sciendo

Serum homocysteine level and severity of coronavirus disease-2019 (COVID-19)

JELITA SIREGAR[1], DARMADI DARMADI[2]

[1] Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Dr Mansyur 5, Medan, Indonesia
[2] Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Dr Mansyur 5, Medan, Indonesia

Introduction: Coronavirus disease-2019 (COVID-19) is still a global health problem nowadays. A particular COVID-19 patients develop severe symptoms. Some biomarkers can be used to determine disease severity and improve outcome. Homocysteine is one of the novel biomarkers. The objective of this study is to determine the role of serum homocysteine level in stratifying severity of COVID-19.

Methods: A cross-sectional study was conducted in Medan, Indonesia from May to December 2021. Subjects were obtained using consecutive sampling method. Inclusion criteria was COVID-19 patients aged 18 years or older and willing to participate in the study. Patients with malignancy, chronic kidney disease, thyroid disease, coronary heart disease, and who consume several medications including cholestyramine, metformin, methotrexate, fibrate, and contraceptive pill, were excluded. Data regarding demographic, laboratory, and biomarker were gathered from each subject. Statistical analyses were conducted at 95% confidence interval.

Results: A total of 100 patients were enrolled. Most subjects were males (59%) and from Batak ethnicity (64%). Twenty percent subjects had severe COVID-19. The levels of serum high-sensitivity C-reactive protein (hs-CRP), D dimer, homocysteine, and procalcitonin were significantly higher in severe COVID-19 subjects. D dimer had the highest sensitivity (91.7%) and specificity (94.7%) in stratifying severe COVID-19, followed by hs-CRP (91.7% and 85.5%, respectively), homocysteine (87.5% and 78.9%, respectively), and procalcitonin (58.3% and 74.0%, respectively).

Conclusion: Homocysteine can be used as a biomarker to determine COVID-19 severity.

Key words: biomarker, coronavirus, COVID-19, homocysteine, severity.

What is new? What is important?

1. Symptoms of COVID-19 varies from mild to severe.
2. Several biomarkers can be utilized to stratify COVID-19 severity.
3. Homocysteine is a biomarker that is sensitive and specific in determining severe COVID-19.

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is firstly reported in the end of 2019 in Wuhan, China. Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is the etiology of the disease. The disease had spread globally in a rapid onset and was declared as a pandemic by World Health Organization (WHO) on 11 March 2020. After almost three years, COVID-19 is still a global health problem at present [1–5]. Data from WHO as of 16th December 2022 show that there are 647,972,911 confirmed cases and 6,642,832 deaths globally. United States has the highest total cases (95,150,745) followed by India (44,601,934) and Brazil (34,684,529). Indonesia is in the 20th position with 6,439,292 total cases [6]. In China, the prevalence rates of severe and critical COVID-19 were 17.84% and 4.9%, respectively. There were 4.9% severe or critical cases in the Silicon Valley region in United states. Severe cases in Egypt and South Korea were reported at 51.6% and 11.96%, respectively [7]. Other literature stated that there were 81% mild or moderate COVID-19 cases, 14% severe cases, and 5% critical cases [8]. The pooled severity and mortality rate are reported to be 18.0% and 3.2%, respectively [1]. The rates are lower compared to two previous coronavirus epidemics [1,9]. However, the burden of disease affects all aspects of life including economy, social, and health [7,10].

The viral entry is initiated by binding of spike (S) glycoprotein of the SARS-CoV-2 with angiotensin-converting enzyme 2 (ACE2) receptor in host's cell. This process triggers inflammatory cascade as the pathogenesis of COVID-19 [1,3,9]. Inflammation is positively associated with severity of COVID-19. Some parameters which are related to inflammation, such as interferons, interleukins, procalcitonin and neutrophil

 DOI: 10.2478/rjim-2023-0002

count, are elevated in severe COVID-19 cases [3,7,11]. Homocysteine is one of novel biomarkers used to stratify COVID-19 severity. It is more specific for detecting cardiovascular damage, one of end organ damages due to COVID-19. Homocysteine is elevated in patients with severe COVID-19 [11]. There is limited publication regarding the role of serum homocysteine level in stratifying COVID-19 severity in Indonesia. In this study, we want to determine the issue particularly in COVID-19 patients in Medan, Indonesia.

