PROGNOSTIČKI ZNAČAJ VREDNOSTI SERUMSKOG FERITINA PRI INICIJALNOJ DIJAGNOZI KOD BOLESNIKA SA AKUTNOM MIJELOIDNOM LEUKEMIJOM

ORIGINALNI RAD

ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF SERUM FERRITIN LEVELS ON INITIAL DIAGNOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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SAŽETAK

Uvod/cilj: Akutna mijeloidna leukemija (AML) je heterogena maligna bolest na čiji tok i ishod utiču brojni prognostički faktori. Serumski feritin (SF) je često povišen kod onkoloških bolesnika, a pokazano je da kod različitih malignitеta snažno utiče na nepovoljan ishod bolesti. Cilj rada je ispitivanje uticaja visokih vrednosti SF-a na ukupno preživljavanje i preživljavanje bez znakova bolesti, kao i ispitivanje povezanosti SF-a sa drugim prognostičkm markerima, kao što su klinički i laboratorijski parametri.

Metode: Retrospektivna analiza obuhvatila je 108 bolesnika kojima je dijagnoza akutne mijeloidne leukemije postavljena na Klinici za hematologiju Kliničkog centra Srbije (KCS), u Beogradu, u periodu od 2017 – 2019. godine. Iz studije su isključeni bolesnici sa akutnom promijelocitnom leukemijom, akutnom leukemijom mešovitih linija, sekundarnom akutnom mijeloidnom leukemijom, kao i bolesnici lečeni palijativnom terapijom. Bolesnici su grupisani prema graničnoj vrednosti od 800 µg/L.

Rezultati: Bolesnici sa višim vrednostima SF-a imali su statistički značajno veću učestalost rane smrti ($p = 0,020$), sepse u indukcionoj fazi lečenja ($p < 0,010$), kao i značajno niže inicijalne vrednosti hemoglobina ($p = 0,040$), u odnosu na bolesnike sa nižim vrednostima SF-a. Analizama preživljavanja pokazano je da je visoka vrednost SF-a pri inicijalnoj dijagnozi značajan nezavisni prediktivni faktor za ukupno preživljavanje ($p = 0,019$) i preživljavanje bez znakova bolesti ($p = 0,040$).

Zaključak: Naša studija pokazala je značajnu povezanost visokih vrednosti SF-a sa pojavom sepse u indukcionoj fazi lečenja, ranom smrću, prosečnim vrednostima hemoglobina, ukupnim preživljavanjem, i preživljavanjem bez znakova bolesti. Identifikacija SF-a, kao nezavisnog prognostičkog faktora i potencijalnog ciljnog mesta delovanja novih lekova, mogla bi da doprinese boljoj prognozi bolesnika sa AML-om.

Ključne reči: malignitet, sepsa, indukcija, rana smrt, ukupno preživljavanje, preživljavanje bez znakova bolesti

ABSTRACT

Introduction/Aim: Acute myeloid leukemia (AML) is a heterogenous malignant disease whose course and outcome are influenced by a number of prognostic factors. Serum ferritin (SF) is often elevated in oncology patients, and it has been shown that it strongly influences an unfavorable outcome in various malignancies. The aim of this study is to assess the effect of high SF values on overall survival and disease-free survival, as well as to assess the correlation of SF values with other prognostic markers, such as clinical and laboratory parameters.

Methods: Retrospective analysis included 108 patients diagnosed with AML at the Clinic for Hematology of the Clinical Center of Serbia (CCS), in Belgrade, in the period 2017 - 2019. Patients with acute promyelocytic leukemia, acute mixed lineage leukemia, secondary AML and patients treated with palliative therapy were excluded from the study. Patients were grouped based on the SF cutoff value of 800 µg/L.

Results: Patients with higher SF values had a significantly higher incidence of early death ($p = 0.020$), sepsis in the induction phase of therapy ($p < 0.010$), and significantly lower initial hemoglobin levels ($p = 0.040$), as compared to patients with lower SF values. SF at diagnosis appeared to be a significant independent predictive factor of overall survival ($p = 0.019$) and of disease-free survival ($p = 0.040$).

Conclusion: Our study showed a significant association of high SF values with sepsis in induction, early death, mean hemoglobin, overall survival, and disease-free survival. Identification of SF as an independent prognostic factor and a potential target site of the action of new drugs could contribute to a better prognosis of AML patients.

Keywords: malignancy, sepsis, induction, early death, overall survival, disease-free survival

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UVOD

Akutna mijeloidna leukemija (AML) je heterogena maligna bolest koja se karakteriše klonalnom proliferacijom neke od prekursorskih ćelija mijeloidne loze. Akumulacijom nezrelih ćelija - blasta, dolazi do progresivne insuficijencije koštane srži i infiltracije krvi i drugih organa [1]. Postizanje dugoročnog preživljavanja je jedan od najvećih izazova u lečenju AML-a, i zavisi od niza prognostičkih faktora koji se odnose na karakteristike bolesti, samog bolesnika ili primenjene terapije. Neki od važnih nezavisnih prognostičkih faktora za ukupno preživljavanje (engl. *overall survival - OS*) su godine starosti, komorbiditeti, opšte funkcionalno stanje procenjeno *ECOG* skalom *(*engl. *Eastern Cooperative Oncology Group performance status*), citogenetski/molekularni profil, i broj leukocita pri dijagnozi. Identifikacija i klasifikacija bolesnika na osnovu prognostičkih faktora je važna jer značajno utiče na izbor terapije, a posredno i na tok i ishod bolesti [2].

