Health, Sports & Rehabilitation Medicine
Vol. 23, no. 2, April-June 2022, 72–75

Biological markers of inflammatory response and coagulopathy in COVID-19 are related with an increased risk of severe disease and death

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Abstract

Background. Determining the risk factors associated with the severity and prognosis of SARS-CoV-2 infection is of crucial importance for a correct diagnosis and management. Severe forms are correlated with increased inflammatory response and procoagulant status, highlighted by increased markers of inflammation and serum level of D-dimers.

Aims. The study aims to evaluate the association between disease severity and the inflammatory syndrome and COVID-19 associated coagulopathy, as well as the existence of a concordance of these parameters in the studied patients.

Methods. We performed a retrospective study, enrolling 99 patients with COVID-19 severe forms admitted to the intensive care unit of Craiova County Emergency Hospital, between 1 January and 1 September 2021. We measured biological markers of inflammation, markers of coagulopathy, serum albumin, LDH.

Results. The patients were divided into two groups: G1–51 patients with severe infection that have deceased, and G2–48 patients with severe infection who survived. All biological markers associated with inflammation showed increased values: leukocyte count, neutrophil-to- lymphocyte ratio, serum fibrinogen andLDH. Significantly increased levels of D-dimers were observed in most of all patients included and a significant association of these parameters with the disease severity. Our best linear regression model correlatedthe COVID-19 associated coagulopathy and the systemic inflammation.

Conclusions. Our study revealed a significant association between markers of the inflammatory response and procoagulant status highlighted by increased D-dimers. Most patients with severe forms of infection had coagulation abnormalities, correlated with the presence of systemic inflammation.

Keywords: SARS-CoV-2 infection, inflammation, coagulopathy.

Introduction

Since 2020, the international medical community has focused its attention on the new coronavirus, called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The virus, first seen in Wuhan, spread immediately nationally and later worldwide (Khan et al., 2020) with over 200 million estimated cases to date.

The World Health Organization has emphasized that one of COVID-19 pandemic's most important aspect is to understand the risk factors for disease severity (Khan et al., 2020). Early diagnosis and correct management of the infection is imperative, monitoring biological markers are of crucial importance.

Leukocytosis occurs in acute infections, leukocyte increasing depending on disease severity, patient age, immune system, and bone marrow efficiency. Most patients with COVID-19, especially those with a severe prognosis have a significantly increased number of leukocytes (Gulati et al., 2020).

An increased neutrophil-to-lymphocyte ratio (NLR) is described. NLR is considered an independent mortality risk factor for COVID-19 patients, especially men (Li et al., 2020).

D-dimers are the most important prognostic factor in monitoring patients with severe forms of COVID-19 (Martynowicz et al., 2021; Xu et al., 2020; Zeng et al., 2020). Elevated levels of D-dimers compared to the reference biological range, (observed at admittance) and their marked increase, up to 3-4 times the initial value, have been associated with an increased mortality, which probably reflects the activation of coagulation, the cytokine storm and the occurrence of multiorgan failure

Received: 2022, May 17; *Accepted for publication:* 2022, May 31
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https://doi.org/10.26659/pm3.2022.23.2.72

(Gulati et al., 2020).

Fibrinogen is used in the monitoring COVID-19 patients, since the onset of the infection, high values of fibrinogen have been observed compared to the reference interval.

In patients with moderate and severe forms of SARS-CoV2 infection, a common pattern of coagulopathy has been described, characterized by increased levels of D-dimers and fibrinogen, correlated with increased C-reactive protein as a marker of inflammation (Martynowicz et al., 2021; Zeng et al., 2020).

Monitoring the immune response is crucial in assessing the risk of COVID-19 disease and in the development of severe forms, especially acute respiratory distress syndrome (ARDS), the main cause of mortality in COVID-19 (Li & Ma, 2020; Martynowicz et al., 2021; Zeng et al., 2020).

Hypothesis

The foundation of this research is the hypothesis that some of the biological characteristics of patients with SARS-CoV-2 infection (especially inflammation and coagulopathy markers) are strong predictors of disease severity and mortality.

Materials and methods

Our study received approval from the Ethics Committee of the University of Medicine and Pharmacy of Craiova. All patients consented to be part of this research.