MATERIALS AND METHODS

An analytical study using cross-sectional design was conducted from May until December 2021 at Haji Adam Malik Hospital and Universitas Sumatera Utara Hospital. Subjects were enrolled using consecutive sampling method from inpatient and outpatient installations. Inclusion criteria was COVID-19 patients aged 18 years or older and willing to participate in the study by signing informed consent. COVID-19 patients were classified into severe based on Indonesian COVID-19 Treatment Guidelines 3rd edition. Patients were diagnosed as severe COVID-19 if they had clinical signs of pneumonia (fever, cough, dyspnea, tachypnea) plus one or more of the following: 1) respiratory rate >30 times/minute, 2) severe respiratory distress, or 3) SpO_2 <93% in room air. Patients with malignancy, chronic kidney disease, thyroid disease, coronary heart disease, and who consume several medications including cholestyramine, metformin, methotrexate, fibrate, and contraceptive pill, were excluded. This study had been approved by Ethical Committee of Universitas Sumatera Utara with a register number of 429/KEPK/USU/202.

Demographic and anthropometric parameters were gathered from each subject. Peripheral blood was drawn as sample for determining serum homocysteine, high-sensitivity C-reactive protein (hs-CRP), procalcitonin, and D-dimer levels. Complete blood count, liver enzymes, and renal function test were also conducted. Serum homocysteine level was determined using Homocysteine Immuniassay Kit (Bio-Rad Lab, Oslo, Norway), hs-CRP using hs-CRP enzyme-linked immunosorbent assay (ELISA) Kit (Aviscera Bioscience, Inc., Santa Clara, CA, USA), while procalcitonin using Human Procalcitonin ELISA Kit (Norcross, GA, USA). Chi-square test was utilized to determine the relationship between categorical variables and severity of COVID-19. Normality test was conducted to determine data distribution of numerical variables. Independent-T test was used to determine the relationship between numerical variables and severity of COVID-19 if the data was distributed normally. Otherwise, we used Mann-Whitney test. Receiver operating characteristic (ROC) curve analysis was applied to determine the cut off points of laboratory parameters in stratifying COVID-19 severity. Diagnostic test was then used to obtain sensitivity and specificity rates of laboratory parameters. All of the statistical analyses were conducted at confidence interval of 95% using SPSS statistical software 22nd version (SPSS Inc., Chicago). P-values of <0.05 were considered significant.

RESULTS

A total of 100 COVID-19 patients were enrolled in this study. Among them, 59% were males, 64% were from Batak ethnicity, and 24% had severe COVID-19. Out of 100 COVID-19 patients, 24 patients were severe, while the other 76 patients were non-severe. There were no significant relationship between gender, ethnicity, and severity of COVID-19 in this study. Median age of patients in severe COVID-19 patients was 60 years and median body mass index (BMI) of patients with severe COVID-19 was 23.4 kg/m^2. However, there was no significant difference in age and BMI between severe and non-severe COVID-19 patients (Table 1).

Table 1

Baseline characteristics of subjects and their association with COVID-19 severity

Variables	COVID-19		P-value
	Severe	Non-severe	
Gender, n (%)			
Male	17 (29)	42 (71)	0.176
Female	7 (17)	34 (83)	
Median age, year (minimum-maximum)	60 (39 – 76)	56.5 (34 – 74)	0.457
Ethnicity			
Batak	18 (28)	46 (72)	0.432
Java	4 (16)	21 (84)	
Others	2 (18)	9 (82)	
Median BMI, kg/m^2 (minimum-maximum)	23.4 (16.1 – 36.3)	22.2 (18 – 35.6)	0.248

BMI body mass index

From laboratory results, there was no significant difference in complete blood count parameters between patients with severe and non-severe COVID-19 patients. Similar findings were observed regarding liver enzymes and renal function test. Inflammatory biomarkers showed significant differences between both groups. Hs-CRP, D dimer, procalcitonin, and homocysteine were significantly higher in severe COVID-19 group compared to its counterpart.

All the significant parameters from previous analyses were involved in ROC curve to determine the cut-off point in discriminating between severe and non-severe COVID-19 patients (Fig. 1).

