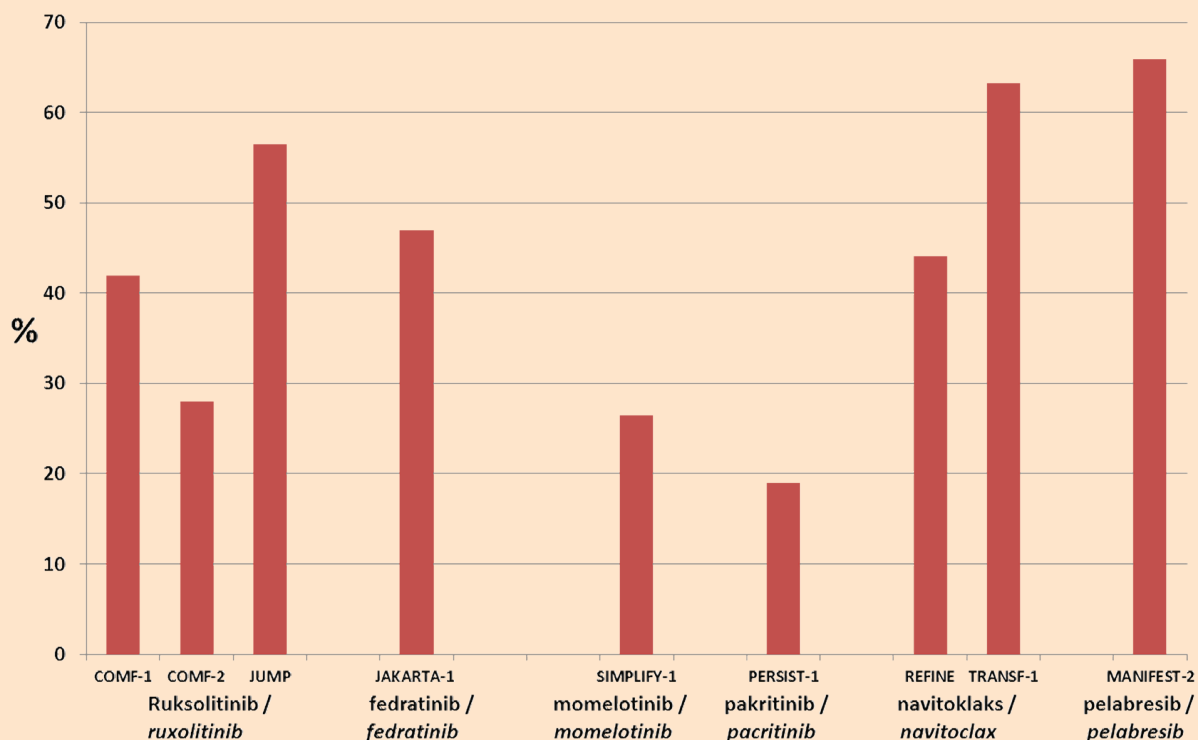
outcomes. For example, the DIPSS+ score stratifies patients into low-risk (15 years survival), moderate-1/intermediate-1 risk (6.6 years), moderate-2/intermediate-2 risk (2.9 years), and high-risk (1.3 years) categories [16].

Following the discovery of the JAK2 mutation, a significant number of drugs targeting the JAK/STAT signaling pathway, known as JAK inhibitors (JAKi), were developed relatively quickly. All registration studies for these drugs initially focused on patients with advanced stages of the disease, including manifest PMF with intermediate-2 and high-risk profiles, and those with manifest myelofibrosis, typically at pathological grades MF-2 and MF-3. Subsequently, therapies with more specific profiles were identified, offering distinct benefits for patients with pronounced anemia (such as momelotinib) or thrombocytopenia (such as pacritinib).

Nowadays, the following JAK inhibitors have been registered in the USA and Europe:

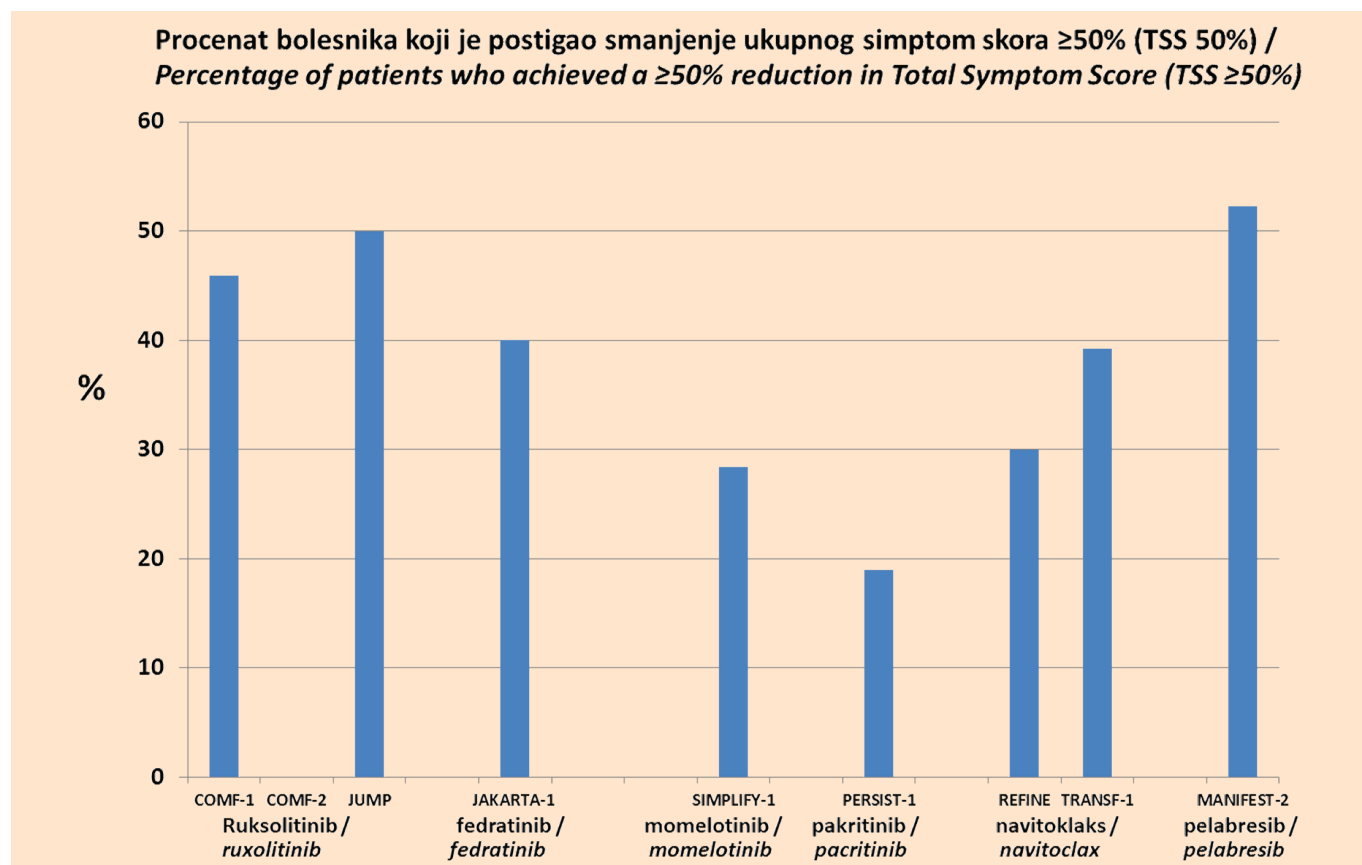
	gene inhibitor:	approved in the USA	approved in Europe
ruxolitinib	JAK1 and JAK2	2011	2012
fedratinib	JAK2, FLT3, Ret, JAK3	2019	2020
momelotinib	JAK1, JAK2, ACVR1	2023	2024
pacritinib	JAK2, Flt-3, ACVR1	2022	No

Procenat bolesnika koji je postigao smanjenje volumena slezine za $\geq 35\%$ (SVS $\geq 35\%$) / Percentage of patients who achieved a decrease in spleen volume by $\geq 35\%$ (SVS $\geq 35\%$)



Grafikon 1. Uporedni rezultati i efikasnost različitih JAKi u kliničkim studijama (stepen redukcije slezine, SVS $\geq 35\%$) (videti dalje tekst)

Graph 1. Comparative outcomes and efficiency of different JAK inhibitors in clinical trials (spleen volume reduction, SVR $\geq 35\%$) (related to following text)



Grafikon 2. Uporedni rezultati i efikasnost različitih JAKi u kliničkim studijama (stepen redukcije ukupnog simptom skora, TSS $\geq 50\%$) (videti dalje tekst)

Graph 2. Comparative outcomes and efficiency of different JAK inhibitors in clinical trials (degree of symptom reduction as Total Symptom Score reduction, TSS $\geq 50\%$) (related to further text)

RUKSOLITINIB (JAKAVI®)

Prvi JAKi koji je ušao u redovnu kliničku primenu je ruxolitinib koji je registrovan 2011. godine u SAD i 2012. godine u Evropi za lečenje PMF u uznapredovanoj fazi bolesti. Njegovi rezultati kroz dve kliničke studije, COMFORT-I (ruxolitinib u odnosu na placebo) [18] i COMFORT-II [19] (ruxolitinib u odnosu na najbolju dostupnu terapiju izuzev JAKi) pokazali su da ovaj lek kod značajnog broja bolesnika postiže kontrolu bolesti u smislu smanjivanja i uklanjanja simptoma koji su posledica povećane inflamacije i smanjivanja veličine odnosno volumena slezine. U COMFORT-I studiji se kod 45,9% bolesnika smanjuju simptomi za više od 50% (mereni specifičnim upitnicima, tzv. *total symptom score*, odnosno TSS $\geq 50\%$). Takođe, postiže se i kontrola bolesti u smislu smanjenja veličine slezine (smanjenje volumena slezine za $\geq 35\%$, SVS $\geq 35\%$, mereno NMR ili CT pregledom) kod 41,9% u COMFORT-I i kod 28% u COMFORT-II studiji. Ovi rezultati su postignuti već u prvih šest meseci primene terapije i neznatno su se popravljali u daljem lečenju. Time je primena ruxolitiniba postala zlatni standard u lečenju bolesnika sa PMF omogućavajući im značajno bolji kvalitet života, a za one koji su imali povoljan odgovor i produženje

RUXOLITINIB (JAKAVI®)

Ruxolitinib, the first JAK inhibitor to enter regular clinical use, was approved in the USA in 2011 and in Europe in 2012 for the treatment of advanced-stage PMF. Results from two clinical studies, COMFORT-I (ruxolitinib vs. placebo) [18] and COMFORT-II [19] (ruxolitinib vs. best available therapy excluding JAK inhibitors), demonstrated that ruxolitinib effectively controls the disease in a significant number of patients by alleviating symptoms associated with increased inflammation and reducing spleen size or volume. In the COMFORT-I study, 45.9% of patients experienced a reduction in symptoms by more than 50%, as measured by the Total Symptom Score (TSS $\geq 50\%$) using specific questionnaires. Additionally, disease control in terms of spleen size reduction (spleen volume reduction of $\geq 35\%$, SVS $\geq 35\%$, measured by NMR or CT) was achieved in 41.9% of patients in COMFORT-I and in 28% in COMFORT-II study. These results were achieved within the first six months of therapy and showed slight improvement with continued treatment. Therefore, ruxolitinib has become the gold standard for treating patients with PMF, significantly improving their quality of life. For those who responded favorably, it also extended

ukupnog preživljavanja (sa 3,8 godina za bolesnike na placebo ili najboljoj terapiji na 5,8 godine kod bolesnika lečenih ruksolitinitibom [20]).

Nakon uspeha ove dve studije, sprovedeno je otvoreno istraživanje u realnoj kliničkoj praksi (JUMP studija) [21,22] koje je uključilo 1546 bolesnika sa primarnom mijelofibrozom nezavisno od incijalnog broja trombocita (broj $Tr < 100 \times 10^9/L$ je bio faktor isključenja bolesnika u obe COMFORT studije). U prvih 6 meseci 56,5% bolesnika je redukovalo veličinu slezine za $\geq 50\%$ palpabilne dužine a do kraja prve godine je to postignuto kod 66,5% bolesnika. Kod većine bolesnika nije određivan volumen slezine. Takođe, kod 50% bolesnika je postignuto smanjenje simptoma bolesti merenih sa nekoliko specifičnih upitnika. To je sve pokazalo da je ruksolitinitib vrlo efikasan lek u pogledu smanjenja tegoba i simptoma bolesnika kao i u smanjenju veličine slezine koja predstavlja najveći terapijski problem ovim bolesnicima.

U Srbiji ruksolitinitib je registrovan 2013. godine, međutim, na listu lekova je stavljen tek 2016. godine sa dosta ograničenim indikacijama preko centralne Komisije RFZO. Pre stavljanja na listu, svega nekoliko bolesnika je lečeno kroz program direktne podrške kompanije Novartis. Poslednja ukupna analiza naših rezultata urađena je 2022. godine i obuhvatila je 155 bolesnika, od toga 129 bolesnika kod kojih je lečenje trajalo najmanje 6 meseci (do prve procene) [23]. Evaluacija bolesnika je obuhvatila procenu smanjivanja opštih simptoma kao i procenu smanjivanja veličine slezine (mereno EHO ili CT pregledom, pri čemu nije bio računat volumen, već smanjenje ukupnog prečnika). Naši rezultati su pokazali da je gubitak opštih simptoma bio izražen (na 6 meseci 111/129 i na 12 meseci 84/90 bolesnika). Smanjenje slezine je uočeno u 91/125 (72,8% bolesnika) u prvih 6 meseci i u 67/90 (74,4%) bolesnika do kraja prve godine. Polovina bolesnika, 29 od 59 evaluiranih bolesnika (49%), je do kraja druge godine smanjila maksimalnu dužinu slezine za $\geq 25\%$, mereno EHO ili CT pregledom.

Ruksolitinitib je lek koji se dosta dobro podnosi, i najveći deo neželjenih reakcija je posledica blokade JAK/STAT puta vezanog za hematopoezu, pa često dolazi do nastanka značajne anemije i trombocitopenije što zahteva korekciju doze leka, a što, opet, često smanjuje ukupne rezultate lečenja. Pored toga, bolesnici na ruksolitinitibu takođe imaju globalno smanjen imuni odgovor, pa su kod njih češće respiratorne i druge infekcije, infekcije herpes virusima, ali su opisani i retki slučajevi aktivacije tuberkuloze [2,21].

Neuspeh terapije ruksolitinitibom kao i gubitak odgovora na ruksolitinitib otvorio je pitanje druge linije primene JAKi. U sklopu toga, sprovedeno je nekoliko

overall survival from 3.8 years for patients on placebo or the best available therapy to 5.8 years for those treated with ruxolitinib [20]).

Following the success of these two studies, an open-label study was conducted in real-world clinical practice (the JUMP study) [21,22] involving 1,546 patients with primary myelofibrosis, regardless of their initial platelet count. A platelet count of $< 100 \times 10^9/L$ was an exclusion criterion in both COMFORT studies. Within the first six months, 56.5% of patients experienced a $\geq 50\%$ reduction in palpable spleen length, and by the end of the first year, this was achieved in 66.5% of patients. In most patients, the spleen volume was not determined. Additionally, 50% of patients experienced a reduction in disease symptoms, as measured by several specific questionnaires. These findings demonstrate that ruxolitinib is highly effective in alleviating patient symptoms and reducing spleen size, which is one of the most challenging therapeutic issues for these patients.

In Serbia, ruxolitinib was registered in 2013 but was included on the list of reimbursed medications in 2016, with very limited indications, by the Central Commission of the Republic Health Insurance Fund. Before being listed, only a few patients were treated through Novartis' direct support program. The most recent overall analysis of our results, conducted in 2022, included 155 patients, of whom 129 had been treated for at least six months (until the first assessment) [23]. The patient evaluation included assessing the reduction of general symptoms and measuring the reduction in spleen size, using ultrasound or CT scans. While the volume was not calculated, the reduction in total spleen diameter was assessed. Our results demonstrated a significant reduction in general symptoms, with 111 out of 129 patients showing improvement at six months and 84 out of 90 patients at twelve months. Spleen reduction was observed in 91 out of 125 patients (72.8%) within the first six months and in 67 out of 90 patients (74.4%) by the end of the first year. By the end of the second year, 29 out of 59 evaluated patients (49%) had reduced the maximum length of their spleen by $\geq 25\%$, as measured by ultrasound or CT scans.

Ruxolitinib is generally well tolerated, but most adverse reactions stem from its inhibition of the JAK/STAT pathway involved in hematopoiesis. This often leads to significant anemia and thrombocytopenia, which may necessitate dose adjustments and can consequently impact overall treatment efficacy. Additionally, patients on ruxolitinib may experience a generally reduced immune response, leading to a higher frequency of respiratory and other infections, including herpesvirus infections. Although rare, cases of tuberculosis activation have also been reported [2,21].

kliničkih studija sa drugim preparatima i prvi koji je u tu arenu ušao jeste fedratinib koji se pokazao efikasnim i kod bolesnika koji su prethodno lečeni ruksolitinibom. Za njim su sprovedena klinička ispitivanja sa momelotinibom (posebno kod bolesnika sa anemijom), kao i sa pakritinibom (kod bolesnika sa trombocitopenijom).

FEDRATINIB (INREBIC®)

Fedratinib je u kliničku praksu ušao 2019. odnosno 2020. godine posle detaljnog praćenja bolesnika u dve kliničke studije, gde se kod retkih bolesnika ispoljio fenomen Vernikeove encefalopatije usled nedostatka vitamina B1, što se kod tih retkih slučajeva dovodilo u vezu sa primenom fedratiniba (*black box* upozorenje) [24]. Međutim, ono što je bitno jeste da je fedratinib u JAKARTA studiji pokazao značajnu efikasnost u odnosu na placebo u pogledu smanjivanja sistemskih tegoba kao i veličine slezine (na kraju šestog terapijskog ciklusa, odnosno posle 6 meseci terapije, kod 47% bolesnika je značajno redukovano volumen slezine, dok je smanjenje simptoma bilo registrovano kod 40% bolesnika [25].

Kod bolesnika prethodno lečenih ruksolitinibom, postignut je takođe vrlo dobar sekundarni odgovor na terapiju jer je kod njih 55% postignuta ponovna redukcija slezine na $\geq 35\%$, mereno NMR ili CT pregledom, kao i dobra kontrola simptoma (26% bolesnika je imalo smanjenje simptoma za $>50\%$), pri čemu je odgovor bio nešto bolji kod bolesnika koji nisu podnosili prethodnu terapiju ruksolitinibom [26].

Detaljna analiza specifične kohorte bolesnika sa incijalno niskim brojem trombocita u okviru JAKARTA studije pokazala je da se slezinski odgovor značajno ne razlikuje između bolesnika koji su incijalno imali $Tr > 100 \times 10^9/L$ i onih sa manjim brojem Tr, od $50-100 \times 10^9/L$ [27].

Na osnovu ovih rezultata fedratinib je uveden kao efikasan lek u lečenju bolesnika koji ne podnose ili nemaju/gube odgovor na prethodnu terapiju ruksolitinibom, čime se značajno poboljšavaju šanse bolesnika za dugotrajnom kontrolom bolesti.

Slično ruksolitinibu, neželjeni efekti fedratiniba su vezani za osnovni mehanizam delovanja, pa se javljaju anemija i trombocitopenija, nešto ređe infekcije, ali i već pomenut deficit vitamina B1 što se uspešno rešava primenom nadoknade [27].

MOMELOTINIB (OMJJARA®)

Momelotinib je najnoviji odobreni JAKi koji je ušao u kliničku primenu 2023. u SAD i 2024. godine u Evropi. Za razliku od ruksolitiniba, i fedratiniba, momelotinib deluje i na aktivin A receptor tipa I koji ima značajnu ulogu u modulaciji delovanja hepcidina čime se omogućava bolja dostupnost gvožđa iz rezervi za aktivnu

The failure of ruxolitinib therapy or loss of response has raised the question of using second-line JAKi. Several clinical studies were conducted to explore alternative treatments, with fedratinib being the first new option to enter the arena. Fedratinib has demonstrated effectiveness even in patients previously treated with ruxolitinib. This was followed by clinical trials of momelotinib, particularly in patients with anemia, and pacritinib, specifically for those with thrombocytopenia.

FEDRATINIB (INREBIC®)

Fedratinib entered clinical practice in 2019 and 2020 following detailed patient monitoring in two clinical studies. In rare cases, the use of fedratinib was associated with Wernicke's encephalopathy due to vitamin B1 deficiency, which has been highlighted in a black box warning [24]. Importantly, the JAKARTA study demonstrated that fedratinib was significantly more effective than placebo in reducing systemic complaints and spleen size. By the end of the sixth treatment cycle (6 months of therapy), 47% of patients had a significant reduction in spleen volume, and 40% experienced a reduction in symptoms [25].

Patients previously treated with ruxolitinib also showed a strong secondary response to fedratinib. Notably, 55% of these patients achieved a $\geq 35\%$ reduction in spleen size, as measured by MRI or CT, and 26% experienced a reduction in symptoms of over 50%. The response was slightly better in patients who had not tolerated previous treatment with ruxolitinib [26].

A detailed analysis within the JAKARTA study of a cohort with initially low platelet counts revealed that the splenic response did not significantly differ between patients with an initial platelet count $> 100 \times 10^9/L$ and those with counts between $50-100 \times 10^9/L$ [27].

Based on these results, fedratinib was introduced as an effective treatment option for patients who cannot tolerate or have not responded to previous ruxolitinib therapy. It significantly enhances the likelihood of achieving long-term disease control.

Like ruxolitinib, fedratinib's side effects are linked to its mechanism of action, including anemia and thrombocytopenia. Infections occur somewhat less frequently, but there is also a risk of vitamin B1 deficiency, which can be effectively managed with supplementation [27].

MOMELOTINIB (OMJJARA®)

Momelotinib is the newest JAK inhibitor, having been approved for clinical use in the US in 2023 and in Europe in 2024. Unlike ruxolitinib and fedratinib, momelotinib also targets the activin A type I receptor, which plays a crucial role in modulating hepcidin activity. This

eritropoezu, a time i smanjuje težina anemije kod bolesnika sa PMF [28,29]. Upravo je zbog toga momelotinib preporučen za primenu kod bolesnika sa PMF koji imaju izraženu anemiju u sklopu bolesti [28]. Dve kliničke studije sa momelotinibom, SIMPLIFY-1 i 2 bile su slične po osnovnom dizajnu, sa time da je SIMPLIFY-1 [30] bila studija poređenja momelotiniba sa ruksolitinibom (po dizajnu *non-inferiority trial*) gde je pokazano da momelotinib nije inferioran u odnosu na standardnu terapiju, ruksolitinib. Ukupan odgovor u prvih 6 meseci u smislu stope značajne redukcije volumena slezine jeste ($SVS \geq 35\%$) od 26,5%, što je komparabilno sa 29% u grupi bolesnika lečenih ruksolitinibom. Smanjenje simptoma kroz redukciju simptom skora ($TSS < 50\%$) takođe je bilo uporedivo, mada je procenat bolesnika lečenih ruksolitinibom sa postignutim $TSS < 50\%$ bio veći, 42,2% u odnosu na broj bolesnika lečenih momelotinibom, gde je 28,4% smanjilo simptome za $> 50\%$. Međutim, ova razlika nije bila značajna, odnosno momelotinib nije bio značajno inferioran i u tom pogledu. Druga studija sa momelotinibom, SIMPLIFY-2 [29,31] je kao kontrolnu grupu koristila ne samo ruksolitinib već je dozvoljavala primenu najbolje dostupne terapije (BAT, *best available therapy*) koja je uključivala i ruksolitinib kod bolesnika koji nisu postigli optimalni odgovor na prethodno lečenje ruksolitinibom. Rezultati ove studije pokazali su da primena momelotiniba nije značajnije uticala na značajno smanjenje volumena slezine, ali je značajno uticala na smanjenje ukupnog simptom skora (43% prema 21%). Obe ove studije su takođe pokazale da značajan broj bolesnika tokom lečenja momelotinibom postaje nezavistan od transfuzija, pa se u grupi lečenih momelotinibom to dogodilo kod 66% bolesnika u odnosu na 49% lečenih ruksolitinibom (SIMPLIFY-1). U drugoj studiji, 43% bolesnika lečenih momelotinibom postiglo je transfuzionu nezavisnost, u odnosu na 21% bolesnika na najboljoj dostupnoj terapiji (SIMPLIFY-2). Shodno tome, sprovedena je i studija koja je uporedila momelotinib sa primenom androgena (danazol) kod bolesnika sa PMF koji su prethodno lečeni primenom JAKi, a koji su razvili značajnu anemiju (MOMENTUM). Ta studija je pokazala da momelotinib smanjuje transfuzionu zavisnost do kraja incijalnih 6 meseci lečenja (31% prema 20% za bolesnike lečene danazolom) [2,29].

Kao i kod drugih JAKi, pri primeni momelotiniba može se javiti prolazno pogoršanje anemije kao i pojava trombocitopenije, o čemu se mora voditi računa tokom lečenja uz titraciju doze leka.

PAKRITINIB (VONJO®)

Pakritinib je poslednji odobreni JAKi. Dostupan je trenutno u SAD gde je odobren kao *orphan* lek 2022.

action enhances the availability of iron from reserves for active erythropoiesis, thereby reducing the severity of anemia in patients with PMF [28,29]. This is why momelotinib is specifically recommended for patients with PMF who have significant anemia as part of their condition [28]. The two clinical trials with momelotinib, SIMPLIFY-1 and SIMPLIFY-2, shared a similar basic design. SIMPLIFY-1 [30] was a non-inferiority trial comparing momelotinib to ruxolitinib, demonstrating that momelotinib was non-inferior to the standard therapy, ruxolitinib. Over the first six months, the overall response rate for significant reduction in spleen volume ($SVR \geq 35\%$) was 26.5% with momelotinib, which is comparable to 29% in the group treated with ruxolitinib. Reduction in symptoms, as measured by a Total Symptom Score ($TSS < 50\%$), was also comparable. However, a higher percentage of patients treated with ruxolitinib achieved $TSS < 50\%$ (42.2%) compared to those treated with momelotinib, where 28.4% experienced a reduction in symptoms by more than 50%. However, this difference was not significant, indicating that momelotinib was not significantly inferior in this regard either. Another study with momelotinib, SIMPLIFY-2 [29,31] compared it not only to ruxolitinib but also allowed for the use of best available therapy (BAT), which could include ruxolitinib for patients who did not achieve an optimal response to their previous treatment. The results of this study indicated that momelotinib did not significantly impact changes in spleen volume but did significantly improve the reduction of the total symptom score, with 43% of patients achieving this outcome compared to 21% in the control group. Both studies also demonstrated that a significant number of patients on momelotinib became transfusion-independent. Specifically, 66% of patients treated with momelotinib achieved this outcome, compared to 49% in the ruxolitinib group (SIMPLIFY-1). In another study, 43% of patients treated with momelotinib achieved transfusion independence, compared to 21% of patients receiving the best available therapy (SIMPLIFY-2). Consequently, a study was conducted to compare momelotinib with the use of the androgen danazol in patients with PMF who had previously been treated with JAK inhibitors and developed significant anemia (MOMENTUM). The study demonstrated that momelotinib reduced transfusion dependence by the end of the initial six months of treatment, with 31% of patients achieving this outcome compared to 20% in the danazol group [2,29].

Like other JAK inhibitors, momelotinib may cause transient worsening of anemia and thrombocytopenia, which should be considered when adjusting the drug dose during treatment.

Godine, ali za sada nije odobren u Evropi. Nekoliko kliničkih ispitivanja je trenutno u toku i ona treba da pokažu realnu vrednost ovog JAKi u kliničkoj praksi, posebno kod bolesnika sa niskim brojem trombocita kod kojih je pakritinib u prethodnim studijama pokazao dobre rezultate. U odnosu na ruksolitinib i fedratinib gde je broj trombocita limitirajući faktor za uvođenje leka i postizanje optimalnog odgovora, pakritinib je u inicijalnoj studiji PERSIST-1 [32] pokazao značajan efekat u smislu smanjenja volumena slezine u odnosu na najbolju dostupnu terapiju (ali bez JAKi) gde je 19% bolesnika postiglo SVS $\geq$ 35% u odnosu na samo 5% u kontrolnoj grupi. Ovi rezultati su dalje ispitivani u studiji PERSIST-2 [33] u koju su uključeni bolesnici sa značajnom trombocitopenijom ($<100 \times 10^9/L$) gde je kontrolna grupa obuhvatala različite terapije, a u najvećoj meri redukovane doze ruksolitiniba (45%). U ovoj studiji pakritinib je pokazao značajno smanjenje kako volumena slezine (SVS $\geq$ 35% 18% prema 3%) tako i ukupnog skora simptoma (kod 25% prema 14% bolesnika).

Rezultati novih studija pokazaće pravu realnu vrednost pakritiniba kod bolesnika sa izraženom trombocitopenijom u PMF.

Kao i drugi JAKi pakritinib može da dovede do anemije, ali su takođe zbog efekta na inhibiciju AVRI receptora dugoročni efekti na bolesnike sa anemijom manji od ruksolitiniba i fedratiniba.

MOGUĆA SEKVENCA PRIMENE JAKI U LEČENJU BOLESNIKA SA PMF

U ovom trenutku u Evropi je dostupno tri a u SAD četiri JAKi koji se mogu kombinovati prema kliničkim i drugim specifičnim hematološkim odlikama, čime smo posle dužeg perioda prestali da budemo ograničeni na samo jedan lek.

Na grafikonu je prikazana orijentaciona shema redosleda primene i izbora JAK inhibitora u kliničkoj praksi [34].

Međutim, jedan od najvažnijih momenata u lečenju bolesnika sa PMF kao i sekundarnim mijelofibrozama (nastalim transformacijom iz policitemije vere ili esencijalne trombocitemije) je adekvatan tajming i pravovremeno uvođenje terapije. Analize podgrupa bolesnika unutar sprovedenih studija [35] kao i primena leka u realnom kliničkom okruženju je pokazali su da su rezultati uspešniji kada se JAKi, prvenstveno za sada ruksolitinib, uvedu ranije u toku bolesti kada bolesnik počinje da ispoljava simptome i tegobe čak i kada njegovi faktori rizika nisu previše ispoljeni (bolesnici u umerenoj/intermediate-1 fazi). Time se omogućava uvođenje leka u situacijama sa boljim bazalnim parametrima krvne slike a isto tako se i omogućuje bolje

PACRITINIB (VONJO®)

Pacritinib is the newest approved JAK inhibitor. It was granted orphan drug status and approved for use in the US in 2022, but it has not been approved in Europe yet. Several clinical trials are currently underway to assess the real-world value of this JAK inhibitor, particularly in patients with low platelet counts, where pacritinib has previously shown promising results. Unlike ruxolitinib and fedratinib, where platelet counts can limit drug use and response, the initial PERSIST-1 study [32] demonstrated that pacritinib had a significant effect on reducing spleen volume compared to the best available therapy (excluding JAK inhibitors). Specifically, 19% of patients treated with pacritinib achieved a spleen volume reduction of $\geq 35\%$ (SVR $\geq 35\%$), compared to just 5% in the control group. These results were further evaluated in the PERSIST-2 study, which included patients with significant thrombocytopenia ($<100 \times 10^9/L$). The control group consisted of various therapies, with a substantial proportion receiving reduced doses of ruxolitinib (45%). In this study, pacritinib demonstrated a significant reduction in both spleen volume (SVR $\geq 35\%$ in 18% of patients compared to 3% in the control group) and overall symptom score (25% of patients achieved a reduction versus 14% in the control group).

