HOMOCISTEIN KAO PREDIKTOR KLINIČKOG ISHODA PACIJENATA HOSPITALIZOVANIH ZBOG COVID-19

ORIGINALNI RAD

ORIGINAL ARTICLE

HOMOCYSTEINE AS A PREDICTOR OF CLINICAL OUTCOMES IN PATIENTS HOSPITALIZED FOR COVID-19

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SAŽETAK

Uvod: Covid-19 i dalje predstavlja zdravstveni, socijalani i ekonomski problem na svetskom nivou. Lečenje ove sistemske infekcije je još uvek izazov. Izučavani su brojni biomarkeri u pravcu potencijalnih prognostičkih parametara, a u najvećoj meri hematološki i parametri inflamacije.

Cilj: Naš glavni cilj je da ispitamo povezanost vrednosti homocisteina sa ishodom bolesti, kao i to da li se njegova vrednost menja tokom bolesti.

Materijal i metode: U našem istraživanju prikupljeni su uzorci krvi 131 pacijenta koji su svi bili pozitivni na SARS-CoV-2 i lečeni hospitalno u UKC Kragujevac. Uključujući kriterijumi su bili sledeći: muškarci i žene stariji od 18 godina, potpisan informisani pristanak za učešće u studiji, prvi put pozitivni na SARS-CoV-2 (brzi Ag test ili PCR test), leče se hospitalno i imaju bilo koju od tri forme bolesti. Krv je uzorkovana prvog, petog i desetog dana hospitalizacije i praćen je tok bolesti. Statistička obrada podataka urađena je korišćenjem programa IBM SPSS Statistics v.21.

Rezultati: Zaključili smo da se vrednost homocisteina menja tokom hospitalizacije, odnosno da ima trend rasta. Postoji statistička značajnost između vrednosti merenih prvog, petog i desetog dana. Pokazali smo da pacijenti koji boluju od hipertenzije (HTA), imaju više vrednosti ispitivanog parametra u odnosu na pacijente koji nisu hipertoničari. Uočeno je i da vrednost homocisteina raste sa godinama. Pokazana je satistička značajnost između vrednosti homocisteina i ishoda bolesti.

Zaključak: Na osnovu naše studije možemo reći da pacijenti kod kojih je izmerena visoka vrednost homocisteina (petog dana hospitalizacije) imaju lošiji ishod bolesti u odnosu na pacijente sa nižim vrednostima ovog markera. Pacijenti sa nižim vrednostima homocisteina imaju veće šanse za oporavak.

Ključne reči: homocistein, Covid-19, ishod bolesti

ABSTRACT

Introduction: COVID-19 is still a global health, social and economic problem. Treating this systemic infection remains a challenge. Numerous biomarkers have been studied so far as potential prognostic parameters, hematological and inflammation parameters most of all.

Aim: Our main goal is to examine the association of homocysteine level with the clinical outcome, as well as whether homocysteine level changes through the course of the disease.

Materials and methods: In our research, blood samples were collected from 131 patients who were SARS-CoV-2 positive and were treated at the University Clinical Center of Kragujevac. The inclusion criteria were as follows: men and women above 18 years of age, a signed informed consent for participation in the study, patients SARS-CoV-2 positive for the first time (which was confirmed by a rapid antigen test or a PCR test), treated in hospital and belonging to any of the three COVID-19 severity categories. Blood samples were taken on the first, fifth and tenth day of the hospitalization period and the course of the disease was monitored. Statistical data processing was done using the IBM SPSS Statistics v.21 program.

Results: Based on our research, we concluded that homocysteine level changed during the hospitalization period and that it had a growing trend. There was a statistical significance between the levels measured on the first, fifth and tenth day. We showed that the patients suffering from hypertension (HTN) had higher levels of the examined parameter compared to the patients who were not hypertensive. It was also observed that homocysteine levels increased with age. There was a statistical significance between homocysteine levels and the outcome of the disease.

Conclusion: Based on our study, we can say that patients with high homocysteine levels (on the fifth day of the hospitalization period) have a worse clinical outcome than patients with lower homocysteine levels. Patients with lower homocysteine levels have a better chance of recovery.

