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The Use of Coagulation Markers to Evaluate the Effectiveness of Coronavirus Disease (COVID-19) Therapeutic Protocols

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ABSTRACT

Background: Patients infected by coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), display various symptoms and severity of the clinical picture. Thus, the therapy and pathophysiology of this disease are a dilemma for health professionals and scientists. **Objective**: This paper aims to evaluate the effectiveness of therapeutic protocols (the use of anticoagulants) in the treatment of COVID-19 patients of various severity of the clinical picture by monitoring coagulation markers (PT, INR, aPTT and D-dimer), as well as the impact of the type and number of comorbidities patients had on these markers. **Methods:** A total of 200 patients with a mild (n=76), moderate (n=70) or severe (n=54) clinical picture was included. Coagulation markers [PT (prothrombin time), INR (international normalized ratio), aPTT (activated partial thromboplastin time), D-dimer] were examined on three occasions: twice during hospitalization and once after hospital discharge. Anticoagulants used intrahospital were fraxiparine, rivaroxaban or unfractionated heparin. Posthospital, patients were taking either rivaroxaban or did not use any anticoagulants. For statistical analysis, SPSS 26.0 and Microsoft Excel 2019 were used, with a level of significance of α=0.05. Nonparametric tests (Kruskal-Wallis, Wilcoxon Signed-Rank and Bonferroni) were applied and effect size (ES) was calculated. **Results:** Three anticoagulants used intrahospital caused a significant decrease in PT, INR and D-dimer and a significant increase in aPTT, especially in patients with a severe clinical picture, but the ES was the biggest with fraxiparine, then rivaroxaban, and lastly unfractionated heparin. Posthospital, rivaroxaban caused a significant decrease in PT, INR and D-dimer and a significant increase in aPTT, especially in patients with a severe clinical picture. Hypertension was the most common comorbidity in all patients, as well as in patients with a severe clinical picture. There was a statistically significant impact of the number of comorbidities patients had on D-dimer, and none on PT, INR and aPTT, but the highest number of comorbidities was in patients with a severe clinical picture. **Conclusion:** The use of anticoagulants, especially fraxiparine intrahospital and rivaroxaban posthospital, is justified in most COVID-19 cases as there is a significant correlation between this disease's pathophysiology and the coagulation process. There is also a positive correlation between the severity of the clinical picture and the number of comorbidities patients have.

Keywords: COVID-19, coagulation markers, anticoagulants, comorbidities.

1. BACKGROUND

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes coronavirus disease (COVID-19), characterized by hypercoagulability, one of the main factors influencing the increased mortality rate in these patients. In COVID-19 patients, coagulation markers, such as prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT) and D-dimer, are frequently modified. The severity

of the clinical picture correlates with these markers. Increased levels of D-dimer (>1 mg/L) are linked with an elevated risk of severe pneumonia and death in these patients. They also present with shorter aPTT and longer PT and INR (1). Recent guidelines give recommendations on the use of therapeutic and prophylactic doses of various anticoagulants for hospitalized and non-hospitalized COVID-19 patients with different severity of the clinical picture (2).

Previous research has shown that patients with the following conditions and comorbidities are more likely to develop a severe clinical picture after contracting COVID-19: cancer, cardiovascular diseases, diabetes mellitus, diseases of the lungs, kidneys and liver and some mental diseases (3).

2. OBJECTIVE

This paper aims to evaluate the effectiveness of therapeutic protocols (the use of anticoagulants) in the treatment of COVID-19 patients of various severity of the clinical picture by monitoring coagulation markers (PT, INR, aPTT and D-dimer), as well as the impact of the type and number of comorbidities patients had on these markers.

3. MATAREIAL AND METHODS

This study was designed as a single-centre retrospective-prospective study. Data were obtained from medical records, discharge summaries and other medical documentation of patients hospitalized in General Hospital Tešanj in the period July 2020 – November 2021.

The study included 200 patients with a mild (n=76), moderate (n=70) or severe (n=54) clinical picture. The inclusion criteria were: age over 18, both genders; diagnosis of COVID-19; hospitalization period of at least two weeks; patients with a mild, moderate or severe clinical picture; signed informed consent.

