DISEMINOVANA INTRAVASKULARNA KOAGULOPATIJA U AKUTNOJ NEPROMIJELOCITNOJ MIJELOIDNOJ LEUKEMIJI – UČESTALOST, KLINIČKO-LABORATORIJSKE KARAKTERISTIKE I PROGNOSTIČKI ZNAČAJ

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DISSEMINATED INTRAVASCULAR COAGULOPATHY IN NON-PROMYELOCYTIC ACUTE MYELOID LEUKEMIA – INCIDENCE, CLINICAL AND LABORATORY FEATURES AND PROGNOSTIC SIGNIFICANCE

Mirjana Cvetković[1], Mirjana Mitrović[1,2]

[1] Medicinski fakultet Univerziteta u Beogradu, Beograd

[2] Klinika za hematologiju Univerzitetskog kliničkog centra Srbije, Beograd, Srbija

[1] *Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

[2] *Clinic for Hematology, Clinical Center of Serbia, Belgrade, Serbia*

SAŽETAK

Uvod: Diseminovana intravaskularna koagulopatija (DIK) je prisutna kod 90% bolesnika sa akutnom promijelocitnom leukemijom (APL). Učestalost DIK-a kod ostalih tipova akutnih mijeloidnih leukemija (ne-APL AML) je znatno manja (10-40%) i do sada ne postoje studije koje su ispitivale uticaj DIK-a na ranu smrt kod ovih bolesnika.

Cilj: Cilj rada bio je da se izvrši analiza učestalosti diseminovane intravaskularne koagulopatije, njenih kliničko-laboratorijskih karakteristika, kao i uticaj na preživljavanje i ranu smrt bolesnika sa ne-APL AML-om.

Materijal i metode: Retrospektivnom analizom je obuhvaćeno 176 bolesnika sa ne-APL AML-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju Univerzitetskog Kliničkog centra Srbije (UKCS) u periodu od 2015. do 2020. godine. Dijagnoza DIK-a je postavljena na osnovu *ISTH* (engl. *International Society on Thrombosis and Haemostasias*) kriterijuma.

Rezultati: Prosečna starost bolesnika iznosila je 53,8±14,5 godina, uz prevalenciju muškog pola (99/176; 56,2%). Manifestna diseminovana intravaskularna koagulopatija konstatovana je kod 74/176 bolesnika (42%), koji su značajno češće imali hemoragijski sindrom ($p = 0,01$). Faktori rizika za nastanak DIK-a bili su: starije životno doba ($p < 0,01$), prisustvo komorbiditeta ($p = 0,01$), leukocitoza ($p < 0,001$) i visoka koncentracija LDH ($p < 0,001$). FAB (engl. French, American and British) podtip ne-APL AML-a, citogenetska grupa rizika i ekspresija CD56 (engl. cluster of differentiation) nisu uticali na nastanak DIK-a ($p > 0,05$). Nije utvrđena razlika u ranoj smrtnosti, ishodu i preživljavanju ne-APL AML bolesnika, sa i bez DIK-a ($p > 0,05$).

Zaključak: Starije životno doba, prisustvo komorbiditeta, leukocitoza i visoke koncentracije LDH nose značajan rizik za razvoj DIK-a, kod bolesnika sa ne-APL AML-om. Prisustvo manifestne diseminovane intravaskularne koagulopatije ne utiče negativno na ranu smrtnost, ishod i preživljavanje bolesnika sa ne-APL AML-om, ukoliko se dijagnoza DIK-a postavi na vreme i preduzme neodložna, adekvatna i intenzivna primena suportivne terapije derivatima i komponentama krvi.

Ključne reči: akutna mijeloidna leukemija, diseminovana intravaskularna koagulopatija, ishod, preživljavanje

ABSTRACT

Introduction: Acute promyelocytic leukemia (APL) has the highest risk for overt disseminated intravascular coagulopathy (DIC), with reported incidence of DIC of up to 90%, as compared to 10-40% in other AML types. The influence of DIC on early death in non-APL AML patients has not been evaluated so far.

Aim: The aim of our study was to analyze the incidence of DIC, its clinical and laboratory characteristics, and the impact on the survival and early death of patients with non-APL AML.

Materials and methods: A total of 176 patients with non-APL AML, diagnosed and treated at the Clinic for Hematology of the Clinical Center of Serbia, between 2015 and 2020, were evaluated retrospectively. The diagnosis of DIC was made on the basis of ISTH (International Society on Thrombosis and Haemostasias) criteria.

Results: The mean age of our patients was 53.8 ± 14.6 years, with 99/176 patients being men (56.2%). DIC was present in 74/176 patients (42.05%), who had a significant prevalence of the hemorrhagic syndrome ($p = 0.01$). The risk factors for overt DIC were the following: older age ($p < 0.01$), comorbidities ($p = 0.01$), leukocytosis ($p < 0.001$) and a high level of LDH ($p < 0.001$). The FAB (French, American and British) type of non-APL AML, the cytogenetic risk group, and CD56 (cluster of differentiation) had no influence on overt DIC ($p > 0.05$). No difference was found in early mortality, outcome, and the survival of non-APL AML patients, with and without DIC ($p > 0.05$).

Conclusion: Older age at diagnosis, comorbidities, leukocytosis, and high LDH concentrations are found to be adverse risk factors for overt DIC in non-APL AML patients. If treated promptly, with immediate, adequate and intensive use of blood derivates and components, DIC has no negative impact on early mortality, outcome, and survival.

Key words: acute myeloid leukemia, disseminated intravascular coagulopathy, outcome, survival

Autor za korespondenciju:
Mirjana Cvetković
Medicinski fakultet, Univerzitet u Beogradu
Čarlija Čaplina 34, 11000 Beograd, Srbija
E-mail: mimamima.cvetkovic@gmail.com

Corresponding author:
Mirjana Cvetković
Faculty of Medicine, University of Belgrade, Serbia
34 Čarlija Čaplina Street, Belgrade, Serbia
E-mail: mimamima.cvetkovic@gmail.com

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UVOD

Akutne mijeloidne leukemije (AML) su heterogena grupa malignih bolesti krvi koje karakteriše klonalna ekspanzija mijeloblasta u koštanoj srži (≥20%), perifernoj krvi i/ili drugim tkivima [1]. AML se ubraja u retke bolesti i čini 1,1% svih malignih bolesti. AML je najčešći tip akutnih leukemija adultnog doba i javlja se sa godišnjom incidencijom od 4,3/100.000 stanovnika, nešto češće kod osoba muškog pola (M:Ž = 5,2/100.000 : 3,6/100.000). AML je bolest starih – prilikom postavljanja dijagnoze, 54% bolesnika ima 65 ili više godina, sa medijanom životnog doba od 68 – 71 godine [2-4]. I pored savremenog lečenja, preživljavanje obolelih od AML-a je veoma kratko (petogodišnje preživljavanje = 24%) [2].

Nastanku AML-a može doprineti prethodna primena hemioterapije, radioterapije i imunosupresivnih lekova, za lečenje malignih ili autoimunih bolesti – kada govorimo o *therapy-related AML* (*t-AML*). Nastanku AML-a takođe može doprineti i okupaciono izlaganje ili izlaganje agensima iz životne sredine, koji oštećuju DNK (dezoksiribonukleinska kiselina). AML može biti i sekundarna, tj. nastati evolucijom hroničnih mijeloproliferativnih neoplazmi (MPN) ili mijelodisplaznih sindroma (MDS). Takođe, utvrđena je i genetska predispozicija za nastanak AML-a (Fankonijeva anemija, Daunov sindrom, Švahman-Dajmondov sindrom, sindromi kongenitalne neutropenije) [1,5]. Međutim, etiologija većine AML-a je nepoznata, kada govorimo o *de novo* AML-u.

