PROGNOSTIČKI I PREDIKTIVNI ZNAČAJ MERLJIVE REZIDUALNE BOLESTI U AKUTNOJ MIJELOBLASTNOJ LEUKEMIJI

ORIGINALNI RAD

ORIGINAL ARTICLE

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

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SAŽETAK

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

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

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

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

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

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

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

ABSTRACT

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

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

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

Results: MRD+ patients who continued treatment with intensive chemotherapy (CHT), using full doses of 3+7 CHT as reinduction therapy, had a significantly longer remission (complete remission – CR) and a longer overall survival (OS). The duration of CR ($p = 0.004$) and OS ($p = 0.019$) were statistically significantly longer in patients who maintained a negative MRD status at the end of treatment. In transplanted patients, overall survival (OS; $p = 0.006$) and duration of remission (CR; $p = 0.002$) were significantly longer (median: OS 20 months; CR 21 months), as compared to the group of non-transplanted patients (median: OS 13 months; CR 8 months).

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

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

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

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UVOD

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

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

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

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

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

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

INTRODUCTION

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

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

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

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

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

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

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

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

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

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

CILJ RADA

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

MATERIJALI I METODE

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

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

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

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

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

STUDY AIM

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

MATERIALS AND METHODS

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

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

Statistička analiza

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

REZULTATI

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

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

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

Statistical analysis

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

RESULTS

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

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

Tabela 1. Inicijalne kliničke karakteristike ispitivanih bolesnika

Table 1. Initial clinical characteristics of the examined patients

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

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

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

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

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

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

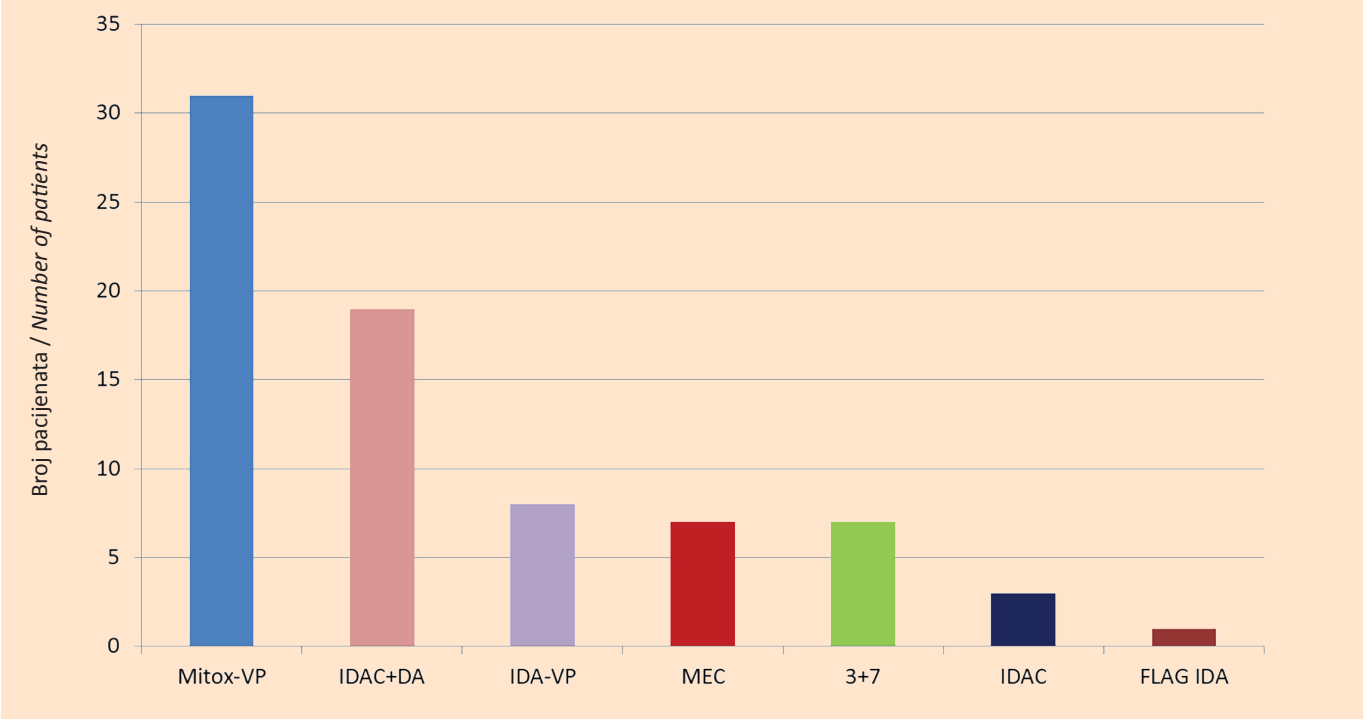
Kod 81 (73%) pacijenta je rađen i *MRD* na kraju lečenja, od kojih je 46 imalo negativan *MRD*, dok je 35 bolesnika imalo pozitivan *MRD*. Takođe, od 50 bolesnika koji su imali *MRD* pozitivnost nakon drugog ciklusa HT, 28 (56%) pacijenata je ostvarilo negativan *MRD* na kraju lečenja. Relaps bolesti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

