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

Mirjana Cvetković¹, Mirjana Mitrović¹,²

¹ Faculty of Medicine, University of Belgrade, Belgrade, Serbia
² Clinic for Hematology, Clinical Center of Serbia, Belgrade, Serbia

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 <0.01) and a high level of LDH (p <0.01). 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 derivatives and components, DIC has no negative impact on early mortality, outcome, and survival.

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

Akutne mijeloidne leukemije (AML) su heterogen 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 spanzija mijeloblasta u koštanoj srži (≥20%), perifernoj pa malignih bolesti krvi koje karakteriše klonalna ekspanzija u međuobolevljama. AML je bolest starih – prilikom postavljanja dijagnoze, 71 godine [2-4]. I pored savremenog lečenja, prežивljanje oboljelih od AML-a je veoma kratko (petogodišnje preživljanje = 24%) [2].

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

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

Savremena klasifikacija akutnih lekmija 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 prognoščni i terapijski značaj [7,8], mada se u svakodnevnoj praksi i dalje koristi FAB (French, American and British) klasifikacijski sistem koji se zasniva na morfološkim i imunofenotipskim karakteristikama lekmijskih celja [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 ranjoj 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, NTRK), 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 sistemskih intravaskularna aktivacija koagulacije. Može dovesti do multiorganne disfunkcije, tromboze i/ei ekscesivnog krvarenja [12,13]. Najčešća stanja koja dovode do DIK-a su sepsa, šok, solidni tumori i maligne bolesti krv – akutne leukemije i Nehočkinovi limfomi. Pod dejstvom proinflamatornih citokina, mononuklearne i endotelne čelijske ekspiriraju tkivni faktor (TF). Kontaktom TF-a sa faktorima koagulacije u krv, započinje koagulacijna kaskada, koja dovodi do generacije trombina i konverzije fibrinogene 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 čelijama, 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) preporučljivo je sistem bodovanja 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 fibrinogene, 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 kreci se od 10% do 40% [16]. Ne postoje studije koje su pratile uticaj vrednosti D-dimera na učestalost DIK-a kod bolesnika sa AML-om.[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 kreci se od 10% do 40% [16]. Ne postoje studije koje su pratile uticaj vrednosti D-dimera na učestalost DIK-a kod bolesnika sa AML-om.[12].

Cilj našeg rada bilo je prikupljanje i analiza podataka 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 dijagnosticovani 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 čelija 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 consequent 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% to 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) čelije. Citogenetska procena rizika (povoljna, intermediarna, 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 i značaj postojećih pridruženih bolesti, tj. komorbiditeta, na osnovu HCT-CI (engl. Hematopoietic cell transplantation specific comorbiditity 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 laktata 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, umerno (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 invravaskularnu koagulopatiju. Kod svih bolesnika određivano je da li su imali kliničke znake prisustva hemoragijinskog 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 komorbiditeten indeksa), i potom konsolidaciju, primenom citozin-arabinozida. Uporedo sa lečenjem AML-a, bolesnici sa menifestnom disinovanoj invravaskularnoj koagulopatijom su lečeni derivatima i komponentama krvi, prema važnim preporukama. Transfuzije trombocita su primenjivane pri vrednostima trombocita <50x10^9/l, kod bolesnika sa manifestnim krvenjem, a u odsustvu krvenja, 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 hipofibri-nogenemijom (<1g/l), koja se nastavljala i pored primene ZSP-a, primali su i krioprecipitat [13]. Praćeni su: ishod lečenja (živ/imro), 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 comorbiditity 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’ regimens, 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 Hik kvadrat test, odnosno Fišerov test tačne verovatnoće. Za analizu preživljavanja, korišćena je Kaplan Mayerova metoda, kao i log-rank test za poredenje preživljavanja među ispitivanim grupama. Vrednosti p < 0,05 smatra- ne 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 prikaza- ne su u Tabeli 1.