U AML-u postoji čitav spektar različitih hromozomskih promena, kao što je translokacija t(15;17) (*PML-RARA),* koja je karakteristična za akutnu promijelocitnu leukemiju (APL). Takođe postoji i niz genetskih mutacija, koje utiču na: signalne puteve (kao što su *FLT3-ITD, KIT, MLL, KRAS, NRAS*), nukleofozmin (*NPM1*), transkripcione faktore (kao što su *CEBPA, RUNX1, GATA-2),* i tumorsku supresiju (*TP53, WT1*). Postoji i niz epigenetskih mutacija, koje dovode do metilacije DNK i modifikacije hromatina (kao što su *TET, IDH1, IDH2, MLL*) [5,6].

Savremena klasifikacija akutnih leukemija Svetske zdravstvene organizacije – SZO (*World Health Organization – WHO*), iz 2016. godine, kao i preporuke *ELN* (*European LeukemiaNet*), upravo se i zasnivaju na molekularnim karakteristikama AML-a, budući da one imaju i prognostički i terapijski značaj [7,8], mada se u svakodnevnoj praksi i dalje koristi *FAB* (*French, American and British*) klasifikacioni sistem koji se zasniva na morfološkim i imunofenotipskim karakteristikama leukemijskih ćelija [9,10].

Klinički, AML nastaje iz „punog zdravlja" i manifestuje se povišenom telesnom temperaturom, anemijom, krvarenjem i rekurentnim infekcijama. Bolesnici sa AML-om često imaju trombocitopeniju i poremećaje hemostaze, tj. koagulopatije, koji značajno komplikuju lečenje i doprinose ranoj smrtnosti ovih bolesnika [11].

INTRODUCTION

Acute myeloid leukemias (AML) are a heterogenous group of malignant diseases of the blood characterized by clonal expansion of myeloblasts in the bone marrow (≥20%), in peripheral blood, and/or in other tissues [1]. AML is a rare disease and accounts for 1.1% of all malignant diseases. AML is the most frequently occurring type of acute leukemia of adult age and has an annual incidence of 4.3 per 100,000 population, occurring somewhat more frequently in men (m : f = 5.2/100,000 : 3.6/100,000). AML is a disease of the elderly – at diagnosis, 54% of the patients are 65 years old or above, with the median age being 68 – 71 years [2-4]. Despite modern treatment, survival of AML patients is very short (five-year survival = 24%) [2].

As far as therapy-related AML (t-AML) is concerned, previous chemotherapy, radiotherapy, and immunosuppressive drugs, for the treatment of malignant and autoimmune diseases, can contribute to its development. Occupational exposure, as well as exposure to agents from the environment, which damage DNA (deoxyribonucleic acid), can also contribute to the occurrence of AML. AML can develop as a secondary disease, i.e., it can occur as the result of the evolution of chronic myeloproliferative neoplasms (MPN) or myelodysplastic syndromes (MDS). Also, a genetic predisposition for the development of AML (Fanconi anemia, Down syndrome, Shwachman-Diamond syndrome, congenital neutropenia syndromes) has been confirmed [1,5]. However, as far as *de novo* AML is concerned, the etiology of most AMLs remains unknown.

In AML, there is a whole array of different chromosomal alterations, such as the translocation t(15;17) (PML-RARA), which is characteristic of acute promyelocytic leukemia (APL). There are also a number of genetic mutations, which affect signal pathways (such as FLT3-ITD, KIT, MLL, KRAS, NRAS), nucleophosmin (NPM1), transcription factors (such as CEBPA, RUNX1, GATA-2), and tumor suppression (TP53, WT1). In AML, there are also epigenetic mutations which lead to the methylation of DNA and the modification of chromatin (such as TET, IDH1, IDH2, MLL) [5,6].

The contemporary classification of acute leukemias, issued by the World health Organization (WHO) in 2016, as well as the European LeukemiaNet (ELN) recommendations are, in fact, based on the molecular characteristics of AML, since they have both prognostic and therapeutic significance [7,8], although, in everyday clinical practice, the French, American and British (FAB) classification system, which is based on morphological and immunophenotypical characteristics of leukemia cells is still in use [9,10].

Diseminovana intravaskularna koagulopatija (DIK) je stečeni sindrom koji karakteriše sistemska intravaskularna aktivacija koagulacije, koja može dovesti do multiorganske disfunkcije, tromboze i/ili ekscesivnog krvarenja [12,13]. Najčešća stanja koja dovode do DIK-a su sepsa, šok, solidni tumori i maligne bolesti krvi – akutne leukemije i Nehočkinovi limfomi. Pod dejstvom proinflamatornih citokina, mononuklearne i endotelne ćelije eksprimiraju tkivni faktor (TF). Kontaktom TF-a sa faktorima koagulacije u krvi, započinje koagulaciona kaskada, koja dovodi do generacije trombina i konverzije fibrinogena u fibrin. Istovremeno, interakcija između trombocita i zida krvnog suda doprinosi stvaranju vaskularnih (ili mikrovaskularnih) ugrušaka. P-selektin iz aktivisanih trombocita dodatno pojačava ekspresiju TF-a. Vezivanje TF-a, trombina i drugih aktivisanih faktora koagulacije (proteaza) za specifične proteaza-aktivirane receptore (PAR), i vezivanje fibrina za *toll-like receptor 4* (*TLR4*) na inflamatornim ćelijama, utiče na inflamaciju posledičnim oslobađanjem pro-inflamatornih citokina i hemokina, što dalje modulira koagulaciju i fibrinolizu [14].

Međunarodno društvo za trombozu i hemostazu (*ISTH – International Society on Thrombosis and Hemostasis*) preporučilo je sistem bodovanja, koji se upotrebljava kod bolesnika koji imaju neki osnovni poremećaj, za koji se zna da je povezan sa razvojem DIK-a, i u kojem se prate četiri laboratorijska parametra: broj trombocita (Tr), protrombinsko vreme (*PT*), koncentracija fibrinogena, i nivo D-dimera [12]. Diseminovana intravaskularna koagulopatija je prisutna kod čak 90% bolesnika sa APL-om [15], dok je učestalost DIK-a kod ostalih tipova AML-a znatno manja, i kreće se od 10% do 40% [16]. Ne postoje studije koje su pratile uticaj vrednosti *ISTH* DIK skora na ranu smrt kod bolesnika sa AML-om.

Cilj našeg rada bilo je prikupljanje i analiza podatka o: učestalosti DIK-a u grupi bolesnika sa ne-APL AML-om, kliničkoj slici, kliničko-laboratorijskim parametrima, odnosno učestalosti krvarenja, trombozi, i ranoj smrti ne-APL AML bolesnika sa DIK-om.

METODE

Rađena je retrospektivna analiza 176 uzastopnih bolesnika sa ne-APL AML-om, koji su dijagnostikovani i lečeni na Klinici za hematologiju Kliničkog centra Srbije, u periodu između 2015. i 2020. godine. Dijagnoza AML-a je postavljena na osnovu citomorfoloških, imunofenotipskih, citogenetskih, i molekularnih karakteristika ćelija koštane srži ili periferne krvi, a u skladu sa preporukama Svetske zdravstvene organizacije, iz 2016. godine [7]. Morfološka dijagnoza je postavljena na osnovu *FAB* klasifikacije [9], a prilikom imunofenotipizacije, tehnikom protočne citometrije, sem standardnih monoklonskih antitela [10], primenjeno je

Clinically, AML develops from "full health" and presents with elevated body temperature, anemia, bleeding and recurrent infections. AML patients often have thrombocytopenia and hemostasis disorders, i.e., coagulopathies, which significantly complicate treatment and contribute to early mortality of these patients [11].