Feritin je protein prisutan u citoplazmi brojnih ćelija sa ulogom u regulaciji metabolizma gvožđa i zaštiti od oksidativnog stresa. Takođe, prisutan je i u nukleusu gde učestvuje u regulaciji transkripcije pojedinih gena. Pored toga, poslednjih godina se stavlja akcenat na njegovu ulogu u angiogenezi, ćelijskoj proliferaciji, ćelijskoj smrti i imunosupresiji. Vrednosti serumskog feritina su povišene kod brojnih maligniteta, što se objašnjava povećanom ekspresijom, koja je stimulisana inflamatornim citokinima (putem aktivacije NF-κB signalnog puta), oksidativnim stresom, faktorima rasta (kao što je insulinu-sličan faktor rasta 1 - IGF1), ili hipoksijom [3,4]. Podaci o korelaciji serumskog feritina (SF) i ishoda lečenja AML-a su još uvek oskudni, mada postoje dokazi da bi visoke vrednosti SF-a pri dijagnozi mogli da budu povezani sa lošim ishodom bolesti [5].

Osnovni cilj ovog rada je ispitivanje prognostičkog značaja inicijalne vrednosti feritina, njegov uticaj na ukupno preživljavanje (*OS*) i period bez znakova bolesti (engl. *disease-free survival - DFS*) u ispitivanoj populaciji bolesnika. Takođe ispitivali smo da li visoke vrednosti SF-a pri dijagnozi koreliraju sa pojedinim kliničkim, laboratorijskim i molekularno-genetskim karakteristikama bolesti.

MATERIJALI I METODE

U istraživanje su uključeni bolesnici kojima je dijagnoza akutne mijeloidne leukemije postavljena na Klinici za hematologiju Kliničkog Centra Srbije (KCS), u periodu 2017 - 2019. Dijagnoza AML-a je postavljena na osnovu kliničke slike, nalaza u perifernoj krvi i koštanoj srži, a prema preporukama Svetske Zdravstvene Organizacije (SZO) i u skladu sa kriterijumima Francusko-američko-britanske grupe za saradnju (engl.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogenous malignant disease which is characterized by clonal proliferation of a precursor cell of the myeloid lineage. The accumulation of immature cells – blasts, leads to progressive insufficiency of bone marrow and the infiltration of the blood and other organs [1]. Achieving long-term survival is one of the greatest challenges in the treatment of AML, and it depends on a series of prognostic factors related to the characteristics of the disease, the characteristics of the patient, or the characteristics of the applied therapy. Some of the important independent prognostic factors for overall survival (OS) are the following: age, comorbidities, Eastern Cooperative Oncology Group (ECOG) performance status, cytogenetic/molecular profile, and the leukocyte count at diagnosis. Identification and classification of patients on the basis of prognostic factors is important as it significantly impacts therapy selection, and indirectly influences the course of the disease [2].

Ferritin is a protein present in the cytoplasm of numerous cells, with a role in the regulation iron metabolism and in the protection against oxidative stress. Also, it is present in the nucleus, where it takes part in the regulation of the transcription of certain genes. Additionally, its role in angiogenesis, cell proliferation, cell death, and immunosuppression has been stressed in recent years. The levels of serum ferritin are elevated in numerous malignancies, which is explained by increased expression, stimulated by inflammatory cytokines (through the activation of the NF-κB signaling pathway), oxidative stress, growth factors (such as the insulin-like growth factor I – IGF1), or hypoxia [3,4]. Data on the correlation between serum ferritin (SF) and AML treatment outcome are still scarce, although there is evidence that high levels of SF at diagnosis may be connected to an unfavorable disease outcome [5].

The main goal of this paper is the analysis of the prognostic significance of the initial ferritin level, its impact on overall survival (OS) and on disease-free survival (DFS), in the analyzed patient population. Also, we analyzed whether high levels of SF at diagnosis correlated with individual clinical, laboratory, molecular and genetic characteristics of the disease.

MATERIALS AND METHODS

Patients diagnosed with acute myeloid leukemia at the Clinic for Hematology of the Clinical Center of Serbia (CCS), in the period 2017 – 2019, were included in the study. The diagnosis of AML was established on the basis of clinical presentation, peripheral blood and bone marrow findings, in keeping with the recommendations of the World Health Organization (WHO) and

French-American-British Cooperative Group - FAB) [6,7]. Od ukupno 201 uključenog bolesnika, podaci o inicijalnim vrednostima SF-a su bili dostupni za 183 bolesnika. Od toga, isključeni su bolesnici koji su imali sekundarnu AML (na terenu mijelodisplazije), jer su u sklopu ranijeg toka bolesti primali transfuzije, što može da utiče na vrednosti SF-a. Pored toga, zbog drugačijeg toka bolesti i specifičnosti u lečenju, pacijenti sa akutnom promijelocitnom leukemijom (APL) i sa akutnom leukemijom mešovitih linija (engl. *acute mixed-lineage leukemia - AMLL*), takođe nisu razmatrani, te je ukupan broj bolesnika koji je obuhvaćen ovom studijom bio 108. Svi oni su lečeni nekim od protokola koji podrazumevaju primenu intenzivne hemioterapije, prema preporukama Evropske radne grupe za leukemiju (engl. *European LeukemiaNet Working Group – ELN*) [8]. Bolesnici su podeljeni, na osnovu vrednosti SF-a, na grupu sa nižim (<800 μg/L) i grupu sa višim vrednostima (>800 μg/L). Prilikom određivanja granične vrednosti, rukovodili smo se radom Lebona i saradnika koji su koristili četvorostruku gornju graničnu vrednost SF-a koja je 200 μg/L (4n = 800 μg/L) [9].