Key words: homocysteine, COVID-19, clinical outcome

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Primljeno • *Received*: November 3, 2022; **Revidirano • *Revised*:** December 6, 2022; **Prihvaćeno • *Accepted*:** December 9, 2022; ***Online first*:** December 25, 2022

DOI: 10.5937/smclk3-40984

UVOD

Krajem 2019. godine, novi virus pod nazivom SARS-CoV-2 identifikovan je kao uzročnik pneumonije u kineskom gradu Vuhanu, a marta 2020. godine Svetska zdravstvena organizacija (SZO) proglasila je stanje pandemije i bolest nazvala koronavirusna bolest 2019 [1]. Bolest se prema kriterijumima SZO iz septembra 2022. godine klasifikuje u tri forme: kritična (eng. *critical COVID-19*), ozbiljna (eng. *severe COVID-19*) i blaga (eng. *mild COVID-19*) [2]. Infekcija je povezana sa rizikom od tromboembolijskih događaja u čijoj osnovi su inflamacija, endotelitis, hiperviskoznost i hiperkoagulabilnost [3]. U literaturi se može pronaći veliki broj istraživanja koja su pokazala visoku incidencu venske tromboembolije kod pacijenata sa teškom kliničkom slikom Covid-19, uprkos primeni tromboprofilakse [4,5]. Ovi pacijenti mogu razviti infarkt miokarda, cerebrovaskularne događaje, arterijsku trombozu ili mikrotrombozu više organa, uključujući i pluća [6]. Koagulopatija je označena kao loš prognostički parametar i zbog toga je tromboprofilaksa savetovana kod svih pacijenata gde ne postoji jasna kontraindikacija [3]. Sprovedene su brojne studije gde je ispitivan i praćen veliki broj markera koji jesu ili mogu biti prediktori prognoze, toka i ishoda ove bolesti [4]. Ispitivani su mnogi parametri inflamacije, kao i brojni imunološki, hematološki i biohemijski parametri [7]. Jedan od ispitivanih biomarkera jeste aminokiselina homocistein, koji od kada je otkriven izaziva veliko interesovanje i predmet je brojnih istraživanja [8,9]. Homocistein je semiesencijalna, sulfhidrilna aminokiselina koja nastaje od esencijalne aminokiseline metionina, ne ulazi u sastav peptida i proteina, i ne unosi se preko hrane [7,8]. Metabolizam homocisteina podrazumeva proces metilacije i transsulfuracije. Za proces metilacije neophodni su vitamin B12, folna kiselina, kao i enzim metiltetrahidroksifolat reduktaza (MTHFR), dok je za proces transsulfuracije potreban vitamin B6 i enzim cistation β-reduktaza [6,10]. Poremećaj metabolizma homocisteina najčešće dovodi do hiperhomocisteinemije koja se definiše kao vrednost homocisteina iznad 15μmol/l [6]. Refsum i sar. su među prvima sproveli veliko istraživanje, na populaciji od preko 18000 ispitanika pri čemu je zaključeno da je hiperhomocisteinemija povezana sa povećanim rizikom od hospitalizacije kod kardiovaskularnih bolesnika, i to u većem procentu kod osoba starijih od 65 godina. Van der Meer i sar. su došli do podatka da je hiperhomocisteinemija povezana sa povećanim rizikom od tromboze [11]. Osim ove povezanosti u literaturi se opisuje i rizik od još nekih oboljenja među kojima su: cerebrovaskularna oboljenja, neurodegenerativna oboljenja, komplikacije u trudnoći, osteoporoza, maligne bolesti, ali i teža klinička slika i loš ishod obolelih

INTRODUCTION

At the end of 2019, a new virus called SARS-CoV-2 was identified as the cause of pneumonia in the city of Wuhan, China, and in March 2020 the World Health Organization (WHO) declared a pandemic and named the disease coronavirus disease 2019 [1]. According to WHO criteria from September 2022, the disease is classified into three categories according to its severity: critical COVID-19, severe COVID-19, and mild COVID-19 [2]. The infection is associated with the risk of thromboembolic events based on inflammation, endotheliitis, hyperviscosity and hypercoagulability [3]. Numerous studies have reported a high incidence of venous thromboembolism in patients with severe clinical picture of COVID-19 despite the use of thromboprophylaxis therapy [4,5]. Such patients can develop myocardial infarction, cerebrovascular events, arterial thrombosis or microthrombosis affecting different organs, including the lungs [6]. Coagulopathy is associated with poor outcomes, so thromboprophylaxis is suggested in all patients with no clear contraindication [3]. Numerous studies have examined many markers that predict or could predict the prognosis, the course and the outcome of this disease [4]. Many inflammatory markers have been examined, as well as numerous immunological, hematological, and biochemical parameters [7]. One of the examined biomarkers is the amino acid homocysteine which has attracted great attention and been widely researched ever since it was discovered [8,9]. Homocysteine is a semi essential sulfur-containing amino acid which is derived from an essential amino acid methionine, is not present in peptides and proteins and is not ingested through food [7,8]. Homocysteine metabolism involves the process of methylation and transsulfuration. The methylation process requires vitamin B12, folic acid and the enzyme methylenetetrahydrofolate (MTHFR), whereas the transsulfuration process requires vitamin B6 and the enzyme cystathionine- β-synthase [6,10]. Impaired homocysteine metabolism usually results in hyperhomocysteinemia which is defined as homocysteine level above 15μmol/l [6]. Refsum et al. were among the first to conduct a large-scale study on a population of over 18,000 subjects and they concluded hyperhomocysteinemia was associated with an increased risk of hospitalization in cardiovascular patients, the risk being higher in patients over 65 years of age. Van der Meer et al. found that hyperhomocysteinemia was associated with an increased risk of thrombosis [11]. In addition to this, the literature describes the risk of some other diseases including cerebrovascular diseases, neurodegenerative diseases, complications in pregnancy, osteoporosis, malignant diseases, but also a more severe clinical