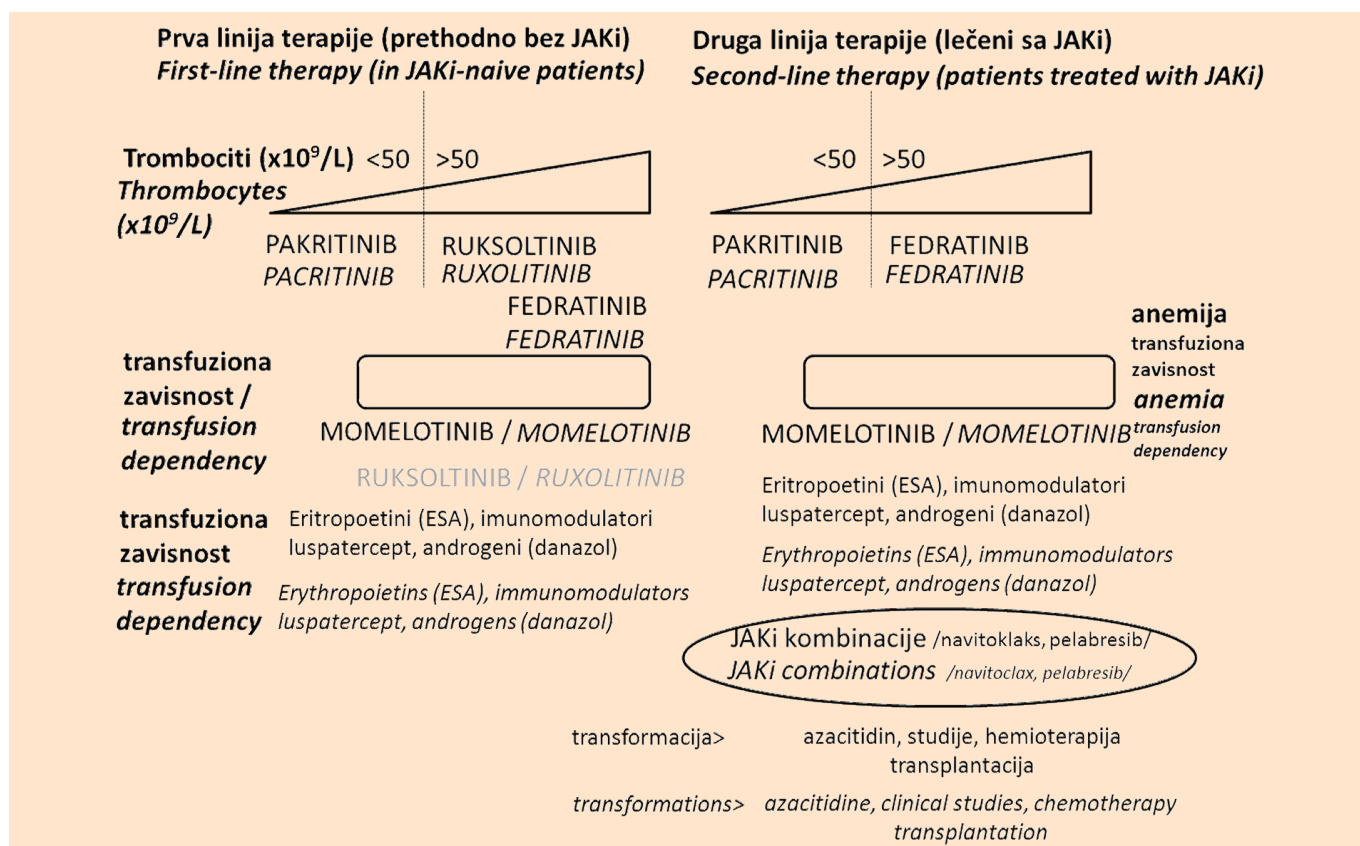
Future studies will reveal the true clinical value of pacritinib for patients with marked thrombocytopenia in PMF.

Like other JAK inhibitors, pacritinib can cause anemia. However, due to its effect on the AVRI receptor, it tends to have less severe long-term effects on anemia compared to ruxolitinib and fedratinib.

POTENTIAL SEQUENCE OF JAK INHIBITOR USE IN THE TREATMENT OF PATIENTS WITH PMF

Currently, three JAK inhibitors are available in Europe and four in the USA. This allows for tailored treatment based on clinical and specific hematological features, ending the long period of reliance on a single drug. The chart provides a guide for the sequence and selection of JAK inhibitors in clinical practice [34].

One of the most crucial aspects of treating patients with primary myelofibrosis (PMF) and secondary myelofibrosis (resulting from transformation of polycythemia vera or essential thrombocythemia) is the appropriate timing and prompt initiation of therapy. Analysis of patient subgroups from conducted studies [35], along with real-world clinical application, indicates that outcomes are more favorable when JAK inhibitors, particularly ruxolitinib, are introduced earlier in the disease course—when symptoms and complaints first appear, even if risk factors are not yet severe (patients in the moderate/intermediate-1 phase). This approach al-



Grafikon 2. Moguća primena odobrenih JAKi prema linijama terapije i kliničkim odlikama bolesnika u lečenju PMF (modifikovano prema Venugopal i sar. [34])

Graph 2. Potential therapy with approved JAK inhibitors based on lines of treatment and clinical features in PMF, modified from Venugopal et al. [34]

i efikasnije delovanje JAKi [22,35]. Za sada nije bilo kliničkih studija sa primenom JAKi u vrlo ranim fazama PMF, dok su rezultati primene ruxolitiniba kod bolesnika sa kasnom fazom policitemije vere pokazali da oni imaju dobar potencijal za kontrolu bolesti pa čak i za smanjivanje stope progresije bolesti u manifestnu fibrotičnu fazu, pa je ruxolitinib i odobren za lečenje bolesnika sa PV u fazi rezistencije na hidroksiureu, kako u SAD tako i u Evropi (EMA).

Kako je mehanizam kliničkih manifestacija u PMF vrlo kompleksan, te kako su često oni međusobno isprepleteni, postoji potreba za kombinovanjem lekova koji deluju na različite molekularne sisteme unutar ćelija hematopoeze kao i na ćelije strome i njihove interakcije. Potencijal kombinovanja različitih agenasa je veoma veliki, od malih molekula kao što su signalni inhibitori drugih unutarćelijskih puteva, inhibitori apoptoze, inhibitori telomerase, do monoklonskih antitela usmerenih na neovaskularizaciju i angiogene i profibrotične faktore. Imajući u vidu da je to predmet brojnih studija od kojih su mnoge u toku, osvrnućemo se samo na dva nova leka koja su do sada u kombinaciji pokazala značajne efekte na smanjenje volumena slezine kao glavnog kliničkog merila efikasnosti lekova za mijelofibrozu [36].

lows for the initiation of treatment with more favorable baseline blood parameters, which can enhance the effectiveness and efficiency of JAKi [22,35]. To date, no clinical studies have investigated the use of JAK inhibitors in the very early stages of PMF. However, results from studies of ruxolitinib in patients with late-stage polycythemia vera have demonstrated its potential for controlling the disease and slowing its progression to the manifest fibrotic phase. Consequently, ruxolitinib is approved for use in PV patients who are resistant to hydroxyurea, both in the USA and in Europe (EMA).

Given the complexity and interrelated nature of clinical manifestations in PMF, there is a need to combine therapies that target different molecular pathways within hematopoietic cells, as well as those affecting stromal cells and their interactions. The potential for combining different agents is substantial, ranging from small molecules like signaling inhibitors targeting various intracellular pathways and apoptosis inhibitors to telomerase inhibitors, as well as monoclonal antibodies that target neovascularization, angiogenic factors, and profibrotic factors. Given that this topic is the subject of numerous ongoing studies, we will focus on just two new drugs that have demonstrated significant effects in reducing spleen volume, which is the primary clinical measure of drug efficacy for myelofibrosis [36].

NAVITOKLAKS

Navitoklaks je aktivator proapoptotskih sistema unutar ćelije koji se zasniva na inhibiciji delovanja antiapoptotskih gena kao što su BCL-2 ali i BCL-XL koji je posebno aktivan kod bolesnika sa mijeloproliferativnim neoplazmama kao što je PMF [37]. Ovaj lek je još od 2007. godine u kliničkom razvoju i sprovedene su brojne studije čiji su dosadašnji rezultati pokazali efikasnost kako samog leka tako i leka u kombinaciji sa drugim citotoksičnim lekovima i signalnim inhibitorima. U PMF, navitoklaks se ispituje u nekoliko studija od kojih su dostupni rani rezultati REFINE i TRANSFORM-1 studije. REFINE studija je bila prva studija koja je primenjivala navitoklaks kod bolesnika koji prethodno nisu imali odgovor na ruxolitinib ili su isti izgubili, (*add on* studija) i dodavanje navitoklaksa je omogućilo da 26,5% bolesnika značajno redukuje volumen slezine kao i da se kod 30% bolesnika postigne značajno smanjenje simptoma merenih kroz specifični uputnik (TSS<50%) [37,38].

Naredna, TRANSFORM-1 studija koja je u toku pokazala je dosta impresivne rezultate koji su prikazani na sastanku Američkog udruženja (ASH) 2023. godine i Evropskog udruženja hematologa 2024. godine [39]. Ova studija je zamišljena kao dvostruko slepa, placebo kontrolisana studija u dve kohorte, pri čemu su svi bolesnici lečeni ruxolitinibom uz dodatak navitoklaksa, odnosno placeba. Preliminarni rezultati pokazali su da se u grupi bolesnika lečenih kombinacijom postiže značajno smanjene volumena slezine gde je 63,2% bolesnika postiglo cilj (SVS $\geq 35\%$) za razliku od 31,5% bolesnika u grupi koja je primala ruxolitinib sa placebo. Rezultati smanjenja TSS skora bili su manje impresivni jer je značajan broj bolesnika imao smanjenje simptoma u celini, a smanjenje TSS<50% je postignuto u približno istoj meri u obe grupe bolesnika, 39,2% u grupi sa navitoklaksom i ruxolitinibom prema 41,7% u grupi na ruxolitinibu sa placebo, što je definitivno neposredni efekat ruxolitiniba na JAK/STAT puteve [37-39].

Jedan od najvećih problema primene navitoklaksa je nastanak trombocitopenije i neophodnost precizne titracije terapije ali u dosadašnjem iskustvu kroz REFINE i TRANSFORM-1 i 2 studiju nije pokazana značajna učestalost krvarenja kao i potreba da se bolesnici isključe zbog izražene trombocitopenije [37].

PELABRESIB

Drugi lek koji obećava je pelabresib, specifični inhibitor bromodomena i ekstra-terminalnih (BET) domena proteina koji bi mogli da nishodno regulišu brojne gene uključene u onkogenezu. Pelabresib je u fazi kliničkog razvoja i u toku su različite studije sa brojnim malignim

NAVITOCCLAX

Navitoclax activates pro-apoptotic pathways by inhibiting anti-apoptotic proteins such as BCL-2 and BCL-XL. This mechanism is particularly effective in patients with myeloproliferative neoplasms, such as PMF [37]. The drug has been under clinical development since 2007, with numerous studies demonstrating its effectiveness both as a standalone treatment and in combination with other cytotoxic drugs and signaling inhibitors. In PMF, navitoclax is being investigated in several studies, with early results available from the REFINE and TRANSFORM-1 trials. The REFINE study was the first to evaluate navitoclax in patients who had either not responded to ruxolitinib or had lost their response (*add-on* study). The addition of navitoclax led to a significant reduction in spleen volume in 26.5% of patients and a notable decrease in symptoms, as measured by a Total Symptom Score (TSS<50%) in 30% of patients [37,38].

The ongoing TRANSFORM-1 study has demonstrated promising results, which were presented at the 2023 American Society of Hematology (ASH) meeting and the 2024 European Hematology Association (EHA) meeting [39]. The study was designed as a double-blind, placebo-controlled trial with two cohorts, where all patients were treated with ruxolitinib, either with the addition of navitoclax or a placebo. Preliminary results indicated that the combination therapy led to a significant reduction in spleen volume, with 63.2% of patients achieving the target (SVR $\geq 35\%$) compared to 31.5% in the ruxolitinib plus placebo group. The reduction in Total Symptom Score (TSS) was less striking. A similar proportion of patients achieved a TSS reduction to below 50% in both groups: 39.2% in the navitoclax plus ruxolitinib group and 41.7% in the ruxolitinib plus placebo group. This indicates that the observed symptom improvement is likely a direct effect of ruxolitinib on the JAK/STAT pathways [37-39].

One of the major challenges with navitoclax is the risk of thrombocytopenia, necessitating careful dose titration. However, based on experience from the REFINE, TRANSFORM-1, and TRANSFORM-2 studies, there has been no significant increase in bleeding incidents or a need to exclude patients due to severe thrombocytopenia [37].

PELABRESIB

Another promising drug is pelabresib, a specific inhibitor of bromodomain and extra-terminal (BET) proteins. By targeting these proteins, pelabresib has the potential to downregulate multiple genes involved in oncogenesis. Pelabresib is currently in clinical development, with ongoing studies across various malignancies. Specifically, two studies, MANIFEST [40] and MANIFEST-2 [40,41],

bolestima, a u mijeloproliferativnim neoplazama sprovedene su dve studije MANIFEST [40] i MANIFEST-2 [40,41], pri čemu je studija MANIFEST-2 bila dvostruko slepa, placebo kontrolisana studija kombinacije ruxolitiniba i pelabresiba, odnosno placeba. Studija MANIFEST-2 je pokazala da dodatak pelabresiba popravljajući ukupni odgovor većine bolesnika na ruxolitinib, jer je SVS $\geq$ 35% postignut kod 65,9% bolesnika u eksperimentalnoj grupi u odnosu na 35,2% bolesnika lečenih ruxolitinibom i placebom [41]. Takođe značajno smanjenje ukupnih simptoma je bilo znatno više u slučaju kombinacije pelabresiba i ruxolitiniba. TSS $<$ 50% je postignut kod 52,3% ispitanika na kombinaciji u odnosu na 46,3% u grupi na ruxolitinibu sa placebom. Ova studija je pokazala da je moguće postići dalju optimizaciju i poboljšanje incijalnog odgovora bolesnika na ruxolitinib čime se otvara mogućnost i za brojne druge kombinacije usmerene na poboljšanje ukupnog terapijskog ishoda bolesnika sa primarnom mijelofibrozo. Kao i drugi JAKi i pelabresib može dovesti do pogoršanja anemije kao i do pojave trombocitopenije, ali najveći broj bolesnika je vrlo dobro podneo ovu kombinovanu terapiju [40].

ZAKLJUČAK

Današnje terapijske mogućnosti u lečenju bolesnika sa mijelofibrozo obećavaju značajno poboljšanje opšteg kvaliteta života, smanjenje simptoma i smanjenje slezine i time vrlo verovatno i produženje života većine bolesnika, posebno ako se terapija uvede na vreme. Razvojem više JAKi dobili smo mogućnost boljeg terapijskog izbora u specifičnim kliničkim situacijama kao što su bolesnici sa citopeničnom mijelofibrozo ili sa izraženom transfuziono zavisnom anemijom čiji je dosadašnji kvalitet života bio neadekvatan. Pored toga, nova saznanja o biologiji bolesti, patofiziološkim i genetskim mehanizmima omogućiću u budućnosti nalaženje i drugih terapijskih ciljeva osim inhibicije JAK/STAT puta, čime će verovatno kombinovana terapija u narednim godinama postati osnova lečenja bolesnika i u prvoj terapijskoj liniji [42].

Nadamo se da će u Srbiji uskoro biti dostupan barem još jedan od registrovanih JAK inhibitora čime bi naši bolesnici imali dodatne terapijske mogućnosti pored učešća u kliničkim studijama čija je dostupnost sporadična.

Sukob interesa: Nije prijavljen.

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have been conducted in myeloproliferative neoplasms. MANIFEST-2 is a double-blind, placebo-controlled trial evaluating the combination of ruxolitinib and pelabresib versus ruxolitinib with placebo. The MANIFEST-2 study demonstrated that adding pelabresib to ruxolitinib significantly enhances the overall response in patients. In the experimental group, 65.9% of patients achieved a spleen volume reduction of $\geq$ 35% (SVR $\geq$ 35%), compared to 35.2% in the ruxolitinib plus placebo group [41]. Additionally, the reduction in overall symptoms was significantly greater with the combination of pelabresib and ruxolitinib. TSS reduction to below 50% was achieved in 52.3% of patients receiving the combination of pelabresib and ruxolitinib, compared to 46.3% in the ruxolitinib plus placebo group. This study showed that it is possible to achieve further optimization and improvement of the patient's initial response to ruxolitinib, which opens the possibility for numerous other combinations aimed at improving the overall therapeutic outcome of patients with primary myelofibrosis. Similar to other JAK inhibitors, pelabresib can cause worsening of anemia and thrombocytopenia. However, the majority of patients tolerated the combination therapy well [40].

CONCLUSION

Current therapeutic options for myelofibrosis offer the potential for significant improvements in overall quality of life, symptom reduction, and spleen size reduction. Timely initiation of therapy is likely to enhance these benefits and potentially extend the lifespan of many patients. The development of additional JAK inhibitors has expanded our therapeutic options, allowing for more tailored treatments in specific clinical scenarios, such as patients with cytopenic myelofibrosis or severe transfusion-dependent anemia. This advancement offers the potential to significantly improve the previously inadequate quality of life for these patients. Moreover, advancing our understanding of the disease's biology, pathophysiological, and genetic mechanisms is expected to reveal new therapeutic targets beyond the JAK/STAT pathway. This progress will likely lead to the adoption of combination therapies as the standard approach for first-line treatment in the coming years [42].

We hope that at least one more of the registered JAK inhibitors will soon be available in Serbia, providing our patients with additional therapeutic options beyond the sporadic availability of clinical studies.

Conflict of interest: None declared.

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KLINIČKE MANIFESTACIJE UDRUŽENE SA PRISUSTVOM ANTIFOSFOLIPIDNIH ANTITELA

PREGLEDNI RAD

REVIEW ARTICLE

CLINICAL MANIFESTATIONS ASSOCIATED WITH THE PRESENCE OF ANTIPHOSPHOLIPID ANTIBODIES

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SAŽETAK

Antifosfolipidna antitela (aPL antitela; engl. *antiphospholipid antibodies*) su heterogena grupa autoantitela koja su usmerena na anjonske fosfolipide ili proteine koji se vezuju za fosfolipide. Mogu biti udružena sa brojnim kliničkim manifestacijama u gotovo svim oblastima medicine, ali je antifosfolipidni sindrom (engl. *antiphospholipid syndrome* – APS) najpreciznije definisani entitet koji se karakteriše prisustvom ovih antitela. Najčešće kliničke manifestacije prisustva aPL antitela su tromboze, u bilo kom delu cirkulacije, kao i komplikacije trudnoće, u formi spontanog gubitka ploda ili prevremenog porođaja usled preeklampsije, eklampsije ili placentalne insuficijencije. Prema modifikovanoj Sapporo klasifikaciji iz 2006. godine, ove manifestacije predstavljaju jedini klinički kriterijum za dijagnozu APS-a. Međutim, kod približno četvrtine bolesnika sa APS-om, prisutne su i druge patološke promene, koje su često udružene sa aPL antitelima, ali koje ne ulaze u zvanične kriterijume za klasifikaciju ove bolesti. Značajno je da ove manifestacije mogu biti udružene sa aPL antitelima i u odsustvu tromboze ili patologije trudnoće, dakle bez zadovoljenih kriterijuma za APS. Prepoznavanje ne-kriterijumskih manifestacija je od velikog značaja pošto bi njihov nalaz trebalo da skrene pažnju na moguće prisustvo aPL antitela i ukaže na postojanje APS-a ili na rizik od njegove pojave. Najnovija klasifikacija, objavljena 2023. godine, od strane Američkog koledža za reumatologiju/Evropske alijanse udruženja za reumatologiju (engl. *American College of Rheumatology/European Alliance of Associations for Rheumatology* – ACR/EULAR), proširila je spisak kliničkih kriterijuma za prepoznavanje antifosfolipidnog sindroma. Ova klasifikacija ima veću specifičnost ali manju senzitivnost u prepoznavanju APS-a u odnosu na ranije kriterijume. Za sada su ACR/EULAR kriterijumi prevashodno namenjeni za odabir bolesnika u kliničkim studijama, a ne za dijagnostikovanje APS-a u svakodnevnoj kliničkoj praksi.

Ključne reči: antifosfolipidna antitela, tromboza, antifosfolipidni sindrom, komplikacije trudnoće, klasifikacija

ABSTRACT

Antiphospholipid antibodies (aPL antibodies) are a heterogeneous group of autoantibodies that target anionic phospholipids or phospholipid-binding proteins. They can be associated with numerous clinical manifestations in almost all areas of clinical medicine, but antiphospholipid syndrome (APS) is the most precisely defined entity. The most common clinical manifestations of aPL are thrombosis in any part of the circulation, as well as pregnancy complications in the form of miscarriage or premature birth due to preeclampsia, eclampsia, or placental insufficiency. According to the modified Sapporo classification of 2006, thrombosis and/or pregnancy complications represent the clinical criteria for diagnosing APS. However, in approximately a quarter of patients with APS, additional clinical manifestations are present, which are not accepted as criteria for APS. Interestingly, these manifestations can be associated with aPL antibodies even in the absence of thrombosis or pregnancy morbidity, i.e., without the presence of the criteria for definitive APS. Recognizing non-criteria manifestations is highly significant because it can draw attention to the possible presence of aPL antibodies and indicate the presence of APS or the risk of its occurrence. The latest classification was published in 2023 by the American College of Rheumatology/European Alliance of Rheumatology Associations (ACR/EULAR). It expanded the list of clinical criteria for the recognition of antiphospholipid syndrome. This classification demonstrates higher specificity but lesser sensitivity in recognizing APS than earlier criteria. At present, the application of the ACR/EULAR criteria is primarily intended for research purposes, i.e., selecting study subjects, rather than for diagnosing APS in everyday clinical practice.

Keywords: antiphospholipid antibodies, thrombosis, antiphospholipid syndrome, pregnancy complications, classification

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UVOD

Poremećaji u regulaciji imunološkog sistema i abnormalna produkcija autoantitela mogu uzrokovati veći broj autoimunih bolesti. Autoantitela koja su usmerena ka specifičnim antigenima obično su udružena sa tipičnom kliničkom slikom kao što je slučaj sa reumatoidnim faktorom kod reumatoidnog artritisa. S druge strane, prisustvo antifosfolipidnih antitela ispoljava se putem brojnih i heterogenih kliničkih manifestacija. Najsnažnija povezanost aPL antitela je dokazana sa pojavom tromboze ili razvojem komplikacija u trudnoći, ali se ova antitela mogu naći i kod čitavog niza drugih oboljenja u čijoj patogenezi ova antitela imaju manje jasnu ulogu [1]. Gotovo da nema oblasti kliničke medicine u kojoj ne postoji neka bolest ili poremećaj koji predstavljaju manifestaciju prisustva aPL antitela. Međutim, i pored uloge u patogenezi nekih od najčešćih bolesti, značaj aPL antitela je u svakodnevnoj praksi prilično potcenjen. Na primer, iako se ova antitela mogu naći kod 10% bolesnika sa infarktom srca, njihovo određivanje često nije deo rutinske prakse čak ni kod mlađih osoba sa trombozom koronarnih arterija [2].

Od posebnog značaja je napomenuti da aPL antitela najčešće predstavljaju perzistentni rizik za pojavu ili progresiju oboljenja sa kojima su udružena. Zbog toga je važno poznavati kliničke manifestacije aPL antitela kako bi se što ranije moglo dijagnostikovati njihovo prisustvo i preduzeti lečenje oboljenja do kojeg dovode.

ANTIFOSFOLIPIDNA ANTITELA

Termin *antifosfolipidna antitela* predstavlja zajednički naziv za heterogenu grupu autoantitela usmerenih ka anjonskim fosfolipidima ili proteinima koji se vezuju za fosfolipide [3]. Mogu biti tranzitorna (prolazna) i perzistentna. Tranzitorna antitela se najčešće javljaju u okviru akutnih virusnih infekcija i iščezavaju u roku od nekoliko nedelja, pri čemu uglavnom ne dovode do kliničkih manifestacija [4]. S druge strane, perzistentna aPL antitela koja su udružena sa kliničkim manifestacijama mogu se javiti kao samostalni (primarni) fenomen ili mogu pratiti autoimune ili neke druge hronične bolesti.

Brojne studije su uverljivo dokumentovale da je nalaz antikardiolipinskih antitela (aCL antitela; engl. *anticardiolipin antibodies*) IgG i IgM klase, anti beta 2 glikoprotein I antitela (anti β 2GPI antitela) IgG i IgM klase i lupusnog antikoagulansa (engl. *lupus anticoagulant* – LAC) značajno povezan sa većim rizikom od nastanka tromboze ili komplikacija u toku trudnoće. Zbog toga je prisustvo bar jednog od ovih antitela neophodan laboratorijski kriterijum za dijagnozu antifosfolipidnog sindroma [5]. Antifosfolipidna antitela mogu biti usmerena i na najmanje 20-tak drugih fosfolipida ili fosfolipid-vezujućih proteina, kao što su fosfatidilserin, protein C,

INTRODUCTION

Disruption in the immune system regulation and abnormal production of autoantibodies can cause many autoimmune diseases. Autoantibodies directed against specific antigens are usually associated with a typical clinical presentation such as the rheumatoid factor in rheumatoid arthritis. On the other hand, the presence of antiphospholipid antibodies is expressed through numerous and heterogeneous clinical manifestations. The strongest association of aPL antibodies has been proven with the occurrence of thrombosis or the development of complications in pregnancy, but these antibodies can also be found in a whole series of other diseases in whose pathogenesis these antibodies have a less clear role [1]. There is almost no area of clinical medicine without any diseases or disorders that are manifestations of the presence of aPL antibodies. However, despite their role in the pathogenesis of some of the most common diseases, the importance of aPL antibodies is rather underestimated in everyday practice. For example, although these antibodies can be found in 10% of heart attack patients, testing for aPL antibodies is often not included in routine practice even in younger patients with coronary artery thrombosis [2].

It is particularly important to note that aPL antibodies most often present a persistent risk for the occurrence or progression of the diseases with which they are associated. Therefore, it is important to understand the clinical manifestations of aPL antibodies in order to diagnose their presence as early as possible and treat the disease they cause.

ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies is an umbrella term for a heterogeneous group of autoantibodies that target anionic phospholipids or phospholipid-binding proteins [3]. They can be transient and persistent. Transient antibodies most often appear in acute viral infections disappearing within several weeks, and they generally do not lead to clinical manifestations [4]. On the other hand, persistent aPL antibodies associated with clinical manifestations may occur as an independent (primary) phenomenon or may accompany autoimmune or other chronic diseases.

Numerous studies have definitively documented that the presence of anticardiolipin antibodies (aCL antibodies) – class IgG and class IgM, anti-beta 2 glycoprotein I antibodies (anti- β 2GPI antibodies) – class IgG and class IgM, and the lupus anticoagulant (LAC) was significantly associated with a higher risk of thrombosis or complications during pregnancy. Therefore, the presence of at least one of these antibodies is a necessary laboratory criterion for the diagnosis of an-

protein S, aneksin II, aneksin V, trombomodulin i drugi. Za ova aPL antitela nije dokazana definitivna povezanost sa kliničkim manifestacijama pa se njihovo određivanje ne preporučuje u redovnoj kliničkoj praksi [5].

Laboratorijska metodologija za dokazivanje antifosfolipidnih antitela obuhvata dve osnovne grupe testova: prisustvo LAC-a se dokazuje koagulacionim metodom dok se aCL antitela i anti β_2 GPI antitela detektuju enzimskim imunosorbentnim esejom (*enzyme-linked immunosorbent assay* – ELISA) ili drugim imunoesejima baziranim na principu vezivanja antitela za kardiolipin ili β_2 GPI u čvrstoj fazi. Ove metode detektuju heterogene populacije aPL antitela koja mogu biti usmerena ka različitim antigenima – u imunoesejima to su kardiolipin ili β_2 GPI, a u slučaju lupusnog antikoagulansa antigeni su fosfolipid-vezujući proteini kao što su β_2 GPI ili protrombin [6]. Novije studije su pokazale da kliničko ispoljavanje prisustva aPL antitela u značajnoj meri zavisi od njihovog profila. Pojava tromboze ili komplikacija trudnoće najsnažnije korelira sa istovremenim postojanjem aCL antitela, anti β_2 GPI antitela i LAC-a (tzv. trostruka pozitivnost) [7]. Pokazano je da antitela usmerena na domen D1 u β_2 GPI molekulu imaju najveći potencijal da dovedu do kliničkih manifestacija antifosfolipidnog sindroma [8].

Prisustvo aPL antitela se može ispoljiti na četiri osnovna načina: kao prisustvo asimptomatskih antitela, kao APS, kao katastrofični APS i putem kliničkih manifestacija koje nisu obuhvaćene kriterijumima za klasifikaciju antifosfolipidnog sindroma.

ASIMPTOMATSKO PRISUSTVO APL ANTITELA

Interesantno je da se prisustvo aPL antitela može dokazati kod približno 1% – 5% osoba u opštoj populaciji, pri čemu učestalost pozitivnog nalaza raste kod starijih osoba, te osoba sa autoimunim bolestima, hroničnim infekcijama i malignitetima [9]. Nije zapažena povezanost između titra i vrste antifosfolipidnih antitela i pojave kliničkih manifestacija kod asimptomatskih osoba [10].

ANTIFOSFOLIPIDNI SINDROM

Antifosfolipidni sindrom je sistemska autoimuna bolest koju karakteriše perzistentno prisustvo aPL antitela sa jedne strane, odnosno dokazana tromboza u bilo kojem delu cirkulatornog sistema i/ili patologija trudnoće, sa druge strane. Prvi, tzv. *Sapporo* kriterijumi za klasifikaciju antifosfolipidnog sindroma publikovani su 1999., a revidirani su 2006. godine (tzv. *Sydney* kriterijumi) [11,5]. Interesantno je da, prema revidiranoj klasifikaciji, brojne kliničke manifestacije za koje se zna da često mogu biti udružene sa prisustvom aPL antitela nisu uvrštene u kriterijume za prepoznavanje definitivnog APS-a. Međutim, klasifikacioni kriterijumi nedavno

tiphosfolipid syndrome [5]. Antiphospholipid antibodies can also target at least 20 other phospholipids or phospholipid-binding proteins, such as phosphatidylserine, protein C, protein S, annexin II, annexin V, thrombomodulin, and other. For these aPL antibodies, no definitive association with clinical manifestations has been proven, therefore it is not recommended to test for them in regular clinical practice [5].

The laboratory methodology for proving antiphospholipid antibodies includes two basic groups of tests: LAC detection is based on functional coagulation assays, while aCL antibodies and anti- β_2 GPI antibodies are detected with the enzyme-linked immunosorbent assay (ELISA) or other immunoassays based on the principle of antibodies binding to cardiolipin or β_2 GPI, in the solid phase. These methods detect heterogeneous populations of aPL antibodies targeting different antigens – in immunoassays, these are cardiolipin or β_2 GPI, and in the case of the lupus anticoagulant, the antigens are phospholipid-binding proteins such as β_2 GPI or prothrombin [6]. Recent studies have shown that the clinical manifestation of the presence of aPL antibodies significantly depends on their profile. The development of thrombosis or pregnancy complications correlates most strongly with the concurrent presence of aCL antibodies, anti β_2 GPI antibodies and LAC (so-called triple positivity) [7]. It has been shown that antibodies targeting the D1 domain in the β_2 GPI molecule have the greatest potential to lead to clinical manifestations of the antiphospholipid syndrome [8].