Svi podaci su sakupljani retrospektivno, analizom istorija bolesti bolesnika, a obuhvatali su socio-demografske, kliničko-laboratorijske, imunološke, citogenetske, i molekularne parametre. Opšte funkcionalno stanje bolesnika procenjeno je prema *ECOG* skali [10]. Pri obrađivanju statističkih podataka, vrednosti ≥2 su smatrane nepovoljnim. Procena komorbiditeta vršena je na osnovu komorbiditetnog indeksa (engl. *hematopoietic cell transplantation-comorbidity index - HCT-CI*), pri čemu su tokom obrade, vrednosti ≥3 smatrane nepovoljnim [11]. SF je određivan spektrofotometrijski u Laboratoriji KCS-a. Citogenetsko-molekularni stepen rizika određen je prema preporukama *ELN*-a [8]. Hematološka obrada podrazumevala je citološku analizu, imunofenotipizaciju, klasičnu citogenetsku analizu, i molekularno-genetska istraživanja. Procena efikasnosti lečenja je sprovođena na kraju indukcionog lečenja prema *ELN* kriterijumima [12]. Naime, kompletna remisija (KR) podrazumevala je: 1) ≤ 5,0% blasta u koštanoj srži, uz odsustvo Auerovih štapića u blastima; 2) odsustvo ekstramedularne bolesti; 3) neutrofile ≥1,5x10^9/L, trombocite ≥100x10^9/L; 4) transfuzionu nezavisnost. Rana smrt je definisana kao smrt u toku 28 dana od početka indukcione hemioterapije. Recidiv bolesti (relaps) definisan je kao: 1) ponovna pojava blasta u koštanoj srži (>5,0%) ili u perifernoj krvi; 2) nastanak ekstramedularne bolesti. *OS* je definisano kao vreme proteklo od dijagnoze do smrti ili datuma poslednjeg praćenja, a *DFS* se definiše kao period koji osoba provede u remisiji do momenta poslednjeg praćenja, relapsa ili smrti [13].

the criteria of the French-American-British Cooperative Group - FAB [6,7]. Of a total of 201 patients, data on initial SF levels were available for 183 patients. Out of this number, patients with secondary AML (in the landscape of myelodysplasia) were excluded from the study, since, in the earlier course of their disease, they received transfusions, which can affect the values of SF. Also, due to the difference in the development of the disease and the specificities in treatment, patients with acute promyelocytic leukemia (APL) and those with acute mixed-lineage leukemia (AMLL), were excluded from the study, which left a total of 108 patients who were involved in the study. All of them were treated with one of the protocols which include the application of intensive chemotherapy, in keeping with the recommendations of the European LeukemiaNet Working Group (ELN) [8]. On the basis of the SF level, patients were divided into the group with lower SF levels (<800 μg/L) and the group with higher SF levels (>800 μg/L). In setting the cut-off value, we were guided by the study by Lebon et al., who applied a quadruple upper cut-off value of SF, amounting to 200 μg/L (4n=800 μg/L) [9].

All data were collected retrospectively, through the analysis of the patients' histories, and they included socio-demographic, clinical and laboratory, immunological, cytogenetic, and molecular parameters. The overall performance status of the patients was assessed according to the ECOG scale [10]. In statistical data processing, levels ≥2 were considered unfavorable. The assessment of comorbidities was performed on the basis of the hematopoietic cell transplantation-comorbidity index (HCT-CI), whereby, during processing, levels ≥3 were considered unfavorable [11]. SF was measured spectrophotometrically in the KCS Laboratory. The cytogenetic and molecular risk level was determined according to ELN recommendations [8]. Hematological processing entailed cytological analysis, immunophenotypization, classical cytogenetic analysis, and molecular and genetic study. The assessment of the efficiency of treatment was made at the end of induction therapy, in keeping with ELN criteria [12]. Namely, complete remission (CR) entailed: 1) ≤ 5.0% blasts in the bone marrow, with an absence of Auer rods in the blasts; 2) absence of extramedullary disease; 3) neutrophils ≥1.5x10^9/L, thrombocytes ≥100x10^9/L; 4) transfusion independence. Early death was defined as death within 28 days of induction therapy initiation. Relapse was defined as: 1) reappearance of blasts in bone marrow (>5.%) or in peripheral blood; 2) occurrence of extramedullary disease. OS is defined as the time elapsing from diagnosis until death or the last date of follow-up, while DFS is defined as the period that a person spends in remission until the moment of the last follow-up, relapse, or death [13].

Statistička obrada izvršena je u programu *SPSS for Windows, Version 16.0*. U cilju obrade prikupljenih podataka, korišćene su deskriptivne analize distribucije kao i testiranje nezavisnosti podataka hi-kvadrat testom i t-testom, uz tabelarno i grafičko prikazivanje podataka. Uticaj serumskog feritina na *OS* i *DFS* ispitivan je univarijantnom analizom. Zbog nepovoljnog odnosa broja ishoda i potencijalnih prediktora, multivarijantna Koksova regresiona analiza nije bila moguća sa svim prediktorima. Sva testiranja su obavljena na nivou značajnosti 0,05.