Disseminated intravascular coagulopathy (DIC) is an acquired syndrome characterized by systemic intravascular activation of coagulation, which can lead to multiorgan dysfunction, thrombosis, and/or excessive bleeding [12,13]. The most common conditions leading to DIC, are the following: sepsis, shock, solid tumors, and malignant diseases of the blood – acute leukemias and non-Hodgkin lymphomas. Under the influence of proinflammatory cytokines, mononuclear and endothelial cells express the tissue factor (TF). Through the contact of TF with coagulation factors in the blood, the coagulation cascade is initiated, which leads to the generation of thrombin and the conversion of fibrinogen into fibrin. At the same time, interaction between thrombocytes and the blood vessel wall contributes to the creation of vascular (or microvascular) thrombi. P-selectin from activated thrombocytes additionally intensifies the expression of TF. The binding of TF, thrombin, and other activated coagulation factors (proteases) to specific protease-activated receptors (PAR), and the binding of fibrin to toll-like receptor 4 (TLR4) on inflammatory cells, affects inflammation through the consequential release of pro-inflammatory cytokines and chemokines, which further modulates coagulation and fibrinolysis [14].

The International Society on Thrombosis and Hemostasis (ISTH) has recommended a scoring system, which is applied in patients with an underlying disorder, known to be linked to the development of DIC, where the following four laboratory parameters are monitored: platelet (thrombocyte) count (Tr), prothrombin time (PT), fibrinogen concentration, and the D-dimer level [12]. Disseminated intravascular coagulopathy is present in as many as 90% of the patients with APL [15], while the frequency of DIC in other types of AML is significantly lower, and ranges from 10% do 40% [16]. There are no studies analyzing the effect of the ISTH DIC on early mortality in patients with AML.

The aim of our study was to collect and analyze data on the following: the incidence of DIC in a group of patients with non-APL AML, the clinical presentation, clinical and laboratory parameters, i.e., the frequency of bleeding, thrombosis, and early death of non-APL AML patients with DIC.

METHODS

A retrospective analysis was performed, involving 176 consecutive patients with non-APL AML, diagnosed

i monoklonsko antitelo za *CD56* (engl. *cluster of differentiation*), karakteristično za *NK* (engl. *natural killer*) ćelije. Citogenetska procena rizika (povoljna, intermedijarna, nepovoljna) je izvršena prema *ELN* preporukama [8] određivanjem kariotipa – pomoću konvencionalne citogenetike, i molekularnih karakteristika – korišćenjem *PCR* (engl. *polymerase chain reaction*) metode. Dijagnoza t-AML-a je postavljena bolesnicima koji su imali pozitivnu ličnu anamnezu i medicinsku dokumentaciju o prethodnoj primeni hemioterapije, radioterapije i imunosupresivnih lekova, za lečenje malignih ili autoimunskih bolesti. Prilikom postavljanja dijagnoze određivan je i značaj postojećih pridruženih bolesti, tj. komorbiditeta, na osnovu *HCT-CI* (engl. *Hematopoietic cell transplantation specific comorbidity index*) skora [17].

Kod svih bolesnika analizirani su sledeći laboratorijski parametri: hemoglobin-Hb (g/l), broj leukocita – Le (x10^9/l), broj Tr (x10^9/l), procenat mijeloblasta u perifernoj krvi, koncentracija laktat dehidrogenaze – LDH (U/l), PT, aktivisano parcijalno tromboplastinsko vreme (aPTT), fibrinogen i D-dimer. Normalne vrednosti za LDH bile su 220 – 460 U/l, dok su za parametre hemostaze bili: 75 – 120%, za PT; 25 – 35 s, za aPTT; 2 – 4 g/l, za fibrinogen; <0,5 μg/ml, za D-dimer. Dijagnoza DIK-a je postavljena na osnovu *ISTH* kriterijuma: broj trombocita (x10^9/l) : >100x10^9/l = 0,<100 = 1, <50 = 2; D-dimer: normalan = 0, umereno (2 – 4 puta) povećan = 2, izrazito visok (≥5 puta) = 3; PT: >75 % = 0, 50 – 75 % = 1, <50 % = 2; fibrinogen: >1 g/l = 0,<1 g/l = 1. Ukupan skor ≥5 ukazuje na manifestnu diseminovanu intravaskularnu koagulopatiju. Kod svih bolesnika određivano je da li su imali kliničke znake prisustva hemoragijskog sindroma prilikom postavljanja dijagnoze.

Svi bolesnici su primali indukcionu kombinovanu citostatsku terapiju (doksorubicin i citozin-arabinozid po šemi '3+7' ili '2+5', u zavisnosti od opšteg funkcionalnog stanja i komorbiditetnog indeksa), i potom konsolidaciju, primenom citozin-arabinozida. Uporedo sa lečenjem AML-a, bolesnici sa menifestnom diseminovanom intravaskularnom koagulopatijom su lečeni derivatima i komponentama krvi, prema važećim preporukama. Transfuzije trombocita su primenjivane pri vrednostima trombocita <50x10^9/l, kod bolesnika sa manifestnim krvarenjem, a u odsustvu krvarenja, ukoliko su trombociti bili <20x10^9/l. Kod bolesnika sa hemoragijskim oblikom DIK-a i produženim PT-om i aPTT-om, primenjivana je zamrznuta sveža plazma (ZSP) u dozi od 15ml/kg. Bolesnici sa teškom hipofibrinogenemijom (<1g/l), koja se nastavljala i pored primene ZSP-a, primali su i krioprecipitat [13]. Praćeni su: ishod lečenja (živ/umro), rana smrtnost (smrtni ishod od prvog dana hospitalizacije do završetka indukcionog lečenja, odnosno otpusta), ukupno preživljavanje

and treated at the Clinic for Hematology of the Clinical Center of Serbia, between 2015 and 2020. AML diagnosis was established on the basis of cytomorphological, immunophenotypical, cytogenetic, and molecular characteristics of bone marrow cells or peripheral blood cells, in keeping with the recommendations of the World Health Organization from 2016 [7]. The morphological diagnosis was based on the FAB classification [9]. During immunophenotypization by means of flow cytometry, in addition to the standard monoclonal antibodies [10], the monoclonal antibody for CD56 (cluster of differentiation), which is characteristic of NK (natural killer) cells, was also applied. The cytogenetic risk assessment (favorable, intermediate, unfavorable) was carried out in keeping with the ELN recommendations [8] through the determination of the karyotype – by means of conventional cytogenetics and molecular characteristics – with the use of the polymerase chain reaction (PCR) method. The diagnosis of t-AML was established in patients with a positive personal anamnesis and medical documentation on previous application of chemotherapy, radiotherapy, and immunosuppressive drugs, for treating malignant or autoimmune diseases. When establishing the diagnosis, the significance of existing associated diseases, i.e., comorbidities was determined, on the basis of the HCT-CI (Hematopoietic cell transplantation specific comorbidity index) score [17].

In all patients, the following laboratory parameters were analyzed: hemoglobin-Hb (g/l), white blood cell count (WBC), i.e., leukocyte count – Le (x10^9/l), platelet count, i.e., thrombocyte count - Tr (x10^9/l), percentage of myeloblasts in peripheral blood, concentration of lactate dehydrogenase – LDH (U/l), PT, activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer. The normal values for LDH were 220 – 460 U/l, while the normal values for hemostasis parameters were: 75 – 120%, for PT; 25 – 35 s, for aPTT; 2 – 4 g/l, for fibrinogen; <0.5 μg/ml, for D-dimer. The diagnosis of DIC was established on the basis of the ISTH criteria: platelet count (x10^9/l) : >100x10^9/l = 0,<100 = 1, <50 = 2; D-dimer: normal = 0, moderately elevated (2 – 4 times) = 2, very high (≥5 times) = 3; PT: >75 % = 0, 50 – 75 % = 1, <50 % = 2; fibrinogen: >1 g/l = 0,<1 g/l = 1. The total score of ≥5 indicates overt disseminated intravascular coagulopathy. In all of the patients, it was assessed whether they had clinical signs of hemorrhagic syndrome at the time of diagnosis.