Upon admission of patients to the hospital, before prescribing therapy, the following coagulation markers were analyzed: PT, INR, aPTT and D-dimer. Analyzes of these markers at the second point were performed intrahospital, within two weeks of patients' admission to the hospital, after prescribing therapy. Analyzes of these markers at the third point were performed posthospital, when patients arrived for control check, approximately one to two months after hospital discharge.

Anticoagulants used intrahospital were fraxiparine, rivaroxaban or UH. Fraxiparine was administered subcutaneously in doses of 2000, 4000, 6000, 8000 or 12000 international units (IU), rivaroxaban orally in doses of 10, 15 or 20 mg once daily, and UH intravenously in doses of 4000, 8000, 10000 or 15000 IU. Posthospital, patients were taking either rivaroxaban orally in doses of 10, 15 or 20 mg once daily or did not use any anticoagulants.

The type and number of comorbidities patients had were also analyzed and their impact on the severity of the clinical picture and coagulation markers was evaluated. Patients were divided into three groups based on the number of comorbidities: 1. no comorbidities; 2. one or two comorbidities; 3. more than two comorbidities.

For statistical analysis, SPSS 26.0 and Microsoft Excel 2019 were used, with a level of significance of α=0.05. Nonparametric tests (Kruskal-Wallis, Wilcoxon Signed-Rank and Bonferroni) were applied. Effect size (ES) (4) was considered small if 0.2–0.49, medium if 0.5–0.79 and large if ≥0.8.

4. RESULTS

In the group of patients with a mild clinical picture, 38 (50%) were men and 38 (50%) women; seven (9.21%) were from 19 to 39, 21 (27.63%) from 40 to 59, and 48 (63.16%) above 60 years of age. In the group of patients with a moderate clinical picture, 49 (70%) were men and 21 (30%) women; six (8.58%) were from 19 to 39, 25 (35.71%) from 40 to 59, and 39 (55.71%) above 60 years of age. In the group of patients with a severe clinical picture, 21 (38.89%) were men and 33 (61.11%) women; 11 (20.37%) were from 40 to 59, and 43 (79.63%) above 60 years of age.

The first measurement of PT, INR and aPTT (p=0.002; p=0.002; p=0.043, respectively) showed a significant difference between patients of various severity of the clinical picture, while D-dimer significantly differed on all three occasions (p<0.001). Patients with a severe clinical picture had the highest value of PT, INR and D-dimer and the lowest value of aPTT at the beginning, but the decrease in PT, INR and D-dimer, as well as the increase

PARAMETER	GROUP	THERAPY				
		1	2	3	4	5
PT	All patients	0.195	0.185	0.146	0.524	0.342
	Mild clinical picture	/	/	/	0.393	0.276
	Moderate clinical picture	/	/	/	0.525	0.306
	Severe clinical picture	0.317	/	0.252	0.583	0.347
INR	All patients	0.195	0.185	0.146	0.524	0.342
	Mild clinical picture	/	/	/	0.393	0.276
	Moderate clinical picture	/	/	/	0.525	0.306
	Severe clinical picture	0.317	/	0.252	0.583	0.347
aPTT	All patients	0.521	0.414	0.359	0.207	0.207
	Mild clinical picture	0.435	0.299	0.292	/	/
	Moderate clinical picture	0.456	0.446	0.337	0.351	/
	Severe clinical picture	0.577	0.464	0.395	0.362	0.318
D-dimer	All patients	0.587	0.440	0.362	0.549	0.351
	Mild clinical picture	0.546	0.378	0.326	0.453	/
	Moderate clinical picture	0.572	0.445	0.347	0.493	0.375
	Severe clinical picture	0.575	0.464	0.371	0.588	0.428

Table 1. ES of anticoagulant therapies on the values of PT, INR, aPTT and D-dimer in all patients and regarding the severity of the clinical picture. 1 – fraxiparine; 2 – rivaroxaban intrahospital; 3 – unfractionated heparin; 4 – rivaroxaban posthospital; 5 – no posthospital therapy; PT – prothrombin time; INR – international normalized ratio; aPTT – activated partial thromboplastin time

in aPTT, were also the biggest in patients with this clinical picture (PT: from 16.054 to 13.030; INR: from 1.3154 to 1.0130; aPTT: from 20.24 to 28.46; D-dimer: from 2547.6174 to 340.1198).