REZULTATI

Od ukupno 108 bolesnika uključenih u istraživanje, 58 (53,7%) pacijenata je bilo muškog pola, dok je 50 (46,3%) pacijenata bilo ženskog pola. Prosečna starost bolesnika bila je 51,9 (opseg: 19 - 75) godina. Broj bolesnika starijih od 60 godina bio je 40 (37,0%). Pri dijagnozi, 33 (30,5%) bolesnika je imalo *ECOG* skor ≥ 2, dok je visok *HCT-CI* skor (≥3) pri dijagnozi imalo 13 (12,0%) bolesnika. Prosečna vrednost broja leukocita bila je 47,2x10^9/L (opseg: 1 - 259 x 10^9/L), a 41 (38,0%) bolesnik je imao vrednosti leukocita preko 30x10^9/L. Prosečna vrednost hemoglobina bila je 99 g/l (opseg: 44 - 153). Prosečna vrednost broja trombocita bila je 71,1x10^9 (opseg: 9 - 421x10^9). Vrednost laktat-dehidrogenaze (LDH) u serumu >450 UI/L je imao 51 (47,2%) bolesnik. Prosečna vrednost blasta u krvi bila je 35,2% (opseg: 0 - 99), dok je prosečna vrednost blasta u koštanoj srži bila 52,9% (opseg: 22 - 94). Prema *ELN* klasifikaciji stepena rizika, najviše je bilo bolesnika sa intermedijarnim rizikom, njih 69 (63,9%), potom sa nepovoljnim, njih 30 (63,9%), a njih 9 (8,3%) su označeni kao bolesnici sa povoljnim rizikom. Četrdeset tri (39,8%) bolesnika je postiglo KR nakon indukcione terapije, od čega je kod njih 22 (20,4%) zabeležen recidiv bolesti. Sepsu u indukcionoj fazi lečenja je imalo 30 (27,8%) bolesnika, dok je rana smrt registrovana kod 24 (22,2%) bolesnika. Alogena transplantacija matične ćelije hematopoeze (alo-TMĆH) je postremisiono sprovođena kod 25 (23,1%) bolesnika, od čega je 7 (6,4%) pacijenata pripadalo grupi sa višim, dok je 18 (16,7%) pacijenata pripadalo grupi sa nižim SF-om.

Prosečna vrednost SF-a bila je 1002,3 µg/L (opseg: 99,7 – 6815,3). Od 108 bolesnika, njih 43 (39,8%) je imalo vrednosti >800 µg/L, dok je preostalih 65 (60,2%) pacijenata imalo vrednosti <800 µg/L. Bolesnici sa višim vrednostima SF-a imali su statistički značajno veći procenat rane smrti (p = 0,020) i sepse u indukciji (p < 0,010), u odnosu na bolesnike sa nižim vrednostima SF-a. Bolesnici sa nižim vrednostima SF-a postizali su veći stopu KR-a (39,8%), u odnosu na bolesnike sa višim vrednostima SF-a (26,9%), ali nije bilo statistički značajne razlike (p = 0,874). Kliničko-demografske karakteristi-

The influence of serum ferritin on OS and DFS was analyzed with univariate analysis. Due to the unfavorable ratio between the number of outcomes and potential predictors, it was not possible to perform Cox regression analysis with all predictors. All tests were performed at the significance level 0.05.

RESULTS

Of a total of 108 patients included in the study, 58 (53.7%) patients were men, while 50 (46.3%) patients were women. The average patient age was 51.9 (range: 19 - 75) years. The number of patients older than 60 years was 40 (37.0%). At diagnosis, 33 (30.5%) patients had an *ECOG* score ≥ 2, while a high *HCT-CI* score (≥3) at diagnosis was registered in 13 (12.0%) patients. The average leukocyte count was 47.2x10^9/L (range: 1 - 259 x 10^9/L), and 41 (38.0%) patients had a leukocyte count above 30x10^9/L. The mean hemoglobin value was 99 g/l (range: 44 - 153). The mean thrombocyte count was 71.1x10^9 (range: 9 - 421x10^9). The serum level of lactate dehydrogenase (LDH) was >450 UI/L in 51 (47.2%) patients. The mean blast value in the blood was 35.2% (range: 0 - 99), while the mean blast value in bone marrow was 52.9% (range: 22 - 94). According to the ELN risk classification, the majority of the patients had an intermediate risk - 69 (63.9%) patients, followed by those with unfavorable risk - 30 (63.9%) patients, while 9 (8.3%) patients were marked as having favorable risk. Forty-three (39.8%) patients achieved CR after induction therapy, of whom 22 (20.4%) recidivated. A total of 30 (27.8%) patients had sepsis in the induction phase of the treatment, while early death was registered in 24 (22.2%) patients. Allogenic hematopoietic stem cell transplantation (allo-HSCT) was carried out, post-remission, in 25 (23.1%) patients, of whom 7 (6.4%) patients belonged to the group with higher SF, while 18 (16.7%) patients belonged to the group with lower SF.

The mean SF level was 1002.3 µg/L (range: 99.7 – 6815.3). Of the 108 patients, 43 (39.8%) of them had levels >800 µg/L, while the remaining 65 (60.2%) of the patients had levels <800 µg/L. Patients with higher levels of SF had a statistically significantly higher percentage of early death (p = 0.020) and sepsis in induction (p < 0.010), as compared to patients with lower levels of SF. Patients with lower levels of SF achieved a higher rate of CR (39.8%), as compared to patients with higher levels of SF (26.9%), but without statistical significance (p = 0.874). Clinical and demographic patient characteristics, as well as their statistical significance in relation to the SF levels have been presented in Appendix I.