All of the patients received combined induction cytostatic therapy (doxorubicin and cytosine arabinoside, following the '3+7' and '2+5' regimen, depending on the general performance status and the comorbidity index), and then consolidation, with the application of cytosine arabinoside. Parallel to the treatment of AML, patients with overt disseminated intravascular coagulopathy

svih bolesnika (engl. *overall survival* – *OS*), kao i da li je prisustvo DIK-a imalo uticaja na ishod i *OS*.

Prilikom statističke analize korišćene su metode deskriptivne statistike: a) za kontinuirane varijable - aritmetička sredina i standardna devijacija (SD), odnosno medijana i opseg i b) za kategoričke varijable – učestalost, izražena u apsolutnim brojevima i procentima. Za određivanje razlike između dve grupe, korišćeni su odgovarajući statistički testovi: parametarski Studentov T-test za dva nezavisna uzorka, odnosno njegova neparametarska paralela – test sume rangova (Man Vitnijev U test). Za ispitivanje razlike učestalosti, korišćeni su Hi kvadrat test, odnosno Fišerov test tačne verovatnoće. Za analizu preživljavanja, korišćena je Kaplan Majerova metoda, kao i log-rank test za poređenje preživljavanja među ispitivanim grupama. Vrednosti $p < 0,05$ smatrane su statistički značajnim.

REZULTATI

U studiju je uključeno 176 bolesnika sa ne-APL AML-om, 99 muškaraca (56,2%) i 77 žena (43,7%) (M:Ž = 1,29), prosečne starosti 53,8 ± 14,6 godina. Demografske, laboratorijske i kliničke karakteristike bolesnika prikazane su u Tabeli 1.

Prilikom postavljanja dijagnoze, hemoragijski sindrom je bio prisutan kod 72/176 bolesnika (40,9%). Analiza parametara krvne slike pokazala je da su bolesnici, u proseku, imali anemiju umerenog stepena (97,3 ± 18,4 g/l), trombocitopeniju gr III (medijana: 44 x10^9/l; raspon: 1 – 421) i leukocitozu (medijana: 18,5 x10^9/l; raspon: 0,6 – 473,2) sa prisustvom blasta u perifernoj krvi (medijana: 16%, raspon: 0 – 99). Vrednost LDH je, u proseku, bila povišena (medijana: 450 U/l, raspon: 102 – 8.840). Parametri hemostaze su pokazali produženo PT (70 ± 18%) i veoma visok D-dimer (medijana: 3,0 µg/ml, raspon: 0,19 – 138). Kriterijume za manifestnu diseminovanu intravaskularnu koagulopatiju je ispunjavalo 74/176 bolesnika (42%), i oni su imali značajno viši *ISTH* skor (grupa I – *ISTH,* medijana: 5, raspon: 5 – 7; grupa II – *ISTH*, medijana: 3, raspon: 0 – 3) (p < 0,001).

Najveći broj bolesnika je imao AML *FAB* tip M4 (n = 66, 37,5%) i pripadao je *ELN* grupi intermedijarnog citogenetskog rizika (n = 93, 52,8%). Pozitivnost *CD*56 je utvrđena kod 62 bolesnika (35,2%). Bolesnici su imali i značajne pridružene bolesti (*HCT-CI* skor, medijana: 1, raspon: 0 – 8).

Bolesnici sa DIK-om su bili značajno stariji (57,4 ± 12,4 godina) u odnosu na bolesnike koji nisu imali DIK (51,2±15,5 godina), $p = 0,006$. Hemoragijski sindrom prilikom postavljanja dijagnoze je bio značajno češći u grupi bolesnika sa DIK-om, 39/74 (52,7%) u odnosu na bolesnike koji nisu imali DIK, 33/102 (32,4%), $p = 0,01$. U pogledu laboratorijskih parametara, were treated with blood derivatives and blood components, as per the current recommendations. Transfusions of thrombocytes were applied when the thrombocyte count was <50 x10^9/l, in patients with manifest bleeding, and when bleeding was absent, in cases when the thrombocyte count was <20 x10^9/l. In patients with the hemorrhagic form of DIC and prolonged PT and aPTT, frozen fresh plasma (FFP) was applied, in the dose of 15ml/kg. Patients with severe hypofibrinogenemia (<1g/l), which persisted despite the application of FFP, also received cryoprecipitate [13]. The following were monitored: treatment outcome (living/deceased), early mortality (lethal outcome occurring between the first day of hospitalization and the conclusion of induction treatment, i.e., discharge from hospital), overall survival of all the patients (OS), as well as whether the existence of DIC had any effect on the outcome and OS.

In statistical analysis, the following methods of descriptive statistics were applied: a) for continuous variables – the arithmetic mean and the standard deviation (SD), i.e., the median and the range, and b) for categorical variables – frequency, expressed in absolute values, and percentages. For determining the difference between the two groups, the appropriate statistical tests were applied, namely: the parametric Student's T-Test for two independent samples, i.e., its non-parametric parallel – the rank sum test (Mann–Whitney U test). For testing the difference in frequency, the chi-square test, i.e., Fisher's exact probability test were used. For analyzing survival, the Kaplan Meier method, as well as the log-rank test for comparing survival amongst the tested groups, were applied. The values $p < 0.05$ were believed statistically significant.

RESULTS

The study included 176 patients with non-APL AML, 99 men (56.2%) and 77 women (43.7%) (M : Ž = 1.29), whose average age was 53.8 ± 14.6 years. The demographic, laboratory and clinical characteristics of the patients are presented in Table 1.

At the time of diagnosis, hemorrhagic syndrome was present in 72/176 patients (40.9%). The analysis of the blood count parameters showed that the patients, on average, had moderate anemia (97.3 ± 18.4 g/l), grade 3 thrombocytopenia (median: 44 x10^9/l; range: 1 – 421), and leukocytosis (median: 18.5 x10^9/l; range: 0.6 – 473.2), with the presence of blasts in peripheral blood (median: 16%, range: 0 – 99). On average, the LDH level was elevated (median: 450 U/l, range: 102 – 8,840). The parameters of hemostasis showed prolonged PT (70 ± 18%) and very high D-dimer (median: 3.0 µg/ml, range: 0.19 – 138). The criteria for overt disseminated intravascular coagulopathy were met

Tabela 1. Modeli multivarijantne logističke regresije u kojima su nezadovoljene potrebe za stomatološkom zdravstvenom zaštitom ishodna varijabla

Table 1. Multivariate logistic regression models with unmet dental health care needs as an outcome variable