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

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

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

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



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

Graph 1. Type of therapy used in MRD-positive patients (after the second cycle of initial therapy)
After two cycles of CHT were administered, MRD negativity was achieved in 35 (31.5%) patients and all of them were subsequently treated with consolidation IDAC therapy. Seventy-six (68.5%) patients had a positive MRD and were treated with different CHT protocols: 31 (40.8%) patients received Mitox-VP; 19 (25%) patients received IDAC+DA; 8 (10.5%) patients received IDA-VP; 7 (9.2%) patients received MEC; 3+7 was received by 7 (9.2%) patients; three (4.0%) patients received IDAC; FLAG IDA was received by one (1.3%) patient.

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

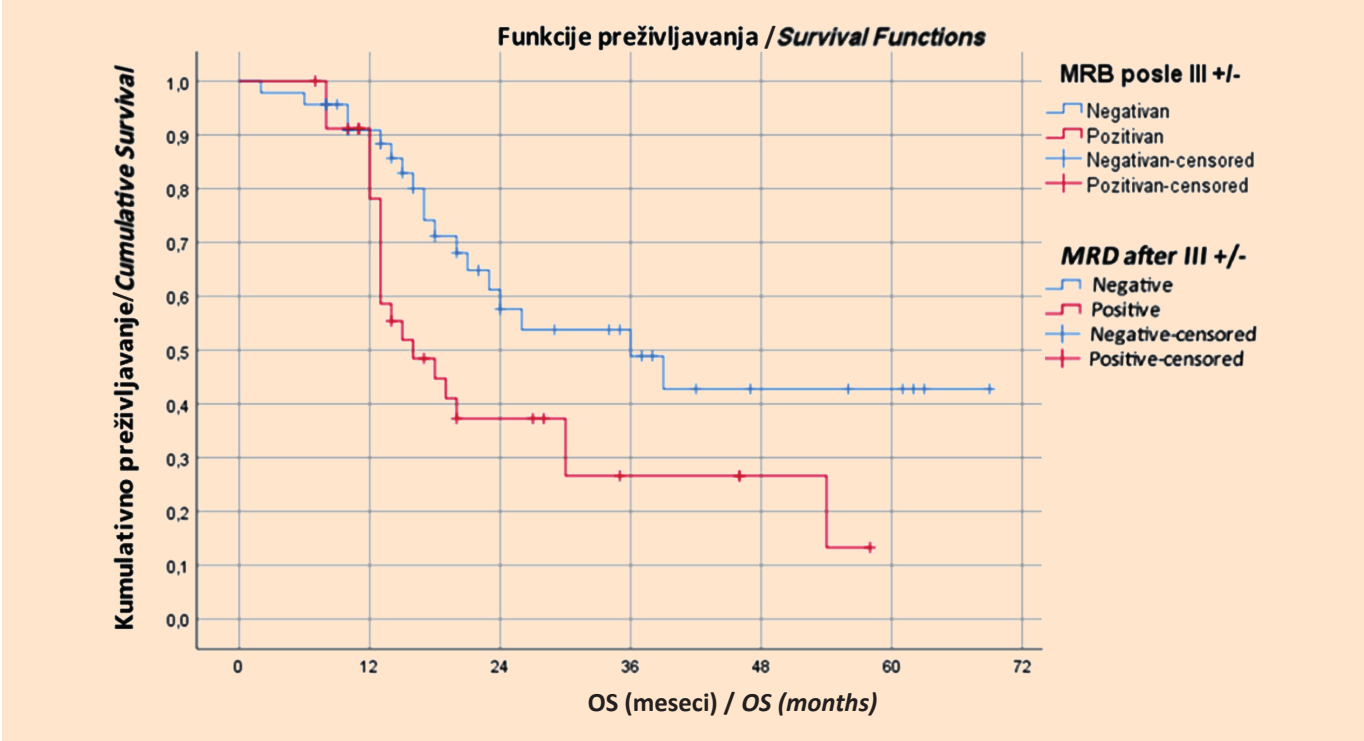
Nije postojala statistički značajna razlika u ukupnom preživljavanju (*OS*) i dužini remisije (*CR*) u odnosu na pol (*p* = 0,245, *HR* = 1,321, 95% *CI* = 0,826-2,114). Nije utvđena statistički značajna razlika u ukupnom preživljavanju (*OS*) i dužini *CR* u odnosu na *MRD* I status (posle prve konsolidacije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

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

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

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

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



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

Graph 2. Kaplan-Meier curve of overall survival (OS) in relation to the MRD status at the end of treatment
Median survival in patients with a positive MRD at the end of treatment was 16.0 months (95% CI = 9.3-22.7), while it was 36.0 months (95% CI = 17.6-54.4) in patients with a negative MRD, which is a statistically significant difference ($p = 0.019$). Subjects with a positive MRD at the end of treatment had more than twice the hazard for a fatal outcome (HR = 2.06, 95% CI = 1.10-3.85; $p = 0.024$).