The presence of aPL antibodies can manifest in four basic ways: as the presence of asymptomatic antibodies, as APS, as catastrophic APS, as well as in the form of clinical manifestations that are not covered by the criteria for the classification of the antiphospholipid syndrome.

ASYMPTOMATIC PRESENCE OF APL ANTIBODIES

Interestingly, the presence of aPL antibodies can be proven in approximately 1% – 5% of people in the general population, with the frequency of positive findings increasing among the elderly, as well as in people with autoimmune diseases, chronic infections, and malignancies [9]. No correlation was observed between the titer and type of antiphospholipid antibodies and the presence of clinical manifestations in asymptomatic individuals [10].

ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome is a systemic autoimmune disease characterized by the persistent presence of aPL antibodies, on the one hand, or proven thrombosis in any part of the circulatory system and/or pregnancy

publikovani od strane ACR/EULAR proširuju spektar kliničkih manifestacija koje se smatraju ispoljavanjem antifosfolipidnog sindroma [12].

Prema ACR/EULAR kriterijumima, boduje se šest kliničkih domena (tromboza vena, tromboza arterija, tromboza u mikrocirkulaciji, obstetričke komplikacije, promene na srčanim zaliscima i hematološke manifestacije) kao i dva laboratorijska domena. U odnosu na modifikovanu Sapporo klasifikaciju u kojoj su svi kriterijumi imali istu težinu, u ACR/EULAR klasifikaciji je izvršeno rangiranje kriterijuma prema važnosti zasnovanoj na specifičnosti za prisustvo APS-a. Tako, na primer, u

pathology, on the other. The first criteria for the classification of the antiphospholipid syndrome, i.e., the Sapporo criteria, were published in 1999. They were revised in 2006 (the Sydney criteria) [11,5]. Interestingly, according to the revised classification, numerous clinical manifestations that are known to be often associated with the presence of aPL antibodies are not included in the criteria for the recognition of definite APS. However, the classification criteria recently published by the ACR/EULAR expand the spectrum of clinical manifestations that are considered to be manifestations of the antiphospholipid syndrome [12].

Tabela 1. Poređenje kriterijuma za antifosfolipidni sindrom po modifikovanoj Sapporo i ACR/EULAR klasifikaciji

Modifikovani Sapporo kriterijumi (2006.)	ACR/EULAR kriterijumi (2023.)
Klinički kriterijumi 1. Tromboze (bilo koji organ ili tkivo) a. Arterija b. Vena c. Mikrocirkulacija 2. Patologija trudnoće a. Tri ili više spontanih gubitaka ploda pre desete nedelje trudnoće b. Spontani gubitak morfološki normalnog fetusa nakon 10-te nedelje trudnoće c. Jedan ili više prevremenih porođaja normalnog neonatusa pre 34. nedelje trudnoće zbog teške preeklampsije, eklampsije ili placentalne insuficijencije	Klinički kriterijumi 1. Tromboze vena – makrovaskularne a. Sa drugim faktorima rizika za trombozu vena: 1 bod b. Bez drugih faktora rizika: 3 boda 2. Tromboze arterija – makrovaskularne a. Sa drugim faktorima rizika za kardiovaskularne bolesti: 2 boda b. Bez drugih faktora rizika: 3 boda 3. Mikrovaskularne tromboze a. <i>Livedo racemoso</i> , livedoidna vaskulopatija, aPL nefropatija, pulmonalna hemoragija (sumnja: 2 boda; potvrđena: 5 bodova) b. Potvrđena adrenalna hemoragija/miokardna bolest: 5 bodova 4. Obstetričke manifestacije a. Tri i više konsektivnih gubitaka ploda pre desete nedelje i/ili smrt fetusa u dobu 10 – 15 nedelja gestacije + 6 dana: 1 bod b. Smrt fetusa u dobu od 16 – 33 nedelja gestacije + 6 dana, u odsustvu teške preeklampsije ili teške placentalne insuficijencije: 1 bod c. Teška preeklampsija ili teška placentalna insuficijencija pre 34. nedelje gestacije, sa ili bez smrti fetusa: 3 boda d. Teška preeklampsija i teška placentalna insuficijencija pre 34. nedelje gestacije, sa ili bez smrti fetusa: 4 boda 5. Srčani zalisci a. Zadebljanje: 2 boda b. Vegetacija: 4 boda 6. Hematološki kriterijumi a. Trombocitopenija: 2 boda
Laboratorijski kriterijumi 1. Perzistentno pozitivan test na LAC i/ili 2. Perzistentno pozitivna antikardiolipinska antitela IgG ili IgM izotipa (u srednjem ili visokom titru, odnosno > 40 GPL ili MPL, odnosno veća od 99. percentila za opštu populaciju) i/ili 3. Perzistentno pozitivna anti β_2 GPI antitela IgG ili IgM izotipa u titru većem od 99. percentila za opštu populaciju	Laboratorijski kriterijumi 1. Pozitivan test na LAC a. Jednom: 1 bod b. Perzistentno: 5 bodova 2. Perzistentno pozitivna aCL i/ili anti β_2 GPI antitela a. Srednje visok ili visok titar IgM aCL i/ili anti β_2 GPI antitela: 1 bod b. Srednje povećan titar (40 – 79 U/ml) IgG aCL i/ili anti β_2 GPI antitela: 4 boda c. Visok titar (> 80 U/ml) IgG aCL ili anti β_2 GPI antitela: 5 bodova d. Visok titar (> 80 U/ml) IgG aCL i anti β_2 GPI antitela: 7 bodova
Dijagnoza: Najmanje jedan klinički i jedan laboratorijski kriterijum unutar pet godina jedan od drugog.	Dijagnoza: Sabira se po jedna karakteristika sa najvišim brojem bodova iz svakog domena. Za dijagnostikovanje APS-a potrebno je najmanje po tri boda u kliničkim i u laboratorijskim domenima. Klinički i laboratorijski kriterijumi moraju biti ustanovljeni unutar tri godine jedan od drugog.

obstetričkim kriterijumima, postojanje ≥ 3 konsekutivna gubitka ploda pre desete nedelje trudnoće se vrednuje jednim bodom i ima manju važnost u odnosu na tešku placentalnu insuficijenciju pre 34. nedelje trudnoće, koja se ocenjuje sa četiri boda. Definitivna dijagnoza se dobija zbirom bodova pojedinačnih kliničkih i laboratorijskih kriterijuma, pri čemu je za definitivnu dijagnozu APS-a neophodno najmanje tri klinička i tri laboratorijska boda. Osnovni cilj ACR/EULAR klasifikacije je da se unapredi specifičnost u prepoznavanju APS-a, kako bi se formirale što homogenije grupe bolesnika za kliničke studije. Međutim, na taj način se zna-

According to the ACR/EULAR criteria, six clinical domains (vein thrombosis, arterial thrombosis, thrombosis in the microcirculation, obstetric complications, heart valve changes, and hematological manifestations) and two laboratory domains are scored. As compared to the modified Sapporo classification, wherein all criteria carry the same weight, in the ACR/EULAR classification, the criteria are ranked according to significance based on specificity for the presence of APS. Thus, for example, in the obstetric criteria, the occurrence of ≥ 3 consecutive pregnancy losses before week 10 of gestation is scored with one point and is

Table 1. Comparison of criteria for antiphospholipid syndrome according to the modified Sapporo and ACR/EULAR classifications

Modified Sapporo criteria (2006)	ACR/EULAR criteria (2023)
Clinical criteria - Thrombosis (any organ or tissue) - Arterial - Venous - Small vessels - Pregnancy morbidity ≥ 3 miscarriages before week 10 of gestation - Unexplained death of a morphologically normal fetus at ≥ 10 weeks of gestation ≥ 1 birth of a morphologically normal neonate < 34 weeks of gestation, due to: (i) eclampsia or severe preeclampsia or (ii) placental insufficiency	Clinical criteria - Macrovascular venous thromboembolism (VTE) - VTE with other high-risk VTE profile: 1 point - VTE without other high-risk VTE profile: 3 points - Macrovascular arterial thrombosis - Arterial thrombosis with high-risk CVD profile: 2 points - Arterial thrombosis without high-risk CVD profile: 4 points - Microvascular thrombosis - Livedo racemosa, livedoid vasculopathy, aPL nephropathy, pulmonary hemorrhage (suspected: 2 points; confirmed: 5 points) - Confirmed adrenal hemorrhage/microvascular myocardial disease: 5 points - Obstetric complications ≥ 3 consecutive prefetal (< 10 weeks of gestation) and/or early fetal death (10 – 15 weeks + 6 days of gestation): 1 point - Fetal death (16 – 33 weeks + 6 d of gestation) in the absence of severe preeclampsia or severe placental insufficiency: 1 point - Severe preeclampsia (< 34 weeks of gestation) or severe placental insufficiency (< 34 weeks of gestation) with/without fetal death: 3 points - Severe preeclampsia (< 34 weeks of gestation) and severe placental insufficiency (< 34 weeks of gestation) with/without fetal death: 4 points - Cardiac valves - Thickening: 2 points - Vegetation: 4 points - Hematological criteria - Thrombocytopenia: 2 points
Laboratory criteria - Persistently positive LAC test and/or - Persistently positive IgG/IgM aCL antibodies (at medium or high ELISA titer, i.e., > 40 GPL or MPL, i.e., higher than the 99th percentile for the general population) and/or - Persistently positive IgG or IgM isotype anti-β_2GPI antibodies in a titer higher than the 99th percentile for the general population	Laboratory criteria - Positive LAC test: - On one occasion: 1 point - Persistently: 5 points - Persistently positive aCL and/or aβ_2GPI antibodies - Moderate or high titer of IgM aCL and/or aβ_2GPI: 1 point - Moderately elevated titer (40 – 79 U/ml) of IgG aCL and/or aβ_2GPI antibodies: 4 points - High titer (≥ 80 U/ml) of IgG aCL or aβ_2GPI antibodies: 5 points - High titer (≥ 80 U/ml) of IgG aCL and aβ_2GPI antibodies: 7 points
Diagnosis: At least one clinical criterion and at least one laboratory criterion. Clinical and laboratory criteria must be detected within five years of each other	Diagnosis: The single highest-scoring feature from each domain is added up. APS is classified as ≥ 3 points in clinical domains and ≥ 3 points in laboratory domains. Clinical and laboratory criteria must be detected within three years of each other.

čajno smanjuje senzitivnost u prepoznavanju APS-a, odnosno povećava verovatnoća neprepoznavanja bolesti kod pojedinačnog bolesnika. U **Tabeli 1** su uporedno prikazani kriterijumi za prepoznavanje APS-a po revidiranoj Sapporo i novoj ACR/EULAR klasifikaciji.

TROMBOZA VELIKIH KRVNIH SUDOVA

Za razliku od urođene trombofilije, tromboze udružene sa aPL antitelima se mogu javiti u bilo kom delu cirkulatornog sistema, odnosno u venama, arterijama i mikrocirkulaciji. Tromboze dubokih vena donjih ekstremiteta, sa ili bez trombozne embolije pluća, predstavljaju najtipičniju kliničku manifestaciju antifosfolipidnog sindroma. U jednoj meta-analizi je pokazano da se kod približno 10% bolesnika sa trombozom dubokih vena može dokazati prisustvo aPL antitela [13]. Bolesnici sa trombozom i aPL antitelima su u visokom riziku od recidiva tromboze nakon obustavljanja antikoagulantne terapije, a mortalitet kod ovih osoba u toku četvorogodišnjeg praćenja je bio značajno veći u odnosu na osobe sa trombozom ali bez aPL antitela [14].

Infarkt mozga i tranzitorni ishemični atak (TIA) predstavljaju najčešće manifestacije tromboze arterija kod bolesnika sa APS-om [15]. Veza između infarkta mozga i prisustva aPL antitela je snažnija kod mlađih nego kod starijih osoba, a moguće je da i etnički faktori imaju važnu ulogu, pri čemu je infarkt mozga posebno čest kod bolesnika azijskog porekla. U jednoj japanskoj studiji, 61% bolesnika sa APL sindromom prezentovalo je infarktom mozga. Treba imati na umu da infarkt mozga ili tranzitorni ishemični atak može biti i posledica embolizacije sa izmenjenih aortalnih ili mitralnih ventila, što nije redak nalaz kod bolesnika sa aPL antitelima.

Infarkt srca nije česta manifestacija antifosfolipidnog sindroma. U jednoj novijoj studiji, prisustvo aPL antitela je nađeno kod 11% od ukupno 805 konsekutivnih bolesnika sa prvim infarktom srca mlađih od 75 godina i kod 1% od ukupno 805 zdravih osoba [16]. Pored toga, pokazano je da prisustvo antifosfolipidnih antitela povećava rizik od rekurentnog ishemijskog događaja kod bolesnika koji imaju koronarnu bolest [17].

PATOLOGIJA TRUDNOĆE

Komplikacije u trudnoći su česta manifestacija prisustva aPL antitela kod žena u reproduktivnom dobu. Najčešće ali najmanje specifične obstetričke manifestacije su ponavljani gubici ploda pre desete nedelje gestacije. Kasniji gubici ploda kao i insuficijencija posteljice koja uzrokuje porođaj pre 34. nedelje trudnoće znatno snažnije sugerišu prisustvo aPL antitela. Kod žena sa aPL antitelima povećan je rizik abrupcije posteljice i pojave sindroma karakterisanog hemolizom,

less significant than severe placental insufficiency before week 34 of gestation, which is scored with four points. A definitive diagnosis is obtained by summing up the points of individual clinical and laboratory criteria, whereby at least three clinical and three laboratory points are necessary for a definitive diagnosis of APS. The primary goal of the ACR/EULAR classification is to improve the specificity in detecting APS, in order to form more homogeneous groups of patients for clinical studies. However, in this way, the sensitivity in recognizing APS is significantly reduced, i.e., the probability of not recognizing the disease in an individual patient increases. **Table 1** compares the criteria for detecting APS according to the revised Sapporo and the new ACR/EULAR classification.

THROMBOSIS OF LARGE BLOOD VESSELS

Unlike congenital thrombophilia, thrombosis associated with aPL antibodies can occur in any part of the circulatory system, i.e., in the veins and arteries, and as microvascular thrombosis. Deep vein thrombosis of the lower extremities, with or without pulmonary embolism, is the most typical clinical manifestation of the antiphospholipid syndrome. In one meta-analysis, it was shown that the presence of aPL antibodies can be detected in approximately 10% of patients with deep vein thrombosis [13]. Patients with thrombosis and aPL antibodies are at high risk of thrombosis recurrence after anticoagulant therapy is discontinued. The mortality in such patients was shown to be significantly higher, as compared to people with thrombosis but without aPL antibodies, during a four-year follow-up [14].

Cerebral infarction and transient ischemic attack (TIA) are the most common manifestations of arterial thrombosis in patients with APS [15]. The association between cerebral infarction and the presence of aPL antibodies is stronger in younger people than in older people, and it is possible that ethnic factors also play an important role, whereby cerebral infarction is especially common in patients of Asian descent. In a Japanese study, 61% of patients with APL syndrome presented with cerebral infarction. It should be noted that cerebral infarction or transient ischemic attack can also be the consequence of embolization originating from malformed aortic or mitral valves, which is not a rare finding in patients with aPL antibodies.

Myocardial infarction is not a common manifestation of the antiphospholipid syndrome. In a recent study, the presence of aPL antibodies was found in 11% of a total of 805 consecutive patients younger than 75 years who had suffered their first myocardial infarction and in 1% of a total of 805 healthy individuals [16]. In addition, it has been shown that the presence of anti-

porastom jetrenih enzima i trombocitopenijom odnosno HELLP sindroma (*Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels – HELLP syndrome*).

Interesantno je da značajan broj žena sa aPL antitelima pokazuje izrazitu sklonost ka obstetričnim komplikacijama bez ispoljavanja sklonosti ka trombozi pa neki autori koriste termine „obstetrični APL sindrom“ i „trombotični APL sindrom“, ali još uvek nema jasnih patofizioloških kriterijuma za ovakvu podelu [18].

TROMBOZA U MIKROCIRKULACIJI

Tromboza sitnih krvnih sudova u različitim organima se javlja kod približno 12% bolesnika sa APS-om i ređa je u odnosu na trombozu arterija i vena [19]. Najčešće se dokazuje patohistološkim pregledom i dominantan je nalaz kod bolesnika sa katastrofičnim antifosfolipidnim sindromom. Može se ispoljiti u velikom broju organa ali su najčešće zahvaćeni koža i bubrezi. U koži se mikrotromboze mogu javiti kao livedoidna vaskulopatija, superficijalna gangrena, subakutni nodusi ili ekhimoza. U srcu i mozgu mikrotromboze mogu rezultovati mikrotrombima i mikroinfarktima dok u plućima mogu rezultovati alveolarnom hemoragijom ili akutnim respiratornim distres sindromom. Mikrotromboze u bubrezima se ispoljavaju kao akutna bubrežna insuficijencija, hipertenzija ili trombotična mikroangiopatija. Infarkti creva, jetre, slezine ili pankreasa mogu biti manifestacije tromboze sitnih krvnih sudova u ovim organima. Do formiranja tromba može doći i u sitnim sudovima retine sa znacima retinalne vaskulopatije.

KOŽNE LEZIJE KOD BOLESNIKA SA APL ANTITELIMA

Koža je često zahvaćena kod bolesnika sa prisutnim aPL antitelima. *Livedo reticularis* je načešća kožna promena kod bolesnika sa APS-om i prisutan je kod približno 24% bolesnika sa ovom bolešću. Njegovo prisustvo bi trebalo redovno da pobudi sumnju na APS, mada zbog male specifičnosti, *livedo reticularis* nije uvršten u kliničke kriterijume APS-a prema novoj ACR/EULAR klasifikaciji [15]. Kod nekih bolesnika, aPL antitela su udružena sa ulceracijama na potkolenicama, gangrenom distalnih delova prstiju i pseudovaskulitom. Interesantno je da se prisustvo aPL antitela može naći kod približno 50% bolesnika sa tzv. Snedonovim sindromom koji podrazumeva postojanje promena na koži po tipu *livedo racemosa* i ponavljanih TIA ili infarkta mozga. *Livedo racemosa* je po morfološkim karakteristikama sličan *livedo reticularis*-u i predstavlja ljubičastu, simetričnu, mrežastu šaru na koži trupa koja ne iščezava pri zagrevanju kože. Kod značajnog procenta bolesnika sa Snedonovim sindromom, prisutni su i arterijska hipertenzija i valvularne mane srca [20].

phospholipid antibodies increases the risk of recurrent ischemic events in patients with coronary disease [17].

PREGNANCY PATHOLOGY

Complications in pregnancy are a frequent manifestation of the presence of aPL antibodies in women of reproductive age. The most common but least specific obstetric manifestation is repeated pregnancy loss before week ten of gestation. Later fetal losses as well as placental insufficiency causing delivery before week 34 of pregnancy strongly indicate the presence of aPL antibodies. In women with aPL antibodies, there is an increased risk of placental abruption and the development of HELLP syndrome (*Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels*).

Interestingly, a significant number of women with aPL antibodies show a marked tendency towards obstetric complications without manifesting a tendency to thrombosis, so some authors use the terms “obstetric APL syndrome” and “thrombotic APL syndrome”, but there are still no clear pathophysiological criteria for this categorization [18].

THROMBOSIS IN THE MICROCIRCULATION

Small blood vessel thrombosis in various organs occurs in approximately 12% of patients with APS and is less common than arterial and venous thrombosis [19]. It is most often proven with pathohistological examination and is the dominant finding in patients with catastrophic antiphospholipid syndrome. It can manifest in many organs, but the skin and kidneys are most often affected. In the skin, microthrombosis can occur as livedoid vasculopathy, superficial gangrene, subacute nodules, or ecchymosis. In the heart and brain, microthrombosis can result in microthrombi and microinfarcts, while, in the lungs, it can result in alveolar hemorrhage or acute respiratory distress syndrome. Microthrombosis in the kidneys manifests as acute renal failure, hypertension, or thrombotic microangiopathy. Infarctions of the intestine, liver, spleen, or pancreas can be manifestations of small blood vessel thrombosis in these organs. Thrombus formation can also occur in the small vessels of the retina with signs of retinal vasculopathy.

SKIN LESIONS IN PATIENTS WITH APL ANTIBODIES

In patients with aPL antibodies, the skin is often affected. *Livedo reticularis* is the most common skin change in patients with APS and is present in approximately 24% of patients with this disease. This is why its presence should always raise suspicion of APS. However, due to low specificity, *livedo reticularis* is not included in the clinical criteria of APS according to the new ACR/

KARDIOLOŠKE MANIFESTACIJE

Promene na srčanim zaliscima, u smislu zadebljanja valvula, disfunkcije zaliska ili prisustva vegetacija mogu se naći kod približno 14% bolesnika sa APS-om [15]. Kod bolesnika sa sistemskim eritemskim lupusom (SEL) i antifosfolipidnim antitelima, promene na valvulama su češće nego kod bolesnika bez SEL-a. U najnovijoj ACR/EULAR klasifikaciji, promene na srčanim zaliscima su uvrštene kao klinički kriterijum u prepoznavanju APS-a.

HEMATOLOŠKE MANIFESTACIJE

Trombocitopenija je najčešći hematološki nalaz kod bolesnika sa APS-om, koji zbog svoje nespecifičnosti nije bio prepoznat kao kriterijum u revidiranoj Sapporo klasifikaciji. Broj trombocita ispod $100 \times 10^9/L$ je prisutan kod približno 30% bolesnika sa APS-om, pri čemu je veća učestalost trombocitopenije kod bolesnika sa sekundarnim APS-om nego kod onih sa primarnim APS-om. Trombocitopenija je najčešće umerenog stepena sa brojem trombocita $50 - 100 \times 10^9/L$. Interesantno je da se prisustvo aPL antitela može dokazati kod približno 40% bolesnika sa primarnom imunološkom trombocitopenijom, bez drugih kriterijuma za APS [15]. Autoimuna hemolizna anemija se može naći kod oko 10% bolesnika sa APS-om. Nalaz neimunološke hemolizne anemije kod bolesnika sa APS-om može ukazivati na mehaničku hemolizu u okviru katastrofičnog antifosfolipidnog sindroma.

BUBREŽNE MANIFESTACIJE

Bubrežne manifestacije, u formi nefropatije koja prati APS, uglavnom su posledica trombotične mikroangiopatije. Najčešće se ispoljavaju u vidu arterijske hipertenzije, nefrotskog sindroma ili češće subnephrotske proteinurije i bubrežne insuficijencije. Pri patohistološkom pregledu se uočavaju hiperplazija i fibroza intime, fokalna kortikalna atrofija i okluzije sitnih arterija [21].

MANIFESTACIJE CENTRALNOG NERVNOG SISTEMA

Pored tranzitornog ishemičnog ataka, infarkta mozga i tromboze venskih sudova mozga, koji ulaze u klasifikacione kriterijume i po revidiranoj Sapporo i po ACR/EULAE klasifikaciji, prisustvo antifosfolipidnih antitela može biti udruženo sa epilepsijom, migrenom i horeom. U retke manifestacije spadaju i amaurosis fugax i transverzni mijelitis [12].

KATASTROFIČNI ANTIFOSFOLIPIDNI SINDROM

Akutna egzacerbacija protrombogenog stanja u krvi sa pojavom mikrotromboza u većem broju organa kod bolesnika sa aPL antitelima naziva se katastrofični APS.

EULAR klasifikacija [15]. In some patients, aPL antibodies are associated with leg ulcers, gangrene of the distal parts of the fingers, and pseudovasculitis. Interestingly, the presence of aPL antibodies can be found in approximately 50% of patients with the so-called Sneddon syndrome, which implies the existence of livedo racemosa-type skin changes and repeated TIA or brain infarction. Livedo racemosa is morphologically similar to livedo reticularis and is a purple, symmetrical, net pattern on the skin of the trunk that does not disappear when the skin is warmed up. In a significant percentage of patients with the Sneddon syndrome, arterial hypertension and valvular heart defects are also present [20].

CARDIOLOGICAL MANIFESTATIONS

Malformations of heart valves, in terms of valve thickening, valve dysfunction, or the presence of vegetation can be found in approximately 14% of patients with APS [15]. In patients with systemic lupus erythematosus (SEL) and antiphospholipid antibodies, valve pathology is more common than in patients without SEL. In the latest ACR/EULAR classification, heart valve malformations are included as clinical criteria for recognizing APS.

HEMATOLOGICAL MANIFESTATIONS

Thrombocytopenia is the most common hematological finding in patients with APS, which, due to its non-specificity, was not recognized as a criterion in the revised Sapporo classification. A platelet count below $100 \times 10^9/L$ is present in approximately 30% of patients with APS, with a higher frequency of thrombocytopenia in patients with secondary APS than in those with primary APS. Thrombocytopenia is most often moderate with a platelet count of $50 - 100 \times 10^9/L$. Interestingly, the presence of aPL antibodies can be demonstrated in approximately 40% of patients with primary immune thrombocytopenia, without other criteria for APS [15]. Autoimmune hemolytic anemia can be found in about 10% of patients with APS. The finding of non-immunological hemolytic anemia in patients with APS may indicate mechanical hemolysis as a part of catastrophic antiphospholipid syndrome.

RENAL MANIFESTATIONS

Renal manifestations, in the form of nephropathy accompanying APS, are mainly the result of thrombotic microangiopathy. They most often manifest as arterial hypertension, nephrotic syndrome, or, more commonly, subnephrotic proteinuria and renal insufficiency. Hyperplasia and fibrosis of the intima, focal cortical atrophy, and occlusion of small arteries are observed during pathohistological examination [21].

Javlja se kod približno 1% bolesnika sa APS-om i najčešće ga pokreću pojava infekcije, hirurške ili druge invazivne procedure ili prekid u primeni antikoagulantne terapije. Katastrofični APS karakteriše brzo nastupajuća insuficijencija većeg broja organa, pri čemu je najčešće kompromitovana funkcija pluća (24% bolesnika), centralnog nervnog sistema (18% bolesnika) i bubrega (18% bolesnika) [22]. Katastrofični APS predstavlja životno ugrožavajuće stanje i, uprkos primeni terapije, smrtnost kod ove bolesti je još uvek oko 30%.

ZAKLJUČAK

Antifosfolipidna antitela su udružena sa širokim spektrom kliničkih manifestacija u gotovo svim medicinskim oblastima. Različite forme tromboze i pojava komplikacija u trudnoći su najtipičnije manifestacije i predstavljaju kliničke kriterijume za utvrđivanje postojanja antifosfolipidnog sindroma kod osoba sa aPL antitelima. S druge strane, povezanost aPL antitela i brojnih drugih kliničkih manifestacija koje ne predstavljaju zvanične kriterijume u klasifikaciji APS-a nije uvek jasna i konzistentna. Međutim, prepoznavanje ovih manifestacija ipak ima veliki značaj jer skreće pažnju na moguće prisustvo aPL antitela i daje povod za temeljno ispitivanje prisustva drugih laboratorijskih i kliničkih kriterijuma za postojanje antifosfolipidnog sindroma. Iako je najnovija klasifikacija APS-a preporučena od strane ACR/EULAR usvojila dodatne kliničke kriterijume koji nisu postojali u prethodnoj klasifikaciji, treba imati u vidu da su sve trenutno važeće preporuke za lečenje APS-a donete na osnovu prethodnih, tzv. revidiranih Sapporo kriterijuma. Zbog toga, klasifikacione ACR/EULAR kriterijume, koji su prevashodno namenjeni za selekciju bolesnika u kliničkim studijama, za sada ne treba koristiti pri postavljanju dijagnoze antifosfolipidnog sindroma kod pojedinačnih bolesnika u svakodnevnoj praksi.

SKRAĆENICE:

aPL antitela – antifosfolipidna antitela (engl. *antiphospholipid antibodies*)
APS – antifosfolipidni sindrom (engl. *antiphospholipid syndrome*)
ACR/EULAR – engl. *American College of Rheumatology/European Alliance of Associations for Rheumatology*
SEL – sistemski eritemski lupus
LAC – engl. *lupus anticoagulant*
ELISA – enzimski imunosorbentni esej (*enzyme-linked immunosorbent assay*)
aCL antitela – antikardiolipinska antitela (aCL antitela; engl. *anticardiolipin antibodies*)
β2GPI – beta 2 glikoprotein I
HELLP sindrom – sindrom karakterisan hemolizom, porastom jetrenih enzima i trombocitopenijom (*Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels – HELLP syndrome*)
TIA – tranzitorni ishemični atak

Sukob interesa: Nije prijavljen.

CENTRAL NERVOUS SYSTEM MANIFESTATIONS

In addition to transient ischemic attack, cerebral infarction, and cerebral venous thrombosis, which are included in the classification criteria of both the revised Sapporo and ACR/EULAR classifications, the presence of antiphospholipid antibodies may be associated with epilepsy, migraine, and chorea. Rare manifestations include amaurosis fugax and transverse myelitis [12].

CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME

Acute exacerbation of the prothrombotic state in the blood with the appearance of microthrombosis in multiple organs in patients with aPL antibodies is called catastrophic APS. It occurs in approximately 1% of patients with APS and is most often triggered by an infection, surgical or other invasive procedures, or discontinuation of anticoagulant therapy. Catastrophic APS is characterized by rapidly occurring failure of multiple organs, with the lung function being the one most commonly compromised (24% of patients), followed by the central nervous system (18% of patients), and the kidneys (18% of patients) [22]. Catastrophic APS is a life-threatening condition and, despite the administration of therapy, mortality in this disease is still around 30%.

CONCLUSION

Antiphospholipid antibodies are associated with a wide range of clinical manifestations in almost all medical fields. Various forms of thrombosis and the development of complications in pregnancy are the most typical manifestations and represent clinical criteria for determining the presence of antiphospholipid syndrome in persons with aPL antibodies. On the other hand, the association of aPL antibodies with numerous other clinical manifestations that do not represent official criteria in the classification of APS is not always clear and consistent. However, recognizing these manifestations is still of great significance because it draws attention to the possible presence of aPL antibodies and indicates the need for a detailed analysis of the presence of other laboratory and clinical criteria proving the existence of antiphospholipid syndrome. Although the latest classification of APS recommended by ACR/EULAR adopted additional clinical criteria that did not exist in the previous classification, it should be noted that all currently valid recommendations for the treatment of APS were made based on the previous criteria, i.e., the revised Sapporo criteria. Therefore, the ACR/EULAR classification criteria, which are primarily intended for the selection of patients in clinical stud-

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ies, should not as yet be used in the diagnosis of antiphospholipid syndrome in individual patients in daily practice.

ABBREVIATIONS AND ACRONYMS:

aPL antibodies – antiphospholipid antibodies
 APS – antiphospholipid syndrome
 ACR/EULAR – American College of Rheumatology/European Alliance of Associations for Rheumatology
 SEL – systemic lupus erythematosus
 LAC – lupus anticoagulant
 ELISA – enzyme-linked immunosorbent assay
 aCL antibodies – anticardiolipin antibodies
 β_2 GPI – beta 2 glycoprotein I
 HELLP syndrome – Hemolysis, Elevated Liver enzyme levels, and Low Platelet levels
 TIA – transient ischemic attack

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LIGHT CHAIN DEPOSITION DISEASE

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SAŽETAK

Bolest taloženja lakih lanaca predstavlja jedan od oblika bolesti taloženja monoklonskih imunoglobulina. Po učestalosti, reč je o retkom entitetu, koji se javlja kod osoba srednjeg životnog doba, češće muškog pola. Najčešće zahvata bubrege, sa kliničkom slikom nefritičkog sindroma, ali može biti lokalizovana i u drugim organima, kao što su jetra, pluća, srce, gastrointestinalni trakt, koža i drugi. U kliničkoj slici dominiraju simptomi i znaci zahvaćenog organa. Dijagnoza se najčešće postavlja biopsijom zahvaćenog organa, pri čemu se bojenjem na Kongo crveno razlikuje od amiloidoze lakih lanaca, a potom se vrši evaluacija koštane srži radi isključivanja drugih plazmocitnih diskrazija. Terapija je bazirana na terapijskim modalitetima za lečenje multiplog mijeloma, uključujući inhibitore proteazoma (bortezomib), uz autologu transplantaciju matičnih ćelija hematopoeze, ali i transplantaciju zahvaćenih organa ukoliko je došlo do potpunog gubitka funkcije. Podaci o terapiji monoklonskim antitelima (daratumumab) otvaraju nove terapijske mogućnosti u lečenju ove bolesti. Dijagnostika i lečenje ove bolesti zahtevaju multidisciplinarni pristup, prvenstveno nefrologa, hematologa i patologa.