Figure 1 a), b) and c) presents that three anticoagulants used intrahospital caused a significant decrease in PT, INR and D-dimer and a significant increase in aPTT [fraxiparine: PT (p=0.012); INR (p=0.012); aPTT (p<0.001); D-dimer (p<0.001); rivaroxaban: PT (p=0.008); INR (p=0.008); aPTT (p<0.001); D-dimer (p<0.001); UH: PT (p=0.048); INR (p=0.048); aPTT (p<0.001); D-dimer (p<0.001)], but the ES was the biggest with fraxiparine, then rivaroxaban, and lastly UH. Regarding the severity of the clinical picture, fraxiparine caused the biggest decrease in PT, INR and D-dimer in patients with a severe clinical picture (ΔPT=2.229; ΔINR=0.2229; ΔD-dimer=3513.7711), then moderate (ΔPT=1.187; ΔINR=0.1187; ΔD-dimer=1022.0915), and lastly mild (ΔPT=0.253; ΔINR=0.0253; ΔD-dimer=686.99), and the biggest increase in aPTT in patients with a severe clinical picture (ΔaPTT=15), then moderate (ΔaPTT=14.71), and lastly mild (ΔaPTT=9.44).

Figure 1 d), e) and f) presents that posthospital, rivaroxaban caused a significant decrease in PT, INR and D-dimer [PT (p<0.001); INR (p<0.001); D-dimer (p<0.001)] and a significant increase in aPTT (p=0.005). In patients who did not take any anticoagulants, outcomes were the same [PT (p<0.001); INR (p<0.001); aPTT (p=0.004); D-dimer (p<0.001)]. Regarding the severity of the clinical picture, rivaroxaban caused the biggest decrease in PT, INR and D-dimer in patients with a severe clinical picture (ΔPT=1.871; ΔINR=0.1871; ΔD-dimer: 201.9079), then moderate (ΔPT=1.691; ΔINR=0.1691; ΔD-dimer: 137.2932), and lastly mild (ΔPT=1.68; ΔINR=0.168; ΔD-dimer: 107.6277), and the biggest increase in aPTT in patients with a severe clinical picture (ΔaPTT=4.75), then moderate (ΔaPTT=2.93), and lastly mild (ΔaPTT=1.11).

Table 1. presents the ES of anticoagulant therapies on the values of PT, INR, aPTT and D-dimer in all patients and regarding the severity of the clinical picture.

In the group of patients with a mild clinical picture, 20 (26.32%) had no comorbidities, 39 (51.32%) had one or two comorbidities, and 17 (22.36%) had more than two comorbidities. In the group of patients with a moderate

MARKER	CLINICAL PICTURE	COMORBIDITIES – GROUPS	x□	H	*p*
PT	Mild	1	13.2	0.762	0.683
		2	13.45		
		3	13.5		
	Moderate	1	13.2	0.294	0.863
		2	13.4		
		3	13.45		
	Severe	1	14.3	0.034	0.983
		2	14.4		
		3	14.5		
INR	Mild	1	1.03	0.762	0.683
		2	1.055		
		3	1.06		
	Moderate	1	1.03	0.294	0.863
		2	1.05		
		3	1.055		
	Severe	1	1.14	0.034	0.983
		2	1.15		
		3	1.16		
aPTT	Mild	1	21	0.476	0.788
		2	21		
		3	19		
	Moderate	1	24	5.780	0.056
		2	21		
		3	20		
	Severe	1	24	2.691	0.260
		2	20		
		3	19		
D-dimer	Mild	1	510.79	18.693	<0.001
		2	889.14		
		3	1444.3		
	Moderate	1	843.025	6.827	0.033
		2	1176.91		
		3	1257.78		
	Severe	1	1091.995	6.606	0.037
		2	1255.41		
		3	1829.48		

Bonferroni *post hoc* test

MARKER	CLINICAL PICTURE	COMORBIDITIES – GROUPS	test statistic	*p*
D-dimer	Mild	1-2	-16.437	0.007
		1-3	-31.362	<0.001
		2-3	-14.925	0.020
	Moderate	1-2	-10.640	0.043
		1-3	-18.154	0.021
		2-3	-7.514	0.323
	Severe	1-2	5.335	0.254
		1-3	14.742	0.017
		2-3	9.407	0.130