od Covid-19 [8,9]. Tri najveće studije o povezanosti Covid-19 i vrednosti homocisteina sprovedene su u Italiji, Kini i Turskoj što je u junu 2022. godine iskorišćeno za meta-analizu, pri čemu se kod ukupno 694 hospitalno lečena pacijenta povišena vrednost homocisteina u serumu pokazala kao loš prognostički znak [6]. Yang i sar. su u svoje istraživanje uključili 273 pacijenta kojima su merili nivo homocisteina prvog dana hospitalizacije uz ostalu propratnu dijaganostiku koja je uključivala CT grudnog koša, a zatim je posle 5-7 dana vršena procena progresije bolesti ponavljanjem CT-a. Pacijenti koji su imali više vrednosti homocisteina na prijemu imali su i progresiju na CT nalazu [12]. Ponti i sar. sproveli su istraživanje na 304 pacijenta gde su merili nivo homocisteina u plazmi prilikom prijema u bolnicu, i pokazali su da je nivo ove supstance bio viši kod pacijenata čije se lečenje završilo smrtnim ishodom [10]. Keskin i sar. su izmerili homocistein u plazmi 117 pacijenata, takođe prilikom prijema, podelivši ih u dve grupe (blaga i teška forma bolesti), i pokazalo se da su pacijenti sa teškom formom bolesti imali viši nivo homocisteina u odnosu na pacijente sa blagom formom [13].

Cilj: Hiperkoagulabilnost i sklonost ka trombozi kod obolelih od Covid-19 privlači veliku pažnju. Sprovedene su studije koje ispituju veliki broj markera hemostaze. U našem istraživanju ispitivali smo parametar koji se ne analizira u rutinskoj praksi već uglavnom kod pacijenata sa sumnjom na stečenu trombofiliju. Odlučili smo da ga analiziramo kroz vreme, odnosno u nekoliko navrata tokom hospitalnog lečenja pacijenata obolelih od Covid-19. Naš glavni cilj je da ispitamo povezanost vrednosti homocisteina sa ishodom bolesti, te da utvrdimo da li se njegova vrednost menja tokom trajanja bolesti.

MATERIJAL I METODE

Uzorkovnje

U našem istraživanju prikupljeni su uzorci krvi 131 pacijenta koji su zbog SARS-CoV-2 infekcije hospitalno lečeni u UKC Kragujevac. Uključujući kriterijumi su bili sledeći: muškarci i žene stariji od 18 godina, potpisan informisani pristanak za učešće u studiji, prvi put pozitivni na SARS-CoV-2 (brzi antigenski test ili PCR test), leče se hospitalno i imaju bilo koju od tri forme bolesti definisane kriterijumima SZO. Krv je uzorkovana prvog, petog i desetog dana hospitalizacije i praćen je tok bolesti. Uzorkovana je jedna epruveta (3ml krvi). Analiza je rađena u hematološkoj laboratoriji Klinike za hematologiju UKC Kragujevac na standardizovanom aparatu. Od 131 ispitanika bilo je 53 žene (40,5%) i 78 muškaraca (59,5%). Ispitanici su imali od 19 do 90 godina pri čemu je prosečna starost iznosila 62,3±16,3 godina.

picture and a poor outcome in patients suffering from COVID-19 [8,9]. The three largest studies on the correlation between COVID-19 and homocysteine levels were conducted in Italy, China and Turkey and their results were used for a meta-analysis in June 2022, whereby in a total of 694 hospitalized patients an elevated serum homocysteine level was a poor prognostic sign [6]. Yang et al. included 273 patients in their research where they measured homocysteine level on the first day of the hospitalization period followed by accompanying diagnostics that included CT of the chest, and then after 5-7 days the disease progression was assessed by repeating CT. The patients with higher homocysteine levels on hospital admission also had progression on CT scan [12]. Ponti et al. conducted a study that included 304 patients whose plasma homocysteine level was measured on hospital admission, and they showed that the level of this substance was higher in patients with lethal outcome [10]. Keskin et al. also measured plasma homocysteine level in 117 patients on hospital admission classifying them into two groups (mild and severe disease) and the results showed that the patients with severe disease had higher homocysteine levels compared to the patients who had mild disease [13].