Ključne reči: bolest deponovanja lakih lanaca, dijagnostika, terapija

ABSTRACT

Light chain deposition disease is one form of monoclonal immunoglobulin deposition disease. In terms of frequency, it is a rare entity that occurs in middle-aged people, more often males. It most often affects the kidneys, with a clinical picture of nephritic syndrome, but it can also be localized in other organs, such as the liver, lungs, heart, gastrointestinal tract, skin, and others. The symptoms and signs of the affected organ dominate the clinical picture. The diagnosis is most often established by biopsy of the affected organ, whereby Congo red staining differentiates it from light chain amyloidosis, and then bone marrow evaluation is performed to rule out other plasmacytic dyscrasias. Therapy is based on therapeutic modalities for treating multiple myeloma, including proteasome inhibitors (bortezomib), autologous hematopoietic stem cell transplantation, and transplantation of the affected organs if there has been a complete loss of function. Data on monoclonal antibody therapy (daratumumab) opens up new therapeutic possibilities for the treatment of this disease. Diagnosis and treatment of this disease require a multidisciplinary approach, primarily by nephrologists, hematologists, and pathologists.

Keywords: light chain deposition disease, diagnosis, therapy

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UVOD

Bolest taloženja lakih lanaca prema 5. reviziji klasifikacije bolesti hematopoetskih i limfoidnih tkiva Svetske zdravstvene organizacije pripada, kao najčešći entitet, grupi bolesti taloženja monoklonskih imunoglobulina, gde osim nje spadaju i bolesti taloženja teških lanaca, kao i bolesti taloženja lakih i teških lanaca [1]. Karakteriše je depozicija lakih lanaca imunoglobulina u različitim organima. Istaloženi laki lanci nemaju karakteristike amiloidne supstance, što ovu bolest razlikuje od amiloidoze lakih lanaca [2].

Bolest je prvi put dokazana 1976. godine na biopsijama bubrega dva pacijenta u Sjedinjenim Američkim Državama. Oba pacijenta su imala bubrežnu slabost, a veoma brzo su se razvile i druge manifestacije, najpre slabost jetre i neurološka simptomatologija, a zatim i srčano popuštanje, gastrointestinalne i endokrine manifestacije [3].

S obzirom na doskora limitirane mogućnosti za preciznu detekciju lakih lanaca učestalost ove bolesti je nepoznata. Češće se javlja kod muškaraca, a medijana starosti pri dijagnozi je 58 godina, dok je čak 36% pacijenata mlađe od 50 godina [4].

ETIOLOGIJA I PATOGENEZA

Etiologija ove bolesti je nepoznata.

U osnovi bolesti, mali patološki klon plazmocita niskog proliferativnog potencijala kao patološki protein proizvodi lake lance, dominantno kappa tipa [5]. Slobodni monoklonski laki lanci talože se u različitim organima kao neorganizovani, amorfni agregati, bez stvaranja depozita u vidu fibrilnih niti, što je i glavna razlika na mikroskopskom nivou između amiloidoze lakih lanaca i bolesti taloženja lakih lanaca [6]. Bolest taloženja lakih lanaca najčešće se manifestuje na bubrezima (kod oko 85% pacijenata), ređe u jetri, srcu, plućima, a vrlo retko u drugim organima [4].

Patofiziološki mehanizam oštećenja funkcije najbolje je izučen kod bubrežnih manifestacija, gde amorfni agregati lakih lanaca započinju kaskadu događaja koji vode ka mezangijalnoj ekspanziji i uvećanju ekstracelularnog matriksa neuobičajenog sastava (bogatog tenascinom), formirajući mezangijalne čvoriće koji zamenjuju normalni matriks bogat kolagenom IV [5,6]. Kao ređa, ali ponekad i prva manifestacija bolesti deponovanja lakih lanaca, u literaturi se pominje i tubulointersticijska bolest bubrega. Najčešće se depoziti lakih lanaca viđaju duž tubularne bazalne membrane, uz linearne glomerularne i vaskularne depozite [7]. Retko, agregati lakih lanaca i Tamm Horsfalovog proteina mogu dovesti do zapušanja distalnih tubula i akutne bubrežne insuficijencije [8,9]. Iako promene na bubrežnim strukturama

INTRODUCTION

Light chain deposition disease, according to the 5th revision of the classification of hematopoietic and lymphoid tissue diseases of the World Health Organization, belongs, as the most common entity, to the group of monoclonal immunoglobulin deposition diseases, which also includes heavy chain deposition diseases, as well as light and heavy chain deposition diseases [1]. The deposition of immunoglobulin light chains in various organs characterizes it. Precipitated light chains do not have the characteristics of an amyloid substance, distinguishing this disease from light chain amyloidosis [2].

The disease was first proven in 1976 in the kidney biopsies of two patients in the United States of America. Both patients had kidney failure, and other manifestations developed very quickly, first liver weakness and neurological symptoms, and then heart failure and gastrointestinal and endocrine manifestations [3].

Given the recently limited possibilities for precise detection of light chains, the frequency of this disease is unknown. It occurs more often in men, and the median age at diagnosis is 58, while as many as 36% of patients are younger than 50 [4].

ETIOLOGY AND PATHOGENESIS

The etiology of this disease is unknown.

At the basis of the disease, a small pathological clone of plasma cells with a low proliferative potential produces light chains, predominantly of the kappa type, as a pathological protein [5]. Free monoclonal light chains are deposited in various organs as disorganized, amorphous aggregates without forming deposits in the form of fibrillar strands, which is the main difference at the microscopic level between light chain amyloidosis and light chain deposition disease [6]. The disease of deposition of light chains is most often manifested in the kidneys (in about 85% of patients), less often in the liver, heart, and lungs, and very rarely in other organs [4].

The pathophysiological mechanism of impaired function is best studied in renal manifestations, where amorphous aggregates of light chains initiate a cascade of events leading to mesangial expansion and enlargement of an extracellular matrix of unusual composition (rich in tenascin), forming mesangial nodules that replace the standard matrix rich in collagen IV [5,6]. Tubulointerstitial kidney disease is also mentioned in the literature as a rarer but sometimes the first manifestation of light chain deposition disease. Most often, deposits of light chains are seen along the tubular basement membrane, along with linear glomerular and vascular deposits [7]. Rarely, aggregates of light chains and Tamm Horsfall protein can lead to

moгу biti detektovane i svetlosnom mikroskopijom, zlatni standard dijagnoze predstavlja elektronska mikroskopija [10]. Na preparatima pod elektronskim mikroskopom mogu se videti sitni granulirani gusti depoziti u svim delovima bubrega. U zidu glomerulskih kapilara depoziti formiraju tanku traku prema lamini rara interna, a veći depoziti se gomilaju u subendotelijalnom regionu. Depoziti se mogu videti i spolja od tubularne bazalne membrane [10].

Što se tiče patofiziološkog mehanizma oštećenja drugih organa u bolesti deponovanja lakih lanaca, najčešće su opisivane promene na biopsijama jetre i srca, koje pokazuju taloženja amorfnih agregata u vaskularnim strukturama [11]. Na preparatima biopsije jetre viđeni su masivni homogeni eozinofilni infiltrati, koji su uglavnom locirani perisinusoidalno. Infiltrati se boje pozitivno na PAS bojenje, ali negativno na Kongo crvenu boju [12]. Biopsije srca uglavnom ne pokazuju promene na svetlosnoj mikroskopiji, dok se na elektronskoj mikroskopiji vide karakteristični linearni depoziti u intresticijalnom prostoru, u vidu tamnih granuliranih gustih depozita, koji su imunofluorescentnim bojenjem dokazani kao monoklonski laki lanci [11].

Prisustvo monoklonskih lakih lanaca u serumu, iako može biti idiopatsko, češće je udruženo sa nekih drugim oboljenjima limfocita i plazmocita, najčešće multiplim mijelomom, limfoplazmocitnim limfomom ili drugim ređim plazmocitnim diskrazijama. Prema literaturnim podacima ova bolest se nalazi udružena sa multiplim mijelomom u oko 65% slučajeva, makroglobulinemijom (oko 2%), i drugim limfoproliferativnim oboljenjima (2-3%) [13]. Takođe u sklopu monoklonske gamopatije nepoznatog značaja (MGUS), bolest deponovanja lakih lanaca se može manifestovati u organima u čak 32-85% slučajeva. Učestalost idiopatskog oblika bolesti deponovanja lakih lanaca nije utvrđena [14].

KLINIČKA SLIKA I LABORATORIJSKI NALAZI

Kliničkom slikom bolesti deponovanja lakih lanaca dominiraju simptomi i znaci zahvaćenog organa. Prema literaturnim podacima, najčešće zahvaćen organ je bubrež, kod oko 85% pacijenata [14]. Kod oko 35% pacijenata, zahvatanje bubrega nije jedina lokalizacija, te se uz bubrežne poremećaje javljaju i simptomi i znaci zahvatanja drugih organa. Izolovane ekstrarenalne manifestacije uz očuvanu bubrežnu funkciju retko su prisutne [4]. Zahvatanje drugih ograna najčešće podrazumeva zahvaćenost jetre (oko 23% slučajeva), zahvaćenost srca, pluća, perifrenog i centralnog nervnog sistema, kože, gastrointestinalnog trakta, slezine, štitaste žlezde i drugih [12,15-20].

distal tubule obstruction and acute renal failure [8,9]. Although changes in renal structures can be detected by light microscopy, the gold standard of diagnosis is electron microscopy [10]. On preparations under the electron microscope, small, granular, dense deposits can be seen in all kidney parts. In the wall of the glomerular capillaries, deposits form a thin strip towards the lamina rara interna, and larger deposits accumulate in the subendothelial region. Deposits can also be seen outside the tubular basement membrane [10].

As for the pathophysiological mechanism of damage to other organs in light chain deposition disease, the most frequently described changes in liver and heart biopsies show the deposition of amorphous aggregates in vascular structures [11]. Liver biopsy samples showed massive homogeneous eosinophilic infiltrates, mainly located perisinusoidally. Infiltrates stain is positive for PAS staining but negative for Congo red [12]. Heart biopsies generally show no changes on light microscopy, while electron microscopy shows characteristic linear deposits in the interstitial space in the form of dark granular dense deposits, proven by immunofluorescence staining to be monoclonal light chains [11].

Although it can be idiopathic, the presence of monoclonal light chains in the serum is more often associated with some other diseases of lymphocytes and plasma cells, most often multiple myeloma, lymphoplasmacytic lymphoma, or other rarer plasmacytic dyscrasias. According to literature data, this disease is associated with multiple myeloma in about 65% of cases, macroglobulinemia (about 2%), and other lymphoproliferative diseases (2-3%) [13]. Also, as part of monoclonal gammopathy of unknown significance (MGUS), light chain deposition disease can manifest in organs in as many as 32-85% of cases. The frequency of the idiopathic form of light chain deposition disease has not been determined [14].

CLINICAL PICTURE AND LABORATORY FINDINGS

The symptoms and signs of the affected organ dominate the clinical picture of light chain deposition disease. According to literature data, the most frequently affected organ is the kidney in about 85% of patients [14]. In about 35% of patients, involvement of the kidneys is not the only localization, and symptoms and signs of involvement of other organs appear along with kidney disorders. Isolated extrarenal manifestations with preserved renal function are rarely present [4]. Involvement of other branches most often involves involvement of the liver (about 23% of cases), involvement of the heart, lungs, peripheral and central nervous system, skin, gastrointestinal tract, spleen, thyroid gland, and others [12,15-20].

Tabela 1. Najčešće zahvaćeni organi u bolesti deponovanja lakih lanaca sa prikazom simptoma, znakova bolesti i nalaza dodatnih dijagnostičkih procedura

Organ/Sistem organa	Simptomi i znaci	Laboratorijske analize/dijagnostički nalazi
Bubreg	Povišen krvni pritisak, testasti otoci, umor, malaksalost	Povišene vrednosti uree, kreatinina, proteinurija (nefritičkog ili nefrotskog ranga), nalaz cilindara u urinu, pozitivni Bence-Jones protein / Biopsija bubrega
Srce	Uvećanje srca, restriktivna kardiomiopacije, srčana insuficijencija	Povišene vrednosti Moždanog natriuretskog peptida (proBNP), kao i troponina I, izmene elektrokardiograma (srčana hipertrofija) i ehokardiograma / Biopsija srca
Pluća	Restriktivni plućni poremećaj, dispnea, hemoptizije, bol u grudima	Restriktivan nalaz spirometrije, na multislajs kompjuterizovanoj tomografiji (MSCT) specifičan nalaz intersticijskih promena u vidu čvoriča, cista
Jetra	Hepatomeglij, insuficijencija jetre, portalna hipertenzija	Povišene vrednosti AST, ALT, GGT, ALP, bilirubin, LDH, Ultrazvuk stomaka / Biopsija jetre
Slezina	Splenomegalija, bol pod levim rebarnim lukom	Ultrazvuk stomaka
Nervni sistem	Periferna polineuropatija, senzorna neuropatija, disfunkcija autonomnog nervnog sistema	Elektromioneurografija / Opciono biopsija nerva
Koža	Bulozne kožne promene, sitnoznasti osip	Biopsija kože
Štitaste žlezde	Uvećanje štitaste žlezde	Visoke vrednosti tireostimulišućeg hormona (TSH), niže vrednosti tireoidnih hormona (fT4, T3) / Ultrazvuk štitaste žlezde

Table 1. The most frequently affected organs in light chain deposition disease with a presentation of symptoms, signs of the disease, and findings of additional diagnostic procedures

Organ/Organ system	Symptoms and signs	Laboratory analyses/diagnostic findings
Kidney	High blood pressure, swelling, fatigue, weakness	Increased values of urea, creatinine, proteinuria (nephritic or nephrotic grade), finding of cylinders in urine, positive Bence-Jones protein / Kidney biopsy
Heart	Enlargement of the heart, restrictive cardiomyopathy, heart failure	Elevated values of Brain natriuretic peptide (proBNP), as well as troponin I, electrocardiogram (cardiac hypertrophy) and echocardiogram changes / Heart biopsy
Lungs	Restrictive pulmonary disorder, dyspnea, hemoptysis, chest pain	Restrictive finding of spirometry, on multislice computed tomography (MSCT) specific finding of interstitial changes in the form of nodules, cysts
Liver	Hepatomegaly, liver failure, portal hypertension	Elevated values of AST, ALT, GGT, ALP, bilirubin, LDH, Abdominal ultrasound / Liver biopsy
Spleen	Splenomegaly, pain under the left rib cage	Abdominal ultrasound
Nervous system	Peripheral polyneuropathy, sensory neuropathy, autonomic nervous system dysfunction	Electromyoneurography / Optional nerve biopsy
Skin	Bullous skin changes, fine-grained rash	Skin biopsy
Thyroid gland	Enlargement of the thyroid gland	High values of thyroid-stimulating hormone (TSH), low values of thyroid hormones (fT4, T3), / Ultrasound of the thyroid gland

DIJAGNOSTIKA

Postavljanje dijagnoze bolesti taloženja lakih lanaca sastoji se iz nekoliko koraka. Ukoliko se na osnovu kliničke slike i manifestacija bolesti postavi sumnja na bolest lakih lanaca, prvi korak je svakako biopsija zahvaćenog organa, najčešće bubrega, ali i jetre, srca, pluća, kože, nerava [21]. Na patohistološkom preparatu vide se amorfni linearni depoziti lakih lanaca, a

DIAGNOSTICS

Diagnosing light chain deposition disease consists of several steps. If light chain disease is suspected based on the clinical picture and manifestations of the disease, the first step is a biopsy of the affected organ, most often the kidneys, but also the liver, heart, lungs, skin, and nerves [21]. Amorphous linear deposits of light chains can be seen on the pathohistological

negativnim bojenjem na Kongo crveno isključuje se amiloidoza lakih lanaca [7]. Potvrda da je zaista reč o monoklonskim lakim lancima sprovodi se određivanjem koncentracije lakih lanaca u serumu i urinu, zajedno sa elektroforezom i imuno elektroforezom proteina seruma i urina. Na ovim ispitivanjima potvrđuje se da su laki lanci monoklonalni, ali se ispituje i da li je reč samo o lakim lancima ili se oni kao paraprotein sintetišu uz teške lance [22]. Poslednji korak u dijagnostici predstavlja i ispitivanje koštane srži patohistološkom analizom, gde se detektuje broj plazmocita, ali i njihove karakteristike na imunohistochemijskom bojenju i fluorescentnoj in situ hibridizaciji (FISH) [4,21,23]. Alternativno, protočnom citometrijom u aspiratu koštane srži mogu se ispitati imunofenotipske karakteristike plazmocita [2,4,21].

TOK, PROGNOZA I LEČENJE

Bolest taloženja lakih lanaca pokazuje prirodno progresivni tok sa krajnjim gubitkom funkcije zahvaćenog organa [24]. Kod skoro 70% pacijenta obolelih od bolesti taloženja lakih lanaca postoji udruženost sa plazmocitom diskrazijom (najčešće multiplim mijelomom) ili nekim drugim limfoproliferativnim oboljenjem [13]. Prognozni parametri koji utiču na dalji tok bolesti su starost pacijenta, prisutnost drugih plazmocitnih diskrazija ili limfoproliferativne bolesti, kao i prisustvo ekstrarenalnog oboljenja, što predstavlja značajan negativan prognozni parametar [24]. Ukoliko razmatramo bubrežne prognozne parametre, najveći značaj ima brzina razvoja bubrežne insuficijencije, dok se kod zahvatanja srca prate biohemijski parametri kao što su proBNP i troponin I [25].

Lečenje ovog entiteta, ukoliko je udružen sa multiplim mijelomom ili drugim plazmocitnim limfoproliferativnim bolestima, lečimo prema protokolima za lečenje tih bolesti. Kada je reč o idiopatskom obliku, ne postoje zvanični konsenzusi o terapiji [26]. U literaturi se uglavnom pominju male grupe lečenih pacijenata, a kontrolisane studije, zbog retkosti entiteta, nisu sprovedene. Prema dostupnim literaturnim podacima, pacijenti su uglavnom lečeni protokolima baziranim na terapijama za lečenje multiplog mijeloma, uz potporu visokodoznom hemioterapijom i autologom transplantacijom matičnih ćelija. Procena efekta lečenja zasniva se na postizanju hematološkog odgovora (procena monoklonskih plazmocita u kostnoj srži), merenju koncentracije slobodnih lakih lanaca u serumu, kao i praćenju funkcije zahvaćenog organa [14,26].

Lečenje pacijenata je inicijalno bazirano na primeni kortikosteroidne terapije, koja je kod prvih dokazanih slučajeva primenjivana najpre kao mono-

preparation, and light chain amyloidosis is ruled out by negative Congo red staining [7]. Confirmation that it is indeed monoclonal light chains is carried out by determining the concentration of light chains in serum and urine, together with serum and urine protein electrophoresis and immunoelectrophoresis. These tests confirm that the light chains are monoclonal, but whether it is only about the light chains or whether they are synthesized as a paraprotein along with the heavy chains [22] is also examined. The last step in the diagnosis is the examination of the bone marrow by pathohistological analysis, where the number of plasma cells is detected, as well as their characteristics on immunohistochemical staining and fluorescent in situ hybridization (FISH) [4,21,23]. Alternatively, immunophenotypic characteristics of plasma cells can be examined by flow cytometry in bone marrow aspirate [2,4,21].

COURSE, PROGNOSIS, AND TREATMENT

Light chain deposition disease shows a naturally progressive course with eventual loss of function of the affected organ [24]. In almost 70% of patients suffering from light chain deposition disease, there is an association with plasmacytic dyscrasia (most often multiple myeloma) or some other lymphoproliferative disease [13]. Prognostic parameters that affect the further course of the disease are the age of the patient, the presence of other plasmacytic dyscrasias or lymphoproliferative diseases, as well as the presence of extrarenal disease, which represents a significant negative prognostic parameter [24]. If we consider renal prognostic parameters, the speed of development of renal insufficiency has the most significant importance, while in the case of cardiac involvement, biochemical parameters such as proBNP and troponin I are monitored [25].

The treatment of this entity, if it is associated with multiple myeloma or other plasmacytic lymphoproliferative diseases, is treated according to the protocols for treating these diseases. When it comes to the idiopathic form, there are no official consensuses on therapy [26]. Small groups of treated patients are primarily mentioned in the literature, and controlled studies have not been conducted due to the entity's rarity. According to available literature data, patients were mostly treated with protocols based on therapies for the treatment of multiple myeloma, supported by high-dose chemotherapy and autologous stem cell transplantation. The assessment of the effect of treatment is based on achieving a hematological response (estimation of monoclonal plasma cells in the bone marrow), measuring the concentration of free light chains in the serum, as well as monitoring the function of the affected organ [14,26].

terapija, sa postizanjem efekta stabilizacije funkcije zahvaćenog organa i postizanjem parcijalnog hematološkog terapijskog odgovora [23]. Osim kortikosteroidne terapije, kao monoterapija, primenjivani su i kombinovani hemioterapijski režimi sa vinkristinom, adriablastinom (VAD) ili vinkristinom, melfalanom i ciklofosfamidom (VMCP) [5,23]. Nakon toga najpre je uz kortikosteroidnu terapiju primenjivana i terapija talidomidom sa ili bez ciklofosfamida, kod manjeg broja pacijenata. Podaci o efikasnosti ove terapije uglavnom su bazirani na pojedinačnim prikazima slučajeva, gde detektujemo kako refrakternost na terapiju, tako i postizanje kompletne dugotrajne remisije [27,28].

Najveći broj pacijenata, prema literaturi je do sada lečen protokolima baziranim na bortezomibu, i to bortezomib+ciklofosfamid+dexametazon (VCD) ili bortezomib+dexametazon (VD), sa postizanjem dobrog hematološkog odgovora. Kompletan odgovor (CR) postignut je kod oko 55,6%, a veoma dobar parcijalni odgovor (VGPR) i parcijalni odgovor (PR) kod ostalih tretiranih pacijenata [26]. Primena autologne transplantacije u lečenju ovih pacijenata nakon inicijalnog lečenja dovodi do dugotrajnije i stabilnije remisije, s povećanjem kompletnog odgovora na 61,5% pacijenata [20].

Lečenje bolesti deponovanja lakih lanaca sprovedeno je i kod pojedinačnih slučajeva uz primenu terapijskih modaliteta sa lenalidomidom. Literaturni podaci govore o većem broju različitih terapijskih režima, te je lenalidomid korišćen u kombinaciji sa prednizonom, sa dexametazonom, melfalanom i prednizonom, kao i deksametazonom i ciklofosfamidom. Postignuti efekti su zabeleženi kao PR ili VGPR, uz održavanje odgovora od 1-4 godine [29].

Poslednjih godina pojavljuju se podaci o pacijentima lečenim daratumumabom kao monoterapijom u inicijalnom lečenju, kod kojih se postiže i održava dugotrajna hematološka remisija [30]. Daratumumab je posmatran i u formi konsolidacije nakon lečenja protokolima baziranim na bortezomibu, gde je kod svih pacijenata zabeleženo poboljšanje hematološkog odgovora (iz PR u VGPR, odnosno iz VGPR u CR) [31].

Kao potporna terapija kod ovih pacijenata pominje se i transplantacija zahvaćenog organa kod terminalne insuficijencije, ali je za sprovođenje ove procedure neophodno prethodno postizanje hematološkog odgovora i smanjenje koncentracije lakih lanaca u serumu, jer u suprotnom dolazi do ponovnog oštećenja funkcije transplantiranog organa [32].

The treatment of patients was initially based on the application of corticosteroid therapy, which in the first proven cases was applied as monotherapy, stabilizing the affected organ's function and achieving a partial hematological therapeutic response [23]. In addition to corticosteroid therapy, combined chemotherapy regimens with vincristine, adriablastin (VAD), or vincristine, melphalan, and cyclophosphamide (VMCP) were used as monotherapy [5,23]. After that, therapy with thalidomide with or without cyclophosphamide was first applied in addition to corticosteroid therapy in a smaller number of patients. Data on the effectiveness of this therapy are mainly based on individual case reports, where we detect both refractoriness to treatment and the achievement of complete long-term remission [27,28].

The largest number of patients, according to the literature, have so far been treated with bortezomib-based protocols, namely bortezomib+cyclophosphamide+dexamethasone (VCD) or bortezomib+dexamethasone (VD), achieving a good hematological response. Complete response (CR) was achieved in about 55.6%, and very good partial response (VGPR) and partial response (PR) in other treated patients [26]. The use of autologous transplantation in treating these patients after the initial treatment leads to a longer and more stable remission, with an increase in complete response to 61.5% of patients [20].

Treatment of light chain deposition disease was also carried out in individual cases using therapeutic modalities with lenalidomide. Literature data speak of many different therapeutic regimens, and lenalidomide was combined with prednisone, dexamethasone, melphalan, and prednisone, as well as dexamethasone and cyclophosphamide. The achieved effects were recorded as PR or VGPR, with response maintenance of 1-4 years [29].

In recent years, data have appeared on patients treated with daratumumab as monotherapy in the initial treatment, in whom long-term hematological remission is achieved and maintained [30]. Daratumumab was also observed in consolidation after treatment with protocols based on bortezomib, where the hematological response was improved in all patients (from PR to VGPR or from VGPR to CR) [31].

Transplantation of the affected organ in case of terminal insufficiency is also mentioned as a supportive therapy in these patients, but in order to carry out this procedure, it is necessary to achieve a hematological response and reduce the concentration of light chains in the serum, because otherwise the function of the transplanted organ will be damaged again [32].

ZAKLJUČAK

Bolest deponovanja lakih lanaca predstavlja redak entitet i dijagnostički izazov kod većine obolelih pacijenata. Za sprovođenje adekvatne dijagnostike, praćenja i terapije pacijenata potreban je multidisciplinarni pristup koji obuhvata nefrologa, hematologa, patologa, kao i subspecijalista drugih grana kod ekstrarenalnih manifestacija.

Sukob interesa: Nije prijavljen.

CONCLUSION

Light chain deposition disease is a rare entity and diagnostic challenge in most affected patients. A multidisciplinary approach, including nephrologists, hematologists, pathologists, and subspecialists of other branches in case of extrarenal manifestations, is needed to carry out adequate diagnostics, follow-up, and therapy of patients.

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FERROPTOSIS AND ITS CLINICAL SIGNIFICANCE

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SAŽETAK

Uvod: Ferroptaza, koju su prvi put spomenuli Dixon i saradnici, 2012. godine, jeste put ćelijske smrti zavisen od gvožđa, uz neophodno prisustvo lipidnih peroksida. Mehanizam procesa i signalni putevi koji u njemu učestvuju se razlikuju u odnosu na ranije poznatu apoptozu, nekrozu i autofagiju. Dolazi i do zapaljenske reakcije, što ovaj put ćelijske smrti dodatno razlikuje od apoptotskog puta. Ferrostatin dovodi do inhibicije ovog procesa.

Metode: Urađen je pregled literature dobijene pretraživanjem *Medline* baze podataka sa posebnim osvrtom na radove koji su se bavili značajem ferroptaze u kliničkoj medicini, prvenstveno hematologiji.

Rezultati: Metabolizam gvožđa u malignoj i zdravoj ćeliji se razlikuje. Maligne ćelije dobro tolerišu oksidativni stres i izbegavaju ferroptozu. Po literaturnim podacima, različiti ispitivani agensi stimulišu ferroptozu i time postaju mogući terapijski agensi. Neki geni povezani sa metabolizmom gvožđa pokazali su prognostički značaj kod obolelih od difuznog B krupnoćelijskog limfoma.

Zaključak: Otkrivanje novih mehanizama ćelijske smrti i signalnih puteva koji su u taj proces uključeni dovodi do potencijalno nove ciljne terapije. Iako obećavaju, ovi rezultati zahtevaju validaciju kroz dalja istraživanja.

Ključne reči: gvožđe, limfom, terapijski agensi, ćelijska smrt

ABSTRACT

Introduction: Ferroptosis, mentioned as such for the first time in 2012 by Dixon et al., is an iron-dependent type of cell death that occurs in the presence of lipid peroxides. The mechanism of the process and the signaling pathways involved in it differ from the previously known apoptosis, necrosis, and autophagy. An inflammatory reaction also occurs, which further distinguishes this type of cell death from apoptosis. Ferrostatin inhibits this process.

Methods: A review of the literature obtained by searching the Medline database was performed, with a special focus on studies concerned with the importance of ferroptosis in clinical medicine, primarily hematology.

Results: Iron metabolism in malignant and healthy cells differs. Malignant cells tolerate oxidative stress well and avoid ferroptosis. According to literature data, various tested agents stimulate ferroptosis and thus become possible therapeutic agents. Some genes linked to iron metabolism have shown prognostic significance in patients with diffuse large B-cell lymphoma.

Conclusion: The discovery of new mechanisms of cell death and the signaling pathways involved in this process leads to potentially new target therapy. Although promising, these results require validation through further research.

Keywords: iron, lymphoma, therapeutic agent, cell death

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UVOD

Ferroptaza, prvi put pomenuta u radu Diksona i saradnika, 2012. godine, jeste neapoptotski, nenekrozn i neautofagni put ćelijske smrti [1]. Dolma i saradnici su, 2003. godine, otkrili da erastin može da izazove nov način ćelijske smrti, a potom su Jagoda i Jang objavili da helatori gvožđa inhibiraju ovaj proces u čijoj osnovi se nalazi disfunkcija mitohondrija [2-4].

Kao što i naziv sugeriše, ferroptaza zavisi od gvožđa, dešava se u prisustvu lipidnih peroksida, a ferostatin je inhibira. Za razliku od apoptoze, u ovom procesu dolazi do privlačenja inflamatornih ćelija i reakcije zapaljenja. U procesu apoptoze, ćelijska membrana bubri i dolazi do kondenzacije hromozoma. U ferroptoz i se mitohondrije smanjuju, njihove membrane su hiperdenznije a mitohondrijalne kriste su manje ili ih nema [1,5-9]. Biohemijski posmatrano, koncentracija glutationa u ćeliji je manja. Glutation peroksidaza 4 (engl. *glutathione peroxidase* – GPX4) je enzim koji dovodi do redukcije lipidnih peroksida, a čija aktivnost je u ovom procesu takođe smanjena. Jon Fe^{2+} oksidiše lipide, koncentracija reaktivnih kiseoničnih radikala (engl. *reactive oxygen species* – ROS) raste i pokreće se ferroptaza [5]. U ovom putu su važni i transmembranski kanali u ćelijskim membranama kroz koje se vrši razmena cistina i glutamata. Cistin ulazi u ćeliju i u njoj se redukuje u cistein, neophodan u sintezi glutationa [1,5]. Ovo je i mesto na kojem deluju induktori ferroptoze. Helatori gvožđa inhibiraju ovaj proces, ali i ferostatin 1 (Fer-1), vitamin E i liproksatin 1.