Table 2. The impact of the number of comorbidities patients had on coagulation markers PT – prothrombin time; INR – international normalized ratio; aPTT – activated partial thromboplastin time; 1 – no comorbidities; 2 – one or two comorbidities; 3 – more than two comorbidities

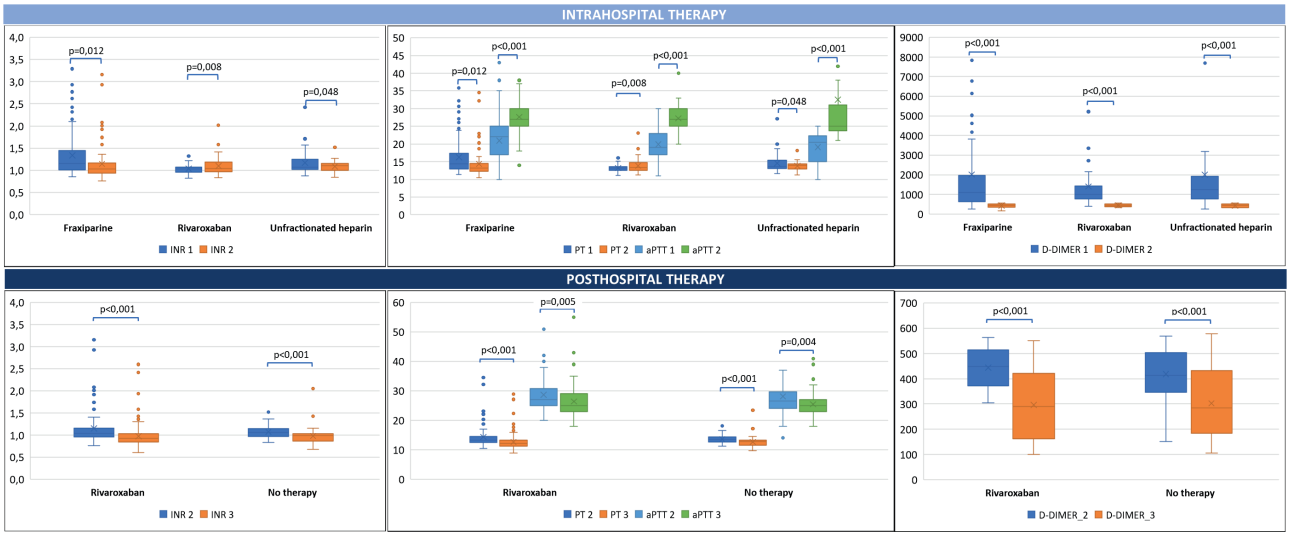

Figure 1. Impact of anticoagulants on coagulation markers

clinical picture, 26 (37.14%) had no comorbidities, 35 (50%) had one or two comorbidities, and nine (12.86%) had more than two comorbidities. In the group of patients with a severe clinical picture, nine (16.67%) had no comorbidities, 22 (40.74%) had one or two comorbidities, and 23 (42.59%) had more than two comorbidities. The highest number of comorbidities in one patient was 11, and that patient was a 70-year-old man with a severe clinical picture.

Hypertension was the most common comorbidity, with 84 (42%) patients having that diagnosis. It was followed up by type 2 diabetes mellitus (DMT2) [52 (26%)], chronic obstructive bronchitis [30 (15%)], chronic gastritis [27 (13.5%)], chronic cardiomyopathy [26 (13%)]. In patients with a severe clinical picture, all of these comorbidities were the most prevalent [hypertension: 29 (53.7%); DMT2: 18 (33.33%); chronic obstructive bronchitis: 11 (20.37%); chronic gastritis: eight (14.81%); chronic cardiomyopathy: 11 (20.37%)].

Results showed that there was a statistically significant impact of the number of comorbidities patients had on D-dimer ($H=22.174$, $p<0.001$), and none on PT ($H=0.509$, $p=0.775$), INR ($H=0.509$, $p=0.775$) and aPTT ($H=1.378$, $p=0.502$) (first measurements). With regard to the number of comorbidities, there was a statistically significant difference in D-dimer in patients with a mild ($H=18.693$, $p<0.001$), moderate ($H=6.827$, $p=0.033$) and severe clinical picture ($H=6.606$, $p=0.037$). Bonferroni post hoc test was also done (Table 2).