After obtaining the cut-off point for each parameter, we conducted a diagnostic test and determined sensitivity and specificity of each parameter in determining severity of COVID-19. The result showed that D dimer had the highest sensitivity and specificity, followed by hs-CRP, homocysteine, and procalcitonin (Table 3).

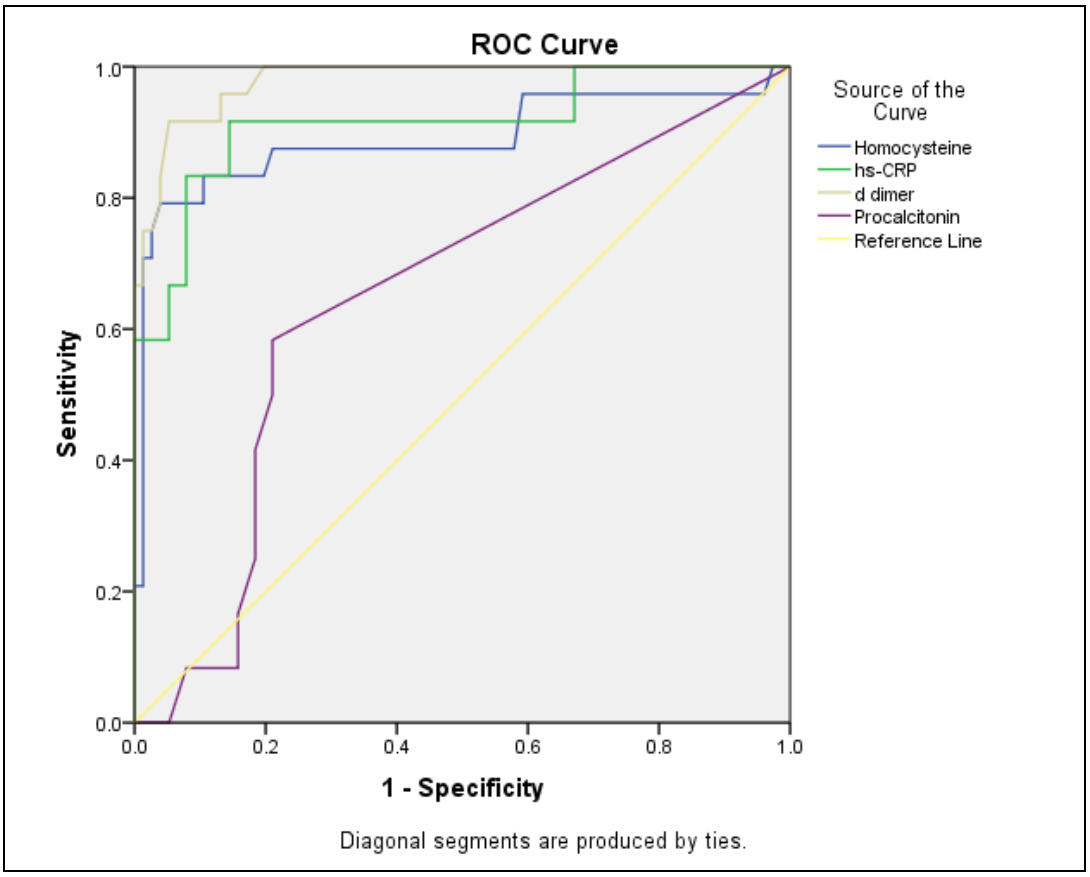


Fig. 1. ROC curve of hs-CRP, D dimer, homocysteine, and procalcitonin in predicting severe COVID-19.