Ferroptaza zavisi od gvožđa, te je očekivano da faktori koji učestvuju u metabolizmu gvožđa ili u regulaciji njegovog metabolizma imaju ili već dokazanu ili moguću ulogu u regulaciji ovog puta ćelijske smrti, čineći ćeliju manje ili više osetljivom na ferroptozu [6,10,11].

Metabolizam gvožđa u zdravoj i malignoj ćeliji

Kada govorimo o regulaciji metabolizma gvožđa reč je o kontroli na ćelijskom nivou i sistemskoj regulaciji metabolizma gvožđa. Homeostaza gvožđa u ćeliji se reguliše uglavnom posttranskripcionom kontrolom gena putem interakcije elemenata i belančevina senzitivnih na koncentraciju gvožđa (engl. *iron-responsive elements* – IRE; *iron-responsive proteins* – IRP) [12].

Sistemska homeostaza je kontrolisana hepcidinom, jetrenom belančevinom čija se koncentracija, između ostalog, povećava u inflamaciji, pod dejstvom interleukina-6 i dovodi do internalizacije i degradacije ferroportina. Ferroportin je, po podacima iz dostupne literature u ovom momentu, jedini eksporter gvožđa iz ćelija [12].

Hranom uneseno gvožđe izlazi iz enterocita putem ferroportina, u trovalentnom obliku, i vezan za transfer-

INTRODUCTION

Ferroptosis, first mentioned in a study by Dixon et al. in 2012, is a non-apoptotic, non-necrotic, and non-autophagic form of cell death [1]. In 2003, Dolma et al. discovered that erastin can cause a new type of cell death, upon which Jagoda and Yang reported that iron chelators inhibited this process, which is based on mitochondrial dysfunction [2-4].

As the name suggests, ferroptosis is iron-dependent, it occurs in the presence of lipid peroxides, and is inhibited by ferrostatin. Unlike apoptosis, this process involves the involvement of inflammatory cells and an inflammatory reaction. In the process of apoptosis, the cell membrane swells and chromosomes condense. In ferroptosis, mitochondria are reduced, their membranes are hyperdense, and mitochondrial cristae are smaller or absent [1,5-9]. In terms of biochemistry, the concentration of glutathione in the cell is lower. Glutathione peroxidase 4 (GPX4) is an enzyme that causes the reduction of lipid peroxides, and whose activity is also reduced in this process. The Fe^{2+} ion oxidizes lipids, the concentration of reactive oxygen species (ROS) increases, and ferroptosis is triggered [5]. Transmembrane channels in cell membranes through which cystine and glutamate are exchanged are also important in this process. Cystine enters the cell and is reduced to cysteine, which is necessary for the synthesis of glutathione [1,5]. This is also the site where ferroptosis inducers act. Iron chelators inhibit this process, but so do ferrostatin 1 (Fer-1), vitamin E, and liproxstatin 1.

Ferroptosis depends on iron, which is why it can be expected that factors involved in iron metabolism or in the regulation of its metabolism have either already proven or potential roles in the regulation of this type of cell death, making the cell more or less sensitive to ferroptosis [6,10,11].

Iron metabolism in healthy and malignant cells

When the regulation of iron metabolism is concerned, we speak of control at the cellular level and of systemic iron metabolism regulation. Iron homeostasis in the cell is regulated mainly by post-transcriptional control of genes via the interaction of iron-responsive elements (IRE) and iron-responsive proteins (IRP) [12].

Systemic homeostasis is controlled by hepcidin, a liver protein whose concentration, among other things, increases in inflammation, under the influence of interleukin-6, leading to the internalization and degradation of ferroportin. According to currently available literature data, ferroportin is the only exporter of iron from cells [12].

Dietary iron leaves the enterocytes via ferroportin, as trivalent iron, and is transported to tissues bound

in se prenosi do tkiva. Kompleks gvožđe-transferin se vezuje za transferinski receptor 1 (TfR1) i procesom endocitoze ulazi u ćelije. U ćeliji prelazi iz trovalentnog u divalentni oblik i ugrađuje se u različite enzime, ulazi u sastav hema, učestvuje u oksidativnoj fosforilaciji i sintezi dezoksiribonukleotida. Suvišno gvožđe se skladišti u feritinu.

Pokazalo se da dve belančevine – ZIP 8 i ZIP 14, s poznatom ulogom u prenošenju cinka, učestvuju i u transportu gvožđa koje nije vezano za transferin (engl. *non-transferrin-bound iron* – NTBI) kroz ćelijsku membranu [12-16], a imaju značaja i u hemohromatozi [16]. Njihovo mesto u drugim stanjima s nakupljanjem gvožđa, poput maligniteta i nekih anemija, ostaje nejasno [12]. Proteini PCBP1 i PCBP2 (engl. *poly(rC) binding protein 1*; *poly(rC) binding protein 2*) su šaperoni (engl. *chaperones*) – oni prate divalentno gvožđe u citoplazmi i imaju ulogu u njegovoj distribuciji u ćeliji [12,17,18], dok koaktivator jedarnog receptora 4 (engl. *nuclear receptor coactivator 4* – NCOA4) učestvuje u autofagiji feritina, te tako deluje na iskoristivost gvožđa koje je u njemu uskladišteno [12,19-22].

Gvožđe koje se nalazi u ćeliji deponovano u feritinu je netoksično ali je, dok je na taj način vezano, nedostupno za ćelijske potrebe. Da bi bilo iskoristivo, mora se osloboditi, obično degradacijom u lizozomima [19-22]. Izgleda da je način degradacije feritina isti i u stanjima s nakupljanjem gvožđa, a i kad postoji njegov nedostatak, ali se lizozomima isporučuje na drukčiji način [12,20]. Kad postoji deficit gvožđa, dolazi do autofagije, a kad postoji nakupljanje gvožđa, aktivni su drugi putevi [20]. NCOA4 je bitan činilac u autofagiji feritina kod nedostatka gvožđa i za regulaciju homeostaze gvožđa u slezini [23,24]. Ako nema nedostatka gvožđa, neautofagni putevi razgradnje feritina su manje aktivni u malignim ćelijama nego u zdravim, te su zato i maligne ćelije otpornije na oštećenja koje izaziva višak gvožđa [20]. Reaktivacija tih puteva bi mogla povećati toksičnost gvožđa za neoplastičnu ćeliju i možda biti nova terapijska strategija [12].

Ćelije kancera menjaju metabolizam tiola, koji su intraćelijski redoks puferi, a oksidativni stres zbog viška gvožđa povezan je sa karcinogenezom [25]. Već smo spomenuli da maligne ćelije zadržavaju veću količinu gvožđa, u lizozomima, ali i u citoplazmi i mitohondrijama. To dovodi do poremećaja njihove funkcije, preuzima se više glukoze i u prisustvu kiseonika, u procesu aerobne glikolize, produkuju se laktati. Ovaj fenomen je poznat kao Varburgov efekat. Osobina većeg preuzimanja glukoze od strane malignih ćelija koristi se u primeni pozitronske emisije tomografije (PET) u dijagnostici i proceni terapijskog efekta kod bolesnika sa malignim oboljenjima [26].

to transferrin. The iron-transferrin complex binds to the transferrin receptor 1 (TfR1) and enters cells via endocytosis. In the cell, trivalent iron is transformed into divalent iron and, as such, it is incorporated into various enzymes, it enters the composition of heme, and participates in oxidative phosphorylation and synthesis of deoxyribonucleotides. Excess iron is stored in ferritin.

It has been shown that two proteins – ZIP 8 and ZIP 14, with a known role in zinc transport, also participate in the transport of non-transferrin-bound iron (NTBI) through the cell membrane [12-16], and they are also important in hemochromatosis [16]. Their role in other conditions characterized by iron overload, such as malignancies and some forms of anemia, remains unclear [12]. Poly(rC) binding protein 1 (PCBP1) and poly(rC) binding protein (PCBP2) are iron chaperones – they accompany divalent iron in the cytoplasm and play a role in its distribution in the cell [12,17,18], while nuclear receptor coactivator 4 (NCOA4) participates in the autophagy of ferritin, thus affecting the utilization of iron stored in it [12,19-22].

The iron found in the cell deposited in ferritin is non-toxic, however, while bound in such a way, it is unavailable for cellular needs. For it to be usable, it must be released, usually by degradation in the lysosomes [19-22]. It seems that the method of ferritin degradation is the same in conditions characterized by iron overload and those characterized by iron deficiency, however, it is delivered to lysosomes differently [12,20]. When there is iron deficiency, autophagy occurs, and when there is iron overload, other pathways are active [20]. NCOA4 is an essential factor in ferritin autophagy in iron deficiency, as well as in the regulation of iron homeostasis in the spleen [23,24]. If there is no iron deficiency, non-autophagic pathways of ferritin degradation are less active in malignant cells than in healthy ones, and therefore malignant cells are more resistant to damage caused by excess iron [20]. The reactivation of these pathways could increase the toxicity of iron to the neoplastic cell and potentially be a new therapeutic strategy [12].

Cancer cells alter the metabolism of thiols, which are intracellular redox buffers, and oxidative stress, due to excess iron, is associated with carcinogenesis [25]. We have already mentioned that malignant cells retain excess iron in lysosomes, but also in the cytoplasm and mitochondria. This leads to the disruption of their function, the uptake of glucose is increased, and, in the presence of oxygen, in the process of aerobic glycolysis, lactates are produced. This phenomenon is known as the Warburg effect. The property of higher uptake of glucose by malignant cells is used in the application of positron emission tomography (PET) in the diagnostics and assessment of the therapeutic effect of treatment in patients with malignant diseases [26].

Agensi koji utiču na ferroptozu

Iz svega prethodno navedenog, vidi se da maligna ćelija, bez obzira na stalnu izloženost oksidativnom stresu, ima sposobnost da ga toleriše i izbegne ferroptozu [25]. Erastin i sulfasalazin blokiraju ulazak cistina kroz kanale u fosfolipidnom delu ćelijske membrane. Na taj način, kao i drugi agensi koji utiču na smanjenje produkcije glutaciona u eksperimentalnim uslovima, dovode do supresije tumorskog rasta i ferroptoze [25,27-29]. Testiranja su pokazala da promena metabolizma cistina i glutaciona dovodi do povišene koncentracije slobodnih kiseoničnih radikala, a antioksidativna terapija prevenira ćelijsku smrt [10]. Erastin je jak induktor ferroptoze koji inhibira transmembranske antiportere za razmenu intraćelijskog glutamata i ekstraćelijskog cistina. Glutathion učestvuje u održavanju redoks ravnoteže i, kad ga nema u dovoljnoj koncentraciji, nagomilavaju se slobodni kiseonični radikali, a već je rečeno da je za njegovu sintezu neophodan ulazak cistina u ćeliju [30-32]. Dikson i saradnici navode da sulfasalazin i sorafenib blokiraju ovaj antiporter i dovode do selektivne ferroptoze, s tim da je, u ovoj studiji, erastin 2.500 puta snažnije inhibirao ovaj sistem od sulfasalazina. Dodatni pokazatelj značaja gvožđa u ferroptozu je i to što deferoksamin, koji je helator gvožđa, suprimira ćelijsku smrt indukovanu erastinom, dok je dodatni unos gvožđa potencira [1,27,33]. Ispitivanja na kulturi ćelija difuznog B krupnoćelijskog limfoma su pokazala značajnu osetljivost na ćelijsku smrt indukovanu erastinom [34]. U drugom istraživanju je pokazano da erastin povećava senzitivnost leukemijskih ćelija akutne limfocitne leukemije (ALL) na efekat SMAC mimetika LCL-161 (engl. *second mitochondria-derived activator of caspases – SMAC mimetic LCL-161*) i da sinergističkim delovanjem dolazi do smrti leukemijske ćelije [35]. Drugi autori navode dozno zavisani efekat erastina na ćelije akutne mijeloidne leukemije, jer je u njihovim istraživanjima na kulturi ćelija dovodio do supresije njihovog rasta, ali i mešanog tipa ćelijske smrti. Supresija ovog efekta se ostvarila ferostatinom 1, inhibitorom ferroptoze ili nekrostatinom 1, inhibitorom nekroptoze, ali ne i inhibitorom kaspaznog puta, koji deluje na apoptozu, niti hlorohinomom, koji inhibiše autofagiju. Na kulturi ćelija hronične mijeloidne leukemije, akutne T limfoblastne leukemije i akutne promijelocitne leukemije, ovi efekti nisu postignuti. Ćelije akutne mijeloidne leukemije (AML) su, uz male doze erastina, bile značajno osetljivije na citozin arabinozid i antracikline [36], uz napomenu da je upotrebljen doxorubicin, a ne daunoblastin. U nedavno objavljenom radu, druga grupa autora je na *in vitro* modelu pokazala erastinom povećanu senzitivnost ćelija akutne mijeloidne leukemije na venetoklaks [37]. Fibroblasti i dendritske ćelije

Agents affecting ferroptosis

Based on all the above, it is evident that malignant cells, regardless of constant exposure to oxidative stress, can tolerate it and avoid ferroptosis [25]. Erastin and sulfasalazine block the entry of cystine through channels in the phospholipid part of the cell membrane. In this way, just like other agents affecting the reduced production of glutathione in experimental conditions, they lead to tumor growth suppression and ferroptosis [25,27-29]. Tests have shown that change in the metabolism of cystine and glutathione leads to an increased concentration of free oxygen radicals, while antioxidant therapy prevents cell death [10]. Erastin is a strong inducer of ferroptosis that inhibits transmembrane antiporters for intracellular glutamate and extracellular cystine exchange. Glutathione participates in maintaining the redox balance and when its concentration is insufficient, free oxygen radicals accumulate, and it has already been said that cystine entry into the cell is necessary for its synthesis [30-32]. In their study, Dixon et al. reported that sulfasalazine and sorafenib blocked this antiporter leading to selective ferroptosis, however, erastin inhibited this system 2,500 times more potently than sulfasalazine. An additional indicator of the importance of iron in ferroptosis is that deferoxamine, an iron chelator, suppresses erastin-induced cell death, while additional iron intake promotes it [1,27,33]. Studies on diffuse large B-cell lymphoma cell cultures have shown significant sensitivity to erastin-induced cell death [34]. In another study, it was shown that erastin increased the sensitivity of leukemic cells in acute lymphocytic leukemia (ALL) to the effect of the second mitochondria-derived activator of caspases (SMAC) mimetic LCL-161 and that the synergistic action led to the death of leukemic cells. [35]. Other authors have reported a dose-dependent effect of erastin on acute myeloid leukemia cells, since, in their studies of cell cultures, it led to the suppression of their growth, but also to a mixed type of cell death. The suppression of this effect was achieved by ferrostatin 1, an inhibitor of ferroptosis, or necrostatin 1, an inhibitor of necroptosis, but not by an inhibitor of the caspase pathway, which acts on apoptosis, nor by chloroquine, which inhibits autophagy. These effects were not achieved in chronic myeloid leukemia, acute T lymphoblastic leukemia, and acute promyelocytic leukemia cell cultures. Acute myeloid leukemia (AML) cells were significantly more sensitive to cytosine arabinoside and anthracyclines [36] when low doses of erastin were administered, where doxorubicin and not daunoblastin was used. In a recent study, another group of authors demonstrated, in an *in vitro* model, that erastin increased the sensitivity of acute myeloid leukemia cells to venetoclax [37]. Fibroblasts and dendritic cells make cystine, which lymph

stvaraju cistin od kojeg zavise limfoidne ćelije, jer moraju da ga unesu da bi sintetisale cistein. Pokazano je da sulfasalazin, delujući na sistem kanala kroz koji se cistin transportuje u kulturi limfomskih ćelija, dovodi do prekida njihove proliferacije, a kasnije je na životinjskom modelu sa transplantiranim limfomom potvrđena supresija tumorskog rasta; za organizam domaćina nije bilo veće štetnosti [38]. Indukcija ferroptoze je postignuta na kulturi ćelija 60 tumorskih linija delovanjem derivata artemisinina. Geni odgovorni za kodiranje belančevina koje učestvuju u metabolizmu gvožđa, poput transferina, transferinskih receptora 1 i 2, označeni su kao prediktori osetljivosti na artemisinin [39].

Ferroptoza u krupnoćelijskom B limfomu

Krupnoćelijski B limfom (engl. *large B-cell lymphoma* – *LBCL*) čini oko trećinu svih ne-Hoćkinovih limfoma, što znači da predstavlja najčešću limfoproliferativnu neoplazmu kod odraslih pacijenata [40]. Prvom terapijskom linijom, odnosno imunohemioterapijom po *R-CHOP* (rituksimab-ciklofosamid, doksorubicin, vinkristin, prednizolon) protokolu, kompletna remisija se postiže u oko 60% bolesnika, ali lečenje pacijenata sa rezistentnom ili relapsirajućom (R/R) bolešću je i dalje klinički problem. Nisu svi bolesnici sa R/R *LBCL* podobni za autolognu transplantaciju matičnih ćelija, niti je *CAR T* ćelijska terapija (engl. *chimeric antigen receptors (CAR) T-cell therapy*) široko dostupna. Bispecifična antitela, u kombinaciji s drugim lekovima, uglavnom su dostupna kroz kliničke studije, a često se desi da bolesnici sa R/R bolešću ne ispunjavaju kriterijume za uključivanje u studiju ili su studije nedostupne. Široko primenjivani standardni skorovi rizika su prognostički alati kreirani da procene šansu za preživljavanje pre i u eri lečenja rituksimabom [41,42]. Uz to, *LBCL* je heterogena bolest koja je i u novoj klasifikaciji Svetske zdravstvene organizacije (SZO) predstavljena sa više entiteta [43], gde u tumačenjima nekad postoje preklapanja i različito viđenje izazova, iz ugla patologa i kliničara [44]. Postoji potreba za razvojem novih prognostičkih skorova, ali i terapeutika koji bi delovali drugim mehanizmima.

Gvožđe je neophodno za mnoge procese, uključujući i ćelijsku proliferaciju [45,46]. Devin i saradnici su razvili novi skor, *IS* (engl. *iron score*) zasnovan na profilu ekspresije gena odgovornih za metabolizam gvožđa. U dve nezavisne kohorte pacijenata sa *LBCL*, koje su obuhvatile 233 i 181 ispitanika, 11 gena je pokazalo prognostički značaj. Visoka ekspresija osam gena je definisala grupu sa lošom prognozom. *IS* je dodatno validiran u tri kohorte u odnosu na ukupno preživljavanje (engl. *overall survival* – *OS*) [47]. *IS* je bio značajno viši u grupi *LBCL* porekla aktivisanih B limfocita (engl. *activated B-cell* – *ABC*) u odnosu na one porekla germi-

hoid cells depend on because they must absorb it in order to synthesize cystine. It has been demonstrated that sulfasalazine, acting on the system of channels through which cystine is transported in a culture of lymphoma cells, leads to the interruption of their proliferation, and later the suppression of tumor growth was confirmed in an animal model with transplanted lymphoma; there was no major harm to the host organism [38]. Induction of ferroptosis was achieved on a cell culture of 60 tumor lines by the action of artemisinin derivatives. Genes responsible for coding proteins involved in iron metabolism, such as transferrin, transferrin receptors 1 and 2, have been identified as predictors of sensitivity to artemisinin [39].

Ferroptosis in large B-cell lymphoma

Large B-cell lymphoma (LBCL) accounts for about a third of all non-Hodgkin's lymphomas, which makes it the most common lymphoproliferative neoplasm in adult patients [40]. With first-line treatment, i.e. immunochemotherapy with the R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, prednisolone) regimen, complete remission is achieved in about 60% of patients, however, the treatment of patients with resistant or relapsing (R/R) disease remains a clinical problem. Not all patients with R/R LBCL are eligible for autologous stem cell transplantation, nor is chimeric antigen receptors (CAR) T-cell therapy widely available. Bispecific antibodies, combined with other drugs, are mostly available through clinical studies, however, it often happens that patients with R/R disease do not meet the criteria for inclusion in a study or that the studies are unavailable. Widely used standard risk scores are prognostic tools designed to estimate the probability of survival before and during the rituximab treatment era [41,42]. Furthermore, LBCL is a heterogeneous disease represented by multiple entities in the new World Health Organization (WHO) classification [43], where there are sometimes overlapping interpretations and different views of the challenges, from the point of view of pathologists and clinicians [44]. New prognostic scores, as well as therapeutic agents that would act via other mechanisms, need to be developed.

Iron is necessary for many processes, including cell proliferation [45,46]. Devin et al. developed a new score – iron score (IS), based on the expression profile of genes responsible for iron metabolism. In two independent cohorts of LBCL patients, which included 233 and 181 subjects, 11 genes showed prognostic significance. High expression of eight genes defined the poor prognosis group. IS was additionally validated in three cohorts in relation to overall survival (OS) [47]. IS was significantly higher in the activated B-cell-

nativnog centra (engl. *germinal-center B-cell-like* – GCB) [47]. Isti autori su na ćelijskim linijama LBCL, odvojeno ABC i GCB tipa, testirali efekat helatora gvožđa, deferasiroksa i deferoksamina, na vijabilnost limfomskih ćelija i videli, u zavisnosti od koncentracije, smanjenu vijabilnost ćelija nakon tretmana helatorima gvožđa. U istom radu je testiran i terapijski efekat irinomicina i ustanovljeno da u nanomolarnim koncentracijama inhibira rast tumorskih ćelija. Suplementacija gvožđem je inhibirala efekat helatora na limfomske ćelije, ali ne i efekat irinomicina, što je sugerisalo drukčiji mehanizam dejstva. Naime, helatori gvožđa su doveli do apoptoze limfomskih ćelija a irinomicin je dovođio do sekvestracije gvožđa u lizozomima, deplecije gvožđa u ćelijskom citosolu i degradacije ferroportina autofagijom u lizozomima, te produkcije reaktivnih kiseoničnih radikala koji su vodili ka ferroptozu [47].

Šmit i saradnici, u radu objavljenom 2021. godine, pokazuju da dimetilfumarat, lek odobren u terapiji multiple skleroze i psorijaze, izazivajući ferroptozu i slabeći NF- κ B/STAT 3 signalni put deluje na LBCL ćelije i ABC i GCB tipa [48].

Grupa naučnika iz Kine je, pre godinu dana, objavila da je povišen nivo glutamina povezan sa lošijim ishodom kod obolelih od LBCL, a da je koncentracija derivata glutamina, α -keto-glutarata, obrnuto korelirala sa agresivnošću LBCL [49]. Akumulacija α -keto-glutarata je dovođila do oksidativnog stresa u dabl hit limfomu (engl. *double-hit lymphoma* – DHT). Visok nivo slobodnih kiseoničnih radikala izazivao je akumulaciju lipidnih peroksida, došlo je do aktivacije TP53 i ferroptoze [49].

ZAKLJUČAK

Sve je više radova koji se bave ferroptozom u različitim malignitetima, pa i hematološkim. Ispituju se potencijalno nove uloge već poznatih lekova i mogućnosti njihove šire primene i sinergističkog dejstva sa standardnom terapijom. Otkrivanje novih mehanizama ćelijske smrti i signalnih puteva koji su u taj proces uključeni dovođi do otkrivanja potencijalno nove ciljne terapije. Sve ovo iz ugla kliničara deluje vrlo interesantno i ohrabrujuće, ali su neophodne dalje kliničke studije i validacija rezultata dobijenih *in vitro* i na laboratorijskim životinjama.

NAPOMENA: Osnove metabolizma gvožđa i ferroptoze su ispitivane u okviru doktorske disertacije prvog autora, odbranjene 2022. godine.

Sukob interesa: Nije prijavljen.

like (ABC) LBCL group, as compared to the germinal-center B-cell-like (GCB) LBCL group [47]. The same authors tested the effect of iron chelators deferasiroksa and deferoksamina on the viability of lymphoma cells on LBCL cell lines (separately ABC and GCB types) and, depending on the concentration, observed reduced cell viability after treatment with iron chelators. In the same study, the therapeutic effect of irinomycin was tested. It was found that nanomolar concentrations of irinomycin inhibited tumor cell growth. Iron supplementation inhibited the effect of chelators on lymphoma cells, but not the effect of irinomycin, suggesting a different action mechanism. Namely, iron chelators led to lymphoma cell apoptosis, while irinomycin led to iron sequestration in lysosomes, iron depletion in the cell cytosol, ferroportin degradation by autophagy in lysosomes, and the production of reactive oxygen radicals leading to ferroptosis [47].

In a study from 2021, Schmidt et al. showed that dimethyl fumarate, a drug approved for the treatment of multiple sclerosis and psoriasis, acted on both ABC LBCL and GCB LBCL cells by causing ferroptosis and weakening the NF- κ B/STAT 3 signaling pathway, [48].

A year ago, a group of scientists from China announced that an elevated level of glutamine was associated with a poorer outcome in LBCL patients, and that the concentration of the glutamine derivative, α -keto-glutarate, was inversely correlated with the aggressiveness of LBCL [49]. Accumulation of α -keto-glutarate led to oxidative stress in double-hit lymphoma (DHT). A high level of free oxygen radicals caused the accumulation of lipid peroxides, leading to the activation of TP53 and to ferroptosis [49].

CONCLUSION

There is an increasing number of studies dealing with ferroptosis in various malignancies, including hematological malignancies. The potential new roles of already-known drugs and the possibilities of their wider application and synergistic effect with standard therapy are being examined. The discovery of new cell death mechanisms and the signaling pathways involved in that process leads to the discovery of potentially new target therapies. From the clinician's point of view, all this seems very interesting and promising, however, further clinical studies and validation of the results obtained *in vitro* and on laboratory animals are necessary.

NOTE: The basic principles of iron metabolism and ferroptosis were investigated in the doctoral dissertation of the first author of this study. The thesis was defended in 2022.

Conflict of interest: None declared.

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HOČKINOV LIMFOM U TRUDNOĆI: PRIKAZ SLUČAJA I PREGLED LITERATURE

PRIKAZ SLUČAJA

CASE REPORT

HODGKIN'S LYMPHOMA IN PREGNANCY: CASE REPORT AND REVIEW OF LITERATURE

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SAŽETAK

Uvod/Cilj: Incidenca Hoćkinovog limfoma (HL) je visoka u reproduktivnom periodu tako da problem javljanja ovog maligniteta tokom trudnoće predstavlja značajan problem u svakodnevnoj kliničkoj praksi. Cilj ovog rada je da se kroz prikaz slućaja prikaže karakteristićni klinićki tok i da se kroz pregled literature analiziraju do sada objavljeni podaci.

Prikaz slućaja: Trudnica, starosne dobi 33 godine, tokom trećeg trimestra trudnoće razvija otok vrata, noćno preznojavanje, svrab uz gubitak telesne mase. Ultrazvućnim pregledom otkrivena je znaćajna limfadenopatija u supraklavikularnoj regiji. Hirurška ekstirpacija limfnog ćvora uz patohistološku verifikaciju potvrdila je dijagnozu Hoćkinovog limfoma. S obzirom na trudnoću i ogranićenu primenu radiografske dijagnostike, magnetna rezonanca (MR) je uraćena i potvrdila je generalizovanu limfadenopatiju s posledićnom hidronefrozom desnog bubrega. Nakon postavljanja dijagnoze uznapredovalog stadijuma HL, odlućeno je da se zapoćne specifićno hematološko lećenje primenom citostatske terapije. Porodaj je sproveden u 37. nedelji gestacije prirodnim putem, bez komplikacija po majku i novoroćdenće. Nakon porodaja, sprovedena je kompletna reevalucija bolesti, uz odluku da se lećenje nastavi do ukupno šest ciklusa hemioterapije prema ABVD (adriablastin, bleomicin, vinblastin, dakarbazin) protokolu. Nakon šest ciklusa hemioterapije, postignuta je metabolićka remisija bolesti.

Zakljućak: Lećenje Hoćkinovog limfoma tokom trudnoće predstavlja kompleksan izazov koji zahteva koordinisan multidisciplinarni pristup i personalizovani plan lećenja.

Kljućne reći: Hoćkinov limfom, trudnoća, ABVD

ABSTRACT

Introduction/Objective: The incidence of Hodgkin lymphoma (HL) is high during the reproductive years, making the occurrence of this malignancy during pregnancy a significant issue in everyday clinical practice. The aim is to present a case study demonstrating the characteristics of clinical courses and analyze published data through a literature review.

Case report: A 33-year-old pregnant woman in her third trimester developed neck swelling, night sweats, itching, and weight loss. Ultrasound examination revealed significant lymphadenopathy in the supraclavicular region. Surgical excision of the lymph node and pathological verification confirmed the diagnosis of Hodgkin lymphoma. Given the pregnancy and the limited use of radiographic diagnostics, magnetic resonance imaging (MR) was performed and confirmed generalized lymphadenopathy with resultant hydronephrosis of the right kidney. After diagnosing advanced-stage HL, it was decided to initiate specific hematologic treatment with chemotherapy. Delivery was carried out at 37 weeks of gestation via vaginal delivery, with no complications for the mother or newborn. Following delivery, a complete disease reevaluation was conducted, and it was decided to continue treatment with a total of six cycles of chemotherapy according to the ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) protocol. After six cycles of chemotherapy, metabolic remission of the disease was achieved.

Conclusion: Managing Hodgkin lymphoma during pregnancy presents a complex challenge requiring a coordinated multidisciplinary approach and a personalized treatment plan.

Keywords: Hodgkin lymphoma, pregnancy, ABVD

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UVOD

Hoćkinov limfom predstavlja hronično maligno limfoproliferativno oboljenje sa bimodalnom distribucijom javljanja, i to u trećoj deceniji života, kao i u šestoj deceniji [1]. Imajući u vidu da prvi pik obuhvata i žene u reproduktivnom periodu, literaturna incidenca javljanja za vreme trudnoće je oko 3% ukupnih pacijenata. [2]. Prema sistematskom pregledu literature objavljenom 2022. godine gde je cilj ispitivanja bio procena krajnjeg ishoda kod trudnice i ploda nakon postavljanja dijagnoze Hoćkinovog limfoma za vreme trudnoće, zaključak je bio da se incidenca kongenitalnih malformacija kao i spontanijih pobačaja statistički značajno ne razlikuje kod trudnica sa HL u odnosu na trudnice kod kojih navedena bolest nije bila dijagnostikovana. Od ostalih mogućih ishoda izdvojila se veća učestalost prevremenih porođaja, anemijskog sindroma koji zahteva primenu krvnih komponentata, kao i venskog tromboembolizma [3]. Cilj ovog rada je da se kroz prikaz slučaja prikaže karakteristični klinički tok i da se kroz pregled literature analiziraju do sada objavljeni podaci.