Aim: Hypercoagulability and a tendency to thrombosis in COVID-19 patients have attracted great attention. Studies examining numerous markers of homeostasis have been conducted. In our research we examined a parameter that is not routinely analyzed except in patients suspected to have acquired thrombophilia. We decided to analyze this parameter over time, i.e. several times during the hospitalization period of COVID-19 patients. Our main aim was to examine the association of homocysteine levels with the outcome of the disease, as well as to find out if the levels changed during the course of the disease.

MATERIALS AND METHODS

Sampling

In our research, blood samples were collected from 131 patients hospitalized due to SARS-CoV-2 infection at the University Clinical Centre of Kragujevac. The inclusion criteria were as follows: men and women above 18 years of age, a signed informed consent for participation in the study, patients SARS-CoV-2 positive for the first time (which was confirmed by a rapid antigen test or a PCR test), treated in hospital and belonging to any of the three severity categories according to WHO criteria. Patients' blood was sampled on the first, fifth, and tenth day of the hospitalization period and the course of the disease was monitored. One test tube of blood (3 ml) was sampled in each case. The analysis was performed in the hematology laboratory of the

Statistička obrada podataka

Statistička obrada podataka urađena je korišćenjem programa IBM SPSS Statistics v.21. Normalnost raspodele podataka analizirana je korišćenjem Kolmogorov-Smirnov testa normalnosti. Za analizu vrednosti homocisteina izmerenih prvog, petog i desetog dana korišćen je Fridmanov test. Za analizu vrednosti homocisteina izmerenih prvog, petog i desetog dana u odnosu na kategorijske promenljive sa dva odgovora korišćen je t-test za nezavisne uzorke ukoliko su podaci po kategorijama pratili normalnu raspodelu i Man-Vitnijev U test ukoliko podaci po kategorijama nisu pratili normalnu raspodelu. Za analizu vrednosti homocisteina izmerenih prvog, petog i desetog dana u odnosu na kategorijske promenljive sa više od dva odgovora korišćena je jednofaktorska ANOVA za različite grupe ukoliko su podaci po kategorijama pratili normalnu raspodelu i Kruskal-Volisov test ukoliko podaci po kategorijama nisu pratili normalnu raspodelu. Za analizu vrednosti homocisteina izmerenih prvog, petog i desetog dana u odnosu na numeričku promenljivu (starost ispitanika) korišćen je metod korelacije i regresije, odnosno tumačena je vrednost Spirmanovog koeficijenta korelacije. Rezultati su smatrani statistički značajnim ukoliko je značajnost (p vrednost) bila manja ili jednaka 0,05.

REZULTATI

Za deskriptivnu statističku analizu neprekidnih promenljivih korišćene su najmanja i najveća vrednost kao i prosečna vrednost i standardno odstupanje. Rezultati ove analize prikazani su u Tabeli 1. Za deskriptivnu statističku analizu kategorijskih promenljivih korišćene su apsolutna i relativna učestalost. Rezultati ove analize prikazani su u Tabeli 2. Za analizu vrednosti homocisteina izmerenu prvog, petog i desetog dana korišćen je Fridmanov test. Primenom Fridmanovog testa utvrdili smo da postoji statistički značajna razlika (Chi-Square statistics=45,319, df=2, $p < 0,001$) u vrednostima homocisteina izmerenih u tri različita trenutka. Da bismo utvrdili između kojih konkretno trenutaka postoji statistički značajna razlika, za naknadne testove korišćen

Clinic for hematology of the University Clinical Centre of Kragujevac using a standardized machine. Out of 131 participants, there were 53 women (40.5%) and 78 men (59.5%). The participants were 19 to 90 years old, the average age being 62.3±16.3 years.