Table 2

Laboratory parameters and their differences based on COVID-19 severity

Laboratory parameters	COVID-19		P-value
	Severe	Non-severe	
Median hemoglobin, g/dL (minimum-maximum)	12.3 (6.7 – 15.6)	12.1 (8.5 – 16)	0.357
Median WBC, mm^{-3} (minimum-maximum)	13,385 (3,100 – 21,170)	11,840 (1,960 – 28,500)	0.680
Mean platelet, mm^{-3} (SD)	253,250 (139,549)	304,500 (135,506)	0.112
Median AST, U/L (minimum-maximum)	52.5 (16.0 – 160.0)	42.0 (13.0 – 337.0)	0.053
Median ALT, U/L (minimum-maximum)	50.5 (12.0 - 312.0)	44.0 (12.0 – 199.0)	0.492
Median ureum, mg/dL (minimum-maximum)	38.0 (10.0 – 107.0)	46.5 (10.0 – 304.0)	0.424
Median creatinine, mg/dL (minimum-maximum)	0.96 (0.46 – 4.26)	1.09 (0.40 – 28.07)	0.611
Median hs-CRP, mg/L (minimum-maximum)	13.0 (1.4 – 20.0)	3.9 (0.5 – 12.0)	<0.001*
Median D dimer, ng/mL FEU (minimum-maximum)	2,000 (560 – 14,000)	400 (100 – 1,350)	<0.001*
Median procalcitonin, ng/mL (minimum-maximum)	2.1 (0.5 – 3.6)	0.5 (0.5 – 4.6)	0.006*
Median homocysteine, µmol/L (minimum-maximum)	16.2 (11.0 – 17.9)	13.3 (10.6 – 15.8)	<0.001*

WBC white blood cell, SD standard deviation, AST aspartate aminotransferase, ALT alanine aminotransferase, hs-CRP high-sensitivity C-reactive protein

Table 3

Sensitivity and specificity of biomarkers in stratifying COVID-19 severity

Biomarkers	AUC	P-value	Cut-off point	Sensitivity	Specificity
hs-CRP	0.914	<0.001	12.35 mg/L	91.7% (88.9–94.5)	85.5% (82–89)
D dimer	0.979	<0.001	1550 ng/ml FEU	91.7% (88.9–94.5)	94.7% (92.5–96.9)
Homocysteine	0.889	<0.001	15.4 µmol/L	87.5% (84.2–90.8)	78.9% (74.8–83)
Procalcitonin	0.650	0.027	2.55 ng/mL	58.3% (53.4–63.2)	74.0% (69.6–78.8)

AUC area under the curve, hs-CRP high-sensitivity C-reactive protein

DISCUSSION

The ongoing COVID-19 pandemic is still a global issue at present [12,13]. The clinical course of COVID-19 varies greatly from mild to severe even death in some cases. Clinicians must be vigilant in monitoring disease progression [13–15] to reduce morbidity and mortality by giving prompt intervention. Inflammation due to rapid viral replication and cellular destruction in COVID-19 activates immune response [5,7]. Hyperinflammation, hypoxia, and hypoperfusion are the culprits of organ damages and patients' deterioration [5,8,15,16]. The proportion of severe COVID-19 is smaller compared to mild and moderate cases. Ghahramani *et al.* reported 26.9% severe cases in their meta analysis [8]. Sole *et al.* found a smaller proportion of severe cases (21.33%) in their study, [13] while Tong-Minh *et al.* found 32% severe cases in their study [5]. The result of our study is in line with previous literatures. We found 24% severe COVID-19 cases.

Previous literature found that severe COVID-19 patients have increased serum AST, ALT, and creatinine levels [8]. Similar findings were reported by Tong-Minh *et al.* even though the elevation of ALT was not significant [5]. Males were dominating in previous report with 44.8% were aged 60 years or older [17]. In the study of Keskin *et al.*, males were also dominating with older age compared to mild group [18]. Patients' age was higher in severe compared to non-severe COVID-19 while male gender was a risk factor for developing severe disease with odds ratio (OR) of 1.22 compared to female gender [13]. In a study by Tong-Minh et al., patients in severe group were dominated by males with a mean age of 64.9 years [5]. In our study, males were dominating in severe COVID-19 group. However, there was no significant association between gender and age with COVID-19 severity in our study. Similar findings were observed in liver enzymes and renal function test. The levels of serum AST, ALT, and creatinine were higher in severe COVID-19 groups but not statistically significant compared to non-severe one.