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

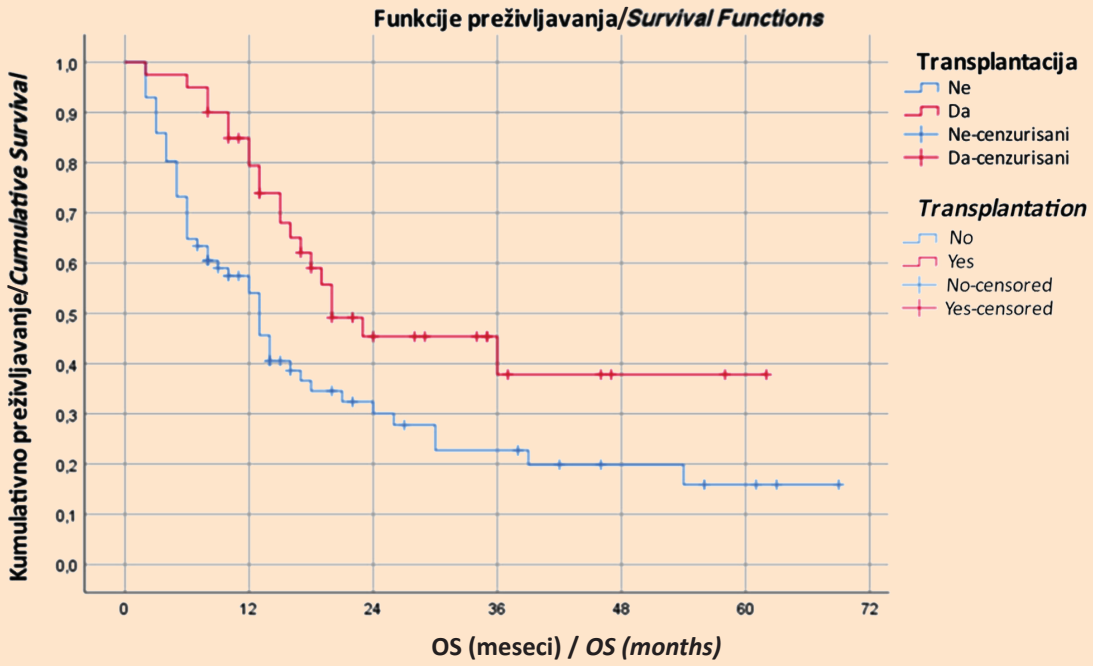
Ukupno preživljavanje (*OS*) je bilo značajno duže u grupi sa intermedijarnim rizikom leukemije (medijana 18 meseci) prema *ELN* klasifikaciji, u odnosu na grupu sa povoljnim (medijana 13 meseci) i nepovoljnim (medijana 6 meseci) rizikom ($p = 0,013$). Takođe, dužina trajanja remisije (*CR*) je bila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

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

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

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

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



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

Graph 3. Kaplan-Meier curve of overall survival (OS) in patients on chemotherapy in relation to transplant patients
The median survival in transplant patients was 20 months (95% CI = 4.0-36.0), while in non-transplant patients it was 13 months (95% CI = 10.1-15.9), which is a statistically significant difference ($p = 0.006$). Subjects who had received a transplant had a 50% lower hazard for the fatal outcome (HR = 0.498, 95% CI = 0.296-0.839; $p = 0.009$).

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

DISKUSIJA

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

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

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

DISCUSSION

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

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

MRD. Optimalni ciljevi za molekularno merenje *MRD* takođe nisu definisani. Izolovana detekcija mutacija uobičajenih u klonskoj hematopoezi povezanoj sa starenjem (engl. *age-related clonal hematopoiesis*), npr. *DNMT3A*, *TET2*, ili *ASKSL1*, ili kod sindroma nasledne predispozicije (engl. *germline predisposition syndromes*), npr. *DDKS41, RUNKS1* ili *GATA2*, nužno ne predstavljaju rezidualnu AML [17-20].

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

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

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

ZAKLJUČAK

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

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

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

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

Sukob interesa: Nije prijavljen.

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

Isolated detection of mutations common in age-related clonal hematopoiesis, e.g. DNMT3A, TET2, or ASKSL1, or in germline predisposition syndromes, e.g. DDKS41, RUNKS1, or GATA2, do not necessarily constitute residual AML [17-20].

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

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

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

CONCLUSION

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

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

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

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

Conflict of interest: None declared.

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