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Laboratory inflammatory parameters depending on the COVID-19 test result

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ABSTRACT

Introduction: The diagnosis of coronavirus disease 2019 (COVID-19) disease is necessary for the further treatment of patients with the present symptoms, and molecular diagnostics is considered the gold standard. However, it is already known that patients with symptoms of the disease can have a negative test due to various factors. The purpose of this study is to evaluate the laboratory parameters in patients with symptoms of COVID-19 infection who have both positive and negative test results.

Methods: The study included 101 samples from patients who tested positive for COVID-19 and 101 samples from patients who tested negative, both groups presenting symptoms of COVID-19. Data on the complete blood count, the absolute values of the differential blood count, and the D-dimer values were collected from the samples that were taken. Using blood count data, neutrophil-to-lymphocyte ratios, platelet-to-lymphocyte ratios, and systemic immune-inflammatory index (SII) were calculated.

Results: Based on the examination and research, a significant increase and change in the values of inflammatory indices and D-dimer were determined. In addition to the increase of these values above the reference value, a positive correlation was confirmed between the inflammation index value and the D-dimer value.

Conclusion: COVID-19-negative patients with characteristic symptoms of COVID-19 had higher values of lymphocytes and the determination of platelets and SII in these patients can be added to the diagnostic algorithm.

Keywords: Inflammatory parameter; coronavirus disease 2019 test; coronavirus disease 2019 infection

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread to a global pandemic within a few months of its appearance in one locality (1). The risk of spreading the disease is greatest in direct contact with an infected person. The aerogenous route is the most common form of transmission (cough and speech). Other people are infected if the virus enters the mucous membrane of their nose, mouth, eye, etc. (2).

Early diagnosis, treatment, and isolation should be carried out whenever possible. Dynamic monitoring of the development of radiological pictures, oxygenation index, and cytokine level is useful for early identification of patients who could develop more severe clinical pictures of the disease (3). The gold standard for proving COVID-19 is a positive test for the presence of SARS-CoV-2 nucleic acid (4). However, considering the possibility of false negative findings suspected patients with typical computed tomography findings can be treated as confirmed cases even if the initial test is also negative. In such cases, isolation and multiple testing of multiple samples should be performed. The confirmed case is based on the epidemiological history, clinical manifestations (temperature and respiratory symptoms), radiological images of the lungs, and the result of the reverse transcription polymerase chain reaction (RT-PCR) test (5).

Clinical established practice for laboratory diagnosis and monitoring of the course of COVID-19 includes some of the basic laboratory analyses: Leukocytes and their differentiation, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), laboratory indicators of coagulation, and other inflammatory parameters and their indices (6,7).

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Therefore, the purpose of this study is to investigate laboratory parameters in patients with symptoms of COVID-19 infection who have both positive and negative test results.

METHODS

This retrospective, descriptive study included patients admitted to the Konjic Health Center and the Konjic General Hospital over a period of 7 months (April 1st–October 31st, 2020). The study was conducted with the consent of the Ethical Committee of the Konjic Health Center (No. 14-347/2023) and the Ethics Committee of Konjic General Hospital (No. 01-04-93/2023). When conducting the study, we followed the instructions and guidelines of the Declaration of Helsinki and good laboratory practice.

The respondents were patients who came to the health center and hospital due to symptoms of the COVID-19 disease and who were COVID-19 tested. The research included 202 patients, of which 101 subjects positive for COVID-19 made up the study group, and 101 subjects admitted to the Konjic Health Center and the Konjic General Hospital with symptoms (fever, cough, headache, shortness of breath, and fatigue), but in whom SARS-CoV-2 was not identified by the RT-PCR test.

Data on absolute values of differential blood count and D-dimer values were collected. Using blood count data, neutrophil to lymphocyte ratios (NLR) – neutrophils/lymphocytes, platelet to lymphocyte ratios (PLR) – platelets/lymphocytes, and systemic immune-inflammatory index (SII) – (neutrophils x thrombocytes)/lymphocytes were calculated.

For the purpose of this study, the results of the examined patients, obtained by processing their venous blood, were used in the Department for Laboratory Diagnostic of the Konjic General Hospital and the Konjic Health Center. Blood samples for performing laboratory tests were taken by venipuncture of the cubital vein in vacutainer tubes with appropriate anticoagulants upon admission of the patients. To determine the differential blood count – from the parameters of which the inflammatory indices are calculated, the blood was collected in a test tube containing EDTA anticoagulant. For the determination of D-dimer, citrate plasma is used, which is obtained by centrifuging a sample of venous blood taken in a test tube with Na-citrate, as an anticoagulant. Differential blood counts were performed on a hematology analyzer D-Cell 60, Diagon Ltd., and MISPA-i2, Agappe Diagnostics, were used to determine D-dimer.