Statistical analysis

Statistical data processing was done using the IBM SPSS Statistics v.21 program. Normal distribution of data was analyzed using Kolmogorov-Smirnov test for normality. The Friedman test was used to analyze homocysteine levels measured on the first, fifth and tenth day. To analyze homocysteine levels measured on the first, fifth and tenth day in relation to categorical variables with two responses t-test was used for independent samples if the data by category followed a normal distribution, whereas the Mann-Whitney U test was applied if the data by category did not follow a normal distribution. For the analysis of homocysteine levels measured on the first, fifth, and tenth day in relation to categorical variables with more than two responses one-factor ANOVA was used for different groups if the data by category followed a normal distribution, whereas the Kruskal-Wallis test was chosen if the data by category did not follow a normal distribution. Correlation and regression were used for the analysis of homocysteine levels measured on the first, fifth, and tenth day in relation to a quantitative variable (participants' age), i.e. Spearman's Rank correlation coefficient (Spearman's rho) was interpreted. The results were considered statistically significant if the significance (p) was less than or equal to 0.05.

RESULTS

In descriptive statistical analysis of continuous variables, the lowest and the highest values were taken into consideration, as well as the mean and standard deviation. The results of this analysis are presented in Table 1. In descriptive statistical analysis of categorical variables, absolute frequency and relative frequency were considered. The results of this analysis are shown in Table 2. The Friedman test was used to analyze homocysteine levels measured on the first, fifth,

Tabela 1. Deskriptivne vrednosti homocisteina tokom trajanja bolesti

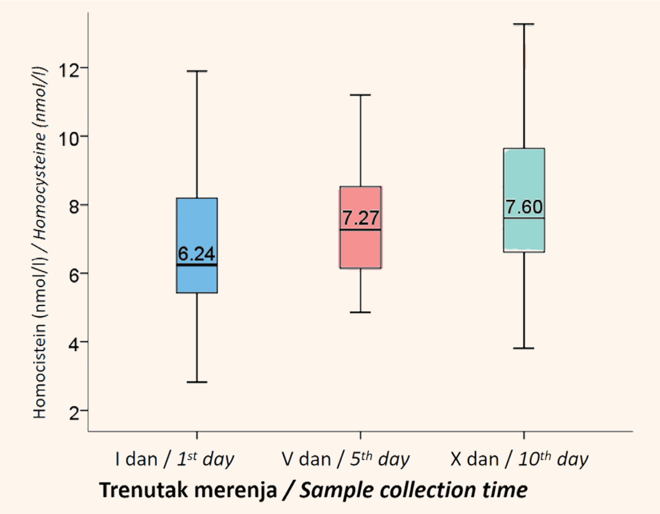
Table 1. Descriptive homocysteine levels during the course of the disease

	Najniža vrednost / *The lowest level*	Najviša vrednost / *The highest level*	Prosečna vrednost / *The mean*	Standardno odstupanje / *Standard deviation*
Homocysteine (nmol/l) I dan / *Homocysteine (nmol/l) 1st day*	2.82	29.48	7.69	4.80
Homocysteine (nmol/l) V dan / *Homocysteine (nmol/l) 5th day*	4.86	29.52	8.07	3.45
Homocysteine (nmol/l) X dan / *Homocysteine (nmol/l) 10th day*	3.81	30.00	9.04	4.51

Tabela 2. Deskriptivna statistička analiza uzorka pacijenata

Table 2. Descriptive statistical analysis of patients' samples

		Broj ispitanika / *No. of respondents*	**Procenat ispitanika / *Percentage of respondents***
Ishod / *Clinical outcome*	Oporavak / *Recovery*	111	84.7%
	Exitus-letalis / *A lethal outcome*	20	15.3%
Forma bolesti / *Disease severity category*	Blaga / *Mild*	29	22.1%
	Ozbiljna / *Severe*	77	58.8%
	Kritična / *Critical*	25	19.1%
Hipertenzija / *Hypertension*	Ima / *Yes*	104	79.4%
	Nema / *No*	27	20.6%



Grafikon 1. Vrednosti homocisteina tokom hospitalizacije

Figure 1. Homocysteine levels during the hospitalization period

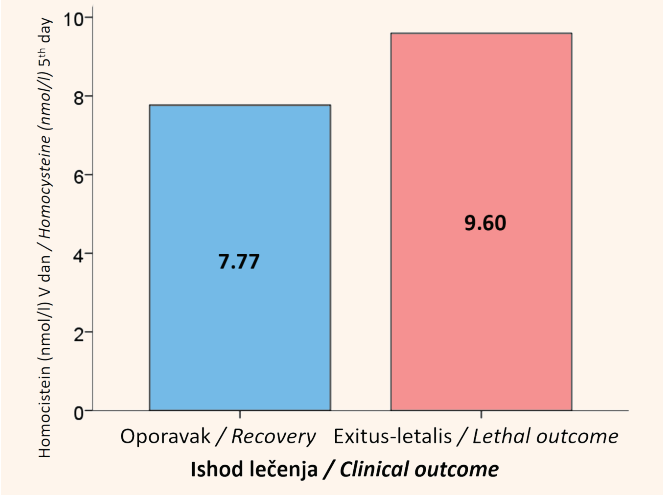
je Vilkoksonov test. Primenom Vilkoksonovog testa utvrdili smo da postoji statistički značajna razlika u vrednostima homocisteina između sva tri merenja. Grafički prikaz ovog rezultata predstavljen je na Grafikonu 1. Za analizu vrednosti homocisteina u odnosu na pol ispitanika korišćen je Man-Vitnijev U test. Primenom ovog testa utvrdili smo da ne postoji statistički značajna razlika u vrednostima homocisteina izmerenih pr-