Prilikom postavljanja dijagnoze, hemoragijski sindrom je bio prisutan kod 72/176 bolesnika (40,9%). Na analizu krvne slike pokazala je da su bolesnici, u prosеку, imali statistički značajku merenog stepena (97,3 ± 18,4 g/l), trombocitopeniju gr III (medijana: 44 x109/l; raspon: 1 – 421) i leukocitozu (medijana: 18,5 x109/l; raspon: 0,6 – 473,2) sa prisustvom blasta u perifernoj krvi (medijana: 16%, raspon: 0 – 99). Vrednost LDH je, u prosеку, bila povišena (medijana: 450 U/l, raspon: 102 – 8,840). Parametri hemostaze su pokazali pro- duženo PT (70 ± 18%) i veoma visok D-dimer (medijana: 3,0 μg/ml, raspon: 0,19 – 138). Kriterijume za manifestnu diseminovana intravaskularna koagulopatija u akutnoj nepromijelocitnoj mijeloidnoj leukemiji, kao i da li je prisustvo DIK-a imalo uticaja na ishod i OS.

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In statistical analysis, the following methods of de- scriptive statistics were applied: a) for continuous vari- ables – 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 ap- plied. The values $p < 0.05$ were believed statistically sig- nificant.

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 demo- graphic, 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 x109/l; range: 1 – 421), and leukocytosis (median: 18.5 x109/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 pro- longed PT (70 ± 18%) and very high D-dimer (medi- an: 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

| Parametar / Parameter | Vrednost / Value | Manifestna DIK (ISTH DIK skor ≥5) / Overt DIC (ISTH DIC score ≥5) | P vrednost / P value |
|-----------------------|-----------------|-------------------------------------------------|---------------------|
|                       |                 | Grupa I - DIK da / Grupa II - DIK ne /          |                     |
|                       |                 | Group I - DIC yes (n = 74/176; 42%) / Grupa II - DIC no (n = 102/176; 58%) |                     |

**Pol – n, % / Sex – n, %**
- Muški: 99 (56.25)
- Ženski: 77 (43.75)

**Starost – srednja vrednost (godine), SD / Age – mean (years), SD**
- 53.8 ± 14.6
- 57.4 ± 12.4
- 51.2 ± 15.5

**Hb – hemoglobin, g/L, SD / Hb – median (g/L), SD**
- 70 ± 18
- 61 ± 16
- 78 ± 15

**Tr – platelets, x10⁹/L, SD / Tr – median (x10⁹/L), range**
- 5.4 ± 1.9
- 5.2 ± 2.0
- 5.6 ± 1.8

**Fibrinogen – srednja vrednost (g/l), SD / Fibrinogen – median (g/L), SD**
- 29.6 ± 5.9
- 30.2 ± 6.1
- 29.2 ± 5.8

**LDH – lactate dehydrogenase, U/L, range / LDH – median (U/L), range**
- 450 (102 – 8,840)
- 591.5 (102 – 5,786)
- 383 (108 – 8,840)

**Dimer – srednja vrednost (μg/mL), SD / Dimer – median (μg/ml), range**
- 3.0 (0.2 – 138)
- 6.2 (0.8 – 138)
- 1.32 (0.2 – 74)

**Tip AML (n, %) / AML type (n, %)**
- FAB M0: 10 (5.7)
- FAB M1: 23 (13.1)
- FAB M2: 34 (19.3)
- FAB M4: 66 (37.5)
- FAB M5: 21 (11.9)
- FAB M6: 0
- FAB M7: 22 (12.5)

**Citogenetska grupa rizika (n, %) / Cytogenetic risk group (n, %)**
- Povoljna / Favorable: 18 (10.2)
- Intermedijarna / Intermediate: 93 (52.8)
- Nepovoljna / Unfavorable: 45 (25.6)
- Nema podataka / Data missing: 20 (11.4)

**CD56 (n, %) /**
- Da / Yes: 62 (35.2)
- Ne / No: 82 (46.6)
- Nema podataka / Data missing: 32 (18.8)

**Hematološki simptomi (n, %) /**
- Da / Yes: 72 (40.9)
- Ne / No: 104 (59.1)
- Nema podataka / Data missing: 35 (47.3)

**HCT-CI (medijana, raspon, %) /**
- 1 (0 – 8)
- 2 (0 – 8)
- 1 (0 – 6)

**Legenda:** APL – akutna promijelocitna leukenija; 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 British; HCT-CI – Hematopoietic cell transplantation specific comorbidity index
bolesnici sa DIK-om su imali značajno niži broj Tr (grupa I – medijana: 32,5 x10⁹/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 – medijana: 32,5 x10⁹/l, raspon: 1 – 151, grupa II – medijana: 61,5, raspon: 2 – 421; 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: 61,6 ± 16%; grupa II: 78 ± 15%; p < 0,001), 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). 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: 32.5x10⁹/l, range: 1 – 151, as compared to group II – median: 61.5, range: 2 – 421; p < 0.001), 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).
DISKUSIJA