Parametar / *Parameter*	**Vrednost / *Value***	**Manifestna DIK (*ISTH* DIK skor ≥5) / *Overt DIC (ISTH DIC score ≥5)***		**P vrednost / *P value***
		Grupa I - DIK da / *Group I - DIC yes* (n = 74/176; 42%)	**Grupa II - DIK ne / *Group II - DIC no*** (n = 102/176; 58%)	
Pol – n, % / *Sex – n, %*				
Muški / *Male*	99 (56.25)	47 (63.5)	52 (51)	
Ženski / *Female*	77(43.75)	27 (36.5)	50 (49)	0.124
Starost- srednja vrednost (godine), SD / *Age – mean (years), SD*	53.8 ± 14.6	57.4 ± 12.4	51.2 ± 15.5	0.006
Le – medijana (x10^9/l), raspon / *WBC – median (x10^9/L), range*	18.5 (0.6-473.2)	32.1 (0.6-451)	13.6 (1.09-473.2)	0.001
Hb – medijana (g/l), SD / *Hb – median (g/L), SD*	97.3 ± 18.4	97.1 ± 17.4	97.6 ± 19.2	0.874
Tr – medijana (x10^9/l), raspon / *Plt – median (x10^9/L), range*	44 (1 – 421)	32.5 (1 – 151)	61.5 (2 – 421)	0.001
% blasta u perifernoj krvi – medijana, raspon / *% peripheral blood blasts- median, range*	16 (0 – 99)	15.5 (0 – 97)	17 (0 – 99)	0.741
LDH – medijana (U/l), raspon / *LDH – median (U/L), range*	450 (102 – 8,840)	591.5 (102 – 5,786)	383 (108 – 8,840)	0.001
PT – srednja vrednost (%), SD / *PT – mean (%), SD*	70 ± 18	61 ± 16	78 ± 15	0.001
aPTT – srednja vrednost (s), SD / *aPTT – mean (s), SD*	29.6 ± 5.9	30.2 ± 6.1	29.2 ± 5.8	0.175
Fibrinogen– srednja vrednost (g/l), SD / *Fibrinogen – mean (g/L), SD*	5.4 ± 1.9	5.2 ± 2.0	5.6 ± 1.8	0.270
D-dimer – medijana (µg/ml), raspon / *D-dimer – median (µg/mL), range*	3.0 (0.2 – 138)	6.2 (0.8 – 138)	1.32 (0.2 – 74)	0.001
Tip AML (n,%) / *AML type (n, %)*				
FAB M0	10 (5.7)	5 (6.8)	5 (4.9)	
FAB M1	23 (13.1)	10 (13.5)	13 (2.7)	
FAB M2	34 (19.3)	13 (17.6)	21 (20.6)	
FAB M4	66 (37,5)	33 (44.6)	33 (33.4)	0.228
FAB M5	21 (11.9)	9 (12.2)	12 (10.8)	
FAB M6	0	0	0	
FAB M7	0	0	0	
t-AML	22 (12.5)	4 (5.3)	18 (17.8)	
Citogenetska grupa rizika (n,%) / *Cytogenetic risk group (n, %)*				
Povoljna / *Favorable*	18 (10.2)			
Intermedijarna / *Intermediate*	93 (52.8)	5 (8.3)	13 (13.5)	
Nepovoljna / *Unfavorable*	45 (25.6)	37 (61.7)	56 (58.3)	0.612
Nema podataka / *Data missing*	20 (11.4)	18 (30)	27 (28.2)	
CD56 (n,%) /				
Da / *Yes*	62 (35.2)	28 (45.9)	34 (41)	
Ne / *No*	82 (46.6)	33 (54.1)	49 (59)	0.674
Nema podataka / *Data missing*	32 (18.8)			
Hemoragijski sindrom (n,%) /				
Da / *Yes*	72 (40.9)	39 (52.7)	33 (32.4)	0.01
Ne / *No*	104 (59.1)	35 (47.3)	69 (67.6)	
HCT-CI (medijana, raspon) / *HCT-CI (median, range)*	1 (0 – 8)	2 (0 – 8)	1 (0 – 6)	0.01
Konačan ishod (n,%) / *Outcome (n, %) / Bleeding-hemorrhagic syndrome (no, %)*				
Živ / *Living*	48 (27.3)	18 (24.3)	30 (29.4)	
Umro / *Deceased*	124 (70.4)	54 (73)	70 (68.6)	
Nema podataka / *Data missing*	4 (2.3)	2 (2.70)	2 (12)	0.496
Rana smrt (n, %) /				
Da / *Yes*	41 (23.3)	20 (27)	21 (20.6)	
Ne / *No*	128 (72.7)	50 (67.6)	78 (76.4)	
Nema podataka / *Data missing*	7 (4)	4 (5.4)	3 (3)	0.281

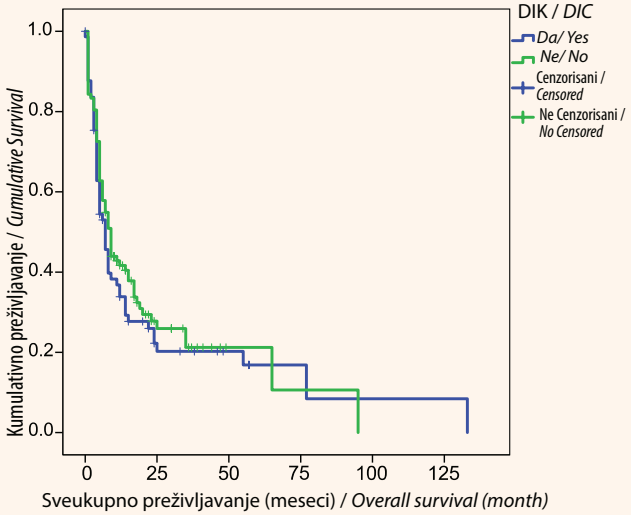
Legenda: APL – akutna promijelocitna leukemija; DIK – diseminovana intravaskularna koagulopatija; Hb – hemoglobin,; Le – leukociti; Tr – trombociti, LDH – laktat dehidrogenaza, PT – protrombinsko vreme; aPTT- aktivisano parcijalno tromboplastinsko vreme; ISTH – International Society for Thrombosis and Haemostasias; FAB – French, American and Bristish; HCT CI – Hematopoietic cell transplantation specific comorbidity index

Legend: *APL – acute promyelocytic leukemia; DIC – disseminated intravascular coagulation; Hb –hemoglobin; WBC – leukocyte count; Plt – platelets, LDH – lactate dehydrogenase, PT – prothrombin time; aPTT – activated partial thromboplastin time; ISTH – International Society for Thrombosis and Haemostasias; FAB – French, American and British; HCT CI – hematopoietic cell transplantation specific comorbidity index*

bolesnici sa DIK-om su imali značajno niži broj Tr (grupa I – medijana: 32,5 x10^9/l, raspon: 1 –151, u odnosu na grupu II – medijana: 61,5, raspon: 2 – 421; $p < 0,001$), značajno više vrednosti LDH (grupa I – medijana: 591,5 U/l, raspon: 102 – 5.786; grupa II – medijana: 383 U/l, raspon: 108 – 8.840; $p < 0,001$), značajno duže PT (grupa I: 61,6 ± 16%; grupa II: 78 ± 15%; $p < 0,001$), i značajno veće vrednosti D-dimera (grupa I – 6,2 µg/ml, raspon: 0,82 – 138; grupa II – 1,32 µg/ml, raspon: 0,2 – 74; $p < 0,001$). Bolesnici sa DIK-om su imali veći broj komorbiditeta (grupa I – prosečan *HCT-CI* skor: 2, raspon: 0 – 8) u odnosu na one koji nisu imali DIK (grupa II – prosečan *HCT-CI* skor: 1, raspon: 0 – 6), $p = 0,01$. Tip AML-a, *ELN* citogenetska grupa rizika, i pozitivnost *CD56* nisu uticali na razvoj manifestne diseminovane intravaskularne koagulopatije ($p > 0,05$).

U pogledu ishoda, od 176 ispitivanih bolesnika, do kraja praćenja živih je bilo 48 ne-APL AML bolesnika (27,7%). Prisustvo DIK-a nije uticalo na ishod (živi, grupa I – 18/74 (24,3%); živi, grupa II – 30/102 (29,4%), $p = 0,496$). Indukciona smrt je zabeležena kod 41/176 bolesnika (23,3%), i nije se značajno razlikovala između dve ispitivane grupe (grupa I – n = 20/74 (27%); grupa II – n = 21/102 (27%); $p = 0,291$).

Ukupno preživljavanje svih bolesnika iznosilo je 7 meseci (raspon: 0 – 57), i premda je bilo kraće kod bolesnika sa manifestnom diseminovanom intravaskularnom koagulopatijom (grupa I – 5 meseci, raspon: 0 – 57), u odnosu na bolesnike bez koagulopatije (grupa II – 7 meseci, raspon: 0 – 49), ova razlika nije pokazala statističku značajnost (log-rank: 0,518), što je i prikazano Kaplan Majerovom krivom preživljavanja (Grafikon 1).



Grafikon 1. Preživljavanje (meseci) 176 bolesnika sa ne-APL AML-om, u odnosu na prisustvo manifestne diseminovane intravaskularne koagulopatije (DIK)

Figure 1. Survival (months) of 176 patients with non-APL AML, in relation to overt DIC

by 74/176 patients (42%), and they had a significantly higher ISTH score (group I – ISTH, median: 5, range: 5 – 7; group II – ISTH, median: 3, range: 0 – 3) ($p < 0.001$).