5. DISCUSSION

Previous studies have shown that COVID-19 patients are usually men (5, 6), which correlates with our results: 108 (54%) of patients were men, but more patients with a severe clinical picture were women [33 (61.11%)]. In the study conducted by Zhang et al. (2021), it was suggested that the severity of the clinical picture correlates with age (7), which is consistent with our results: 187 (93.5%) patients were older than 40, and no patients younger than 40 had a severe clinical picture. The number of comorbidities represents the risk factor for developing a severe clinical picture and is usually higher in older patients. In our study, in the group of patients with a severe clinical picture, 79.63% of patients were older than 60.

Results of our study demonstrated that three anticoagulants used intrahospital caused a significant decrease in PT, INR and D-dimer and a significant increase in aPTT, but ES was the biggest with fraxiparine. All coagulation markers were modified the most in patients with a severe clinical picture. In a retrospective study conducted by Volteas et al. (2022), in COVID-19 patients, therapeutic compared to prophylactic doses of either LMWH or UH showed no survival benefit, but UH was associated with a significantly higher mortality rate compared to LMWH (66% and 28%; $p=0.001$). D-dimer was not significantly different between therapeutic and prophylactic doses of either LMWH or UH. The peak of D-dimer occurred early, matching the administration time of anticoagulants, and gradually decreased during hospitalization (8). A meta-analysis conducted by Abdel-Maboud et al. (2021) included five studies and showed a positive effect of the prophylactic dose of heparin in COVID-19 patients with D-dimer >3 μg/L, regardless of age, gender or comorbidities (9).

Results of our study demonstrated that posthospital, rivaroxaban caused a significant decrease in PT, INR and D-dimer and a significant increase in aPTT, while in patients who did not take any anticoagulants, outcomes were the same. All coagulation markers were modified the most in patients with a severe clinical picture. MICHELLE was a randomized controlled trial in 318 COVID-19 patients with an increased risk of VTE, who received either rivaroxaban orally 10 mg once daily for 35 days or no anticoagulant after hospital discharge. Cardiovascular death or VTE within 35 days occurred in five (3%) patients receiving rivaroxaban and 15 (9%) patients not receiving an anticoagulant ($p=0.029$). Clinical outcomes were significantly improved with rivaroxaban (10).

COVID-19 patients with different medical disorders, such as chronic kidney disease, diabetes mellitus, lung and liver disease, cardiovascular diseases, obe-

sity, anxiety, immunodeficiency and mental illnesses, have a substantially higher risk of acquiring a severe clinical picture (11). When compared to people without chronic disorders, those with one comorbidity are 1.5 times more likely to die, while those with more than 10 comorbidities 3.8 times (12). Although the precise processes by which extant disorders have an impact on the severity of the clinical picture in COVID-19 patients are unknown, coagulation and hormonal pathways are thought to be implicated (13). D-dimer was compared in 31 COVID-19 patients without any comorbidities and 38 comorbid patients in a study conducted by Tuna et al. (2022). Comorbid patients did not have significantly higher D-dimer compared to patients without any comorbidities (14). In our study, results showed that patients with a severe clinical picture were the most common in the group with more than two comorbidities and the least common in the group with no comorbidities. The most prevalent comorbidities in all patients, likewise in patients with a severe clinical picture, were hypertension, DMT2, chronic obstructive bronchitis, chronic gastritis and chronic cardiomyopathy, the diagnoses that are risk factors for having a severe clinical picture. There was a statistically significant impact of the number of comorbidities patients had on D-dimer in all patients and regarding the severity of the clinical picture, which differs from the results obtained in the study conducted by Tuna et al. (2022). In patients with a severe clinical picture, the difference in D-dimer was statistically significant between patients with one or two comorbidities and patients with more than two comorbidities. Although there was no statistically significant impact of the number of comorbidities patients had on PT, INR and aPTT in all patients and regarding the severity of the clinical picture, the highest number of comorbidities was in patients with a severe clinical picture.

6. CONCLUSION

The use of anticoagulants, especially fraxiparine intrahospital and rivaroxaban posthospital, is justified in most COVID-19 cases as there is a significant correlation between this disease's pathophysiology and the coagulation process. There is also a positive correlation between the severity of the clinical picture and the number of comorbidities patients have.