and tenth day. Applying the Friedman test, we found that there was a statistically significant difference (chi-squared statistics=45.319, df=2, $p < 0.001$) between homocysteine levels measured at three different times. For subsequent measurements we used the Wilcoxon test in order to determine the exact moments between which there is a statistically significant difference. Using the Wilcoxon test, we concluded that there was a statistically significant difference between homocysteine levels at all three measurements. A graphical presentation of this result can be seen in Chart 1. The Mann-Whitney U test was used to analyze homocysteine levels in relation to gender. Using this test, we concluded that there was no statistically significant difference between homocysteine levels measured on the first, fifth, and tenth day in relation to gender. To analyze the relationship between homocysteine levels measured on the first, fifth, and tenth day and participants' age, correlation and regression method was used, i.e. Spearman's Rank correlation coefficient was interpreted. Using the correlation method, i.e. interpreting Spearman's Rank correlation coefficient, we concluded that there was a moderately weak, positive and statistically significant correlation between homocysteine levels measured on the fifth day and the participants' age. The results of this analysis are shown in Table 3. To analyze homocysteine levels in relation to

Tabela 3. Značajnost vrednosti homocisteina izmerenih petog dana hospitalnog lečenja u odnosu na starost, ishod bolesti i prisustvo hipertenzije

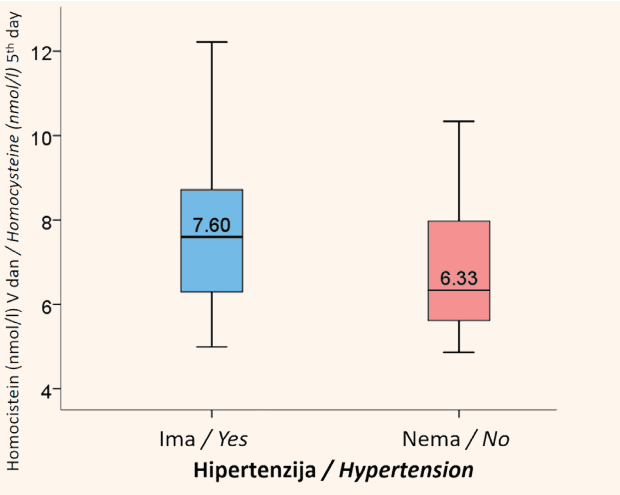
Table 3. The significance of homocysteine levels measured on the fifth day of the hospitalization period in relation to age, clinical outcome, and the presence of hypertension

Homocysteine (nmol/l) V dan / *Homocysteine (nmol/l) 5th day*	**Spirmanovo Rho/ *Spearman's rho* Statistika testa / *Test statistics***	**N/ df**	**Značajnost / *Significance***
Starost ispitanika / *Age*	0.201	104	0.041
Ishod bolesti / *Clinical outcome*	2.022	102	0.046
Prisustvo hipertenzije / *The presence of hypertension*	2.117	-	0.034



Grafikon 2. Prosečna vrednost homocisteina u V danu u odnosu na ishod bolesti

Figure 2. The average homocysteine level on the 5th day in relation to the clinical outcome



Grafikon 3. Vrednost homocisteina u V danu u odnosu na prisustvo hipertenzije

Figure 3. Homocysteine level on the 5th day in relation to the presence of hypertension