The greatest number of patients had AML FAB type M4 (n = 66, 37.5%) and belonged to the ELN group of intermediate cytogenetic risk (n = 93, 52.8%). CD56 positivity was established in 62 patients (35.2%). Patients also had significant associated diseases (HCT-CI score, median: 1, range: 0 – 8).

Patients with DIC were significantly older (57.4 ± 12.4 years), as compared to patients without DIC (51.2 ± 15.5 years), $p = 0.006$. Hemorrhagic syndrome at the time of diagnosis was significantly more common in the group of patients with DIC, 39/74 (52.7%), as compared to patients without DIC, 33/102 (32.4%), $p = 0.01$. As to laboratory parameters, patients with DIC had a significantly lower platelet count (group I – median: 32.5x10^9/l, range: 1 – 151, as compared to group II – median: 61.5, range: 2 – 421; $p < 0.001$), significantly higher levels of LDH (group I – median: 591.5 U/l, range: 102 – 5,786; group II – median: 383 U/l, range: 108 – 8,840; $p < 0.001$), significantly longer PT (group I: 61.6±16%; group II: 78±15%; $p < 0.001$), and significantly higher levels of D-dimer (group I – 6.2 µg/ml, range: 0.82 – 138; group II – 1.32 µg/ml, range: 0.2 – 74; $p < 0.001$). Patients with DIC had a greater number of comorbidities (group I – average HCT-CI score: 2, range: 0 – 8), as compared to the patients without DIC (group II – average HCT-CI score: 1, range: 0 – 6), $p = 0.01$. The type of AML, the ELN cytogenetic risk group, and CD56 positivity, did not affect the development of overt disseminated intravascular coagulopathy ($p > 0.05$).

As to the outcome, by the end of the follow-up period, of the 176 subjects, there were 48 living non-APL AML patients (27.7%). The occurrence of DIC did not affect the outcome (living, group I – 18/74 (24.3%); living, group II – 30/102 (29.4%), $p = 0.496$). Induction death was registered in 41/176 patients (23.3%), and it did not significantly differ between the two analyzed groups (group I – n = 20/74 (27%); group II – n = 21/102 (27%); $p = 0.291$).

The overall survival of all patients was 7 months (range: 0 – 57), and though it was shorter in patients with overt disseminated intravascular coagulopathy (group I – 5 months, range: 0 – 57), as compared to patients without coagulopathy (group II – 7 months, range: 0 – 49), this difference did not show statistical significance (log-rank: 0.518), which has been presented with the Kaplan Meier survival curve (Figure 1).

DISCUSSION

The pathophysiological mechanism of DIC development in acute leukemias is complex, and it simultaneously includes the following: a) coagulation activation caused by the exposure of the tissue factor (TF) to blood;

DISKUSIJA

Patofiziološki mehanizam nastanka DIK-a u akutnim leukemijama je kompleksan, i podrazumeva istovremeno: a) aktivaciju koagulacije uzrokovanu izlaganjem tkivnog faktora (TF) krvi; b) poremećaj kontrole antikoagulantih mehanizama, i c) supresiju fibrinolize putem povećane ekspresije *PAI-1* (*plasminogen activator inhibitor-1*). Ove promene udruženo uzrokuju endotelijalnu disfunkciju i mikrovaskularne tromboze, koje dovode do disfunkcije organa i značajno negativno utiču na prognozu osnovne bolesti [14]. Leukemijske ćelije oslobađaju TF, a takođe sekretuju i proinflamatorne citokine, pre svega IL-6 (interleukin) i *TNF* alfa (*tumor necrosis factor*), koji oštećuju endotel krvnih sudova. Oštećenje endotela, s jedne strane, dovodi do povećane ekspresije TF-a, kao i *PAI-1*, a sa druge strane dovodi do smanjene ekspresije trombomodulina (TM) koji konvertuje protein C (PC) u aktivisani PC (APC), i inhibira koagulaciju. Leukemijske ćelije oslobađaju mnoge mikropartikule, koje, osim TF-a, sadrže i kancer-prokoagulant, koji ima aktivnost serin proteaze, i koji direktno, aktivacijom faktora X (FX) započinje koagulacionu kaskadu i generisanje trombina.

DIK karakteriše i poremećaj fibrinolize, koji, u fiziološkim uslovima, ima za cilj da degradacijom fibrinskih depozita spečava insuficijenciju periferne cirkulacije. U akutnim leukemijama, proinflamatorni citokini uzrokuju povećanu eksperesiju *PAI-1*, koji inhibicijom aktivatora plazminogena sprečava nastanak plazmina (sekundarna fibrinoliza). U akutnim leukemijama, veoma je pojačana i primarna fibrinoliza. Sve ovo dovodi do izražene hipofibrinogenemije i porasta fibrinogen/fibrin degradacionih proizvoda (FDP) i D-dimera [18]. Oko 15% bolesnika sa ne-APL AML-om dodatno razvije DIK u toku indukciono-remisione terapije. Maligne ćelije, pod dejstvom citotoksičnih lekova, podležu apoptozi, uz oslobađanje intranuklearnih proteina, poput histona H3 i *HMGB1* (*high-mobility group box-1*), što doprinosi nastanku DIK-a i sindroma lize tumora (engl. *tumor lysis syndrome – TLS*) [19].

Incidencija DIK-a u akutnim leukemijama je varijabilna. Najveća je kod APL-a, a najmanja kod B-ćelijske akutne limfoblastne leukemije [16]. Diseminovana intravaskularna koagulopatija je veoma ispitivana kod APL-a, ali su podaci o njenoj učestalosti i značaju u ne-APL AML-u veoma oskudni i raznoliki. Tako se saopštena učestalost DIK-a, na osnovu *ISTH* kriterijuma, kod bolesnika sa ne-APL AML-om, prilikom postavljanja dijagnoze, kreće od 6,4% [20] do 25,2% [16]. Liburel i saradnici [21] su saopštili da je učestalost diseminovane intravaskularne koagulopatije, određene prema *ISTH* kriterijumima, bila veća kod mlađih bolesnika (18 – 65 godina) (8,5%), u odnosu na starije bolesnike sa ne-APL

b) the disruption of the anticoagulant mechanism control, and c) the suppression of fibrinolysis through increased expression of PAI-1 (plasminogen activator inhibitor-1). These changes jointly cause endothelial disfunction and microvascular thromboses, which lead to organ dysfunction, and have a significantly negative effect on the prognosis of the underlying disease [14]. Leukemia cells release TF, and they also secrete proinflammatory cytokines, primarily IL-6 (interleukin) and TNF alfa (tumor necrosis factor), which damage the endothelium of blood vessels. Endothelial damage, on the one hand, leads to increased expression of TF and PAI-1, and, on the other hand, it causes decreased expression of thrombomodulin (TM), which converts protein C (PC) into activated PC (APC), and inhibits coagulation. Leukemia cells release many microparticles, which, in addition to TF, also contain cancer procoagulant, which has the activity of serine proteas, and which initiates the coagulation cascade as well as the generating of thrombin through direct activation of factor X (FX).

DIC is characterized by fibrinolysis disruption, whose purpose, in physiological conditions, is to prevent insufficiency in peripheral circulation through the degradation of fibrin deposits. In acute leukemias, proinflammatory cytokines cause the increased expression of PAI-1, which, through the inhibition of plasminogen activators, prevents the synthesis of plasmin (secondary fibrinolysis). In acute leukemias, primary fibrinolysis is also very intensified. All of this leads to marked hyperfibrinogenemia and the increase in fibrinogen/fibrin degradation products (FDP) and D-dimer [18]. Around 15% of patients with non-APL AML additionally develop DIC during induction remission therapy. Malignant cells, under the influence of cytotoxic drugs, undergo apoptosis, releasing intranuclear proteins, such as histone H3 and HMGB1 (high-mobility group box-1), which contributes to the development of DIC and the tumor lysis syndrome (TLS) [19].