Several biomarkers have been used to determine COVID-19 severity. Hematologic biomarkers (e.g. lymphopenia, neutrophil to lymphocyte ratio, thrombocytopenia), biochemical biomarkers (e.g. bilirubin, creatine kinase, troponin), inflammatory biomarkers (e.g. C-reactive protein, interleukins, procalcitonin), and coagulation biomarkers (D-dimer, prothrombin time) are among the commonly used biomarkers [11,14,17]. There are novel biomarkers in determining COVID-19 severity such as homocysteine and angiotensin. Homocysteine is discovered in 1932 and closely related to cardiovascular damage [11,19]. Elevated homocysteine level (hyperhomocysteinemia) is observed in cardiovascular diseases [11,15,18]. Homocysteine is also involved in platelet reactivity and thrombogenesis [15,18]. It is observed to be increased in many viral infections such as human immunodeficiency virus, hepatitis virus, and human papilloma virus [18].

Homocysteine, together with age, monocyte to lymphocyte ratio, and disease onset, might predict severe pneumonia on chest CT scan at first week in COVID-19 patients. However, the result did not mention other organ involvement [11]. Mean homocysteine level in severe COVID-19 patients was 1.75 µmol/L higher compared to non-severe ones and this was followed by unfavorable disease progression [15]. Population with polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene has defective homocysteine metabolism. The elevated homocysteine level due to the polymorphism causes deterioration and increased mortality rate in COVID-19 patients. The polymorphism itself is more prominent in the Latino population [20]. Analysis by Keskin et al. showed that homocysteine has AUC of 0.835 with sensitivity of 81.8% and specificity of 82.4% in stratifying severe COVID-19 cases. If combined with monocyte to lymphocyte ratio and D dimer, the AUC was increased to 1,000, which could be stated as perfect predictors (100% sensitivity and specificity) [18]. Our result is in accordance with previous studies. We found that serum homocysteine level is significantly higher in severe COVID-19 groups (P <0.001). Higher AUC was also found with sensitivity of 87.5% and specificity of 78.9% in stratifying severity of COVID-19.

Significant increase in D dimer level is observed in severe compared to non-severe COVID-19 cases [5,18]. COVID-19 patients with D dimer levels above 1000 ng/mL were reported to be at risk for severe disease and death [14]. According to Huang *et al.*, D dimer of higher that 0.5 mg/L has a 58% sensitivity, 69% specificity, and 0.69 AUC in predicting severe COVID-19 [15]. Patients with higher D dimer levels

had 13.89 times higher risk for mortality compared to COVID-19 patients with lower D dimer levels [17]. Sole *et al.* confirmed the previous results and found that elevated D dimer levels in patients have OR of 5.67 for severe disease [13]. Our study utilized higher cut-off point of D dimer and found an AUC of 0.979, sensitivity of 91.7% and specificity of 94.7% in determining severe from non-severe cases. Our results were also in line with previous literatures where D dimer serum level were significantly higher in severe COVID-19 than in non-severe COVID-19.

High level of hs-CRP is associated with severe COVID-19 and death [12,14,20]. Patients with higher hs-CRP levels had adjusted OR for death of 13.39 compared to those with lower hs-CRP levels [17]. This data is supported by results form a meta-analysis by Ghahramani *et al.* and Tong-Minh *et al.* However, the reported ORs were lower [5,8] A CRP level of 10 mg/L or higher has a 51% sensitivity, 68% specificity, and 0.84 AUC in predicting severe COVID-19 [16]. Patients in severe COVID-19 group in our study had higher hs-CRP levels compared to their counterparts ($P < 0.001$). We also found higher sensitivity and specificity of hs-CRP in determining severe from non-severe cases using higher cut-off point.

Procalcitonin is also increased in severe COVID-19 cases [8,12]. With an AUC 0.88, procalcitonin has 88% sensitivity and 68% specificity at a cut-off of 0.5 ng/mL in differentiating severe from mild and moderate COVID-19 patients [16]. A study in The Netherlands showed that high serum procalcitonin level may predict severe COVID-19 with OR of 4.19 and AUC of 0.82 compared to low serum procalcitonin level [5]. Another literature used a lower cut-off point (0.05 ng/mL). Patients with procalcitonin levels above the cut-off point had 2.91 times higher risk for developing severe COVID-19 [21]. A higher odds ratio (8.21) was even reported by Sole *et al.* for patients with higher serum procalcitonin levels [13]. Heer *et al.* in their publication were strongly disagree with antibiotic utilization in severe COVID-19 patients with high serum procalcitonin levels. They came into this conclusion due to the absence of association between bacterial culture and serum procalcitonin level. Higher serum procalcitonin level was more associated with disease severity (OR: 3.2) [22]. This study found that serum procalcitonin level was higher in severe compared to non-severe COVID-19 groups ($P = 0.006$). Using higher cut-off point, we found an AUC of 0.650, sensitivity of 58.3%, and specificity of 74% in stratifying COVID-19 severity. Our results were lower compared to previous study.