Patofiziološki mehanizam nastanka DIK-a u akutnim leukemijama je kompleksan, i podrazumeva istovremeno: a) aktivaciju koagulacije uzrokovano izlaganjem tkinovnog faktora (TF) krvi; b) poremećaj kontrole antikoagulantnih mehanizama, i c) supresiju fibrinolize putem povećane ekspresije PAI-1 (plasminogen activator inhibitor-1). Ove promene udruženo uzrokuju endotelianalnu disfunkciju i mikrovaskularne tromboze, koje dovode do disfunkcije organsa i značajno negativno utiču na progonu osnovne bolesti [14]. Leukemijeske celije oslobađaju TF, a takođe sekretnju i proinflamatorne citokine, pre svega IL-6 (interleukin) i TNF alfa (tumor necrosis factor), koji oštećuju endotel krvnih sudova. Oštećenje endotelja, 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 aktivani PC (APC), i inhibira koagulaciju. Leukemijeske celije oslobađaju mnoge mikropartikule, koje, osim TF-a, sadrže i kancer-prokoagulant, koji ima aktivnost serin proteaza, 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 fibrinških depozita spečava insuficijenciju periferne cirkulacije. U akutnim leukemijama, proinflamatorni citokini uzrokuju povećanu ekspresiju PAI-1, koji inhibicijomaktivatora plasminogenova sprečava nastanak plasmina (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-remisije terapije. Maligne celije, pod dejstvom citotoksičnih lekova, podležu apop tozi, uz oslabljanje 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 varijabila. Najveća je kod APL-a, a najmanja kod B-ćeljske akutne limfoblastne leukemije [16]. Disseminovana 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]. Libourej i saradnici [21] su saopštili da je učestalost disseminovane intravaskularne koagulopatije, određene prema ISTH kriterijumima, bila veća kod mladih 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 dysfunction 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 proteases, 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 plasin (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]. Libourej 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 znatno češće imali hemoragijski sindrom (13%). Učestalost DIK-a kod bolesnika u našoj studiji iznosila je 42%, znatno više, u porječenju sa gorenavezuid podacima. Uz to, naši bolesnici su znatno češće imali manifestno krivanje 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 celije 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 krivanja [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 uticaja 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 odusustvu DIK-a, usled infiltracije koštane sa akutnim leukemijama veoma često imaju trombocitopenije, znatno duže PT i viši D-dimer, kao i AML tip FL3-ITD. Liburel i saradnici [21] objavili su našim rezultatima koje su u pravilu potvrđene u studiji Guo i saradnika [16] koja je pokazala da je prevalencija DIK-a znatno veća kod bolesnika sa normalnim karyotipom i mutacijama NPM1 i/ili FLT3-ITD.

U pogledu bioloških karakteristika ne-APL AML-a i rizika za nastanak DIK-a kod naših bolesnika nije utvrđena razlika između FAB podtijepa AML-a i citogenetske grupe rizika, a i 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 karyotipom i mutacijama NPM1 i FLT3-ITD [27], kao i AML tip FAB5 [28] povezani sa hiperleukocitozom.
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 antifibrinolitika u lečenju DIK-a u AML-u se ne preporučuje, s obzirom na opasnost od promicanja fibrinskog 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 preživljanje 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 preživljanje bolesnika sa ne-APL AML-om, ukoliko se dijagnoza DIK-a postavi na vreme i preuzme neodložna, adekvatna i intenzivna primena suportivne terapije derivatima i komponentama krvi.

**Sukob interesa:** Nije prijavljen.

**CONCLUSION**

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

**Conflict of interest:** None declared.

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