The incidence of DIC in acute leukemias is variable. The highest incidence is in APL, and the lowest incidence is in B-cell acute lymphoblastic leukemia [16]. Disseminated intravascular coagulopathy has been intensively tested and analyzed in APL, however, the data on its frequency and significance in non-APL AML are very limited and varied. Hence, reported frequency of DIC, based on ISTH criteria, in patients with non-APL AML, at diagnosis, ranges from 6.4% [20] to 25.2% [16]. Libourel et al. [21] reported that the frequency of disseminated intravascular coagulopathy, determined according to the ISTH criteria, was higher in younger patients (18 – 65 years) (8.5%), as compared to older patients with non-APL AML (6.3%), while older patients significantly more often had hemorrhagic syndrome (13%). The frequency

AML-om (6,3%), dok su stariji bolesnici značajno češće imali hemoragijski sindrom (13%). Učestalost DIK-a kod bolesnika u našoj studiji iznosila je 42%, znatno više, u poređenju sa gorenavedenim podacima. Uz to, naši bolesnici su znatno češće imali manifestno krvarenje prilikom postavljanja dijagnoze, preko 50% bolesnika sa DIK-om (39/74) imalo je hemoragijski oblik DIK-a.

Leukocitoza (>20x10^9/l) nosi sa sobom veći rizik za razvoj DIK-a u AML-u. Leukemijske ćelije koje, za razliku od eritrocita, nisu elastične i savitljive, stvaraju agregate u mikrocirkulaciji, što, sa jedne strane, dovodi do vaskularne okluzije i dodatnog oštećenja endotela, a sa druge, do pojačanog oslobađanja citokina, mikropartikula i intranuklearnih proteina iz agregiranih blasta [16,22]. Sem izražene leukocitoze, u grupi naših bolesnika sa DIK-om, registrovane su i značajno više vrednosti LDH. Visoka koncentracija LDH je prediktor visokog rizika od krvarenja [23].

Važno je istaći da su bolesnici sa DIK-om bili znatno stariji i imali su značajno više komorbiditeta, u odnosu na bolesnike sa ne-APL AML-om koji nisu razvili DIK. Samo po sebi, starije životno doba se smatra stanjem hronične inflamacije [24], a prisutne pridružene bolesti dodatno mogu uticati na razvoj DIK-a. Analizu uticaju komorbiditeta na razvoj DIK-a nismo našli u dostupnoj literaturi.

Bolesnici sa DIK-om, iz naše studije, imali su teži stepen trombocitopenije, značajno duže PT i viši D-dimer, u odnosu na bolesnike koji nisu imali DIK, a to su i parametri koji se prate u *ISTH* DIK skoru. Postavljanje adekvatne dijagnoze DIK-a u AML često predstavlja izazov, s obzirom na samu prirodu bolesti [11]. Kako bolesnici sa akutnim leukemijama veoma često imaju trombocitopenije, i u odsustvu DIK-a, usled infiltracije koštane srži i primene citotoksične terapije, preporučen je novi *JMWH* (*Japanese Ministry of Health and Welfare*) sistem bodovnja, za dijagnozu DIK-a, u kom se boduje i osnovna bolest (akutne leukemije nose 1 bod), a modifikovani su bodovni kriterijumi za trombocitopeniju, koncentraciju fibrinogena i FDP-a [25].

U pogledu bioloških karakteristika ne-APL AML-a i rizika za nastanak DIK-a, kod naših bolesnika nije utvrđena razlika između *FAB* podtipova AML-a i citogenetske grupe rizika, a ni ekspresija *CD56*, za koju je saopšteno da nosi lošu prognozu u AML-u [26], nije uticala na razvoj DIK-a. Naši rezultati se razlikuju od rezultata Guo i saradnika [16], koji su utvrdili da je prevalencija DIK-a značajno veća kod bolesnika sa normalnim kariotipom i mutacijama *NPM1* i/ili *FLT3-ITD*. Liburel i saradnici [21] su objavili najveću učestalost DIK-a u *FAB* tipu M5. Interesantno je da su mutacije *NPM*1 i *FLT3-ITD* [27], kao i AML tip *FAB*5 [28] povezani sa hiperleukocitozom.

of DIC in the patients from our study was 42%, which is significantly higher, as compared to the abovementioned data. Additionally, our patients more often had manifest bleeding at diagnosis, more than 50% of the patients with DIC (39/74) had hemorrhagic syndrome.

Leukocytosis (>20x10^9/l) carries a higher risk for the development of DIC in AML. Leukemia cells, which, conversely to erythrocytes, are not elastic and flexible, create accumulations in the microcirculation, which, on one hand, leads to vascular occlusion and additional damage to the endothelium, and, on the other hand, it leads to increased release of cytokines, microparticles, and intranuclear proteins from aggregated blasts [16,22]. In addition to marked leukocytosis, in our group of patients with DIC, significantly higher levels of LDH were also registered. A high concentration of LDH is a predictor of a high risk of bleeding [23].

It is important to emphasize that patients with DIC were significantly older and that they had significantly more comorbidities, as compared to patients with non-APL AML who did did not develop DIC. Elderly age, in itself, is considered to be a state of chronic inflammation [24], while present associated diseases can additionally affect the development of DC. We were not able to find analyses of the influence of comorbidities on the development of DIC in available literature.

The patients with DIC, from our study, had a more severe degree of thrombocytopenia, significantly longer PT, and a higher D-dimer, as compared to patients without DIC, and these are the parameters that are monitored on the ISTH DIC score. Establishing the correct diagnosis of DIC in AML is often a challenge, bearing in mind the nature of the disease itself [11]. As patients with leukemia very often suffer from thrombocytopenia, even in the absence of DIC, due to the infiltration of bone marrow and the application of cytotoxic therapy, a new JMWH (Japanese Ministry of Health and Welfare) scoring system has been proposed for DIC diagnosis. This system scores the underlying disease, as well (acute leukemias are scored with one point), however, the scoring criteria for thrombocytopenia, fibrinogen concentration, and FDP have been modified [25].

As far as the biological characteristics of non-APL AML and the risks for the development of DIC are concerned, in our patients, no difference was determined between the FAB subtypes of AML and the cytogenetic group of risks. Additionally, the expression of CD56, which has been reported to carry an unfavorable prognosis in AML [26], did not affect the development of DIC. Our results differ from the results obtained in the study by Guo et al. [16], who found that the prevalence of DIC was significantly higher in patients with a normal karyotype and the mutations NPM1 and/or FLT3-ITD. Libourel et al. [21] reported the highest frequency of DIC

Udruženost DIK-a i AML-a nosi lošu prognozu [11,15,18-22]. Bolesnici sa DIK-om iz naše studije su, uporedo sa lečenjem osnovne bolesti, primali i suportivnu terapiju derivatima i komponentama krvi [11,13]. Primena antifibrinolitka u lečenju DIK-a u AML-u se ne preporučuje, s obzirom na opasnost od promocije stvaranja fibrinskih depozita [11]. U Japanu je, za lečenja DIK-a u akutnim leukemijama, odobrena primena rekombinantnog solubilnog trombomodulina (rTM), koji vezivanjem za trombin, inaktivira koagulaciju [29]. Ishod, rana smrtnost i preživljavanje kod naših ne-APL AML bolesnika sa prisutnim DIK-om, nisu se značajno razlikovali u odnosu na bolesnike koji nisu imali DIK, što je posledica opisanog terapijskog pristupa.

ZAKLJUČAK

Na osnovu sprovedenog istraživanja, možemo zaključiti da starije životno doba, prisustvo komorbiditeta, leukocitoza, i visoke koncentracije LDH, nose značajan rizik za razvoj DIK-a kod bolesnika sa ne-APL AML-om. Prisustvo manifestne diseminovane intravaskularne koagulopatije ne utiče negativno na ranu smrtnost, ishod i preživljavanje bolesnika sa ne-APL AML-om, ukoliko se dijagnoza DIK-a postavi na vreme i preduzme neodložna, adekvatna i intenzivna primena suportivne terapije derivatima i komponentama krvi.