vog, petog i desetog dana u odnosu na pol ispitanika. Za analizu povezanosti vrednosti homocisteina izmerenih prvog, petog i desetog dana i starosti ispitanika korišćen je metod korelacije i regresije, odnosno tumačena je vrednost Spirmanovog koeficijenta korelacije. Primenom metoda korelacije, odnosno tumačenjem vrednosti Spirmanovog koeficijenta korelacije, utvrdili smo da postoji umereno slaba, pozitivna i statistički značajna povezanost između vrednosti homocisteina izmerenih petog dana i starosti ispitanika. Rezultati ove analize prikazani su u Tabeli 3. Za analizu vrednosti homocisteina u odnosu na ishod bolesti ispitanika korišćen je t test za nezavisne uzorke. Primenom t testa za nezavisne uzorke utvrdili smo da ne postoji statistički značajna razlika u vrednostima homocisteina izmerenih prvog i desetog dana u odnosu na ishod bolesti ispitanika, ali da postoji u odnosu na vrednosti izmerene petog dana. Rezultati su prikazani u Tabeli 3 i na Grafikonu 2. Za analizu vrednosti homocisteina u odnosu na formu bolesti ispitanika korišćen je Kruskal-Volisov test. Primenom Kruskal-Volisovog testa utvrdili smo da ne postoji statistički značajna razlika u vrednostima homocisteina izmerenih prvog, petog i desetog dana u odnosu na formu bolesti ispitanika. Za analizu vrednosti homocisteina u odnosu na prisustvo hipertenzije kod ispitanika korišćen je Man-Vitnijev U test. Utvrdili smo da postoji statistički značajna razlika u vrednostima homocisteina izmerenih petog dana u odnosu na prisustvo hipertenzije kod ispitanika. Rezultati ove analize prikazani su u Tabeli 3. Značajno više vrednosti homocisteina izmerene petog dana imaju ispitanici sa hipertenzijom. Grafički prikaz vrednosti homocisteina izmerenih petog dana u odnosu na prisustvo hipertenzije kod ispitanika predstavljen je na Grafikonu 3.

the clinical outcome the independent samples t-test was used. Applying the independent samples t-test we found that there was no statistically significant difference between homocysteine levels measured on the first and tenth day in relation to the clinical outcome, but that there was a statistically significant difference in relation to homocysteine levels measured on the fifth day. The results are shown in Table 3 and Chart 2. To analyze homocysteine levels in relation to disease severity, the Kruskal-Wallis test was applied. Using the Kruskal-Wallis test we found that there was a statistically significant difference between homocysteine levels measured on the first, fifth, and tenth day in relation to disease severity. To analyze homocysteine levels in relation to the presence of hypertension in participants, the Mann-Whitney U test was applied. We concluded that there was a statistically significant difference between homocysteine levels measured on the fifth day in relation to the presence of hypertension in participants. The results of this analysis are shown in Table 3. Significantly higher homocysteine levels measured on the fifth day were found in participants suffering from hypertension. A graphical presentation of homocysteine levels measured on the fifth day in relation to the presence of hypertension is shown in Chart 3.

DISCUSSION

So far, homocysteine levels have been analyzed on hospital admission in order to determine their correlation with the outcome of the disease and the clinical picture [6]. In our study, we checked homocysteine levels through the hospitalization period, i.e. through the course of the disease. Patients' blood was sampled on the first day of the hospitalization period and then

DISKUSIJA

Do sada su u literaturi analizirane vrednosti homocisteina prilikom prijema pacijenta u cilju korelacije sa ishodom i kliničkom slikom [6]. U našoj studiji pratili smo vrednosti homocisteina kroz dane hospitalizacije, odnosno kroz tok bolesti. Uzorkovana je krv prvog dana hospitalizacije, zatim petog i desetog dana. Uključili smo 131 pacijenta oba pola (53 žene i 78 muškaraca) sa sve tri fome bolesti. Ispitanici su imali od 19 do 90 godina pri čemu je prosečna starost iznosila 62,3±16,3 godina. Blagu formu bolesti imalo je 29 pacijenata (22,1%), ozbiljnu 77 (58,8%), a kritičnu 25 pacijenata (19,1%). Na kućno lečenje otpušteno je 111 pacijenata (84,7%), dok je kod 20 pacijenata ishod bio letalan (15,3%). Zbog velikog broja istraživanja o povezanosti homocisteina i kardiovaskularnih bolesti pacijente smo podelili i na osnovu prisustva HTA [13,14]. 104 pacijenta je bolovalo od HTA (79,4%), a 27 nije imalo HTA (20,6%). Dosadašnja istraživanja pokazala su da postoji povezanost između povišenih vrednosti homocisteina prvog dana hospitalizacije i težine kliničke slike, kao i ishoda bolesti. Viši nivo homocisteina registrovan je kod pacijenata sa teškom formom bolesti u odnosu na pacijente sa lakšom formom, kao i kod pacijenata sa smrtnim ishodom u odnosu na one koji su se oporavili [10,11]. Takođe, više vrednosti povezane su i sa progresijom bolesti na CT-u [10]. U našoj studiji pokazana je statistička značajnost izmerenih vrednosti homocisteina analiziranih kroz vreme, tačnije tokom bolesti vrednost se povećavala. Najniža izmerena vrednost homocisteina prvog dana iznosi 2,82, a najviša 29,4 (prosečna vrednost 7,49, standardno odstupanje 4,80). U petom danu najniža izmerena vrednost je 4,86, a najviša 29,52 (prosečna vrednost 8,07, standardno odstupanje 3,45). Utvrdili smo da postoji statistička značajnost između vrednosti homocisteina izmerenih petog dana hospitalizacije i ishoda bolesti ($p < 0,05$). Pacijenti kod kojih se bolest završila smrtnim ishodom imali su značajno više vrednosti ovog biomarkera petog dana hospitalizacije u odnosu na pacijente koji su se oporavili. Primetili smo da pacijenti koji boluju od HTA imaju više vrednosti homocisteina od pacijenata koji nisu hipertoničari. Postoji statistički značajna razlika u vrednostima homocisteina izmerenih petog dana u odnosu na prisustvo hipertenzije kod ispitanika ($p < 0,05$). Takođe, primećeno je da vrednost ispitivanog parametra raste sa starošću ispitanika. Postoji statistički značajna povezanost između vrednosti homocisteina izmerenih petog dana i starosti ispitanika ($p < 0,05$). Kao najznačaji podatak pokazala se vrednost homocisteina izmerena petog dana, koja je statistički značajno povezana sa ishodom bolesti, prisustvom hipertenzije i starošću ispitanika.