PRIKAZ SLUČAJA

Trudnica, starosne dobi 33 godine, tokom trećeg trimestra treće trudnoće razvija otok vrata, noćno preznojavanje, svrab uz gubitak telesne mase. Ultrazvučnim pregledom mekih tkiva vrata opisana je signifikantna limfadenopatija dominantno u donjem jugularnom spratu i supraklavikularno levo, promera 30x24 mm i 43x20 mm. Tada se u laboratorijskim analizama u nalazu KKS izdvojila leukocitoza sa neutrofilijom i monocitozom (Le 23,9x10⁹/L, Neu 20,3 x10⁹/L, Mon 1,6x10⁹/L), mikrocitna anemija srednje teškog stepena (Hgb 86 g/L, MCV 72,4 fL), trombocitoza (Tr 626x10⁹/L), a u biohemijskim analizama hipoalbuminemija (albumin 30g/L), hipoferemija uz hiperferitinemiju (Fe 4,1 umol/L, TIBC 59,1 umol/L, feritin 798,7 ug/L), povišen nivo alkalne fosfataze (ALP 274 U/L), laktat dehidrogenaze (LDH 674 U/L), beta 2 mikroglobulina (beta 2 M 3,59 mg/L), C reaktivnog proteina (CRP 162,9 mg/L), i u testovima hemostaze hiperfibrinogenemija (fibrinogen 8,1 g/L). Ostali nalazi su bili u granicama referentnih vrednosti. Ginekološki pregled je bio uredan. Odlučeno je da se navedeni limfni nodus supraklavikularne regije levo ekstirpira u celosti. Na osnovu pristiglog histopatološkog nalaza postavljena je dijagnoza HL, tipa nodularne skleroze, sa karakterističnim imunofenotipom: LCA-, CKPAN-, OCT2-, BOB1-, Pax-5-/+, CD79alfa -, CD20-, CD19-, CD15-/+, CD30+, Fascin +, MUM-1+, EBV-, bcl-6+, CD34-, CD3-, CD5-, CD4-, CD8-, ALK-. U okviru dopunske dijagnostike urađen je pregled vrata, grudnog koša, abdomena i male karlice magnetnom rezonan-

INTRODUCTION

Hodgkin's lymphoma is a chronic malignant lymphoproliferative disease with a bimodal distribution of occurrence in the third decade of life as well as in the sixth decade [1]. Considering that the first peak also includes women in the reproductive period, the literature incidence of occurrence during pregnancy is about 3% of total patients. [2]. According to a systematic review of the literature published in 2022, where the study aimed to assess the final outcome of pregnant women and the fetus after the diagnosis of Hodgkin's lymphoma during pregnancy, the conclusion was that the incidence of congenital malformations as well as spontaneous abortions is not statistically significantly different in pregnant women with HL compared to pregnant women in whom the mentioned disease was not diagnosed. Among the other possible outcomes, a higher frequency of premature births, anemic syndrome requiring the use of blood components, and venous thromboembolism [3] were singled out. This paper aims to present a characteristic clinical course through a case report and analyze the data published so far through a literature review.

CASE REPORT

A pregnant woman, aged 33, during the third trimester of her third pregnancy, developed swelling of the neck, night sweats, and itching with loss of body weight, an ultrasound examination of the soft tissues of the neck described significant lymphadenopathy dominantly in the lower jugular floor and supraclavicular left, diameter 30x24mm, and 43x20mm. Then, in the laboratory analysis, leukocytosis with neutrophilia and monocytosis (Le 23.9x10⁹/L, Neu 20.3x10⁹/L, Mon 1.6x10⁹/L), microcytic anemia of medium severity (Hgb 86 g/L, MCV 72.4 fL) were distinguished., thrombocytosis (Tr 626x10⁹/L), and in biochemical analyzes hypoalbuminemia (albumin 30g/L), hypoferrinemia with hyperferritinemia (Fe 4.1 umol/l, TIBC 59.1 umol/L, ferritin 798.7 ug/L), elevated level of alkaline phosphatase (ALP 274 U/L), lactate dehydrogenase (LDH 674 U/L), beta 2 microglobulin (beta 2 M 3.59 mg/L), C reactive protein (CRP 162.9 mg/L), and in hemostasis tests hyperfibrinogenemia (fibrinogen 8.1 g/L). Other findings were within reference values. The gynecological examination was in order. It was decided to extirpate the said lymph node of the left supraclavicular region in its entirety. Based on the received histopathological findings, a diagnosis of HL, a type of nodular sclerosis, with a characteristic immunophenotype: LCA-, CKPAN-, OCT2-, BOB1-, Pax-5-/+, CD79alfa -, CD20-, CD19-, CD15-/+, CD30+, Fascin +, MUM-1+, EBV-, bcl-6+, CD34-, CD3-, CD5-, CD4-, CD8-, ALK-. As part of additional diagnostics, an examination

com (MR), gde je uočena limfadenopatija supraklavikularno (najvećeg promera do 44x22 mm, mediastinalno u svim grupama (najvećeg promera do 28x15 mm), abdominalno u vidu konglomerata dominantno u hilusu jetre (promera 61x28x46 mm), paraaortalno (promera 55x24x39 mm) i parailijačno (promera 31x18x30 mm), sa opisanom hidronefrozom gr. II desnog bubrega. Ehokardiografski pregled je bio uredan uz očuvanu globalnu sistolnu funkciju leve komore (ejekciona frakcija 70%). Odluka o specifičnom hematološkom lečenju je nakon sprovedene dijagnostike bila odložena zbog SARS-CoV-2 pozitivnosti i blaže kliničke slike Covid-19. Pacijentkinja je lečena primenom empirijske antibiotske terapije širokog spektra uz tromboprolaksu niskomolekularnim heparinom i ostalu simptomatsko - suportivnu terapiju. Po oporavku, kod pacientkinje sa dijagnozom HL, tipa nodularne skleroze, u IIIB kliničkom stadijumu, EORTC/LYSA i GHSG - "advanced stage", odlučeno je da se započne lečenje osnovne bolesti prema protokolu ABVD, inicijalno primenom dva ciklusa nakon čega je planirana procena interim PET/CT pregledom, uz redovno praćenje sa perinatološke strane (biometrija, cervikometrija i praćenje stanja ploda) neposredno pre primene antineoplastične terapije. Dve nedelje nakon sprovedenog d15 II ciklusa ABVD protokola bio je planiran porođaj carskim rezom u 37. nedelji gestacije, ali se pacientkinja u tom terminu porodila prirodnim putem, bez pratećih komplikacija. Od strane neonatologa konstatovan je uredan i klinički i laboratorijski nalaz kod ploda. Interim PET/CT pregled je urađen februara 2024. i ukazao je na diskretno povišen metabolizam glukoze u limfnim čvorovima u IV jugularnoj grupi u vratu obostrano, mediastinalnim limfnim čvorovima retrosternalno i limfnim čvorovima hepatogastrično - Deauville skor 3. Imajući u vidu navedeni nalaz, kao i inicijalni stadijum bolesti, odlučeno je da se lečenje nastavi sa još četiri ciklusa hemioterapije prema ABVD protokolu. Terapiju je dobro podnosila i nije bilo pratećih komplikacija niti indikovanih hospitalizacija između ciklusa. Lečenje je kompletirano sa ukupno šest ciklusa ABVD protokola, a po završetku lečenja kontrolni PET/CT je ukazao na metaboličku remisiju osnovne bolesti.

DISKUSIJA

Hoćkinov limfom je jedan od najčešćih maligniteta u trudnoći, s obzirom na činjenicu da je incidenca njegovog javljanja najčešća u reproduktivnom periodu. Prema Korkontzelos I. i saradnicima [4], incidence javljanja je 1 slučaj HL na 6000 trudnoća. Karakteristike ovog maligniteta se ne razlikuju kod trudnica, u odnosu na opštu populaciju. Pacijentkinje sa dijagnozom HL u trudnoći imaju trogodišnje preživljavanje od 85% [5].

of the neck, chest, abdomen, and pelvis was performed with magnetic resonance imaging (MR), where lymphadenopathy was observed supraclavicularly (with the largest diameter up to 44x22 mm, mediastinally in all groups (with the largest diameter up to 28x15 mm), abdominally in the form of conglomerates, dominantly in hilus of the liver (diameter 61x28x46 mm), paraaortic (diameter 31x18x30 mm), with described hydronephrosis gr. II of the right kidney. The echocardiographic examination was regular, with a preserved global systolic function (ejection fraction of 70%). Hematological treatment was postponed due to SARS-CoV-2 positivity and a milder clinical picture of COVID-19. The patient was treated with empiric broad-spectrum antibiotic therapy, thromboprophylaxis, low molecular weight heparin, and other symptomatic-supportive therapy. Type of nodular sclerosis, in the IIIB clinical stage, EORTC/LYSA and GHSG - "advanced stage," it was decided to start the treatment of the underlying disease according to the ABVD protocol, initially by applying two cycles, after which an interim PET/CT examination is planned, with regular monitoring from the perinatal side (biometry, cervicometry and monitoring of the condition of the fetus) immediately before the application of antineoplastic therapy. Two weeks after the d15 II cycle of the ABVD protocol, cesarean delivery was planned in the 37th week of gestation, but the patient gave birth naturally at that time without complications. The neonatologist noted that the clinical and laboratory findings of the fetus were normal. An interim PET/CT examination was performed in February 2024 and indicated discretely elevated glucose metabolism in lymph nodes in the IV jugular group in the neck bilaterally, mediastinal lymph nodes retrosternal and hepatogastric lymph nodes - Deauville score 3. Considering the above finding, as well as the initial stage of the disease, it was decided to continue the treatment with four more cycles of chemotherapy according to the ABVD protocol. She tolerated the therapy well, and there were no accompanying complications or indicated hospitalizations between cycles. The treatment was completed with a total of six cycles of the ABVD protocol. At the end of the treatment, the control PET/CT indicated a metabolic remission of the underlying disease.

DISCUSSION

Hodgkin's lymphoma is one of the most common malignancies in pregnancy, considering the fact that the incidence of its occurrence is most common in the reproductive period. According to Korkontzelos I. and colleagues [4], the incidence of occurrence is 1 case of HL per 6000 pregnancies. The characteristics of this

Samo postavljanje dijagnoze HL tokom trudnoće može biti značajno prolongirano, imajući u vidu da simptomi koji ukazuju na postojanje HL mogu biti pripisani tegobama koje se i inače javljaju u trudnoći (zamaranje, malaksalost, dispneja, noćno preznojava-nje). Sličan sled događaja se desio i kod naše pacijent-kinje, imajući u vidu da je od pojave prvih simptoma do definitivnog postavljanja dijagnoze proteklo nešto manje od dva meseca. Drugi problem predstavlja li-mitiranost dostupnosti različitih dijagnostičkih proce-dura. Dok biopsija limfne žlezde ne predstavlja rizik ni za majku, ni za fetus, određene radiografske metode, poput kompjuterizovane tomografije (CT) i pozitron-ske emisije tomografije (PET) mogu u određenoj meri biti rizične, pre svega za fetus [6]. Bezbednim se smatra primena magnetne rezonance i ultrazvučnih metoda, a eventualna radiografija se može razmotriti u cilju procene proširenosti bolesti ili kod nekih akutnih stanja, uz obaveznu upotrebu zaštitne kecelje. Ipak, od krucijalne važnosti je sprovesti planiranu dijagnostiku i evaluaciju hematološkog statusa, u cilju adekvatne odluke o lečenju. Multidisciplinarni pristup je neop-hodan radi procene optimalnog trenutka započinjanja specifičnog hematološkog lečenja, uz personalizovan pristup svakom pacijentu i koordinaciju hematologa, ginekologa i neonatologa, gde je potrebno uzeti u ob-zir potencijalnu korist i rizik od primene ili odlaganja ciljane terapije. Svakako je neophodno poštovati želju pacijentkinje za nastavkom ili prekidom trudnoće u da-tom trenutku. Ukoliko se pacijentkinja odluči za prekid trudnoće, pristup lečenju je identičan kao i kod opšte populacije.

Kod ranih stadijuma bolesti (IA i IIA po Ann Arbor klasifikaciji), ne postoji zvaničan konsenzus o primeni antineoplastične terapije. Preporuke su da, ukoliko je to moguće, lečenje bude odloženo do porođaja. Tho-mas, P.R. i saradnici [7] su pokazali na studiji od 19 pa-cijentkinja da odlaganje terapije do porođaja zaista predstavlja adekvatan izbor, ne ugrožavajući ni majku ni fetus. Sa druge strane, Aviles, A. i saradnici [8] su u svojoj studiji koja je obuhvatila 44 pacijentkinje sa di-jagnozom HL tokom trudnoće u ranom stadijumu bo-lesti, ukazali na značaj primene ABVD protokola, koji predstavlja zlatni standard u lečenju HL. Rezultati stu-dije su pokazali da nije bilo kongenitalnih malforma-cija, kao i da su PFS (period do progresije bolesti) i OS (ukupno preživljavanje) tokom medijane praćenja od 120,4 meseca iznosili 95%, odnosno 93%, redom.

Kod uznapredovalih stadijuma bolesti, odlaganje primene specifične terapije može, pak, predstavljati veći rizik od započinjanja specifičnog lečenja. I u ovoj grupi pacijenata, ABVD protokol još uvek predstavlja terapiju izbora. Alternativu može predstavljati A-AVD

malignancy do not differ in pregnant women com-pared to the general population. Patients diagnosed with HL during pregnancy have a three-year survival rate of 85% [5].

Making a diagnosis of HL during pregnancy can be significantly prolonged, bearing in mind that symp-toms indicating the existence of HL can be attributed to complaints that also occur during pregnancy (fatigue, weakness, dyspnea, night sweats). A similar sequence of events happened with our patient, bearing in mind that less than two months passed from the appearance of the first symptoms to the definitive diagnosis. An-other problem is the limited availability of various di-agnostic procedures. While biopsy of the lymph gland poses no risk to either the mother or the fetus, certain radiographic methods, such as computed tomography (CT) and positron emission tomography (PET), can be somewhat risky, primarily for the fetus [6]. The appli-cation of magnetic resonance and ultrasound meth-ods is considered safe, and possible radiography can be considered to assess the spread of the disease or, in some acute conditions, with the mandatory use of a protective apron. Nevertheless, it is crucial to carry out planned diagnostics and evaluation of the hematolog-ical status to make an adequate decision on treatment. A multidisciplinary approach is necessary to assess the optimal moment of starting a specific hematological treatment, with a personalized approach to each pa-tient and the coordination of hematologists, gynecolo-gists, and neonatologists, where it is necessary to take into account the potential benefits and risks of apply-ing or delaying targeted therapy. It is certainly essen-tial to respect the patient's wish to continue or termi-nate the pregnancy at a given moment. If the patient decides to terminate the pregnancy, the approach to treatment is identical to that of the general population.

In the early stages of the disease (IA and IIA, ac-cording to the Ann Arbor classification), there is no official consensus on the use of antineoplastic thera-py. Recommendations are that, if possible, treatment should be delayed until childbirth. Thomas, P.R. et al. [7] showed in a study of 19 patients that postponing therapy until childbirth really represents an adequate choice without endangering either the mother or the fetus. On the other hand, Aviles, A. et al. [8] in their study, which included 44 patients diagnosed with HL during pregnancy in the early stages of the disease, pointed out the importance of applying the ABVD protocol, which represents the gold standard in the treatment of HL. The study's results showed no congenital malfor-mations and that PFS (period to disease progression) and OS (overall survival) during a median follow-up of 120.4 months were 95% and 93%, respectively.

protokol (brentuksimab vedotin, adriablastin, vinblastin, dakarbazin), ali za sada ne postoji dovoljno podataka o potencijalnom teratogenom efektu brentuksimaba [9,10]. Alkilirajući agensi su poznati kao izuzetno teratogeni, te njih svakako treba izbegavati kod trudnica, posebno u prvom trimestru trudnoće [11]. Imajući u vidu činjenicu da se tokom prvog trimestra odvija organogeneza i da je tada fetus izuzetno vulnerabilan, ne preporučuje se primena bilo kakve antineoplastične terapije u ovom periodu. U većem broju studija, primena ABVD protokola u drugom i trećem trimestru trudnoće kod trudnica sa HL pokazala se bezbednom, ne izazivajući komplikacije kako kod majke, tako ni kod fetusa [12-18]. Ipak, Cotteret, C. i saradnici su došli do zaključka da primena doksorubicina tokom trudnoće može dovesti do prolazne disfunkcije leve komore kod novorođenčeta, uz kompletnu restituciju mesec dana nakon porođaja [19]. Studija [20] koja je obuhvatila 405 pacijentkinja sa dijagnozom HL lečenih BEACOPP (bleomicin, etopozid, doksorubicin, ciklofosfamid, vinkristin, prokarbazin, prednison) protokolom, došla je do zaključka da je čak 51,4% pacijentkinja razvilo permanentnu amenoreju, s višom stopom kod pacijentkinja lečenih eskalirajućim BEACOPP protokolom u odnosu na standardni. Zbog svega navedenog, ABVD protokol se smatra relativno bezbednim za lečenje HL u trudnoći.

Radioterapija nosi sledstveni rizik od kongenitalnih anomalija kod odojčadi i adolescenata koji su bili izloženi zračenju *in utero*, i to uglavnom ako uzmemo u obzir doze veće od 50 Gy. Samim tim, primena radioterapije u trudnoći ne predstavlja apsolutnu kontraindikaciju, posebno kada se radi o supradijafragmalnoj lokalizaciji tumora, ali i ovo je jedna od odluka koja mora biti multidisciplinarno sagledana zbog potencijalnih rizika koje nosi [21].

Još jedan od izazova predstavlja i izmenjena farmakokinetika tokom trudnoće. Hipoalbuminemija, koja se može javiti u trudnoći, povećava slobodnu frakciju lekova u plazmi, te tako menja njihovu distribuciju. Treba imati u vidu da se brojni citostatici izlučuju putem mleka, te je dojenje tokom primene citostatika kontraindиковano. Određeni antineoplastični lekovi mogu perzistirati u mleku određeni period nakon primene leka, te se savetuje da se dojenje odloži minimum 3-6 nedelja po kompletiranju lečenja.

Prilikom odluke o idealnom terminu porođaja neophodan je personalizovan pristup. Ukoliko je to moguće, porođaj treba odložiti najmanje do 35. nedelje gestacije. Takođe, zbog mijelosupresivnog efekta određenih citostatika i potencijalne jatrogene aplazije koštane srži, porođaj treba planirati minimum tri nedelje po kompletiranju terapije, odnosno, kada se postigne

In advanced stages of the disease, delaying the application of specific therapy may, on the other hand, represent a greater risk than starting specific treatment. Even in this group of patients, the ABVD protocol is still the therapy of choice. An alternative may be the A-AVD protocol (brentuximab vedotin, adriablastine, vinblastine, dacarbazine), but for now, there is not enough data on the potential teratogenic effect of brentuximab [9,10]. Alkylating agents are known to be highly teratogenic and should be avoided in pregnant women, especially in the first trimester of pregnancy [11]. Given the fact that organogenesis takes place during the first trimester and that then the fetus is highly vulnerable, it is not recommended to use any antineoplastic therapy during this period. In a large number of studies, the application of the ABVD protocol in the second and third trimesters of pregnancy in pregnant women with HL has been shown to be safe without causing complications in either the mother or the fetus [12-18]. Nevertheless, Cotteret, C., and co-workers concluded that the administration of doxorubicin during pregnancy can lead to transient left ventricular dysfunction in the newborn, with complete restitution one month after delivery [19]. A study [20] that included 405 patients diagnosed with HL treated with BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) protocol concluded that as many as 51.4% of patients developed permanent amenorrhea, with a higher rate in female patients treated with an escalating BEACOPP protocol compared to the standard one. Because of all the above, the ABVD protocol is considered relatively safe for the treatment of HL in pregnancy.

Radiotherapy carries a consequent risk of congenital anomalies in infants and adolescents who were exposed to radiation in utero, especially if we consider doses higher than 50 Gy. Therefore, the use of radiotherapy during pregnancy does not represent an absolute contraindication, especially when it comes to supradiaphragmatic tumor localization, but this is also one of the decisions that must be considered multidisciplinary because of the potential risks it carries [21].

Another challenge is the altered pharmacokinetics during pregnancy. Hypoalbuminemia, which can occur in pregnancy, increases the free fraction of drugs in plasma, thus changing their distribution. Many cytostatics are excreted through milk, so breastfeeding during the use of cytostatics is contraindicated. Certain antineoplastic drugs can persist in milk for a certain period after the drug is administered, so it is advised to postpone breastfeeding for at least 3-6 weeks after completing the treatment.

odgovarajući oporavak hematoloških parametara. Porođaj je kod naše pacijentkinje planiran prema aktuelnim preporukama, tri nedelje nakon primene d15 drugog ciklusa ABVD protokola, u 37. nedelji gestacije, kada je po uvidu u klinički i laboratorijski status završen prirodnim putem, bez pratećih komplikacija.

Sistematski pregled Houlihana i saradnika [3] je ukazao da ne postoji statistički značajna razlika u stepenu javljanja kongenitalnih malformacija i spontanih pobačaja kod trudnica sa dijagnozom HL u odnosu na trudnice u opštoj populaciji. Ipak, kod pacijentkinja sa dijagnozom HL češće se javljaju prevremeni porođaji, što može biti povezano sa planiranim pretermijskim završavanjem trudnoće, češće nego spontanim, bilo kao odluka tima lekara ili odluka same pacijentkinje kako bi se izbegla primena terapije tokom trudnoće. Takođe, češći su venski tromboembolijski događaji (VTE), što je očekivano, s obzirom na činjenicu da sama trudnoća doprinosi hiperkoagulabilnosti i venskoj stazi. Kod naše pacijentkinje je po započinjanju lečenja primenjivana i profilaktička doza niskomolekularnog heparina, imajući u vidu i dobro poznat pojam tromboza udruženih sa kancerom, gde aktivni malignitet i primena antineoplastične terapije dodatno povećavaju rizik od VTE.

ZAKLJUČAK

Hoćkinov limfom predstavlja hematološki malignitet sa dobrom prognozom i visokom stopom izlečenja. Lečenje pacijentkinja sa HL tokom trudnoće nosi sa sobom brojne izazove, polazeći od dijagnostike, preko izbora odgovarajuće terapije i momenta započinjanja lečenja, do donošenja odluke o terminu završavanja trudnoće. Kompleksnost lečenja potvrđuje i činjenica o neophodnosti prisustva multidisciplinarnog tima u cilju razmatranja prethodno navedenih odluka. Studije ukazuju na obećavajuće rezultate kod trudnica lečenih ABVD protokolom. Ipak, dodatna istraživanja su neophodna za dalja saznanja, kao i dugoročno praćenje potencijalnih postterapijskih posledica kod dece rođene od majki sa dijagnozom HL koje su lečene tokom trudnoće.

Sukob interesa: Nije prijavljen.

A personalized approach is necessary when deciding on the ideal date of birth. If possible, childbirth should be postponed until at least the 35th week of pregnancy. Also, due to the myelosuppressive effect of certain cytostatics and potential iatrogenic bone marrow aplasia, childbirth should be planned at least three weeks after the completion of therapy; that is when appropriate recovery of hematological parameters is achieved. The birth of our patient was planned according to current recommendations, three weeks after the application of d15 of the second cycle of the ABVD protocol, in the 37th week of gestation, when, according to the clinical and laboratory status, it was completed naturally, without accompanying complications.

A systematic review by Houlihan et al. [3] indicated that there is no statistically significant difference in the incidence of congenital malformations and spontaneous abortions in pregnant women diagnosed with HL compared to pregnant women in the general population. However, in patients diagnosed with HL, premature births occur more often, which may be related to planned premature termination of pregnancy, more often than spontaneous, either as a decision of the medical team or the decision of the patient herself to avoid the use of therapy during pregnancy. Also, venous thromboembolic events (VTE) are more common, which is expected, given the fact that pregnancy itself contributes to hypercoagulability and venous congestion. Our patient was given a prophylactic dose of low-molecular-weight heparin after the start of treatment, bearing in mind the well-known concept of thrombosis associated with cancer, where active malignancy and the use of antineoplastic therapy additionally increase the risk of VTE.

CONCLUSION

Hodgkin's lymphoma is a hematological malignancy with a good prognosis and a high cure rate. Treatment of patients with HL during pregnancy brings with it numerous challenges, starting from diagnosis, through the selection of appropriate therapy and the moment of starting treatment, to deciding on the date of termination of pregnancy. The complexity of the treatment confirmed the fact of the necessity of the presence of a multidisciplinary team to review the aforementioned decisions. Studies indicate promising results in pregnant women treated with the ABVD protocol. However, additional research is necessary for further knowledge, as well as long-term monitoring of potential post-therapy consequences in children born to mothers diagnosed with HL who were treated during pregnancy.

Conflict of interest: None declared.

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MULTIPLI MIJELOM SA DISEMINACIJOM U CENTRALNI NERVNI SISTEM – PRIKAZ SERIJE SLUČAJA

PRIKAZ SLUČAJA

CASE REPORT

MULTIPLE MYELOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT – CASE SERIES

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SAŽETAK

Uvod: Multipli mijelom sa diseminacijom u centralni nervni sistem (CNS-MM) predstavlja veoma redak entitet koji čini manje od 1% ekstrapedularnih multiplih mijeloma, a manifestuje se spektrom neuroloških deficita. Modaliteti lečenja mogu biti lokalni, uključujući intratekalnu hemioterapiju i radioterapiju, kao i sistemski, uključujući autolognu/alogeni transplantaciju matičnih ćelija hematopoeze (ATMČH).

Cilj: Prikazujemo iskustvo našeg centra u lečenju ovog retkog entiteta.

Prikaz bolesnika: Kod prve pacijentkinje sa dijagnozom multiplog mijeloma BJ lambda CS IIIA R-ISS 2, verifikovana je tumorska masa u bazi lobanje sa destrukcijom desne sfenoidne i temporalne kosti, koja se manifestovala paralizom *n. facialis-a*. Pacijentkinja je lečena po CVD (ciklofosfamid, bortezomib, deksametazon) protokolu sa postignutom parcijalnom remisijom, tumorska promena je palijativno ozračena, potom je sprovedena autologna transplantacija matičnih ćelija hematopoeze. Lečenje je nastavljeno po DaraRd (daratumumab, lenalidomid, deksametazon) protokolu sa ciljem sprovođenja druge autologne transplantacije matičnih ćelija hematopoeze po produbljivanju remisije. Dosadašnje ukupno preživljavanje iznosi 20 meseci. Kod druge pacijentkinje sa dijagnozom multiplog mijeloma BJ kappa CS IIIA ISS 2, diseminacija u CNS se desila u prvom relapsu bolesti. Sprovedena je zračna terapija endokranijuma, potom je ordinirano sedam ciklusa po PAD (bortezomib, doksorubicin, deksametazon) protokolu sa efektom progresije bolesti i kasnije smrtnim ishodom. Ukupno preživljavanje iznosilo je 48 meseci; preživljavanje od dijagnostikovanja infiltracije CNS-a je bilo 25 meseci.

Zaključak: Uprkos napretku u dijagnostici i lečenju, prognoza za CNS-MM ostaje loša zbog kompleksnog i agresivnog kliničkog ponašanja, a zbog niske incidencije i ograničenosti podataka, postoji potreba za daljim studijama sa ovom malom grupom pacijenata.

Ključne reči: ekstrapedularni multipli mijelom, radioterapija, hemioterapija, transplantacija matičnih ćelija hematopoeze

ABSTRACT

Introduction: Central nervous system involvement in multiple myeloma (CNS-MM) is a very rare entity accounting for less than 1% of all extramedullary multiple myeloma, which manifests as a variety of neurological deficits. Treatment modalities can be locally administered therapy, including intrathecal chemotherapy and radiotherapy, as well as systemic therapy, including autologous/allogeneic hematopoietic stem cell transplantation (AHSCT).

The aim: This article aims to present the experience of our center in the treatment of this rare entity.

Case reports: The first patient was diagnosed with multiple myeloma BJ lambda CS IIIA R-ISS 2 with a tumor mass located at the base of the skull, causing right facial nerve paralysis. The patient underwent six treatment cycles of the CVD (cyclophosphamide, bortezomib, dexamethasone) regimen, achieving partial remission, followed by palliative radiation and autologous stem cell transplantation. The treatment was continued with the DaraRd (daratumumab, lenalidomide, dexamethasone) regimen aimed at a second stem cell transplant which is to be carried out upon deepening remission. The overall survival of the patient, so far, is 20 months. The second patient with multiple myeloma BJ kappa CS IIIA ISS 2 developed CNS involvement with the first recurrence of the disease. Cranial radiation and seven cycles of the PAD (bortezomib, doxorubicin, dexamethasone) regimen were carried out, leading to disease progression and death. The overall survival of the patient was 48 months, with 25 months survival since the diagnosis of CNS infiltration.

Conclusion: Despite advances in diagnosis and treatment, the prognosis for CNS-MM remains poor because of its complex and aggressive clinical behavior. Due to its low incidence, available data are limited indicating the need for further studies involving this small group of patients.

Keywords: extramedullary multiple myeloma, radiotherapy, chemotherapy, hematopoietic stem cell transplantation

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UVOD

Multipli mijelom sa diseminacijom u centralni nervni sistem (CNS-MM) predstavlja veoma redak entitet sa incidencijom manjom od 1% svih ekstramedularnih multiplih mijeloma [1]. U toku protekle decenije primećen je porast incidencije ovog entiteta, prevashodno zbog poboljšanja dijagnostike, lečenja i posledičnog produženja preživljavanja obolelih [2]. Može se dijagnostikovati na početku bolesti, ali se češće utvrđuje u relapsu, odnosno, tokom progresije bolesti [1]. Diseminacija može biti hematogenim putem ili direktnom invazijom, a lezije mogu biti intraparenhimalne i/ili leptomeningealne, sa spektrom neuroloških manifestacija [3]. Pokazano je da je zahvaćenost moždanih ovojnica povezana sa najlošijom prognozom, sa medijanom ukupnog preživljavanja (engl. *median overall survival* – *mOS*) od dva do šest meseci [1,3,4], dok u ostalim slučajevima *mOS* iznosi do 25 meseci [5,6].

Dijagnostička obrada, pored one standardne za multipli mijelom, podrazumeva CT/MR endokranijuma i kičme, lumbalnu punkciju i analizu cerebrospinalne tečnosti, kao i biopsiju tumorskog tkiva [4,7]. Modaliteti lečenja mogu biti lokalni, uključujući intratekalnu hemioterapiju i radioterapiju, te sistemski, uključujući autolognu/alogenu transplantaciju matičnih ćelija hematopoeze (ATMČH) [4].

Uprkos napretku u dijagnostici i lečenju, prognoza za CNS-MM ostaje loša zbog kompleksnog i agresivnog kliničkog ponašanja, nastalog najčešće na terenu progresivne bolesti i usled prethodno sprovedenih terapijskih modaliteta [4,8–10]. Zbog niske incidencije, dostupni podaci su ograničeni, što ukazuje na potrebu za daljim studijama usmerenih na ovu malu grupu pacijenata.

Cilj ovog rada je da prikaže iskustvo našeg centra u lečenju ovog retkog entiteta.

Prikaz slučaja 1

Kod pacijentkinje starosti 62 godine se bolest manifestovala patološkom frakturom leve klavikule i levog humerusa decembra 2022. godine. Na učinjenom CT pregledu opisane su difuzne osteolitičke promene na dugim kostima, vratnim i torakalnim kičmenim pršljenovima i lobanji. Istog meseca je na Institutu za ortopediju Banjica učinjena resekcija i stabilizacija levog humerusa sa biopsijom. Patohistološkim nalazom postavljena je dijagnoza dobro diferentovanog plazmocitoma. Na Institutu za onkologiju i radiologiju Srbije je sprovedena dopunska dijagnostika, marta 2023. godine. U bioptatu koštane srži utvrđena je infiltracija sa 60% patoloških plazmocita. Elektroforezom i imunofiksacijom seruma i urina registrovani su monoklonski lambda laki lanci. Citogenetskom *FISH*

INTRODUCTION

Central nervous system involvement in multiple myeloma (CNS-MM) is a very rare entity with an incidence lower than 1% of all extramedullary multiple myeloma [1]. Over the past decade, an increase in the incidence of this entity was observed, primarily due to the improvement of diagnostics, treatment, and consequent prolongation of survival time of the affected patients [2]. It can be diagnosed at the onset of the disease, but it is more often determined in recurrence, i.e., during the progression of the disease [1]. Dissemination can be hematogenous or by direct invasion, and the lesions can be intraparenchymal and/or leptomeningeal, with an array of neurological manifestations [3]. It has been shown that meningeal involvement is associated with the worst prognosis, with a median overall survival (*mOS*) of two to six months [1,3,4], while in other cases *mOS* is up to 25 months [5,6].