Criteria for inclusion in the study: Patients hospitalized in the Konjic General Hospital, patients with complete laboratory tests and medical documentation, patients with proven COVID-19 disease, PCR test, and patients with symptoms but without identified SARS-CoV-2 virus. Patients with treated and controlled hypertension and hyperlipidemia were also included. Exclusion criteria: patients who were not hospitalized in the General Hospital of Konjic, patients with incomplete laboratory tests and incomplete medical documentation, patients with diabetes, pregnant women, and patients younger than 18 years.

After data collection, statistical data processing was done using the statistical program IBM SPSS Statistics 23.0. Microsoft Word and Excel programs were used for data preparation and presentation. Descriptive statistics was used to calculate the mean, standard deviation, and percentages. The Chi-squared test was used to examine differences between genders and age groups and differences between the selection of patients based on the results of laboratory results. The student t-test was used to examine statistically significant differences in the examined hematological and coagulation parameters. Numerous correlations between the examined parameters were proven using the Pearson correlation coefficient. $p < 0.05$ is considered significant.

RESULTS

Descriptive data are presented in Table 1 for patients with regard to gender and COVID-19 test. In our sample, there was an equal number of patients who were COVID-19-positive patients (CPP) (n = 101) or COVID-19-negative patients (CNP) (n = 101) on the COVID-19 test, where 64 (60.4%) men and 37 (38.5%) women were positive for COVID-19, whereas 42 (39.6%) men and 59 (61.5%) women tested negative for the COVID-19 test. The χ^2 test shows that in our sample there was a significant difference in the results of the COVID-19 test with regard to gender ($\chi^2 = 9.608$, $p = 0.002$). Of the 60 patients who were younger than 60 years, 17 (28.3%) were positive on the COVID-19 test, and 43 (71.7%) were negative on the COVID-19 test. Out of 75 patients who were 60–70 years old, 42 (56.0%) were positive on the COVID-19 test, and 33 (44.0%) were negative on the COVID-19 test, out of 67 respondents who were older than 70 years, 42 (62.7%) were positive on the COVID-19 test, and 25 (37.3%) were negative on the COVID-19 test. The χ^2-test shows that there was a significant difference with regard to age categories and the COVID-19 test ($\chi^2 = 16.660$, $p < 0.01$).

The t-test showed a significant difference in the number of lymphocytes between subjects with regard to the COVID-19 test ($p < 0.001$), that is, patients from the control group (patients in whom the SARS-CoV-2 virus was not detected) showed a slightly higher value of lymphocytes, and less variability of the results. The CPP group had higher D-dimer values, with greater variability of results compared to the control group, and there was a significant difference in D-dimer values between patients with regard to the COVID-19 test ($p < 0.001$). A significant difference in the values of neutrophils and platelets was not observed when comparing these two groups.

We found that a higher percentage (33.67%) of CPP had elevated neutrophil values, while at the same time, a higher percentage (77.82%) of respondents from the control group had neutrophil values within the reference values, significantly ($p = 0.019$), as shown in Table 2. A much higher percentage, even 98 (97.03%) of CNP had neutrophil values within the reference interval. About 25.73% of CPP group had lymphocyte values below the reference values, significantly ($p < 0.001$). We have shown that a much higher percentage (85.15%) of CPP have D-dimer values above the reference values, while at the same time, a higher

TABLE 1. Differences in sex, age, and laboratory analyzes in COVID-19 patients

Variables	COVID-19 positive (%)	COVID-19 negative (%)	Test value	p-value
Male	64 (60.4)	42 (39.6)	9.608	0.002
Female	37 (38.5)	59 (61.5)		
<60 years	17 (28.3)	43 (71.1)	16.660	<0.001
60-70 years	42 (56)	33 (44)		
>70 years	42 (62.7)	25 (37.3)		
Neutrophils (2,06-6,5*10^9/L)	6.29±4.820	5.42±2.656	1.587	0.114
Lymphocytes (1,2-3,4*10^9/L)	1.24±0.981	1.86±0.713	-5.143	<0.001
Platelets (150-400*10^9/L)	240.50±104.659	242.69±109.002	-0.146	0.884
D-dimer (0-5 μg/mL)	2.29±1.73	1.05±1.23	5.857	<0.001

TABLE 2. Differences in the values of laboratory parameters according to reference intervals

Parameter (reference range)	Reference interval	COVID-19 positive	COVID-19 negative	χ^2	p-value
Neutrophil count (2,06-6,5*10^9/L)	Under	6	2	7.944	0.019
	In	61	79		
	Above	34	20		
Lymphocyte count (1,2-3,4*10^9/L)	Under	26	3	22.590	<0.001
	In	74	98		
	Above	1	0		
Platelets count (150-400*10^9/L)	Under	18	11	1.987	0.370
	In	79	86		
	Above	4	4		
D-dimer (0-5 μg/mL)	Under	0	0	23.830	<0.001
	In	15	47		
	Above	86	54		

percentage (46.53%) of the CNP group have D-dimer values below the limit, significantly ($p < 0.001$).