Research protocol

a) Period and place of research

This retrospective study analyzes the data from patients hospitalized in the Emergency Clinical County Hospital, Craiova, Romania between 1 January - 1 September 2021.

b) Subjects and groups

We performed an intermediate analysis of 99 patients diagnosed with severe forms of COVID-19, hospitalized in the Intensive Care Clinic, analyzing the laboratory parameters with prognostic significance, as well as their dynamics. All patients had a positive nasopharyngeal RT-PCR test for SARS-CoV-2. Patients with severe forms of SARS-CoV-2 infection were selected. Patients were divided into 2 groups: group G1 included deceased patients with severe forms of COVID-19 (51 patients), and group G2 included patients who survived severe forms of COVID-19 (48 patients).

c) Applied tests

In both groups, biological markers were analyzed and correlated with the disease severity and prognosis - leukocyte, lymphocyte and thrombocyte counts, neutrophil/lymphocyte ratio, ESR, fibrinogen, C-reactive protein, serum albumin and lactate dehydrogenase.

d) Statistical processing

The variables were presented as means (± standard deviations). F-test was used to determine the variance of each variable. Mann-Whitney test and t-test were used to assess the differences in means between groups. In order to compare the relative odds of mortality based on the values of different variables, Odds ratio and 95% confidence intervals were computed. Linear regression models were fitted to test the association of coagulopathy with continuous variables, included in the univariate and multivariate models because of their known or suspected association with SARS-CoV-2 associated coagulopathy. Variables with a p value of ≤ 0,1 were included in multivariate regression analysis. The correlation coefficient (R) was used in the evaluation of linear correlation between dependent and independent variables.

Results

Table I presents the biological characteristics of the patients.

G1 patients showed significantly increased values of leukocyte count (21.55 ± 11.41 vs. 7.88 ± 3.91 x10^3/cm^3, p = 0.00), neutrophils (13.3 ± 6.86 vs. 6.68 ± 2.96 x10^3/cm^3, p = 0.00), Ne/Ly (15.34 ± 8.03 vs. 4.24 ± 1.71, p = 0.00), CPR (25.80 ± 14.71 vs. 16.66 ± 12.42 mg/dl, p = 0.01), LDH (535,77 ± 165,57 vs. 280,60 ± 81,26 U/l, p = 0,00) compared to G2.

Table I

Biological characteristics of study patients.

Marker	Total patients	Deceased patients (G1)	Survivors (G2)	P
N	99	51	48	
Leukocytes (x 10^3/cm^3)	14.56 ± 10.85	21.55 ± 11.41	7.88 ± 3.91	0.00*
Lymphocytes (x 10^3/cm^3)	1.31 ± 0.68	0.93 ± 0.56	1.66 ± 0.6	0.00*
Neutrophils (x 10^3/cm^3)	9.94 ± 6.21	13.3 ± 6.86	6.68 ± 2.96	0.00*
Thrombocytes (x 10^3/cm^3)	199.62 ± 122.5	146.24 ± 119.96	225.28 ± 98.89	0.00*
Ne/Ly	9.59 ± 8.01	15.34 ± 8.03	4.24 ± 1.71	0.00*
Albumine (g/dl)	2.88 ± 0.66	2.49 ± 0.66	3.36 ± 0.55	0.00*
D-dimers (µg/ml)	5.10 ± 6.90	7.66 ± 7.65	1.46 ± 1.37	0.00*
CRP (mg/dl)	22.01 ± 14.44	25.80 ± 14.71	16.66 ± 12.42	0.01*
Fibrinogen (mg/dl)	517.80 ± 274.65	628.66 ± 296.74	391.55 ± 179.92	0.00*
ESR (mm/h)	50.20 ± 33.62	61.71 ± 38.67	40.43 ± 25.25	0.01*
LDH (U/l)	426.81 ± 165.57	535.77 ± 165.57	280.60 ± 81.26	0.00*

Data is shown as mean ± standard deviation.

*p<0,05 Group 1 vs. Group 2. p values based on t-test for equality of means or Mann-Whiteny test.

In G1 patients there was a decrease in the number of lymphocytes (0.93 ± 0.56 vs. 1.66 ± 0.6 $x10^3/cm^3$, p = 0.00), thrombocytes (146.24 ± 119.96 vs. 225.28 ± 98.89 $x10^3/cm^3$, p = 0.00), serum albumin value (2.49 ± 0.66 vs. 3.36 ± 0.55 g/dl, p = 0.00), compared to G2.

Significant elevated levels of serum D-dimers were identified in 79% of patients. Elevated levels of D-dimers are associated with increased mortality, with an OR of 10.2 (95% CI = 2.06 - 50.3; p = 0.00).

Serum fibrinogen values were increased in G1 patients compared to G2 (628.66 ± 296.74 vs. 391.55 ± 179.92 µg/ml, p = 0.00). Elevated fibrinogen levels are associated with increased mortality, with an OR of 3.69 (95% CI = 1.34 - 10.15).