Diagnostics, in addition to the standard procedures for multiple myeloma, include CT/MR of the endocranium and spine, lumbar puncture and analysis of cerebrospinal fluid, as well as tumor tissue biopsy [4,7]. Treatment modalities can be local, including intrathecal chemotherapy and radiotherapy, or systemic, including autologous/allogeneic hematopoietic stem cell transplantation (HSCT) [4].

Despite developments in diagnostics and treatment, the prognosis for CNS-MM remains poor due to its complex and aggressive clinical behavior, stemming most often from the progression of the disease and previously applied therapeutic modalities [4,8–10]. Due to low incidence, available data are limited, indicating the need for further studies aimed at this small group of patients.

This paper aims to present the experience of our center in the treatment of this rare entity.

Case report 1

In the case of a 62-year-old patient, the disease manifested as a pathological fracture of the left clavicle and left humerus, in December 2022. CT imaging revealed diffuse osteolytic lesions in the long bones, cervical and thoracic spinal vertebrae, and the skull. In the same month, resection and stabilization of the left humerus with biopsy were performed at the Institute for Orthopedic Surgery *Banjica*. Pathohistological findings verified the diagnosis of well-differentiated plasmacytoma. Additional diagnostic procedures were carried out at the Institute of Oncology and Radiology of Serbia, in March 2023. Bone marrow biopsy revealed a 60% pathological plasma cell infiltration. Monoclonal lambda light chains were registered with electrophoresis and immunofixation of serum and urine. Cyto-

analizom (engl. *fluorescence in situ hybridization*) detektovane su delecija 13q u 80% nukleusa i delecija TP23 u 25% nukleusa. Postavljena je definitivna dijagnoza multiplog mijeloma *BJ lambda CS IIIA, R-ISS 2*. U daljem toku bolesti došlo je do razvoja periferne paralize desnog *n. facialis-a*, zbog čega je učinjen CT endokranijuma na kome je opisana tumorska promena baze lobanje sa destrukcijom dela desne sfenoidne i temporalne kosti. Otpočeto je lečenje po CVD protokolu (ciklofosamid, bortezomib, deksametazon); ordinirano je šest ciklusa zaključno sa septembrom 2023. godine. Pri reevaluaciji bolesti, u bioptatu koštane srži utvrđeni su: infiltracija sa 25% patoloških plazmocita, vrednosti lambda lakih lanaca u serumu, proteinurija, te vrednost beta2 mikroglobulina u padu za više od 50%. Terapijski efekat procenjen je kao parcijalna remisija. Sprovedena je palijativna radioterapija zaostale promene baze lobanje sa 25 Gy u 10 frakcija, zaključno sa oktobrom 2023. godine. U daljem toku lečenja, pacijentkinja je podvrgnuta autolognoj transplantaciji matičnih ćelija hematopoeze, koja je sprovedena februara 2024. godine. Na proceni terapijskog efekta nakon sprovedene transplantacije, održava se parcijalna remisija. Juna 2024. godine, po ulasku monoklonskog anti-CD38 antitela daratumumaba na pozitivnu listu lekova Republičkog fonda za zdravstveno osiguranje u našoj zemlji, lečenje je nastavljeno po *DaraRd* protokolu (daratumumab, lenalidomid, deksametazon) sa ciljem produbljivanja remisije i sprovođenja druge ATMČH. U toku je drugi ciklus pomenute terapije. Dosadašnje ukupno preživljavanje iznosi 20 meseci.

Prikaz slučaja 2

Kod pacijentkinje starosti 40 godina, bolest se manifestovala naglo nastalom paraparezom, 2012. godine. Učinjen je MR pregled kičme na kome je opisana infiltracija *Th4* pršljenkog tela sa ekstraosealnom paravertebralnom i epiduralnom propagacijom, značajnom stenozom lumena vertebralnog kanala i posledičnom kompresijom kičmene moždine. Tokom jula meseca 2012. godine, na Institutu za ortopediju Banjica, učinjena je dekompresija i stabilizacija segmenta torakalne kičme *Th3-5* uz biopsiju infiltrisanog tela *Th4*. Patohistološki je verifikovan dobro diferentovani plazmocitom. Na Institutu za onkologiju i radiologiju Srbije, sprovedena je kompletna dijagnostička obrada i u bioptatu koštane srži utvrđena je infiltracija patološkim plazmocitima od 80%. Elektroforezom i imunofiksacijom seruma i urina detektovani su monoklonski laki *kappa* lanci. Citogenetska *FISH* analiza nije rađena. Postavljena je dijagnoza multiplog mijeloma *BJ kappa, CS IIIA, ISS 2*. Avgusta i septembra iste

netic *FISH* analysis (fluorescence in situ hybridization) detected 13q deletion in 80% of nuclei and TP23 deletion in 25% of nuclei. A definitive diagnosis of multiple myeloma *BJ lambda CS IIIA, R-ISS 2* was established. In the further course of the disease, peripheral paralysis of the right *n. facialis* developed, which is why a CT scan of the endocranium was performed, revealing a tumorous lesion in the base of the skull with the destruction of a segment of the right sphenoid and temporal bones. The patient was treated with six cycles of the CVD (cyclophosphamide, bortezomib, dexamethasone) regimen, ending in September 2023. Upon reevaluation of the disease, the following were found in the bone marrow biopsy: infiltration with 25% of pathological plasma cells, values of lambda light chains in the serum, proteinuria, and a value of beta2 microglobulin reduced by more than 50%. The therapeutic effect was evaluated as partial remission. Palliative radiotherapy of residual changes in the base of the skull was carried out with 25 Gy in 10 fractions, concluding in October 2023. In the further course of treatment, the patient underwent autologous hematopoietic stem cell transplantation, which was carried out in February 2024. When the therapeutic effect after transplantation was evaluated, the patient was found to be in partial remission. In June 2024, after the monoclonal anti-CD38 antibody daratumumab was added to the positive list of drugs of the Republic Fund of Health Insurance of Serbia, the treatment of this patient was continued with the *DaraRd* (daratumumab, lenalidomide, dexamethasone) regimen, with the aim of deepening remission and conducting a second cycle of AHSCT. The second cycle of this therapy is now in progress. The patient's overall survival to date is 20 months.

Case report 2

In a 40-year-old female patient, the disease manifested as sudden paraparesis, in 2012. An MRI examination of the spine was performed, which revealed infiltration of the *Th4* vertebral body with extraosseous paravertebral and epidural propagation, significant stenosis of the lumen of the vertebral canal, and consequent compression of the spinal cord. In July 2012, at the Institute of Orthopedic Surgery *Banjica*, decompression and stabilization of the *Th3-5* thoracic spine segment were performed, along with a biopsy of the infiltrated *Th4* body. A well-differentiated plasmacyte was verified pathohistologically. A complete diagnostic work-up was carried out at the Institute of Oncology and Radiology of Serbia. Bone marrow biopsy showed an 80% pathological plasma cell infiltration. Monoclonal light kappa chains were detected with electrophoresis

godine, sprovedena je palijativna radioterapija zahvaćenog pršljena sa 40 Gy u 20 frakcija. Otpočeta je sistemska terapija po CTD protokolu (ciklofosamid, talidomid, deksametazon); ordinirano je osam ciklusa sa postignutom parcijalnom remisijom (infiltracija koštane srži sa 40% patoloških plazmocita; pad vrednosti κ lanaca za 50%). Usledila je terapija održavanja talidomidom u trajanju od 10 meseci. U junu 2014. godine, dolazi do razvoja neuroloških simptoma u vidu glavobolja, diplopije i nestabilnosti pri hodu. Zbog kliničke sumnje na diseminaciju u CNS učinjena je kompletna dijagnostička obrada; radiografski su opisane difuzne fokalne lezije lobanje uz progresiju nalaza na torakalnim i lumbalnim kičmenim pršljenovima. Na MR pregledu endokranijuma opisana je mekotkivna tumorska promena fronto-parijetalno levo sa infiltracijom moždanog parenhima. U daljem toku je sprovedena zračna terapija endokranijuma u pet frakcija. Lečenje je nastavljeno po PAD protokolu (bortezomib, doxorubicin, deksametazon), ordinirano je sedam ciklusa sa efektom progresije bolesti. Smrtni ishod nastupio je jula 2016. godine. Ukupno preživljavanje iznosilo je 48 meseci, dok je preživljavanje od dijagnoze infiltracije centralnog nervnog sistema do smrtnog ishoda bilo 25 meseci.

DISKUSIJA

Uvidom u dostupnu literaturu ustanovljeno je da u većini studija medijana starosti iznosi preko 60 godina što korelira sa predilekcionom životnom dobu za nastanak multiplog mijeloma u opštoj populaciji [11,12]. Međutim, nekoliko studija je pokazalo da se CNS-MM može javiti i kod mlađih pacijenata [1,5,13,14]. U studiji Čena i saradnika, medijana starosti je iznosila 53 godine [4]. Kod naše pacijentkinje je bolest dijagnostikovana u 40. godini života, što je čini jednom od najmlađih pacijenata sa dijagnostikovanim CNS-MM-om u literaturi. Obolevanje u mlađem životnom dobu može biti povezano sa agresivnijim tokom bolesti. Bolest se dominantno javlja kod ženskog pola [14].

Infiltracija centralnog nervnog sistema se, u većini slučajeva, viđa u relapsu/progresiji bolesti [4,5,7,9,10,13], međutim, u malom broju studija je kod većeg broja pacijenata dijagnostikovana inicijalno [14]. Ono što ovaj entitet čini terapijskim izazovom u slučajevima javljanja u kasnijem toku bolesti jeste refraktornost na prethodno primenjeno lečenje. Agresivnijem kliničkom toku doprinosi i postojanje visoko rizičnih citogenetskih mutacija od kojih su delecija 13q i delecija 17p najčešće [15]. Čang i saradnici su u svojoj studiji dokazali deleciju 17p kod 89% pacijenata [10], dok su Đurčisin i saradnici otkrili deleciju 13q kod 39%, a deleciju 17p kod 23% pacijenata [9]. Kod

and immunofixation of serum and urine. Cytogenetic FISH analysis was not performed. A diagnosis of multiple myeloma BJ kappa, CS IIIA, ISS 2 was established. In August and September of the same year, palliative radiotherapy of the affected vertebra with 40 Gy in 20 fractions was performed. The patient was put on systemic therapy with the CTD regimen (cyclophosphamide, thalidomide, dexamethasone) – she underwent eight cycles achieving partial remission (infiltration of the bone marrow with 40% of pathological plasma cells; decrease in the value of kappa chains by 50%). This was followed by maintenance thalidomide therapy for 10 months. In June 2014, the patient developed neurological symptoms – headaches, diplopia, and unsteady gait. Due to the clinical suspicion of CNS involvement, a complete diagnostic work-up was performed. Radiographically, diffuse focal lesions of the skull were described with progression to the thoracic and lumbar spinal vertebrae. MRI examination of the endocranium revealed a soft tissue tumorous lesion in the left frontoparietal area with infiltration of the brain parenchyma. In the further course, radiation therapy of the endocranium was carried out in five fractions. The patient was treated with seven cycles of the PAD protocol (bortezomib, doxorubicin, dexamethasone), resulting in disease progression. In July 2016, the disease ended in a fatal outcome. The overall survival of the patient was 48 months, while her survival time from diagnosis of central nervous system involvement until death was 25 months.

DISCUSSION

A review of the currently available literature reveals that in most studies the median age of patients is above 60 years, which correlates with the predilection age for the onset of multiple myeloma in the general population [11,12]. However, several studies have shown that CNS-MM can occur in younger patients as well [1,5,13,14]. In a study by Chen et al., the median age was 53 years [4]. Our patient was diagnosed with the disease at the age of 40, which makes her one of the youngest patients diagnosed with CNS-MM documented in literature. The onset of CNS-MM at a younger age may be associated with a more aggressive course of the disease. The disease predominantly occurs in women [14].

Infiltration of the central nervous system is, in most cases, seen in relapse/progression of the disease [4,5,7,9,10,13]. However, a small number of studies show it being diagnosed at the onset in a larger number of patients [14]. What makes this entity a therapeutic challenge in its late stage is its refractoriness to previously applied treatment. The presence of high-risk cytogenetic mutations contributes to a more aggres-

jedne od naših pacijentkinja otkrivena je delecija 13q, dok kod druge pacijentkinje, u vreme dijagnostikovanja, citogenetska analiza nije spadala u dijagnostički standard. Na agresivniji tok bolesti i potencijalnu diseminaciju u CNS može ukazati i povišena vrednost laktat-dehidrogenaze (LDH) koja je jedan od parametara *R-ISS* sistema bodovanja (engl. *Revised International Staging System*) [16]. Naša druga pacijentkinja je imala inicijalno višestruko povišenu vrednost LDH, ali zbog nedostatka *FISH* analize, *R-ISS* skor nije bilo moguće izračunati.

Kliničkom slikom može da dominira spektar neuroloških deficita, kao posledica infiltracije kičmene moždine, kičmenih korenova, moždanih ovojnica ili moždanog parenhima. Kod polovine pacijenata, dominantni simptom je glavobolja praćena vizuelnim smetnjama [14], što je bila manifestacija bolesti jedne od naših pacijentkinja. Neurološka simptomatologija može biti povezana sa metaboličkim stanjem, najčešće hiperkalcemijom, što je često inicijalna manifestacija novodijagnostikovanog multiplog mijeloma [17,18].

Standard lečenja nije jasno definisan i zasniva se na iskustvima sa malim brojem pacijenata. Osim niske incidencije, infiltracija CNS-a često predstavlja kriterijum za isključivanje iz velikih studija koje su usmerene na ispitivanje novih terapijskih modaliteta u lečenju multiplog mijeloma [2,8,14]. Okosnicu sistemskog lečenja čine monoklonska antitela i imunomodulatori koji, za razliku od najčešće korišćenih inhibitora proteazoma, prolaze hematoencefalnu barijeru [19–22]. Radioterapija, kao lokalna terapija, ima najveću primenu u brzju kontroli neuroloških simptoma [23]. Transplantacija matičnih ćelija hematopoeze predstavlja standard lečenja kod novodijagnostikovanih pacijenata sa multiplim mijelomom koji ispunjavaju kriterijume za transplantaciju, ali u ovoj ograničenoj grupi pacijenata njena efikasnost, način i vreme sprovođenja nisu jasno definisani. Razlog za to jesu kratko vreme preživljavanja, efikasnost kondicionih režima u pogledu penetracije hematoencefalne barijere i očekivane komplikacije same procedure [24,25].

Medijana ukupnog preživljavanja se razlikuje među studijama i u dostupnoj literaturi se kreće od dva do 40 meseci [3,6,9]. Razlike u *mOS*-u su posledica vremena javljanja infiltracije CNS-a, kao i lokalizacije bolesti. U *GIMEMA* studiji je pokazano da su bolje preživljavanje imali pacijenti sa osteoduralnom infiltracijom (*mOS* 25 meseci) naspram pacijenata sa leptomeningealnom infiltracijom (*mOS* 6 meseci) [6]. Kod naših pacijentkinja *mOS* iznosi 34 meseca, dok *mOS* od momenta dijagnostikovanja infiltracije CNS-a iznosi 22,5 meseca, što korelira sa podacima o preživljavanju pacijenata koji

sive clinical course, of which 13q deletion and 17p deletion are the most common [15]. In their study, Chang et al. demonstrated a 17p deletion in 89% of patients [10], while Jurczyszyn et al. found a 13q deletion in 39% and a 17p deletion in 23% of patients [9]. In one of our patients, a 13q deletion was detected, while in the other patient, at the time of diagnosis, the cytogenetic analysis did not fall within the diagnostic standard. An increased value of lactate dehydrogenase (LDH), which is one of the parameters of the *R-ISS* scoring system (*Revised International Staging System*) [16], can also indicate a more aggressive course of the disease and potential CNS involvement. Initially, our second patient had a multiply elevated LDH level, but due to the lack of *FISH* analysis, the *R-ISS* score could not be calculated.

The clinical presentation can be dominated by an array of neurological deficits, resulting from an infiltration of the spinal cord, spinal roots, meninges, or brain parenchyma. In half of the patients, the dominant symptom is headache accompanied by visual disturbance [14], which was the manifestation of the disease in one of our patients. Neurological symptomatology can be related to the metabolic state, most often hypercalcemia, which is often the initial manifestation of newly diagnosed multiple myeloma [17,18].

The standard of treatment is not clearly defined and is based on experience with a small number of patients. Apart from low incidence, CNS involvement is often an exclusion criterion for large studies aimed at investigating new therapeutic modalities in the treatment of multiple myeloma [2,8,14]. The basis of systemic treatment consists of monoclonal antibodies and immunomodulators, which, unlike the most commonly used proteasome inhibitors, can cross the blood-brain barrier [19–22]. Radiotherapy, as a local therapy, is mostly applied in the rapid control of neurological symptoms [23]. Hematopoietic stem cell transplantation represents the standard of treatment in newly diagnosed patients with multiple myeloma who are eligible for transplantation, however, in this limited group of patients, its effectiveness, as well as its method and time of administration, are not clearly defined. The reason for this is the short survival time, the effectiveness of conditioning regimens in terms of penetration of the hematoencephalic barrier, and the expected complications of the procedure itself [24,25].

Median overall survival varies among studies and ranges from two to 40 months in the available literature [3,6,9]. Differences in *mOS* are the result of the time of occurrence of CNS infiltration, as well as the localization of the disease. In the *GIMEMA* study, it was shown that patients with osteodural infiltration (*mOS*

nisu imali leptomeningealnu infiltraciju.

S obzirom na nisku incidenciju javljanja infiltracije CNS-a, ali agresivnu i prirodu brze progresije, potrebno je svaki novonastali neurološki simptom detaljno evaluirati. Poseban diferencijalno dijagnostički izazov predstavlja neurotoksičnost sistemske terapije. Kod naše pacijentkinje kod koje se diseminacija u CNS javila u relapsu bolesti, neurološka manifestacija je diferencijalno dijagnostički mogla odgovarati i neurotoksičnosti primenjene terapije. U literaturi je opisano nekoliko slučajeva razvoja progresivne multifokalne leukoencefalopatije (PML) tokom primene daratumumaba [21]. Najučestalija neurotoksičnost inhibitora proteazoma i imunomodulatora je periferna neuropatija [26,27], posebno tokom kombinovane primene. Trombocitopenije i protrombogene stanja izazvana imunomodulatorima mogu, u retkim slučajevima, biti neposredno povezane sa simptomatologijom moždanog udara [28].

ZAKLJUČAK

Iako redak entitet, CNS-MM predstavlja veliki dijagnostički, klinički i terapijski izazov koji iziskuje dalja ispitivanja u cilju produžetka preživljavanja obolelih.

Sukob interesa: Nije prijavljen.

25 months), compared to patients with leptomeningeal infiltration (mOS 6 months), had a better survival time [6]. In our patients, the mOS is 34 months, while the mOS from the moment of diagnosis of CNS involvement is 22.5 months, which correlates with the survival data of patients who did not have leptomeningeal infiltration.

Given the low incidence of CNS involvement, but also the aggressive nature of its rapid progression, it is necessary to evaluate each new neurological symptom in detail. The neurotoxicity of systemic therapy is a particular differential diagnostic challenge. In our patient, in whom CNS involvement occurred during the relapse of disease, the neurological manifestation could also correspond to the neurotoxicity of the administered therapy. Several cases of the development of progressive multifocal leukoencephalopathy (PML) during the administration of daratumumab have been described in literature [21]. The most common neurotoxicity of proteasome inhibitors and immunomodulators is peripheral neuropathy [26,27], especially during combined administration. Thrombocytopenia and prothrombogenic states caused by immunomodulators can, in rare cases, be directly related to stroke symptomatology [28].

CONCLUSION

Although a rare entity, CNS-MM represents a major diagnostic, clinical, and therapeutic challenge that requires further investigation for the purpose of prolonging the survival time of patients.

Conflict of interest: None declared.

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PROGRESIJA BOLESTI KOD PACIJENATA SA PRIMARNOM MIJELOFIBROZOM NISKOGRIZIKA – PRIKAZ SLUČAJA

PRIKAZ SLUČAJA

CASE REPORT

DISEASE PROGRESSION IN PATIENTS WITH LOW-RISK PRIMARY MYELOFIBROSIS – CASE REPORT

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SAŽETAK

Uvod: Medijana preživljavanja (OS) pacijenata sa primarnom mijelofibrozom (PMF) niskog rizika je preko 15 godina, ali prema „MOST” prospektivnoj studiji, progresiju bolesti ima skoro 60% ovih pacijenata.

Cilj: Predstaviti tok bolesti i lečenje pacijenata sa PMF-om niskog rizika.

Prikaz slučaja: Svi pacijenti su imali utvrđenu PMF, međunarodni prognostički skoringsistem (IPSS) niskog rizika i normalnu početnu citogenetiku.

Slučaj 1: Pacijent, muškarac starosti 61 godina, primljen je u maju 2016. godine sa Tr 772x10⁹/L, LDH 566U/L, bez splenomegalije, JAK2V617F+. Prvobitno je lečen hidroksiureom (HU) od 2016. do 2020. godine. Od 2021. godine uveden je ruxolitinib zbog razvoja splenomegalije i leukocitoze, uz progresiju bolesti 18 meseci kasnije (slezina 26cm, Plt 20x10⁹/L, 10% blasta u srži, složen kariotip: -5, del 7q, mar+). Uveden je azacitidin, ali zbog sepse dolazi do smrtnog ishoda u oktobru 2022. godine. OS je 66 meseci.

Slučaj 2: Pacijentkinja, žena starosti 47 godina, primljena je u julu 2011. godine sa Tr 899x10⁹/L, LDH 899U/L, slezinom 15x7cm, JAK2V617F+, ASXL+. Inicijalno je lečena sa HU, a od oktobra 2013. godine ruxolitinibom, zbog progresije splenomegalije (slezina 19,3cm, LDH 1881U/L), uz potonju normalizaciju veličine slezine, broja Tr i LDH. Pacijentkinja je u remisiji. OS je 126 meseci.

Slučaj 3: Pacijent, muškarac starosti 64 godine, primljen je u maju 2012. godine sa Tr 1457x10⁹/L, LDH 631U/L, graničnom splenomegalijom, JAK2V617F-, MPL+, SRSF2+, U2AF1+, ASXL1+. Inicijalno je lečen sa HU (maj 2012. godine), a od aprila 2019. godine danazolom zbog pojave transfuzione zavisnosti. Zbog srčane insuficijencije, smrtni ishod je nastupio u februaru 2020. godine. OS je 96 meseci.

Zaključak: Neki pacijenti sa PMF-om niskog rizika imaju progresiju bolesti, a buduća istraživanja će pokazati da li rana sekvencioniranja sledeće generacije (NGS) analiza non-driver mutacija i rano uvođenje terapije doprinose promeni toka bolesti.

Ključne reči: mijeloproliferativna neoplazma, primarna mijelofibroza, prognoza

ABSTRACT

Introduction: The median survival (OS) of patients with low-risk primary myelofibrosis (PMF) is over 15 years, but according to the “MOST” prospective study, disease progression occurs in almost 60% of these patients.

Aim: to present the disease course and treatment outcome of patients with low-risk PMF.

Case report: All patients were diagnosed with PMF, low-risk International Prognostic Scoring System (IPSS), and normal initial cytogenetics.

Case 1: A 61-year-old male patient was admitted in May 2016 with Tr 772x10⁹/L, LDH 566U/L, no splenomegaly, JAK2V617F+. He was initially treated with hydroxyurea (HU) from 2016 to 2020. In 2021, he was introduced to ruxolitinib due to the development of splenomegaly and leukocytosis, with disease progression 18 months later (spleen 26cm, Plt 20x10⁹/L, 10% blasts in the marrow, complex karyotype: -5, del 7q, mar+). Azacitidine was introduced, but death occurred due to sepsis in October 2022. OS is 66 months.

Case 2: A 47-year-old female patient was admitted in July 2011 with Tr 899x10⁹/L, LDH 899U/L, spleen 15x7cm, JAK2V617F+, and ASXL+. She was initially treated with HU and has been treated with ruxolitinib since October 2013 due to the progression of splenomegaly (spleen 19.3cm, LDH 1881U/L), with subsequent normalization of spleen size, number of Tr, and LDH. The patient is in remission. OS is 126 months.

Case 3: A 64-year-old male patient was admitted in May 2012 with Tr 1457x10⁹/L, LDH 631U/L, borderline splenomegaly, JAK2V617F-, MPL+, SRSF2+, U2AF1+, ASXL1+. He was initially treated with HU (May 2012) and since April 2019 with danazol due to the emergence of transfusion dependence. Due to heart failure, death occurred in February 2020. OS is 96 months.

Conclusion: Some patients with low-risk PMF have disease progression and future studies will show whether early Next Generation Sequencing (NGS) analysis of non-driver mutations and early initiation of therapy contribute to changing the course of the disease.

Keywords: myeloproliferative neoplasm, primary myelofibrosis, prognosis

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UVOD

Primarna mijelofibroza (PMF) predstavlja stečeni klonalni poremećaj matične ćelije hematopoeze uz abnormalnu proliferaciju mijeloidnih ćelija. PMF je Bcr-Abl (Breakpoint cluster region protein-Abelson tyrosine-protein kinase, eng.) negativna mijeloproliferativna neoplazma (MPN) [1,2]. Karakteriše se prisustvom konstitucionalnih simptoma, splenomegalijom usred ekstramedularne hematopoeze i mogućnošću razvoja leukemijske transformacije [1]. Većina pacijenata ima prisutnu mutaciju gena za Janus kinazu (JAK)2/V617F [3]. Dijagnoza PMF se određuje prema ICC kriterijima (2022.). Prisustvo nukleiranih eritrocita, nezrelih granulocita i dakriocita je tipično, ali prefibrotični PMF ne mora da pokaže jasnu leukoeritroblastozu. Fibroza koštane srži je obično povezana sa mutacijama JAK2, CALR ili MPL mutacijama. Kariotip najčešće pokazuje abnormalnosti po tipu +9, del(13k), del(20k) i hromozoma br 1 [4-6]. Prognostički model baziran je na razvoju međunarodnog prognostičkog scoring sistema (IPSS) iz 2009. godine, koji se koristi u inicijalnoj dijagnostici i uključuje starost >65god., hemoglobin <100g/l, Le >25x10⁹/l, prisustvo > 1% cirkulišućih blasta i razvoj konstitucionalnih simptoma. U zavisnosti od broja prisustvnih štetnih faktora, pacijenti su statifikovani u grupe niskog, srednjeg-1, srednjeg-2 i visokog rizika bolesti [7]. DIPSS (dinamički IPSS skor) koristi iste prognostičke varijable, ali je primenljiv bilo kad u toku bolesti [8]. Konvencionalni tretman je ograničenog uticaja na preživljavanje pacijenata i uključuje "watch and wait" za asimptomatske pacijente, stimulator eritropoeze, androgene ili imunomodulatore za anemiju, primenu citoreduktivnih lekova (hidroksiurea kod razvoja splenomegalije i konstitucionalnih simptoma), u pojedinačnim slučajevima i splenektomiju ili radioterapiju [9]. Ciljana molekularna terapija (JAK inhibitori) je efikasna i kod JAK2+ i JAK2- pacijenata. Ruksolitinib je terapija izbora za PMF sa splenomegalijom i/ili razvojem konstitucionalnih simptoma. Alogena transplantacija matičnih ćelija ostaje jedini način izlječenja PMF, ali je ograničena na pacijente sa visokim i srednjim 2 rizikom. U toku su mnoge studije kombinacije JAK inhibitora sa drugim agensima [9]. Više studija je pokazalo da lečenje mijelofibroze u ranijem toku bolesti, dok je pacijent niskog, ili srednjeg-1 rizika ili što je pre moguće ukoliko ima srednji i visoki rizik, rezultira većom relativnom efikasnošću i manjom toksičnošću u poređenju sa kasnijim započinjanjem lečenja [10]. Cilj ovog rada je bio da prikažemo bolesnike sa PMF niskog stepena rizika, kod kojih je došlo do biološke progresije bolesti, i da istaknemo njihove klinčke i biološke karakteristike.

INTRODUCTION

Primary myelofibrosis (PMF) is an acquired clonal disorder of hematopoietic stem cells with abnormal proliferation of myeloid cells. PMF is a Bcr-Abl (Breakpoint cluster region protein-Abelson tyrosine-protein kinase, Eng.) negative myeloproliferative neoplasm (MPN) [1,2]. It is characterized by the presence of constitutional symptoms, splenomegaly in the midst of extramedullary hematopoiesis, and the possibility of leukemic transformation [1]. Most patients have a Janus kinase gene mutation (JAK)2/V617F [3]. The diagnosis of PMF is determined according to the ICC criteria (2022). The presence of nucleated erythrocytes, immature granulocytes, and dacryocytes is typical, but prefibrotic PMF does not necessarily show clear leukoerythroblastosis. Bone marrow fibrosis is usually associated with JAK2, CALR, or MPL mutations. The karyotype most often shows abnormalities of type +9, del(13k), del(20k), and chromosome number 1 [4-6]. The prognostic model is based on the development of the International Prognostic Scoring System (IPSS) from 2009, which is used in the initial diagnosis and includes age >65 years, hemoglobin <100g/l, Le >25x10⁹/l, presence > 1% of circulating blasts and development of constitutional symptoms. Depending on the number of harmful factors present, patients were stratified into groups of low, medium-1, medium-2, and high risk of the disease [7]. DIPSS (dynamic IPSS score) uses the same prognostic variables but is applicable at any time during the disease [8]. Conventional treatment has a limited impact on patient survival and includes "watch and wait" for asymptomatic patients, erythropoiesis stimulators, androgens or immunomodulators for anemia, the use of cytoreductive drugs (hydroxyurea in the development of splenomegaly and constitutional symptoms), and in individual cases, splenectomy or radiotherapy [9]. Targeted molecular therapy (JAK inhibitors) is effective in both JAK2+ and JAK2- patients. Ruxolitinib is the therapy of choice for PMF with splenomegaly and/or the development of constitutional symptoms. Allogeneic stem cell transplantation remains the only cure for PMF but is limited to high- and intermediate-2-risk patients. Many studies of the combination of JAK inhibitors with other agents are underway [9]. Several studies have shown that treatment of myelofibrosis earlier in the course of the disease, while the patient is at low or intermediate-1 risk, or as early as possible if he has intermediate and high risk, results in greater relative efficacy and less toxicity compared to later initiation of treatment [10]. This work aimed to present patients with low-risk PMF, in whom biological progression of the disease occurred, and to highlight their clinical and biological characteristics.

PRIKAZI SLUČAJEVA

Prvi slučaj

Kod pacijenta, muškog pola, starosti 61 godinu, u maju 2016. godine u krvnoj slici konstatovana je leukocitoza i trombocitoza (Hb 158 g/L, MCV 90fl, Le $12,9 \times 10^9/L$, Tr $792 \times 10^9/L$, u diferencijalnoj formuli neutrofili 66%, limfociti 26%, bez leukoeritroblastoze), a u biohemijskim analizama povišen LDH (556U/L). Ultrazvuk abdomena nije pokazao prisustvo splenomegalije. Pacijent nije imao nikakve tegobe na prikazu. U sprovedenoj hematološkoj dijagnostici, nalaz biopтата koštane srži pokazao je fibrozu gr I, uočen je spontani razvoj formacija eritroidnih kolonija, konvencionalna citogenetika je bila uredna (20 metafaza). Konstatovana je JAK2V617F mutacija (PCR). Nivo serumskog eritropetina bio je uredan. Postavljena je dijagnoza prefibrotičnog PMF-a, niskog stepena rizika prema IPSS skor, uz MPN 10 skor 10/100.