Tabela 1. Kliničko-demografske karakteristike pacijenata prema vrednostima feritina

Table 1. Patients' clinical and demographic characteristics based on ferritin levels

Karakteristika / *Characteristic*	**Ukupno / *Total***	**SF<800 µg/L / *SF<800 µg/L***	**SF >800 µg/L / *SF >800 µg/L***	**p**
Pol (n,%) / *Gender (n,%)*	108 (100.0%)			0.343
M / *M*	58 (53.7%)	32 (55.2%)	26 (44.8%)	
Ž / *F*	50 (46.3%)	33 (66.0%)	17 (34.0%)	
Prosečna starost / *Mean age*	51.9	50.3	54.1	0.168
>60 god (n,%) / *>60 years (n,%)*	40 (37.0%)	22 (20.4%)	18 (16.7%)	0.399
ECOG ≥ 2 (n,%) / *ECOG ≥ 2 (n,%)*	33 (30.6%)	16 (14.8%)	17 (15.7%)	0.151
HCT-CI ≥ 3 (n,%) / *HCT-CI ≥ 3 (n,%)*	13 (12.0%%)	6 (5.6%)	7 (6.5%)	0.424
Prosečna vrednost leukocita (x10^9/L) / *Mean leucocyte value (x10^9/L)*	47.3	48.4	45.4	0.810
>30 (*n*, %)	41 (38.0%)	27 (25.0%)	14 (13.0%)	0.460
Prosečna vrednost hemoglobina (g/L) / *Mean hemoglobin levels (g/L)*	99	102.2	94.6	0.040
Prosečna vrednost trombocita (x10^{9}g/L) / *Mean platelet levels (x10^{9}g/L)*	71.1	76.1	63.5	0.366
LDH >450 (UI/L) / *LDH >450 (UI/L)*	51	32	19	0.751
Blasti u krvi (%) / *Blasts in blood (%)*	35.2	32.8	39	0.341
Blasti u koštanoj srži (%) / *Blasts in bone marrow (%)*	52.9	57.6	61.5	0.397
ELN (n,%) / *ELN (n,%)*				0.483
Povoljan / *Favorable*	9 (8.3%)	4 (3.7%)	5 (4.6%)	
Intermedijarni / *Intermediate*	69 (63.9%)	44 (40.7%)	25 (23.1%)	
Nepovoljan / *Unfavorable*	30 (27.8%)	17 (15.7%)	13 (12.0%)	
Remisija (n,%)/ *Remission (n,%)*	43 (39.8%)	29 (26.9%)	14 (13.0%)	0.874
Rana smrt (n,%) / *Early Death(n,%)*	24 (22.2%)	9 (8.3%)	15 (13.9%%)	0.020
Sepsa u indukciji (n,%) / *Sepsis in induction (n,%)*	30 (27.8%)	8 (7.4%)	22 (20.4%)	<0.010
Relaps (n,%) / *Relapse (n,%)*	22 (20.4%)	12 (11.1%)	10 (9.3%)	0.224
Alo-TMĆH (n,%) / *Allo-HSCT (n,%)*	25 (23.1%)	18 (16.7%)	7 (6.5%)	0.060

ECOG - Eastern Cooperative Oncology group; HCT-CI – Hematopoietic cell transplantation comorbidity index; LDH - Laktat dehidrogenaza; *FAB - French-American-British Cooperative Group; ELN - EuropeanLeukemiaNet;* Alo-TMĆH - alogena transplantacija matične ćelije hematopoeze

ECOG - Eastern Cooperative Oncology group; HCT-CI – Hematopoietic cell transplantation comorbidity index; LDH – Lactate dehydrogenase; FAB - French-American-British Cooperative Group; ELN - EuropeanLeukemiaNet; Allo-HSCT- Allogeneic hematopoietic stem cell transplantation

ke pacijenata, kao i njihova statistička značajnost prema vrednostima SF-a, predstavljene su u Prilogu I.

Prosečno *OS* je bilo 14,8 meseci (opseg: 11,5 - 18,8), trogodišnje *OS* je bilo 16,4%, dok je prosečno *DFS* bilo 22 meseca (opseg: 12,6 - 31,2). U grupi bolesnika sa nižim vrednostima SF-a, prosečno *OS* je bilo značajno duže (18,8 meseci, 95%CI: 11,6 - 31,2) u odnosu na grupu sa višim vrednostima SF-a (12,2 meseca, 95%CI: 7,3 - 15,4) (p = 0,030). Naša studija je pokazala, da je prosečno *DFS* u grupi sa nižim SF-om bilo 29 meseci (95%CI: 16,9 - 41,1), dok je u grupi sa višim SF-om bilo 12 meseci (95%CI: 7,2 - 16,1), što je bilo statistički značajno (p = 0,048). Pored SF>800 µg/L, univarijantnom analizom ispitivali smo i uticaj drugih karakteristika na *OS* i *DFS*. Naime, ovom analizom pokazano

The average OS was 14.8 months (range: 11.5 – 18.8), three-year OS was 6.4%, while average DFS was 22 months (range: 12.6 – 31.2). In the group of patients with lower levels of SF, average OS was significantly longer (18.8 months, 95%CI: 11.6 – 31.2), as compared to the group with higher SF levels (12.2 months, 95%CI: 7.3 – 15.4) (p = 0.030). Our study showed that the average DFS in the group with lower SF was 29 months (95%CI: 16.9 – 41.1), while it was 12 months (95%CI: 7.2 – 16.1) in the group with higher SF, which proved to be statistically significant (p = 0.048). In addition to SF>800 µg/L, univariate analysis was used to analyze the influence of other characteristics on OS and DFS. Namely, this analysis showed that the following were significant predictors of poor OS: age