Regardless of the results obtained in this study, there are several limitations to acknowledge. First, this study was conducted in a single region with nearly homogenous population therefore extrapolation should be done prudently. Second, we did not determine end organ involvement in each patients which may affect serum biomarker levels. For instance, homocysteine is more specific in cardiovascular damage. Third, we did not uniformly measure the biomarkers at a same point of time. This may affect the level of biomarkers. Further study overcoming the above issues is mandatory to confirm the results of this study.

CONCLUSION

Disease progression in COVID-19 is variable and a particular proportion of patients may deteriorate rapidly. Homocysteine is a novel biomarker that can be used to determine COVID-19 severity. Other biomarkers are also promising such as hs-CRP, D dimer, and procalcitonin. Early detection of disease progression may reduce morbidity and mortality by administering prompt management.

Introducere: *COVID-19 reprezintă o problemă mondială de sănătate publică. O parte dintre pacienți dezvoltă simptome severe. Anumiți biomarkeri pot fi utili pentru startificarea bolii, homocisteina fiind unul dintre aceștia. Scopul acestui studiu a fost de a evalua rolul homocisteinei în stratificarea severității COVID-19.*

Metode: *A fost realizat un studiu transversal în Medan, Indonezia, din mai până în decembrie 2021. Criteriile de includere au fost reprezentate de pacienți adulți cu COVID-19 ce au acceptat participarea. Pacienții cu cancer, boală renală cronică, boală tiroidiană, boală coronariană și cu medicație de tipul colestiraminei, metforminului, metotrexatului, fibraților și anticoncepționalelor au fost excluși. Au fost prelucrate datele clinice și de laborator.*

Rezultate: *Au fost incluși 100 de pacienți, 59% au fost bărbați și 64% de etnicitate Batak. 20% dintre pacienți au avut COVID-19 formă severă. Nivelurile hs-CRP, d-dimerilor, homocisteinei și procalcitoniei au fost semnificativ mai mari la pacienții cu formă severă. Pentru stratificarea severității, D-Dimerii au avut cea mai mare sensibilitate (91,7%) și specificitate (94,7%), apoi hs-CRP (91,7% respectiv 85,5%), homocisteina (87,5%, respectiv 78,9%) și procalcitonina (58,3% și respectiv 74%).*

Concluzii: *Homocisteina poate fi utilizată ca biomarker pentru stratificarea severității COVID-19.*

Correspondence to: Jelita Siregar, M.D, Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Dr Mansyur 5, Medan, Indonesia
Telephone: 62-8211612006,
E-mail: jelitasiregar1978@gmail.com; jelitasiregarusu@gmail.com

Funding: This research received financial support from TALENTA, Universitas Sumatera Utara, Indonesia.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