on the fifth and tenth day. We included 131 patients of both sexes (53 women and 78 men) suffering from all three disease severity categories of COVID-19. The participants were 19 to 90 years old, the average age being 62.3±16.3 years. Mild COVID-19 was found in 29 patients (22.1%), 77 patients (58.8%) suffered from severe COVID-19 and 25 patients (19.1%) had critical COVID-19. 111 patients (84.7%) were discharged to home for home health care, whereas 20 patients (15.3%) had a lethal outcome. Due to the large number of studies on the association between homocysteine and cardiovascular diseases, we also classified patients according to the presence of hypertension (HTN) [13,14]. 104 patients suffered from HTN (79.4%), whereas 27 patients did not have HTN (20.6%). Previous studies showed a correlation between elevated homocysteine levels on the first day of the hospitalization period and the severity of the clinical picture, as well as the final outcome. Higher levels of homocysteine were found in patients with severe COVID-19 compared to patients with mild disease, as well as in patients with lethal outcome in comparison with those who recovered [10,11]. Moreover, higher homocysteine levels are associated with disease progression on CT [10]. Our study presented a statistical significance of homocysteine levels analyzed through time; more precisely, during the course of the disease the levels increased. The lowest homocysteine level measured on the first day was 2.82 and the highest level was 29.4 (the mean 7.49; standard deviation 4.80). On the fifth day, the lowest measured level was 4.86 and the highest was 29.52 (the mean 8.07; standard deviation 3.45). We concluded there was a statistically significant correlation between homocysteine levels measured on the fifth day of the hospitalization period and the clinical outcome ($p < 0.05$). The patients with a lethal outcome had significantly higher levels of this biomarker on the fifth day of the hospitalization period in comparison with the patients who recovered. We also noticed that the patients who suffered from HTN had higher homocysteine levels than the patients who were not hypertensive. There is a statistically significant difference between homocysteine levels measured on the fifth day in relation to the presence of hypertension in participants. Apart from this, we noticed that the level of the examined parameter increased with age. There is a statistically significant correlation between homocysteine levels measured on the fifth day and participants' age ($p < 0.05$). Homocysteine level measured on the fifth day was the most significant finding being statistically significantly correlated with the clinical outcome, the presence of hypertension and age.

ZAKLJUČAK

Iz naše studije možemo zaključiti da se vrednosti homocisteina kod pacijenta lečenih hospitalno od Covid-19 menjaju tokom bolesti, odnosno da se vrednost povećava. Pokazali smo da vrednosti homocisteina izmerene petog dana značajno utiču na ishod lečenja. Pacijenti oboleli od Covid-19 kod kojih su izmerene više vrednosti homocisteina imaju lošiji ishod bolesti, odnosno veću šansu za smrtni ishod. Pacijenti sa nižim vrednostima homocisteina imaju bolju prognozu i brži oporavak.

Sukob interesa: Nije prijavljen.

CONCLUSION

Based on the results of our study, we can conclude that homocysteine levels in patients hospitalized for COVID-19 changed through the course of the disease, i.e. that they increased. We showed that homocysteine levels measured on the fifth day significantly affected the outcome of the treatment. COVID-19 patients who had higher homocysteine levels had worse clinical outcomes, i.e. a greater chance of a lethal outcome. Patients with lower homocysteine levels had a better prognosis and recovered more quickly.

Conflict of interest: None declared.

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