PROGNOSTIČKI FAKTORI KOD STARIJIH BOLESNIKA SA AKUTNOM MIJELOIDNOM LEUKEMIJOM

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PROGNOSTIC FACTORS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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SAŽETAK

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

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

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

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

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

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

ABSTRACT

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

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

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

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

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

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

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UVOD

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

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

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

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

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

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

MATERIJALI I METODE

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

INTRODUCTION

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

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

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

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

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

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

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

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

U okviru hematološke dijagnostike učinjena je:

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

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

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

MATERIALS AND METHODS

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

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

The following was performed as part of the hematological diagnostics:

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

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

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

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

REZULTATI

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

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

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

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

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

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

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

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

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

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

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

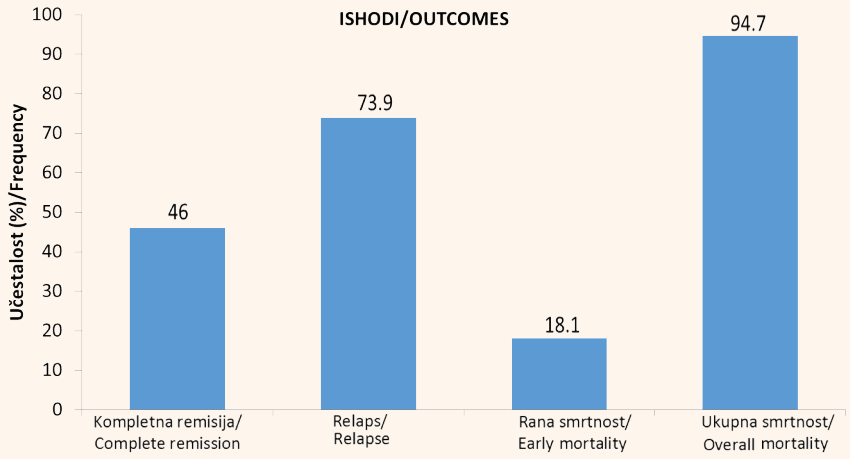
RESULTS

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

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

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

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



Grafikon 1. Učestalost ishoda pacijenata obuhvaćenih studijom

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

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

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

Tabela 1. Uticaj prognostičkih faktora na *OS*

Table 1. Influence of prognostic factors on OS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISKUSIJA

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ZAKLJUČAK

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

Sukob interesa: Nije prijavljen.

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

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

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

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

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

CONCLUSION

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

Conflict of interest: None declared.

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