- **Patient consent form:** All participants were informed about the subject of the study.
- **Author contributions:** N.O. and S.S. gave substantial contributions to the conception or design of the work in the acquisition or interpretation of data. N.O. and H.C. performed a statistical analysis of data. N.O. and S.S. had a part in the article preparing for drafting or revising it critically for important intellectual content. All authors gave final approval of the version to be published.
- **Conflict of interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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REFERENCES

1. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan: A Retrospective Observational Study. Am J Respir Crit Care Med. 2020; 201(11): 1372-1379. doi: 10.1164/rccm.202003-0543OC.
2. National Institutes of Health. COVID-19 Treatment Guidelines. 2023. Access: 21.07.2023. https://www.covid19treatmentguidelines.nih.gov/therapies/antithrombotic-therapy/.
3. Centers for Disease Control and Prevention. Underlying Medical Conditions Associated with Higher Risk for Severe COVID-19: Information for Healthcare Professionals. 2023. Access: 27.07.2023. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html.
4. Cohen J. Statistical Power Analysis for the Behavioral Sciences. New York: Routledge Academic. 1988.
5. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia ZY. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). J Gen Intern Med. 2020; 35(5): 1545-1549. doi: 10.1007/s11606-020-05762-w.
6. Li LQ, Huang T, Wang YQ, Wang ZP, Liang Y, Huang TB, et al. COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. J Med Virol. 2020; 92(6): 577-583. doi: 10.1002/jmv.25757.
7. Zhang H, Wu Y, He Y, Liu X, Liu M, Tang Y, et al. Age-Related Risk Factors and Complications of Patients With COVID-19: A Population-Based Retrospective Study. Front Med (Lausanne). 2022; 8(1): 757459. doi: 10.3389/fmed.2021.757459.
8. Volteas P, Drakos P, Alkadaa LN, Cleri NA, Asencio AA, Oganov A, et al. Low-molecular-weight heparin compared with unfractionated heparin in critically ill COVID-19 patients. J Vasc Surg Venous Lymphat Disord. 2022; 10(5): 1128-1136. doi: 10.1016/j.jvsv.2022.04.019.
9. Abdel-Maboud M, Menshawy A, Elgebaly A, Bahbah EI, El Ashal G, Negida A. Should we consider heparin prophylaxis in COVID-19 patients? a systematic review and meta-analysis. J Thromb Thrombolysis. 2021; 51(3): 830-832. doi: 10.1007/s11239-020-02253-x.
10. Ramacciotti E, Barile Agati L, Calderaro D, Aguiar VCR, Spyropoulos AC, de Oliveira CCC, et al. Rivaroxaban versus no anticoagulation for post-discharge thromboprophylaxis after hospitalisation for COVID-19 (MICHELLE): an open-label, multicentre, randomised, controlled trial. Lancet. 2022; 399(10319): 50-59. doi: 10.1016/S0140-6736(21)02392-8.
11. Pennington AF, Kompaniyets L, Summers AD, Danielson ML, Goodman AB, Chevinsky JR, et al. Risk of Clinical Severity by Age and Race/Ethnicity Among Adults Hospitalized for COVID-19-United States, March-September 2020. Open Forum Infect Dis. 2020; 8(2): ofaa638. doi: 10.1093/ofid/ofaa638.
12. Kompaniyets L, Pennington AF, Goodman AB, Rosenblum HG, Belay B, Ko JY, et al. Underlying Medical Conditions and Severe Illness Among 540,667 Adults Hospitalized With COVID-19, March 2020-March 2021. Prev Chronic Dis. 2021; 18(1): E66. doi: 10.5888/pcd18.210123.
13. Bigdelou B, Sepand MR, Najafikhoshnoo S, Negrete JAT, Sharaf M, Ho JQ, et al. COVID-19 and Preexisting Comorbidities: Risks, Synergies, and Clinical Outcomes. Front Immunol. 2022; 13(1): 890517. doi: 10.3389/fimmu.2022.890517.
14. Tuna Ö, Ermis C, Enez Darcin A, Dagistan E, Salman S. Comparison of inflammation markers and severity of illness among patients with COVID-19, acute psychiatric disorders and comorbidity. Eur J Psychiatry. 2023; 37(2): 125-132. doi: 10.1016/j.ejpsy.2022.01.008.