Tabela 2. Univarijantna analiza uticaja pojedinih karakteristika na ukupno preživljavan (engl. *overall survival - OS*)

Table 2. Univariate analysis showcasing the influence of some characteristics on overall survival (OS)

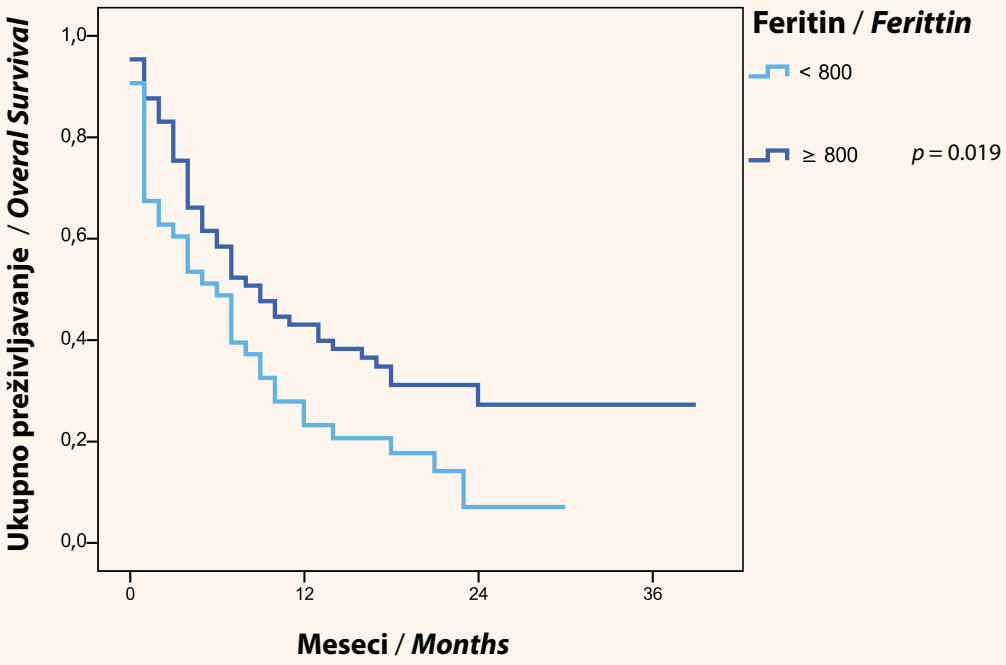
	Univarijantna analiza / *Univariate analysis*	
	HR (95%CI)	*p*
Starost >60godina / *Age>60 years*	1.4 (1.1 - 2.3)	0.049
ECOG skor ≥ 2 / *ECOG score ≥ 2*	1.1 (0.7 – 1.9)	0.351
HCT-CI≥ 3 / *HCT-CI≥ 3*	1.7 (0.9 – 3.4)	0.087
Broj leukocita >30x10^9/L / *Leukocyte count >30x10^9/L*	0.9 (0.6 – 1.5)	0.748
Broj trombocita <50x10^9/L / *Platelet count <50x10^9/L*	0.9 (0.5 – 1.3)	0.531
Hb <100g/L	1.1 (0.7 – 1.7)	0.738
Serumski feritin > 800 µg/L / *Serum ferritin > 800 µg/L*	1.7 (1.1 – 2.8)	0.019
ELN nepovoljan rizik / *Unfavorable ELN risk*	0.9 (0.5 – 1.5)	0.060
ELN intermedijarni rizik / *Intermediate ELN risk*	1.2 (0.7 – 1.9)	0.227
Nesprovođenje alo-TMĆH / *Non-implementation of Allo-HSCT*	4.2 (2.1 – 8.1)	<0.001

ECOG - Eastern Cooperative Oncology group; HCT-CI – Hematopoietic cell transplantation comorbidity index; Hb - hemoglobin*; ELN - EuropeanLeukemiaNet;* Alo-TMĆH - alogena transplantacija matične ćelije hematopoeze

ECOG - Eastern Cooperative Oncology group; HCT-CI – Hematopoietic cell transplantation comorbidity index; Hb-hemoglobin; ELN - EuropeanLeukemiaNet; Allo-HSCT- Allogeneic hematopoietic stem cell transplantation

je da su značajni prediktori za loše *OS* bili: starost >60 godina (p = 0,049), nepovoljan rizik po *ELN* klasifikaciji (p = 0,040), SF>800 µg/L (p = 0,019), i nesprovođenje alo-TMĆH-a (p<0,010) (Prilozi II, III). Takođe, univarijantna analiza pokazala je da su značajni faktori za kraći *DFS* bili: SF >800 µg/L (p = 0,040), broj trombocita <50x10^9/L (p = 0,008) i nesprovođenje alo-TMĆH-a (p = 0,002).

>60 years (p = 0.049), unfavorable risk according to the ELN classification (p = 0.040), SF>800 µg/L (p = 0.019), and absence of allo-HSCT (p<0.010) (Appendices II, III). Also, univariate analysis showed that the following factors were significant for shorter DFS: SF >800 µg/L (p = 0.040), thrombocyte count <50x109/L (p = 0.008), and absence of allo-HSCT (p = 0.002).