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in the FAB type M5. It is interesting to note that the mutations NPM1 and FLT3-ITD [27], as well as the AML type FAB5 [28] have been linked to hyperleukocytosis.

The joint occurrence of DIC and AML carries an unfavorable prognosis [11,15,18-22]. The patients with DIC, from our study, received, not only treatment for their underlying disease, but were simultaneously also given supportive blood derivatives and components therapy [11,13]. The application of antifibrinolytics in the treatment of DIC in AML is not recommended, due to the danger of promoting the creation of fibrin deposits [11]. In Japan, the application of recombinant soluble thrombomodulin (rTM) has been approved, for treating DIC in acute leukemias, as it inactivates coagulation by binding to thrombin [29]. The outcome, early mortality, and survival in our non-APL AML patients with DIC did not significantly differ from the same parameters in our patients without DIC, which is the consequence of the above-described treatment approach.

CONCLUSION

Based on the research conducted within this study, we can conclude that older age, the presence of comorbidities, leukocytosis, and high levels of LDH, carry a significant risk of DIC development in patients with non-APL AML. The occurrence of overt disseminated intravascular coagulopathy does not negatively affect early mortality, the outcome, and overall survival of patients with non-APL AML, if the diagnosis of DIC is established on time, and timely, appropriate and intensive supportive therapy with blood derivatives and components is administered promptly.

Conflict of interest: None declared.

LITERATURA / *REFERENCES*

1. Pollyea DA, Bixby D, Perl A, Bhatt VR, Altman JK, Appelbaum FR, et al. NCCN Guidelines insights: Acute Myeloid Leukemia, Version 2.2021.J Natl Compr Canc Netw. 2021 Jan; 19(1):16-27.
2. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, et al. SEER cancer statistics review, 1975-2016, National Cancer Institute, dostupno na: https://seer.cancer.gov/statfacts/html/amyl.html, pristupljeno: 8. 2. 2021.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7–30.
4. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Rev. 2019 Jul;36:70-87.
5. Martignoles JA, Delhommeau F, Hirsch P. Genetic Hierarchy of Acute Myeloid Leukemia: From Clonal Hematopoiesis to Molecular Residual Disease. Int J Mol Sci. 2018 Dec; 19(12): 3850.
6. Rose D, Haferlach T, Schnittger S, Perglerová K, Kern W, Haferlach C. Subtype-specific patterns of molecular mutations in acute myeloid leukemia. Leukemia. 2017 Jan;31(1):11-7.
7. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016 May; 127(20):2391–405.
8. Döhner H, Estey E, Grimwade D. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017 Jan;129(4):424-47.
9. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med. 1985 Oct; 103(4):620-5.
10. Canaani J, Beohou E, Labopin M, Socié G, Huynh A, Volin L, et al. Impact of FAB classification on predicting outcome in acute myeloid leukemia, not otherwise specified, patients undergoing allogeneic stem cell transplantation in CR1: An analysis of 1690 patients from the acute leukemia working party of EBMT. Am J Hematol.2017 Apr;92(4):344-50.
11. Wang TF, Makar RS, Antic D, Levy JH, Douketis JD, Connors JM, et al. Management of hemostatic complications in acute leukemia: Guidance from the SSC of the ISTH. J Thromb Haemost. 2020 Dec;18(12):3174-83.

12. Toh CH, Hoots WK, SSC on Disseminated Intravascular Coagulation of the ISTH. The scoring system of the scientific and standardisation committee on disseminated intravascular coagulation of the International society on thrombosis and haemostasis: a 5-year overview. J Thromb Haemost. 2007 Mar;5(3):604-6.
13. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. British Committee for Standards in Haematology. Br J Haematol. 2009 Apr;145(1):24-33.
14. Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. Nat Rev Dis Primers. 2016 Jun;2:16037.
15. Mitrovic M, Suvajdzic N, Bogdanovic A, Kurtovic NK, Sretenovic A, Elezovic I, et al. International Society of Thrombosis and Hemostasis Scoring System for disseminated intravascular coagulation ≥ 6: a new predictor of hemorrhagic early death in acute promyelocytic leukemia.Med Oncol. 2013 Mar;30(1):478.
16. Guo Z, Chen X, Tan Y, Xu Z, Xu L. Coagulopathy in cytogenetically and molecularly distinct acute leukemias at diagnosis: Comprehensive study. Blood Cells Mol Dis. 2020 Mar; 81:102393.
17. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005 Oct;106(8):2912-9.
18. Ikezoe T. Advances in the diagnosis and treatment of disseminated intravascular coagulation in haematological malignancies. Int J Hematol. 2021 Jan;113(1):34-44.
19. Uchiumi H, Matsushima T, Yamane A, Doki N, Irisawa H, Saitoh T, et al. Prevalence and clinical characteristics of acute myeloid leukemia associated with disseminated intravascular coagulation. Int J Hematol. 2007 Aug;86(2):137-42.
20. Shahmarvand N, Oak JS, Cascio MJ, Alcasid M, Goodman E, Medeiros BC, et al. A study of disseminated intravascular coagulation in acute leukemia reveals markedly elevated D-dimer levels are a sensitive indicator of acute promyelocytic leukemia. Int J Lab Hematol. 2017 Aug;39(4):375-83.
21. Libourel EJ, Klerk CPW, van Norden Y, de Maat MPM, Kruip MJ, Sonneveldet P, et al. Disseminated intravascular coagulation at diagnosis is a strong predictor for thrombosis in acute myeloid leukemia. Blood Oct. 2016(14);128:1854-61.
22. Giammarco S, Chiusolo P, Piccirillo N, Di Giovanni A, Metafuni E, Laurenti L, et al. Hyperleukocytosis and leukostasis: management of a medical emergency. Expert Rev Hematol. 2017 Feb;10(2):147-54.
23. Naymagon L, Moshier E, Tremblay D, Mascarenhas J. Predictors of early hemorrhage in acute promyelocytic leukemia. Leuk Lymphoma. 2019 Oct;60(10):2394-403.
24. Brábek J, Jakubek M, Vellieux F, Novotný J, Kolář M, Lacina L, et al. Interleukin-6: Molecule in the Intersection of Cancer, Ageing and COVID-19. Int J Mol Sci. 2020 Oct;21(21):7937.
25. Wada H. Disseminated intravascular coagulation. Clinica Chimica Acta. 2004 Jun; 344(1-2):13–21.
26. Pinheiro LHS, Trindade LD, Costa FO, Silva NL, Sandes AF, Nunes MAP, et al. Aberrant Phenotypes in Acute Myeloid Leukemia and Its Relationship with Prognosis and Survival: A Systematic Review and Meta-Analysis. Int J Hematol Oncol Stem Cell Res.2020 Oct;14(4):274-88.
27. Tien FM, Hou HA, Tsai CH, Tang JL, Chen Y, Kuo YY, et al. Hyperleukocytosis is associated with distinct genetic alterations and is an independent poor-risk factor in de novo acute myeloid leukemia patients. Eur J Haematol. 2018 Jul; 101(1):86-94.
28. Inaba H, Fan Y, Pounds S, Geiger TL, Rubnitz JE, Ribeiro RC, et al. Clinical and biologic features and treatment outcome of children with newly diagnosed acute myeloid leukemia and hyperleukocytosis. Cancer. 2008 Aug;113(3):522-9.
29. Ookura M, Hosono N, Tasaki T, Oiwa K, Fujita K, Ito K, et al. Successful treatment of disseminated intravascular coagulation by recombinant human soluble thrombomodulin in patients with acute myeloidleukemia. Medicine (Baltimore). 2018 Nov;97(44):e12981.