Elevated ESR values were present in 69.12% of patients, being associated with an increased mortality, with an OR of 2.08 (95% CI = 0.71 - 6.09; p = 0.01).

A linear regression analysis was performed to determine the model with the best statistical significance in predicting the serum level of D-Dimers. In univariate linear models the following independent variables were significantly correlated and predicted the value of serum D-Dimers: fibrinogen (R = 0.50; p = 0.00) and LDH (R = 0.66; p = 0.00). The independent variables were also analyzed: PCR (R = 0.15; p = 0.01) and ESR (R = 0.23; p = 0.09). The best multivariate regression model that predicted the plasma level of D-Dimers includes two independent variables: LDH and fibrinogen (R = 0.72; R square = 0.52; adjusted R square = 0.50; p = 0.00). All independent variables included in the model revealed a positive correlation with the serum level of D-Dimers.

Discussions

Most patients with a severe prognosis of COVID-19 have a significantly increased number of leukocytes (Gulati et al., 2020), with a higher neutrophil/lymphocyte ratio (NLR) (Li et al., 2020). NLR is an independent risk factor for mortality in patients with severe forms of COVID-19, and appears to be predominant in men (Li et al., 2020). Patients with SARS-CoV-2 infection have higher blood levels of "neutrophil extracellular traps" (NETs), with an increased potential to spread inflammation and microvascular thrombosis – including inside the lungs, with the installation of acute respiratory distress syndrome (Li & Ma, 2020; Yang et al., 2020). Lymphocytes count was low in most infected patients (Frater et al., 2020), the severity of lymphopenia being correlated with the disease severity (Tan et al., 2020). Thrombocytopenia was also very common in SARS-CoV-2 infection (Zhou et al., 2020), being associated with an increased risk of severe disease (Zhou et al., 2020), the mechanisms of thrombocytopenia involving direct infection of bone marrow cells by viral particles and inhibition of thrombocyte synthesis. The cytokine storm destroys thrombocyte precursors in the bone marrow, decreasing the number of peripheral thrombocytes. Other supposed mechanism would be the destruction of thrombocytes by the immune system, and their aggregation in the lungs, a cause of microthrombosis and peripheral thrombocyte consumption (Hanff et al., 2020).

D-dimers are the most important prognostic element in monitoring patients with severe forms of SARS-CoV-2 infection (Martynowicz et al., 2021; Xu et al., 2020; Zeng et al., 2020). The marked increase in serum levels of D-dimers up to 3-4 times the initial value, were associated with increased mortality, which probably reflects the activation of coagulation in sepsis, followed by cytokine storm and multiorgan failure (Gulati et al., 2020; Zhang et al., 2020).

Some patients with severe COVID-19 infection may develop coagulopathy (according to ISTH–The International Society of Thrombosis and Hemostasis) with fulminant activation of coagulation and an increased consumption of coagulation factors, with a marked elevation of D-dimers (Asakura & Ogawa, 2021; Franchini et al., 2020).

Fibrinogen, along with ESR and LDH are used in monitoring patients with COVID-19 disease, increased values of fibrinogen compared to the reference biological range is found since initial disease stage (Ponti et al., 2020).

In patients with severe SARS-CoV-2 infection, a common pattern of coagulopathy has been observed, showing increased levels of D-dimers associated with increased serum fibrinogen levels. This correlates with the elevation of other inflammatory marker (CRP–C reactive protein) (Xu et al., 2020; Zeng et al., 2020). In patients with COVID-19, the value of CRP at admittance correlates with the severity of the disease, being a good predictor of prognosis, too (Fan et al., 2020).

Patients with severe COVID-19 have low serum albumin levels, associated with an increased risk of death (Aziz et al., 2020; Violi et al., 2021). Current studies suggest that albumin therapy could be considered a potential treatment (Violi et al., 2021).

Conclusions

1. There is a significant correlation between the severity of SARS-CoV-2 infection and certain biological markers associated with the presence of systemic inflammation, such as neutrophil/lymphocyte ratio, fibrinogen, LDH, serum D-dimers.
2. A significant association between the coagulopathy profile, the presence of systemic inflammation and the severity of SARS-CoV-2 infection was found in most of all patients with severe forms of COVID-19, these markers being useful in the initial assessment of the disease prognosis.

Author contribution

All authors designed the study, contributed to data collection, analyzed, accessed and verified the data. All authors drafted the initial manuscript, interpreted the data, and critically reviewed the final manuscript and approved the decision to submit the manuscript.

Conflict of interests

None to declare.

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