Grafikon 1. Kaplan-Majerova kriva koja pokazuje razlike u OS kod pacijenata sa SF>800 µg/L naspram SF<800 µg/L

Figure 1. Kaplan-Meier curve showcasing the OS differences in patients with SF>800 µg/L vs SF<800 µg/L

DISKUSIJA

Naša studija je pokazala da su vrednosti SF-a povišene kod skoro svih bolesnika sa AML-om u ispitivanoj populaciji, što su pokazale i druge studije [5,9,14-15]. SF je višestruko povišen kod bolesnika koji su primali transfuzije zbog hroničnog opterećenja gvožđem. Pored toga, smatra se da do hiperferitinemije dovode i subklinička inflamacija i oštećenje jetre [9]. U našoj populaciji bolesnika, prosečna vrednost SF-a bila je 1002,3 μg/L, što je više nego što je saopšteno u studiji Ihlova i saradnika (769 μg/L) [15]. Naime, oni su koristili C-reaktivni protein (CRP) kao korektivni faktor za SF, te je uticaj pomenute inflamacije na vrednosti SF-a smanjen. U našoj studiji, vrednosti CRP-a nisu bili dostupni za sve bolesnike, te korekcija nije mogla biti sprovođena. Ispitivanjem povezanosti nivoa SF-a sa osnovnim kliničkim karakteristikama, ustanovili smo da je u grupi bolesnika sa vrednostima SF>800 μg/L veća incidencija rane smrti (p = 0,020) i sepse u indukcionom lečenju (p < 0,010), dok je prosečna vrednost hemoglobina značajno niža (p = 0,040), u odnosu na grupu bolesnika gde je SF<800 μg/L. Sa druge strane, podaci o vrednostima SF-a, kao prediktivnog faktora za nastanak sepse tokom indukcione terapije, su malobrojni [5,9,15]. Ono što je poznato je da je ishod sepse nepovoljniji ukoliko su vrednosti SF-a veće [16,17]. U drugim studijama pokazano je da su bolesnici sa visokim vrednostima SF-a pri inicijalnoj dijagnozi značajno češće bili muškarci i bolesnici starosti >60 godina, dok u našoj studiji takva povezanost nije uočena (p = 0,343, p = 0,399) [5,9,15]. U drugim studijama, bolesnici sa visokim SF-om su značajno češće imali hiperleukocitozu >30x10^9/L, u odnosu na pacijente sa niskim vrednostima SF-a, dok to nije registrovano u našoj studiji. Bolesnici sa visokim feritinom nisu imali statistički veći broj leukocita u perifernoj krvi (p = 0,460) [5,9]. U našoj studiji nije registrovana statistički značajna razlika u pogledu postizanja KR-a (p = 0,874) i pojave relapsa (p = 0,224) između grupa pacijenata sa visokim i niskim SF-om. Sa druge strane, u studiji Lebona i saradnika, pokazano je da su pacijenti sa visokim vrednostima SF-a značajno češće imali relaps, u poređenju sa pacijentima koji su imali niže vrednosti.

U našoj populaciji bolesnika, prosečno *OS* bilo je 14,8 meseci, sa trogodišnjim preživljavanjem od 12,0%. Naša studija je pokazala da bolesnici sa vrednostima SF<800 μg/L imaju statistički značajno bolje *OS* (18,8 meseci) i *DFS* (29 meseci), u odnosu na grupu bolesnika sa SF> 800 μg/L, gde je prosečno *OS* bilo 12,2 meseca, a *DFS* 12 meseci (p = 0,019, p = 0,040, redom). Ovakvu značajnost su potvrdile i druge studije, gde su pacijenti sa niskim vrednostima SF-a takođe statistički značajnije bolje preživljavali i imali duže *DFS* [5,9,15].

DISCUSSION

Our study showed that the SF levels were elevated in almost all patients with AML in the analyzed population, which has been shown by other studies as well [5,9,14-15]. SF is elevated manifold in patients who had received transfusions, due to chronic iron overload. Additionally, it is believed that hyperferritinemia is also caused by subclinical inflammation and damage to the liver [9]. In our population of patients, the average SF level was 1002.3 μg/L, which is more than was reported in a study by Ihlow et al. (769 μg/L) [15]. Namely, they used C-reactive protein (CRP) as a correction factor for SF, which is why the influence of the abovementioned inflammation on SF levels was reduced. In our study, CRP values were not available for all patients, which is why the correction could not be carried out. Analyzing the link between the SF level and the basic clinical characteristics, we found that, in the group of patients with SF levels >800 μg/L, there was a higher incidence of early death (p = 0.020) and sepsis in induction treatment (p < 0.010), while the mean hemoglobin value was significantly lower (p = 0.040), as compared to the group of patients with SF<800 μg/L. On the other hand, data on SF levels, as a predictive factor for the development of sepsis during induction therapy, is scarce [5,9,15]. What is known is that the higher the SF level - the more unfavorable the outcome of sepsis [16,17]. Other studies have shown that patients with high SF levels at initial diagnosis were significantly more often men and patients aged >60 years, while such a link was not found in our study (p = 0.343, p = 0.399) [5,9,15]. In other studies, patients with high levels of SF significantly more often had hyperleukocytosis >30x10^9/L, as compared to patients with low SF levels, while this was not registered in our study. Patients with high levels of ferritin did not show a statistically significantly higher leukocyte count in peripheral blood (p = 0.460) [5,9]. In our study, a statistically significant difference between the group with high SF and the one with low SF was not registered in relation to the achievement of CR (p = 0.874) and the occurrence of relapse (p = 0.224). On the other hand, a study by Lebon et al. showed that patients with high levels of SF relapsed significantly more frequently, as compared to patients with lower SF levels.