Uz primenu acetilsalicilne kiseline od 100 mg *per os* dnevno, po sprovednoj dijagnostici i daljoj progresiji trombocitoze i leukocitoze, započeto je (jun 2016. godine) lečenje hidroksiureom (HU), 500 mg *per os* dnevno, u periodu 2016. do 2020. godine. Decembra 2019. godine, kontrolni ultrazvuk abdomena i dalje je bio uredan. Međutim, u avgustu 2020. godine (53 meseca od dijagnoze PMF) konstatovana je progresija leukocitoze ($30 \times 10^9/L$, uz Hb 129g/L i Tr $161 \times 10^9/L$), te je doza HU eskalirana na 1000 mg *per os* dnevno. Oktobra 2020. godine (55 meseci od dijagnoze PMF) pacijent prijavljuje mučninu, gubitak od 5 kg telesne težine za mesec dana, noćno znojenje i preznojavanje. Konstatovana je dalja progresija leukocitoze ($50 \times 10^9/L$, 3% blasta u perifernom razmazu) i porast LDH (1627 U/L). Doza HU je eskalirana na 1500 mg *per os* dnevno, uz potonju pojavu trombocitopenije.

U oktobru 2020. godine sprovedena je hematološka reevaluacija. Aspiraciona punkcija nije pokazala akutnu transformaciju MPN-a (2% blasta). U biopšanu koštane srži uočena je fibroza gr II, 4% CD34+ ćelija. Nalaz konvencionalne citogenetike u 20 mitoza bio je uredan. Konstatovano je prisustvo PMF-a, intermediarnog-2 rizika prema DIPSS skor, MPN skor 19/100. U februaru 2021. godine radiografski je konstatovana dalja progresija splenomegalije (22,2 cm), bez splahnične tromboze. Uveden je Ruxolitinib® u dozi 2x20 mg *per os* dnevno, a mesec dana kasnije, zbog leukocitoze $146 \times 10^9/L$ (uz Hb 120 g/L i Tr $101 \times 10^9/L$), ponovo je dodata HU (500 mg *per os* dnevno). Nakon 18 meseci od početka primene ruxolitiniba (avgust 2022. godine), konstatovana je dalja progresija bolesti (produbljivanje trombocitopenije, progresija splenomegalije do 26 cm), uz pojavu aberantnog kariotipa sa 2 nezavisna

CASE REPORTS

First case

In a patient, male, age 61, in May 2016, leukocytosis and thrombocytosis were found in the blood count (Hb 158 g/L, MCV 90fl, Le $12.9 \times 10^9/L$, Tr $792 \times 10^9/L$, in the differential formula neutrophils 66%, lymphocytes 26%, without leukoerythroblastosis), and in biochemical analyzes elevated LDH (556U/L). Abdominal ultrasound did not show the presence of splenomegaly. The patient did not have any symptoms during the examination. In the performed hematological diagnosis, the findings of the bone marrow biopsy showed fibrosis gr I, spontaneous development of erythroid colony formations was observed, and conventional cytogenetics was normal (20 metaphases). JAK2V617F mutation was detected (PCR). The serum erythropoietin level was average. A diagnosis of prefibrotic PMF, low risk according to the IPSS score, with an MPN 10 score of 10/100, was made.

Along with the use of acetylsalicylic acid of 100 mg *per body per day*, after the implementation diagnosis and further progression of thrombocytosis and leukocytosis, treatment with hydroxyurea (HU), 500 mg *per body per day*, was started (June 2016), in the period from 2016 to 2020. In December 2019, the follow-up abdominal ultrasound was still normal. However, in August 2020 (53 months after the diagnosis of PMF), a progression of leukocytosis was noted ($30 \times 10^9/L$, with Hb 129 g/L and Tr $161 \times 10^9/L$), and the dose of HU was escalated to 1000 mg *per person per day*. In October 2020 (55 months after the diagnosis of PMF), the patient reported nausea, weight loss of 5 kg in one month, night sweats, and sweating. Further progression of leukocytosis ($50 \times 10^9/L$, 3% blasts in peripheral smear) and LDH increase (1627 U/L) were constant. The dose of HU was escalated to 1500 mg *per os per day*, with the subsequent occurrence of thrombocytopenia.

In October 2020, a hematological reevaluation was carried out. Aspiration puncture showed no acute transformation of MPN (2% blasts). In the bone marrow biopsy, fibrosis grade II was observed in 4% CD34+ cells. The findings of conventional cytogenetics in 20 mitoses were normal. The presence of PMF, intermediate-2 risk, according to the DIPSS score, and the MPN score of 19/100 was established. In February 2021, further progression of splenomegaly (22.2 cm) was detected radiographically, without splenic thrombosis. Ruxolitinib® was introduced at a dose of 2x20 mg *per body per day*, and a month later, due to leukocytosis $146 \times 10^9/L$ (with Hb 120 g/L and Tr $101 \times 10^9/L$), HU was added again (500 mg *per os per day*). After 18 months from the start of ruxolitinib administration (August

klona (-5, del 7q, +mar). Ponovljena aspiracija srži pokazala je 10% blasta u srži. Kod pacijenta je započeto lečenje primenom azacitidina, ali usled pojave septičnog stanja sa razvojem septičnog šoka dolazi do letalnog ishoda u oktobru 2022. godine. Period ukupnog preživljavanja (OS, Overall Survival, eng.) je iznosio 66 meseci.

Drugi slučaj

Pacijentkinja starosti 47 godina javila se hematologu u julu 2011. godine zbog pojave pruritusa i abdominalnog bola, kao i nalaza krvne slike (Hb 128 g/L, MCV 89fL, Le $8.7 \times 10^9/L$, Tr $772 \times 10^9/L$, u diferencijalnoj formuli neutrofili 66%, limfociti 25%, monociti 5%, bez leukoeritroblastoze). U laboratorijskim analizama LDH je iznosio 899 U/L. Inicijalni ultrazvuk abdomena pokazao je slezinu promera 15x7cm. Sprovedena je hematološka dijagnostika. U bioptatu koštane srži uočena je fibroza Gr I, bio je pozitivan spontani razvoj formacija eritroidnih kolonija, a konvencionalna citogenetika bila je uredna (u 4 analizirane metafaze - nizak mitotski indeks). Konstatovana je JAK2V617F mutacija-homozigot (PCR). NGS (Next Generation Sequencing- mutacioni panel Prof. Skode) analiza utvrdila je prisustvo ASXL1 mutacije. Nivo serumskog eritropoetina je bio uredan. Konstatovana je dijagnoza prefibrotičnog PMF, niskog stepena rizika prema IPSS skoru, MPN 10 skor 25/100.

Inicijalno je uvedena acetilsalicilna kiselina (100 mg *per os* dnevno), a od jula 2012. godine je uvedena HU (500mg *per os* dnevno), s obzirom na progresivnu splenomegaliju (18x7cm) i porast LDH (1221U/L). Tada je utvrđeno prisustvo intermedijarnog-1 rizika prema DIPSS skoru. U oktobru 2013. godine pacijentkinja prijavljuje malaksalost, noćno znojenje, preznojavanje i ponovnu pojavu abdominalnog bola. Slezina se palpirala do nivoa umbilikusa. U krvnoj slici Hb $130 \times 10^9/L$, Le $13 \times 10^9/L$, Tr $563 \times 10^9/L$, postoji tendencija daljeg rasta LDH (1881U/L), uz uredne druge zapaljenske parametre. Kontrolni ultrazvuk abdomena pokazao je dalju progresiju splenomegalije (19,3x7,5cm). Pacijentkinja nije bila motivisana za rebiopsiju koštane srži. Konstatovano je i dalje prisustvo intermedijarnog-1 rizika (DIPSS), uz porast MPN 10 skora na 36/100.

Istog meseca je uveden ruxolitinib u dozi od 2x20 mg *per os* dnevno (u okviru "compassionate" programa). U daljem toku lečenja ruxolitinibom dolazi do potpune regresije splenomegalije (13x5 cm februara 2015. godine), uz potpunu laboratorijskih parametara. Na poslednjoj kontroli, u januaru 2024. godine (i dalje ruxolitinib 2x20 mg *per os* dnevno), u krvnoj slici Hb 124 g/L, Le $4.2 \times 10^9/L$, Tr $413 \times 10^9/L$. LDH 478U/L. Poslednji ultrazvuk abdomena je pokazao promer slezine 10x3 cm. Pacijentkinja nije prijavljivala tegobe na poslednjoj

2022), further progression of the disease was noted (deepening thrombocytopenia, progression of splenomegaly up to 26 cm), with the appearance of an aberrant karyotype with 2 independent clones (-5, del 7q, +mar). A repeat marrow aspiration showed 10% blasts in the marrow. The patient started treatment with azacitidine, but due to the appearance of a septic condition with the development of septic shock, the patient died in October 2022. The overall survival (OS) period was 66 months.

Second case

A 47-year-old patient consulted a hematologist in July 2011 due to the appearance of pruritus and abdominal pain, as well as blood count findings (Hb 128 g/L, MCV 89fL, Le $8.7 \times 10^9/L$, Tr $772 \times 10^9/L$, in the differential formula neutrophils 66%, lymphocytes 25%, monocytes 5%, without leukoerythroblastosis). In laboratory analyses, LDH was 899 U/L. The initial abdomen ultrasound showed a spleen with a diameter of 15x7cm. A hematological diagnosis was carried out. In the bone marrow biopsy, Gr I fibrosis was observed, there was a positive, spontaneous development of erythroid colony formations, and conventional cytogenetics were normal (metaphase in 4 analyzed - low mitotic index). JAK2V617F mutation-homozygote was established (PCR). NGS (Next Generation Sequencing - Prof. Skoda mutation panel) analysis determined the presence of ASXL1 mutation. The serum erythropoietin level was average. The diagnosis of prefibrotic PMF, low risk, according to the IPSS score, and MPN 10 score 25/100 was established.

Acetylsalicylic acid (100 mg *per os* per day) was initially introduced, and from July 2012, HU (500 mg *per os* per day) was introduced, considering progressive splenomegaly (18x7cm) and an increase in LDH (1221U/L). Then, the presence of intermediate-1 risk was determined according to the DIPSS score. In October 2013, the patient reported malaise, night sweats, sweating, and the recurrence of abdominal pain. The spleen was palpated up to the level of the umbilicus. In the blood count, Hb $130 \times 10^9/L$, Le $13 \times 10^9/L$, Tr $563 \times 10^9/L$, there is a tendency for further growth of LDH (1881U/L), along with normal other inflammatory parameters. Control ultrasound of the abdomen showed further progression of splenomegaly (19.3x7.5 cm). The patient was not motivated to undergo a bone marrow rebiopsy. The presence of intermediate-1 risk (DIPSS) was still established, with an increase in the MPN 10 score to 36/100.

In the same month, ruxolitinib was introduced at a dose of 2x20 mg *per os* per day (within the "compassionate" program). In further treatment with ruxolitinib, there is a complete regression of splenomegaly (13x5 cm in February 2015), with complete laboratory pa-

kontroli. Pacijentkinja je u stabilnoj remisiji osnovne bolesti. Period bez progresije bolesti iznosi 126 meseci. Dalje praćenje uz nastavak ruxolitiniba je u toku.

Treći slučaj

Pacijent muškog pola, starosti 64 godine, inicijalno se javio hematologu u maju 2012. godine zbog nalaza trombocitoze u krvnoj slici, uz podatak da je za povišene trombocite znao od novembra 2010. godine (tada $Tr\ 989 \times 10^9/L$), ali se nije ispitivao. Prilikom pregleda, u krvnoj slici su registrovani $Hb\ 107\text{g/L}$, $MCV\ 82\text{fl}$, $Le\ 11,2 \times 10^9/L$, $Tr\ 1457 \times 10^9/L$, u diferencijalnoj formuli neutrofili 62%, limfociti 20%, monociti 5%. U laboratorijskim nalazima LDH je iznosio 631U/L , a feritin 9ug/L , uprkos prethodnoj primeni oralnih preparata gvožđa. Ultrazvuk abdomena iz maja 2012. godine pokazao je slezinu veličine $12 \times 5\text{ cm}$. Pacijent, izuzev blagog svraba kože, nije imao druge tegobe.

U sprovedenoj hematološkoj dijagnostici, nalaz bioptata koštane srži pokazao je fibrozu gr I, uočen je spontani razvoj formacija eritroidnih kolonija. Konvencionalna citogenetika bila je uredna (4 metafazenezak mitotski indeks). PCR metodom nije potvrđena JAK2V617F mutacija. NGS metodom (mutacioni panel Profesora Skode) utvrđeno je prisustvo MPL, SRSF2, U2AF1 i ASXL1 mutacije. Nivo serumskog eritropoetina bio je uredan. Postavljena je dijagnoza prefibrotičnog PMF, niskog stepena rizika prema IPSS skor, uz MPN 10 skor 5/100.

Inicijalno je uvedena acetilsalicilna kiselina (100 mg per os dnevno), ali je obustavljena u maju 2012. godine zbog pojave izražene epistakse (utvrđena aktivnost Von Willebrand-ovog faktora 34%). Istog meseca je uvedena HU (500 mg per os dnevno), čije je doziranje u daljem toku lečenja bilo korigovano u zavisnosti od parametara krvne slike. HU je primenjivana sve do aprila 2019. godine, kada je konstatovano prisustvo transfuziono zavisnog anemijskog sindroma, te je lečenje nastavljeno Danazolom ($2 \times 200\text{ mg per os}$ dnevno).

U oktobru 2019. godine urađena je hematološka reevaluacija. Rebiopsija koštane srži pokazala je prisustvo fibroze, sada gr III, dok aspiraciona punkcija nije pokazala akutnu transformaciju (8% blasta). Uočeno je prisustvo kompleksnog kariotipa, $46\text{ XY, del}(20)\text{q11/}(5), 46\text{ XY/15/}$. PCR za JAK2V617F mutaciju je i dalje bio negativan. U krvnoj slici su zabeleženi $Hb\ 72\text{g/L}$, $Le\ 19,1 \times 10^9/L$, $Tr\ 487 \times 10^9/L$, u perifernom razmazu krvi 6% blasta sa leukoeritroblastozom, retikulociti 5.2%. LDH je iznosio 2498 U/L , a feritin $247,8\text{ mg/L}$. Ultrazvučni nalaz iz oktobra 2019. godine pokazao je slezinu veličine 14 cm .

U daljem toku lečenja pacijent je prijavljivao izraženu malaksalost i zamor, te je nastavljena transfuzi-

rameters. At the last control, in January 2024 (still ruxolitinib $2 \times 20\text{ mg per os}$ per day), in the blood count $Hb\ 124\text{g/L}$, $Le\ 4.2 \times 10^9/L$, $Tr\ 413 \times 10^9/L$. LDH 478U/L . The last abdomen ultrasound showed a diameter of the spleen of $10 \times 3\text{ cm}$. The patient did not report any complaints at the last check-up. The patient is in stable remission of the underlying disease. The period without disease progression is 126 months. Further follow-up with the continuation of ruxolitinib is ongoing.

Third case

A male patient, aged 64, initially consulted a hematologist in May 2012 due to the findings of thrombocytosis in the blood count, with the information that he had known about elevated platelets since November 2010 (then $Tr\ 989 \times 10^9/L$), but he did not examine it. During the initial examination, the blood count showed $Hb\ 107\text{ g/L}$, $MCV\ 82\text{fl}$, $Le\ 11.2 \times 10^9/L$, $Tr\ 1457 \times 10^9/L$, in the differential formula neutrophils 62%, lymphocytes 20%, monocytes 5%. In the laboratory findings, LDH was 631U/L , and ferritin was 9ug/L , despite previous administration of peroral iron medications. An abdominal ultrasound from May 2012 showed a spleen measuring $12 \times 5\text{ cm}$. The patient, except for mild skin itching, had no other complaints.

In the performed hematological diagnosis, the findings of the bone marrow biopsy showed fibrosis grade I, and spontaneous development of erythroid colony formations was observed. Conventional cytogenetics was normal (metaphase 4 - low mitotic index). The PCR method did not confirm the JAK2V617F mutation. The presence of MPL, SRSF2, U2AF1, and ASXL1 mutations was determined using the NGS method (Professor Skoda's mutation panel). The serum erythropoietin level was average. A diagnosis of prefibrotic PMF, low risk according to IPSS score, with an MPN 10 score of 5/100, was made.

Acetylsalicylic acid (100 mg per os per day) was initially introduced, but it was discontinued in May 2012 due to severe epistaxis (Von Willebrand factor activity was determined to be 34%). In the same month, HU (500 mg per os per day) was introduced, the dosage of which was corrected in the further course of treatment depending on the blood count parameters. HU was applied until April 2019, when the presence of transfusion-dependent anemia syndrome was established, and the treatment was continued with Danazol ($2 \times 200\text{ mg per os}$ per day).

In October 2019, a hematological reevaluation was performed. Bone marrow rebiopsy showed the presence of fibrosis, now gr III, while aspiration puncture showed no acute transformation (8% blasts). The presence of a complex karyotype, $46\text{ XY, part}(20)\text{q11/}(5), 46\text{ XY/15/}$, was observed. PCR for the JAK2V617F mutation was still negative. The blood count showed

ona substitucija koncentratima eritrocitima, dva puta mesečno. I pored navedene terapije, konstitucionalni simptomi su se pogoršavali, uz održavanje transfuzione zavisnosti. Nastavljeno je sa primenom Danazola, međutim, usred razvoja srčane insuficijencije, došlo je do smrtnog ishoda u februaru 2020 godine. OS je iznosilo 96 meseci.

DISKUSIJA

Mnogi pacijenti kod kojih je postavljena dijagnoza PMF niskog/ nižeg stepena rizika manifestuju jasne kliničke znake i simptome osnovne bolesti i stoga bi imali koristi od primene aktivnog lečenja [10]. Na prikazu ovi pacijenti najčešće imaju malaksalost (30%), LDH je povećana kod ¼ pacijenata, slezina je palpabilna kod 19%, a leukocitoza je prisutna u 15% slučajeva [10]. Prema nalazima „MOST” observacione studije (The Myelobrosis and Essential Thrombocythemia Observational Study; NCT02953704), u trenutku uključivanja u studiju 41% pacijenata (od 232 ispitanika) pripadalo je grupi niskog rizika prema DIPSS skorom, a 59% grupi intermedijarnog-1 rizika ali je splenomegalija konstatovana kod 41% pacijenata sa niskim rizikom, a manje (31%) kod pacijenata sa intermedijarnim-1 rizikom [11]. Samo jedan pacijent u našoj miniseriji je imao jasne simptome na prikazu, drugi samo blagi pruritus. Interesantno, svi naši pacijenti imali su značajnu trombocitozu na prikazu i samo blagu leukocitozu u dva slučaja. Dva naša pacijenta prikazala su se splenomegalijom, u jednom slučaju graničnom.

Prema aktuelnim preporukama NCCN-a (National Comprehensive Cancer Network, eng.), ruxolitinib predstavlja dobru terapijsku opciju za rano lečenje pacijenata sa PMF nižeg stepena rizika, što dokazuju i podaci JUMP I ROBUST studija pacijenata sa intermedijarnim-1 rizikom, i koje potvrđuju benefit rane primene ruxolitiniba, uz 50% ili veću redukciju splenomegalije kod čak 61% pacijenata (nakon 48 nedelja terapije), uz značajnu redukciju inicijalne simptomatologije [12,13]. Rezultati COMBI studije ukazali su na to da je u podgrupi pacijenata sa PMF (dominantno niskog ili intermedijarnog-1 stepena rizika), kombinovana primena ruxolitiniba i malih doza pegilovanog interferona alfa 2a, dovela do remisije u 44% slučajeva, uz prihvatljivu toksičnost [14]. Svi naši pacijenti imali su inicijalnu dijagnozu prefibrotičnog PMF niskog stepena rizika, svi su inicijalno lečeni primenom antiagregacione terapije, a potom sa HU zbog progresije splenomegalije ili leukocitoze ili trombocitoze. Kod dva pacijenta je uveden ruxolitinib, uz kompletni odgovor u jednom slučaju i aktuelno održavanje remisije bolesti nakon više od 10 godina primene ruxolitiniba, ali i dalje progresije bolesti do smrtnog ishoda u drugom slučaju. Treba

Hb 72g/L, Le $19.1 \times 10^9/L$, Tr $487 \times 10^9/L$, in the peripheral blood smear 6% blasts with leukoerythroblastosis, reticulocytes 5.2%. LDH was 2498 U/L, and ferritin was 247.8 mg/L. The ultrasound findings from October 2019 showed a spleen measuring 14 cm.

In the further course of treatment, the patient reported severe weakness and fatigue, and transfusion substitution with erythrocyte concentrates was continued twice a month. Despite the mentioned therapy, the constitutional symptoms worsened, while the transfusion dependence was maintained. The use of Danazol was continued; however, amid the development of heart failure, there was a fatal outcome in February 2020. OS was 96 months.

DISCUSSION

Many patients diagnosed with low/low-risk PMF manifest clear clinical signs and symptoms of the underlying disease and would, therefore, benefit from active treatment [10]. At presentation, these patients most often have weakness (30%), LDH is increased in ¼ of patients, the spleen is palpable in 19%, and leukocytosis is present in 15% of cases [10]. According to the findings of the “MOST” observational study (The Myelobrosis and Essential Thrombocythemia Observational Study; NCT02953704), at the time of inclusion in the study, 41% of patients (out of 232 subjects) belonged to the low-risk group according to the DIPSS score, and 59% to the intermediate-1 risk group, but splenomegaly was noted in 41% of patients with low risk, and less (31%) in patients with intermediate-1 risk [11]. Only one patient in our miniseria had apparent symptoms at presentation; the other had only mild pruritus. Interestingly, all our patients had significant thrombocytosis at presentation and only mild leukocytosis in two cases. Two of our patients presented with splenomegaly, in one case borderline.

According to the current NCCN (National Comprehensive Cancer Network) recommendations, ruxolitinib represents an excellent therapeutic option for the early treatment of patients with PMF of lower risk, as evidenced by the data of the JUMP and ROBUST studies of patients with intermediate-1 risk, which confirm the benefit early administration of ruxolitinib, with a 50% or more significant reduction of splenomegaly in as many as 61% of patients (after 48 weeks of therapy), with a substantial reduction of initial symptoms [12,13]. The results of the COMBI study indicated that in a subgroup of patients with PMF (predominantly low or intermediate-1 risk), the combined use of ruxolitinib and low-dose pegylated interferon alfa 2a led to remission in 44% of cases, with acceptable toxicity [14]. All our patients had an initial diagnosis of low-risk

napomenuti da je kod drugog pacijenta bila prisutna progresivna leukocitoza koja značajno smanjuje ukupno preživljavanje sa medijanom OS od 13,3 meseca. U ovakvim slučajevima, prema rezultatima nekih retrospektivnih analiza, primena ruxolitiniba pokazala je najveći benefit, sa produženjem medijane OS na 21,2 meseci [15], što ugrubo odgovara i medijani OS kod našeg pacijenta, a po uočenoj progresiji prethodne blage leukocitoze.

Non-driver mutacije imaju značajan uticaj na klinički tok bolesti, ali i ishod lečenja. ASXL1 non-driver mutacije imaju značajnu ulogu u progresiji pre-PMF u pravi PMF, kao i u stepenu fibroze, ali i leukemijskoj transformaciji PMF [15]. SRSF2 mutacije pokazale su tendenciju redukcije klonske proliferacije u toku uočene progresije bolesti. ASXL1 mutacije nisu direktno odgovorne za leukemogenezu, ali dovode do akceleracije akumulacije direktnih leukemogenih faktora, npr. mutiranih produkata RAS-signalnog puta. Stoga, aktuelni literaturni podaci idu u prilog tome da ASXL1 mutacija ima kritičnu ulogu u kliničkom toku PMF, uz uticaj na pogoršanje fibroze i povećanje mogućnosti leukemijske transformacije. Obzirom na svoju značajnu frekvenciju u PMF, ASXL1 mutacije bi u budućnosti mogle biti značajne za target-terapiju [16]. Kod dva naša pacijenta je rađen NGS, uz ASXL1 prisutvo mutacije u oba slučaja sa potpuno drugačijim kliničkim ishodom. Naime, jedna pacijentkinja je u dugogodišnjoj remisiji uz primenu ruxolitiniba, dok je drugi pacijent imao smrtni ishod, ali nije lečen sa JAK inhibitorom, već antiagregacionom terapijom, HU i danazolom po postizanju transfuzione zavisnosti. Ni kod jednog našeg pacijenta nije dokazana leukemijska transformacija.

Klinički tok bolesti kod pacijenata sa PMF nižeg rizika pokazuje nepredvidljivost, što potvrđuju prethodni, ali i najnoviji rezultati „MOST” observacione studije prema kojima 60% pacijenata (medijana praćenja grupe od 232 pacijenta sa MF niskog/intermedijernog-1 rizika je bila 52,9 meseci) ima znakova progresije bolesti, dok je 8.3% pacijenta imalo leukemijsku transformaciju ili umrlo [11,17]. Treba napomenuti da je 84 pacijenta imalo PMF niskog rizika, a kriterijumi progresije bolesti su bili Hb <100 g/L, Tr <100×10⁹/L, konstitucionalni simptomi, pojava ili progresija splenomegalije, prisutvo blasta >1% u perifernoj krvi, Le >25×10⁹/L, smrtni ishod usled progresije bolesti, leukemijska transformacija i više od jedne transfuzije Er. U našoj miniseriji se uočava jasna progresija bolesti u sva tri slučaja, ali uz različite terapijske odgovore i klinički ishod bolesti. Dva pacijenta su imala smrtni ishod, OS su iznosili 66 i 96 meseci, dok je treća pacijentkinja u stabilnoj remisiji sa OS od 156 meseci. Ona je takođe nosilac ASXL1 mutacije (Tabela 1).

prefibrotic PMF; all were initially treated with antiplatelet therapy and then with HU due to the progression of splenomegaly or leukocytosis or thrombocytosis. In two patients, ruxolitinib was introduced, with a complete response in one case and current maintenance of disease remission after more than 10 years of ruxolitinib administration, but still disease progression to death in the other case. Of note, another patient had progressive leukocytosis, which significantly reduced overall survival with a median OS of 13.3 months. In such cases, according to the results of some retrospective analyses, the use of ruxolitinib showed the most significant benefit, with an extension of the median OS to 21.2 months [15], which roughly corresponds to the median OS in our patient, and according to the observed progression of the previous mild leukocytosis.

Non-driver mutations have a significant impact on the clinical course of the disease as well as the outcome of treatment. ASXL1 non-driver mutations play an essential role in the progression of pre-PMF to true PMF, as well as in the degree of fibrosis and the leukemic transformation of PMF [15]. SRSF2 mutations tended to reduce clonal proliferation during the observed disease progression. ASXL1 mutations are not directly responsible for leukemogenesis but lead to an acceleration of the accumulation of direct leukemogenic factors, e.g., mutated products of the RAS signaling pathway. Therefore, current literature data supports that ASXL1 mutation plays a critical role in the clinical course of PMF, affecting worsening fibrosis and increasing the possibility of leukemic transformation. Considering its significant frequency in PMF, ASXL1 mutations could be important for target therapy in the future [16]. In two of our patients, NGS was performed, with ASXL1 mutation in both cases, resulting in a completely different clinical outcome. Namely, one patient is in long-term remission with the use of ruxolitinib, while the other patient had a fatal outcome but was not treated with a JAK inhibitor but with antiaggregation therapy, HU, and danazol after achieving transfusion dependence. No leukemic transformation was proven in any of our patients.

The clinical course of the disease in patients with lower-risk PMF shows unpredictability, which is confirmed by the previous and the most recent results of the “MOST” observational study, according to which 60% of patients (the median follow-up of a group of 232 patients with low/intermediate-1 risk MF was 52.9 months) have signs of disease progression, while 8.3% of patients had leukemic transformation or died [11,17]. It should be noted that 84 patients had low-risk PMF, and the criteria for disease progression were Hb <100 g/L, Tr <100×10⁹/L, constitutional symptoms, the appearance or progression of splenomegaly, the pres-

Tabela 1. Dijagnoza, lečenje i ishod bolesti u našoj grupi pacijenata

Pacijent / Patient	Dijagnoza / Diagnose	JAK2V617F mutacioni status / JAK2V617F mutation	NGS mutacije / NGS mutations	Lečenje / Treatment	Neželjeni događaji / Adverse events	Ukupno preživljavanje / Overall survival (meseci / months)	Ishod / Outcomes
Br. 1 / No1	prePMF IPSS „nizak rizik / IPSS low risk“ MPN 10- 10/100	+	Nije urađen / Not done	ASA HU Rukoslitinib Azacitidin	Progresija 18m nakon uvođenja rukoslitiniba / Disease progression 18mts after ruxo intro- duction	66	Smrtni ishod / Death
Br. 2/ No2	prePMF IPSS „nizak rizik/ IPSS low risk“ MPN 10- 25/100	+	ASXL1+	ASA, HU, Ruksolitinib/ Ruxolitinib	Ne/ None	126	Kompletna remisija / Complete remission
Br. 3/ No3	prePMF IPSS „nizak rizik/ IPSS low risk“ MPN 10- 5/100	-	MPL+, SRSF2+ U2AF1+ ASXL1+	ASA HU Danazol	Epistaksa / Epistaxis Transfuziona zavisnost / Transfusion depen- dence Srčana insuficijencija / Cardiac insufficiency	96	Smrtni ishod / Death

Legenda: PMF– Primarna mijelofibroza; IPSS – Internacionalni Prognostički Skor sistem; MPN 10 – skor procene simptoma mijeloproliferativne neoplazme 10; JAK2V617F – Janus kinaza2 V617F; NGS – Sekvencioniranje sledeće generacije; ASA – Acetilsalicilna kiselina; HU- Hidrokisurea; m- meseci

Table 1. Diagnose, treatment, and disease outcomes in our group of our pts

Legend: PMF– Primary myelofibrosis; IPSS – International Prognostic Score System; MPN 10 – Myeloproliferative neoplasm symptom assessment 10; JAK2V617F – Janus kinase2 V617F; NGS – Next Generation Sequencing; ASA – Acetilsalicylic acid; HU-Hydroxyurea; m- months

ZAKLJUČAK

Aktuelni podaci idu u prilog značajnom prisustvu progresije bolesti i kod pacijenata sa incijalnim PMF niskog rizika, što zahteva regularno praćenje ovih pacijenata uz blagovremeno uključivanje/izmenu aktuelne terapije u slučaju progresije. Naši podaci takođe potvrđuju da je PMF niskog rizika jedan entitet sa mnogo različitih lica. Buduća istraživanja će pokazati da li rana NGS analiza non-driver mutacija, kao i rano uvođenje terapije doprinose promeni toka bolesti, kao i da li će non-driver mutacije biti nova meta u ciljanoj terapiji za PMF.

Sukob interesa: Nije prijavljen.

ence of blasts >1% in peripheral blood, Le >25×10⁹/L, death due to disease progression, leukemic transformation and more than one transfusion Er. In our miniseries, a clear progression of the disease can be observed in all three cases, but with different therapeutic responses and clinical outcomes of the disease. Two patients had a fatal outcome: OS was 66 and 96 months, while the third was a patient in stable remission with an OS of 156 months. She is also a carrier of the ASXL1 mutation (Table 1).

CONCLUSION

Current data supports the significant presence of disease progression in patients with low-risk initial PMF, which requires regular monitoring of these patients with timely inclusion/change of current therapy in case of progression. Our data confirm that low-risk PMF is one entity with many different faces. Future research will show whether early NGS analysis of non-driver mutations and early therapy introduction contribute to changing the course of the disease and whether non-driver mutations will be a new target in targeted therapy for PMF.

Conflict of interest: None declared

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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.

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The sections of the paper are: the cover page, the abstract and keywords, the text of the paper, acknowledgements (optional), literature, and attachments.

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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.

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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 $\pm$ 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.

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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.

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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).

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