All three inflammatory indices (NLR, PLR, and SII) were significantly higher in the CPP group, $p < 0.001$, respectively, as shown in Table 3.

According to the Pearson correlation test, Tables 4 and 5 show correlations for COVID-19 patients with positive and negative tests.

DISCUSSION

It is already known that a small number of patients with characteristic symptoms of the COVID-19 disease may have a negative test result. Some of the proven reasons for the negative results of patients with symptoms of the COVID-19 disease are inadequate collection and/or transport of samples, as well as low viral load during molecular testing. Therefore, it is necessary to test the patients again, and until then, treat them according to their other characteristics (e.g., patient history and radiological diagnosis) (8,9).

The aim of many studies was to examine the course and outcome of COVID-19 infection in patients with a negative SARS-CoV-2 test, as well as to examine the association of laboratory parameters with the test result (10,11). Therefore, through our study, we have shown the connection between these two groups of patients (CPP and CNP) and their laboratory analysis results.

To begin with, in our sample, the number of men was significantly higher than the number of women, which follows the already proven increased risk of COVID-19 infection and more severe clinical symptoms in men. Furthermore, in the group of CPP, there were more male patients. That men are much more vulnerable to COVID-19 than women was also proven in a study of Bwire GM. The immunity of women and men differs physiologically in many ways, which is a determinant of the ability to overcome pathogens. In general, men are more vulnerable in terms of infections. There are numerous determinants that are the reason for this, and some of them are the lower amount of angiotensin-converting enzyme 2 in women, the worse lifestyles of men, and men's failure to take serious preventive measures compared to women. Accordingly, statistics conducted in the Spanish and Italian populations showed a higher mortality rate for men (12).

Comparing the two examined groups of patients, CNP had a significantly higher value of lymphocytes, but the values of lymphocytes were in the reference interval for most patients. However, a smaller number of patients were characterized by lymphopenia, which is a clinical feature of the COVID-19 disease (13).

Another parameter that was significantly lower in the CNP group was D-dimer, which was elevated in 193 patients in this study. It should be noted that patient data were collected after the first symptoms appeared in the first few days. Therefore, our results are expected and follow the results of the Lehmann et al. study, which showed that the peak concentration of D-dimer is 3 days after the onset of the first symptoms of the disease (14).

If we consider only the value of neutrophils with regard to the COVID-19 test, the Student's t-test proved that there were no significant differences in the neutrophil values between the patients with regard to the COVID-19 test, although it was shown that CPP had higher values of neutrophils, with greater variability of results compared to CNP group. Most likely, the obtained results speak in favor of the fact that the control group can also be observed with subjects suffering

TABLE 3. Differences in inflammatory index values between COVID-19 patients

Parameters	COVID-19 positive	COVID-19 negative	t-test value	p-value
NLR	6.14±3.629	3.39±2.351	6.407	<0.001
PLR	240.2±134.82	149.5±92.76	5.570	<0.001
SII	149.38±129.289	78.95±60.212	4.962	<0.001

TABLE 4. Correlations of inflammatory parameters among COVID-19-positive patients

Variables	Neutrophils	Lymphocytes	Platelets	D-dimer	NLR	PLR	SII
Neutrophils	1	0.232*	0.477**	0.250*	0.536**	−0.026	0.946**
Lymphocytes		1	0.369**	−0.109	−0.385**	−0.446**	0.256**
Platelets			1	0.119	0.044	0.257**	0.609**
D-dimer				1	0.305**	0.082	0.124
NLR					1	0.552**	0.402**
PLR						1	0.044
SII							1

TABLE 5. Correlations of inflammatory parameters among COVID-19 negative patients

Variables	Neutrophils	Lymphocytes	Platelets	D-dimer	NLR	PLR	SII
Neutrophils	1	0.91	0.040	0.353**	0.667**	−0.084	0.686**
Lymphocytes		1	0.236*	−0.080	−0.540**	−0.552**	0.241*
Platelets			1	0.001	−0.126	0.522**	0.703**
D-dimer				1	0.389**	0.068	0.207*
NLR					1	0.387**	0.319**
PLR						1	0.264**
SII							1

from COVID-19, although the presence of the virus was not detected by the PCR test, which would be especially significant for hospital settings when triaging patients.