REFERENCES

1. HU Y., SUN J., DAI Z., DENG H., LI X., HUANG Q., *et al. Prevalence and severity of coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis.* J Clin Virol. 2020;**127**:104371.
2. RUSLIE RH., DARMADI D., SIREGAR GA. *Susceptibility of coronavirus disease-19 in pediatric population.* Open Access Maced J Med Sci. 2020;**8**: 363–5.
3. RAHMAN S., MONTERO MTV., ROWE K., KIRTON R., KUNIK JR F. *Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence.* Expert Rev Clin Pharmacol. 2021;**14**(5):601–21.
4. RUSLIE RH., DARMADI D., SIREGAR GA. *Pediatric immunization practice during coronavirus disease-2019 pandemic.* Open Access Maced J Med Sci. 2020;**8**: 258–60.
5. TONG-MINH K., VAN DER DOES Y., ENGELEN S., DE JONG E., RAMAKERS C., GOMMERS D., *et al. High procalcitonin levels associated with increased intensive care unit admission and mortality in patients with a COVID-19 infection in the emergency department.* BMC Infect Dis. 2022;**22**(1):165.
6. World Health Organization. *WHO coronavirus (COVID-19) dashboard.* Available from: covid19.who.int. Accessed 6 October 2022.
7. ZHANG N., XIE T., NING W., HE R., ZHU B., MAO Y. *The severity of COVID-19 and its determinants: a systematic review and meta-analysis in China.* Sustainability. 2021;**13**(9):5305.
8. GHAHRAMANI S., TABRIZI R., LANKARANI KB., KASHANI SMA., REZAEI S., ZEIDI N., *et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis.* Eur J Med Res. 2020;**25**(1):30.
9. LAMERSs MM., HAAGMANS BL. *SARS-CoV-2 pathogenesis.* Nat Rev Microbiol. 2022;**20**(5):270–84.
10. RUSLIE RH., DARMADI D. *Coronavirus disease-2019 and mental health.* Open Access Maced J Med Sci. 2020;**8**: 268–71.
11. PONTI G., MACCAFERRI M., RUINI C., TOMASI A., OZBEN T. *Biomarkers associated with COVID-19 disease progression.* Crit Rev Clin Lab Sci. 2020;**57**(6):389–99.
12. ZENG F., HUANG Y., GUO Y., YIN M., CHEN X., XIAO L., *et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis.* Int J Infect Dis. 2020;**96**:467–74.
13. SOLE FD., FARCOMENI S., LOFFREDO L., CARNEVALE R., MENICHELLI D., VICARIO T., *et al. Features of severe COVID-19: a systematic review and meta-analysis.* Eur J Clin Invest. 2020;**50**(10):e13378.
14. AHMEIDI AA., MUSA A., AHMED HS., ELAHMAR AA., GOOTA RB., AHMED IA., *et al. Inflammatory markers as predictors of mortality in COVID-19 infection.* Afr J Lab Med. 2020;**9**(1):a1298.
15. CARPENE G., NEGRINI D., HENRY BM., MONTAGNANA M., LIPPI G. *Homocysteine in coronavirus disease (COVID-19): a systematic literature review.* Diagnosis (Berl). 2022;**9**(3):306–10.
16. HUANG I., PRANATA R., LIM MA., OEHADIAN A., ALISJAHBANA B. *C-reactive protein, procalcitonin, D-dimer, and feritin in severe coronavirus disease-2019: a meta analysis.* Ther Adv Respir Dis. 2020;**14**:1753466620937175.
17. KHEDAR RS., GUPTA R., SHARMA KK., MITTAL K., AMBALIYA HC., GUPTA JB., *et al. Biomarkers and outcomes in hospitalized covid-19 patients: a prospective registry.* medRxiv. 2022;**20**:22277718.
18. KESKIN A., USTUN GU., ACI R., DURAN U. *Homocysteine as a marker for predicting disease severity in patients with COVID-19.* Biomark Med. 2022;**16**(7):559–68.
19. PONTI G., PASTORINO L., MANFREDINI M., OZBEN T., OLIVIA G., KALECI S., *et al. COVID-19 spreading across world correlates with C677T allele of the methylenetetrahydrofolate reductase (MTHFR) gene prevalence.* J Clin Lab Anal. 2021;**35**(7):e23798.
20. DARMADI D., PAKPAHAN C., RUSLIE RH., REZANO A. *Inflammatory laboratory findings associated with severe illness among hospitalized individuals with COVID-19 in Medan, Indonesia: a cross-sectional study*. F1000Res. 2021; **10**: 1246.
21. HEIDARI-BENI F., VAHEDIAN-AZIMI A., SHOJAEI S., RAHIMI-BASHAR F., SHARIARY A., JOHNSTON TP., *et al. The level of procalcitonin in severe COVID-19 patients: a systematic review and meta-analysis.* Adv Exp Med Biol. 2021;**1321**:277–86.
22. HEER RS., MANDAL AKJ., SZAWARSKI P., MISSOURIS CG. *Procalcitonin is a biomarker for disease severity rather than bacterial co-infection in COVID-19.* Eur J Emerg Med. 2022;**29**(4):315.

Received 2nd November 2022