In our patient population, average OS was 14.8 months, with a three-year survival of 12.0%. Our study showed that patients with SF levels <800 μg/L had statistically significantly better OS (18.8 months) and DFS (29 months), as compared to the patient group with SF> 800 μg/L, where the average OS was 12.2 months, and DFS was 12 months (p = 0.019, p = 0.040, respectively). Such significance has been confirmed by other studies as well, where patients with low levels of SF

Ispitivanjem uticaja pojedinih varijabli na *OS* univarijantnom analizom, utvrdili smo da se kao nezavisni prediktivni faktori loše prognoze izdvajaju starost >60 godina (p = 0,049), SF> 800 µg/L (p = 0,019) i nesprovođenje alo-TMĆH-a (p < 0,010). Neke studije su, pored ovih parametara, pokazale i da drugi parametri, kao što su intermedijarni i nepovoljni rizik po *ELN*-u, *ECOG* skor>1 i hiperleukocitoza >30x10^9/L, imaju statistički značajan uticaj na *OS* [5,8,15]. Ispitivanjem uticaja ovih varijabli na *DFS*, univarijantna analiza pokazala je da su SF>800 µg/L (p = 0,040), trombocitopenija <50x10^9 (p = 0,008) i odsustvo alogene TMĆH (p = 0,002) bili značajni nezavisni prediktivni faktori u našoj populaciji bolesnika. Pored toga, u navedenim studijama utvrđeno je da na *DFS* negativno utiču i *ECOG*>1, nepovoljni rizik po ELN-u i hiperleukocitoza >30x10^9/L [5,9,15].

U tumačenju ovih rezultata moramo biti oprezni jer je vrednost SF-a podložna uticaju različitih faktora, kao što su prisustvo komorbiditeta, disfunkcija jetre, tumor-nezavisna inflamacija, kao i poremećaji metabolizma gvožđa. Standardizacija određenih korektivnih faktora doprinela bi smanjenju uticaja ovih varijabli na vrednost SF-a, a broj studija koje se bave ulogom SF-a u akutnim mijeloidnim leukemijama je nedovoljan.

Ograničenja naše studije su: relativno kratak period praćenja, relativno mali broj ispitanika, odsustvo korekcije vrednosti SF-a, kao i nemogućnost sprovođenja multivarijantne analize.

ZAKLJUČAK

Naša studija je pokazala da visoke vrednosti SF-a pri dijagnozi imaju negativan prognostički uticaj na *OS* i *DFS* kod bolesnika sa AML-om i da su povezane sa značajno većom incidencijom rane smrti i sepse u indukcionoj fazi lečenja. Pokazali smo, takođe, da su bolesnici sa visokim vrednostima SF-a pri dijagnozi značajno češće imali niske vrednosti hemoglobina, u odnosu na bolesnike iz grupe sa niskim vrednostima SF-a. Postojanje većih multicentričnih prospektivnih studija u kojima bi bolesnici bili praćeni duži vremenski period, doprinelo bi boljem razumevanju uticaja hiperferitinemije na tok i ishod bolesti. Identifikacija SF-a, kao nezavisnog prognostičkog faktora i potencijalnog ciljnog mesta delovanja novih lekova, mogla bi da doprinese boljem preživljavanju kod bolesnika sa AML-om.

Sukob interesa: Nije prijavljen.

also had a statistically significantly better survival and a longer DFS [5,9,15].

By testing the influence of individual variables on OS with univariate analysis, we determined that the following were independent predictive factors of an unfavorable prognosis: age >60 years (p = 0.049), SF> 800 µg/L (p = 0.019), and absence of allo-HSCT (p < 0.010). Some studies have shown, in addition to these parameters, that other parameters, such as intermediate and unfavorable risk according to ELN, an ECOG score >1, and hyperleukocytosis >30x10^9/L, have a statistically significant influence on OS [5,8,15]. Analyzing the influence of these variables on DFS, univariate analysis showed that SF>800 µg/L (p = 0.040), thrombocytopenia <50x10^9 (p = 0.008), and the absence of allogenic HSCT (p = 0.002) were significant independent predictive factors in our patient population Additionally, in the aforementioned studies, it has been determined that DFS is negatively influenced by ECOG>1, unfavorable risk according to ELN, and hyperleukocytosis >30x10^9/L [5,9,15].

We must be cautious in interpreting these results, as SF levels are subject to the influence of various factors, such as the existence of comorbidities, liver failure, tumor-independent inflammation, as well as disturbance in the metabolism of iron. Standardization of certain correction factors would contribute to the decrease of the influence of these variables on SF levels, and the number of studies dealing with the role of SF in acute myeloid leukemias is insufficient.

The limitations of our study are, as follows: a relatively short follow-up period, a relatively small number of subjects, the absence of correction of SF values, as well as the inability of carrying out multivariate analyses.

CONCLUSION

Our study showed that high levels of SF at diagnosis had a negative prognostic impact on OS and DFS in patients with AML, and that they were linked to a significantly higher incidence of early death and sepsis in the induction phase of therapy. We also demonstrated that patients with high levels of SF at diagnosis significantly more often had low levels of hemoglobin, as compared to patients belonging to the group with low SF levels. The existence of larger multicentric prospective studies, which would follow patients for longer periods of time, would contribute to a better understanding of the impact of hyperferritinemia on the course and outcome of disease. The identification of SF, as an independent prognostic factor and a potential target site for the action of new drugs, may contribute to a better survival of patients with AML.

Conflict of interest: None declared.

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