Twenty-nine patients had thrombocytopenia, which is not uncommon for the COVID-19 disease, with a slightly higher number of patients with thrombocytopenia in CPP (15).

NLP, PLR, and SII are some of the most studied inflammatory indices. Their calculation in many studies has proven their predictive value to assess the clinical picture and predict the outcome of various diseases. Basically, index calculations are sensitive and specific parameters of inflammatory conditions – neutrophils, lymphocytes, platelets, and others, therefore, their calculation is proven to be significant for monitoring patients (16). Elevated values of these three indices are associated with deterioration of the clinical picture and worse outcomes in COVID-19 patients (17). We notice that all inflammatory indices are significantly higher in CPP, which may indicate that these patients had a stronger immune response to inflammation and higher values of laboratory parameters (18). Moreover, our results showed that the choice of these indices is a good and significant tool for triaging patients with symptoms of COVID-19 infection, independent of the COVID-19 test.

The correlation test showed a number of positive and negative significant correlations between the parameters of two groups of patients – CPP and CNP. However, we emphasize that in CPP, the highest correlation was observed in the absolute value of neutrophils with SII, and in CNP, the highest correlation was observed in the correlation of platelets with SII. We thereby show that the ratio of neutrophils to SII is very significant in CPP and the ratio of platelets to SII in CNP. The above results can be very useful for doctors and clinicians who participate in the triage of patients and for implementing diagnostic protocols for the treatment of patients with symptoms of COVID-19 infection, but with a negative test for the same.

Finally, we believe that the results of our study follow other conducted studies on this topic. Namely, the study by Saad Menezes et al., showed that the CNP group has a better prognosis for the disease (19). Therefore, according to the mean values of the tested parameters in our study, CNP had a larger number of lymphocytes and platelets. Moreover, in several studies that have already been conducted, it has been shown that a lower number of lymphocytes and platelets are associated with a worse prognosis and outcome of COVID-19 (20,21).

Limitations

Our study has several strengths and weaknesses. Namely, the conducted study gave useful and significant results for the not so researched aspect of COVID-19 patients. Our study was conducted in two health institutions, which gives it a special dimension. The main disadvantages are the relatively small number of patients and the small number of included inflammatory parameters. In the coming period, the results of our conducted study direct us to continue research and include more inflammatory parameters (e.g.: ESR, CRP, IL-6, procalcitonin, and others), indices, and examine the influence of primary comorbidity. With the aforementioned settings, we came up with the idea to monitor the prognosis and outcome of the COVID-19 disease, which would build on this study as a base for all subsequent research.

CONCLUSION

Our study showed that the triage of patients with symptoms of COVID-19 disease can be done based on basic

laboratory parameters, in addition to molecular diagnostics as the gold standard. CNP with characteristic symptoms of COVID-19 infection had higher values of lymphocytes and the determination of platelets and SII in these patients can be added to the diagnostic algorithm.

DECLARATION OF INTERESTS

Authors declare no conflict of interest.

REFERENCES

1. Cilović-Lagarija Š, Hasanica N, Mulić M, Radojičić M, Branković S, Pilav A, et al. An overview of human resources and capacity in health care institutions in the federation of Bosnia and Herzegovina in the context of responding to the COVID 19 pandemic. Mater Sociomed 2021;33(1):56-9.

   https://doi.org/10.5455/msm.2021.33.56-59

2. Yazdani S, Heydari M, Foroughi Z, Jabali H. Factors affecting COVID-19 transmission and modelling of close contact tracing strategies. Iran J Public Health 2021;50(10):2121-31.

   https://doi.org/10.18502/ijph.v50i10.7516

3. Rasool G, Riaz M, Abbas M, Fatima H, Qamar MM, Zafar F, et al. COVID-19: Clinical laboratory diagnosis and monitoring of novel coronavirus infected patients using molecular, serological and biochemical markers: A review. Int J Immunopathol Pharmacol 2022;36:03946320221115316.

   https://doi.org/10.1177/03946320221115316

4. Torretta S, Zuccotti G, Cristofaro V, Ettori J, Solimeno L, Battilocchi L, et al. Diagnosis of SARS-CoV-2 by RT-PCR using different sample sources: Review of the literature. Ear Nose Throat J 2021;100(2 Suppl):131S-8S.

   https://doi.org/10.1177/0145561320953231

5. Kanji JN, Zelyas N, MacDonald C, Pabbaraju K, Khan MN, Prasad A, et al. False negative rate of COVID-19 PCR testing: A discordant testing analysis. Virol J 2021;18:13.

   https://doi.org/10.1186/s12985-021-01489-0

6. Battaglini D, Lopes-Pacheco M, Castro-Faria-Neto HC, Pelosi P, Rocco PR. Laboratory biomarkers for diagnosis and prognosis in COVID-19. Front Immunol 2022;13:857573.

   https://doi.org/10.3389/fimmu.2022.857573

7. Hasanefendic B, Sehercehajic E, Dedic A, Tihic-Kapidzic S, Begovic E, Hajro S, et al. Systemic inflammatory indexes (NLR, dNLR, PLR and SII) role in predicting COVID-19 progression. J Health Sci 2023;13(2):113-7.

   https://doi.org/10.17532/jhsci.2023.2155

8. Takahashi H, Ichinose N, Okada Y. False-negative rate of SARS-CoV-2 RT-PCR tests and its relationship to test timing and illness severity: A case series. IDCases 2022;28:e01496.

9. Pecoraro V, Negro A, Pirotti T, Trenti T. Estimate false-negative RT-PCR rates for SARS-CoV-2. A systematic review and meta-analysis. Eur J Clin Invest 2022;52(2):e13706.

   https://doi.org/10.1111/eci.13706

10. Benes J, Dzupova O, Polakova A, Sojkova N. Repeatedly negative PCR results in patients with COVID-19 symptoms: Do they have SARS-CoV-2 infection or not? Epidemiol Mikrobiol Imunol 2021;70(1):3-9.

11. Caramello V, Macciotta A, Bar F, Mussa A, De Leo AM, De Salve AV, et al. The broad spectrum of COVID-like patients initially negative at RT-PCR testing: A cohort study. BMC Public Health 2022;22:45.

    https://doi.org/10.1186/s12889-021-12409-w

12. Bwire GM. Coronavirus: Why men are more vulnerable to covid-19 than women? SN Compr Clin Med 2020;2(7):874-76.

    https://doi.org/10.1007/s42399-020-00341-w

13. Toori KU, Qureshi MA and Chaudhry A. Lymphopenia: A useful predictor of COVID-19 disease severity and mortality. Pak J Med Sci 2021;37(7):1984-8.

    https://doi.org/10.12669/pjms.37.7.4085

14. Lehmann A, Prosch H, Zehetmayer S, Gysan MR, Bernitzky D, Vonbank K, et al. Impact of persistent D-dimer elevation following recovery from COVID-19. PLoS One 2021;16(10):e0258351.

    https://doi.org/10.1371/journal.pone.0258351

15. Wool GD, Miller JL. The impact of COVID-19 disease on platelets and coagulation. Pathobiology 2021;88(1):15-27.

    https://doi.org/10.1159/000512007

16. Sagud M, Madzarac Z, Nedić Erjavec G, Simunovic Filipcic I, Mikulic FL, Rogic D, et al. The associations of neutrophil-lymphocyte, platelet-lymphocyte, monocyte-lymphocyte ratios and immune-inflammation index with negative symptoms in patients with schizophrenia. Biomolecules 2023;13(2):297.

    https://doi.org/10.3390/biom13020297

17. Kosidło JW, Wolszczak-Biedrzycka B, Matowicka-Karna J, Dymicka-Piekarska V, Dorf J. Clinical significance and diagnostic utility of NLR, LMR, PLR and SII in the course of COVID-19: A literature review. J Inflamm Res 2023;16:539-562.

    https://doi.org/10.2147/JIR.S395331

18. Eissa M, Shaarawy S, Abdellateif MS. The role of different inflammatory indices in the diagnosis of COVID-19. Int J Gen Med 2021;14:7843-53.

    https://doi.org/10.2147/IJGM.S337488

19. Saad Menezes MC, Santinelli Pestana DV, Ferreira JC, Ribeiro de Carvalho CR, Felix MC, Marcilio IO, et al. Distinct outcomes in COVID-19 patients with positive or negative RT-PCR test. Viruses 2022;14(2):175.

    https://doi.org/10.3390/v14020175

20. Buchari B, Andayani H, Putri NA. Association between lymphocyte level and severity of coronavirus disease 2019 patients: A cross-sectional study in Indonesia. Open Access Maced J Med Sci 2022;10(B):614-8.

    https://doi.org/10.3889/oamjms.2022.8569

21. Hasanefendic B, Dedic A, Hajro S, Sehercehajic E, Hajrovic A, Alimanovic-Alagic R, et al. Differences between biochemical, hematological and coagulation parameters among patients with mild and severe COVID-19. Sanamed 2023;8(1):27-33.