Le Infezioni in Medicina, Suppl. 1, 89-95, 2020

Prognosis of COVID-19: Changes in laboratory parameters

Maria Lagadinou[1], Elena E Solomou[2], Nicholas Zareifopoulos[1], Markos Marangos[2], Charalambos Gogos[2], Dimitrios Velissaris[1,2]

[1]Emergency Department, University Hospital of Patras, Greece;
[2]Department of Internal Medicine, University Hospital of Patras, Greece

SUMMARY

Introduction: Since December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome - Coronavirus 2 (SARS-CoV-2) emerged in China and has become a global threat. Comparison of hematological parameters between mild and severe cases of SARS-CoV 2 is so far limited, but significant differences in parameters such as interleukin-6, d-dimers, glucose, fibrinogen and C-reactive protein have been already reported.
Purpose: In this study we analyzed the changes observed in easily measured blood biomarkers in the patients and provided evidence of how these markers can be used as prognostic factors of the disease.
Methods: Demographic characteristics, detailed medical history, and laboratory findings of all enrolled SARS-CoV 2 infection positive patients who were referred to Patras University Hospital from the period of March 4th 2020 (when first confirmed case in Greece appeared in our hospital) until April 4th 2020 were extracted from electronic medical records and analyzed.
Results: We provided evidence that some very common laboratory values can be used as independent predictive factors in SARS-CoV 2 infection. Despite the retrospective nature of this study and the small number of subjects analyzed, we showed that NLR, LDH, d-dimers, CRP, fibrinogen and ferritin can be used early at the patient's first visit for SARS-CoV 2 infection symptoms and can predict the severity of infection.
Conclusion: More studies are warranted to further objectively confirm the clinical value of prognostic factors related to SARS-CoV 2 and establish an easy-to-get panel of laboratory findings for evaluating the disease severity.

Keywords: SARS CoV 2, risk factors, disease severity, laboratory tests.

INTRODUCTION

In December 2019, the city of Wuhan in China became the center of an outbreak of pneumonia of unknown reason. The outbreak of Severe Acute Respiratory Syndrome - CoronaVirus 2 (SARS CoV 2) has rapidly spread throughout the world [1]. Although the outbreak is likely to have started from a zoonotic transmission, recent reports have provided evidence for person to person transmission in family and hospital settings via direct contact or through droplets spread by coughing from an infected person [2-4].
The clinical spectrum of the disease varies from asymptomatic infection, mild upper respiratory symptoms to severe pneumonia with respiratory failure and even death. The most common symptoms at onset of SARS-CoV 2 illness are fever, cough, ache, dyspnoea, haemoptysis and diarrhoea. The severe symptoms of SARS-CoV 2 are associated with an increase rate of fatalities [2]. Both clinical and epidemiological features of patients with COVID-19 demonstrate that this kind of infection can cause clusters of severe respiratory illness leading to intensive care unit admissions and high mortality rates [3].
The outbreak of SARS CoV 2-induced coronavirus disease 2019 (COVID-19) has put health authori-

Corresponding author
Maria Lagadinou
E-mail: m_lagad2004@yahoo.gr

ties on high alert in China and across the globe. As a new type of highly contagious viral infection in human, the pathophysiology of unusually high pathogenicity for SARS-CoV 2 has not yet been completely understood [1]. Epidemiological and clinical characteristics of patients with SARS-CoV 2 have been reported but risk factors for mortality and clinical course of illness, including viral shedding, have not been well described.

The aim of this retrospective study was to assess the epidemiology of the disease for the first month period since the first case was identified at the largest reference center in western Greece and evaluate the related to disease blood biomarkers, as information on specific laboratory data between severe and moderate COVID-19 is limited.

MATERIAL AND METHODS

Data collection

Demographic characteristics, detailed medical history, and laboratory findings of all enrolled SARS-CoV 2 infection positive patients who were referred to Patras University Hospital from the period of March 4th 2020 (when first confirmed case in Greece appeared in our hospital) until April 4th 2020 were extracted from electronic medical records and analyzed. Written informed consent was obtained from all patients enrolled in the study.

Demographic characteristics from all study subjects were analyzed. Parameters from peripheral blood counts included white blood cells (WBC), lymphocytes absolute count (ALC) (<1100K/μL or >1100 K/μL), absolute monocyte count (K/μL), coagulation parameters (PLTs, fibrinogen, D-dimers), platelets markers (PDW and MPV), C-reactive protein, lactate dehydrogonase (LDH), creatinine kinase (CPK), and neutrophil to lymphocyte ratio (NLR). The presence of lung infiltrations are also included in the analysis from chest x-rays or CT scan where available. Patients were categorized based on sequential organ failure assessment (SOFA) score. Hospitalization was also included in the analysis and outcome.

Statistical analysis

Statistical analysis of data was performed using SPSS-25 statistical software. Statistical values were expressed as mean ± SD. The minimum value of the level of statistical significance, p-value, in all statistical tests was set at 0.05. The Pearson correlation was used for studying relationship between variables and the Mann-Whitney test was used to investigate the differences of a continuous variable to two different and independent population groups.

RESULTS

In this retrospective, one-center study, a total number of 64 adult patients (≥18 years old) with laboratory-confirmed SARS-CoV 2 using PCR were enrolled. All patients were evaluated at the Emergency Department of Patras University Hospital during the period from 4th March 2020 until 4th April 2020. SOFA score was used to categorize the patients in two groups: those with SOFA score >2 (group 1) were classified as having severe COVID-19 infection and were admitted to the hospital (67.2%) and those who had a SOFA score <2 (group 2), were considered moderate and were treated in an outpatient setting (32.8%).

The median age of all patients was 57.11±16.3 years old. 47.7% were men and 52.3% were women. 58.14% of the severe cases were male. The median age of the severe cases was significantly higher than moderate ones (62.2±13.4 vs 47.1±16.2 years old, p= 0.001). Co-morbidities were present in twenty patients (30.8%). Hematological or other malignancies were observed as the most common comorbidity (30%), followed by hypertension (25%), atrial fibrillation (15%), hyperlipidemia (10%), diabetes (10%) and multiple sclerosis (10%). The most common symptoms on admission were fever and cough. X-ray and/or CT scan abnormalities (lung infiltrations) were found in 29 patients

Table 1 - Patients' demographic characteristics.

Parameters	*Admitted patients*	*Outpatients*
Males(n)/Females(n)	25/23	6/10
Age (yrs)	62±13.4	47±16.2
Comorbidities		
Malignancies	5	-
Hypertension	3	1
Arrhythmia	3	-
Diabetes	1	1
Multiple sclerosis	1	1
Other	3	1
Heterozygous thalassaemia	6	2

(45.3%). All patients' demographic characteristics are shown in table 1.

We then examined the plasma levels of acute reactant proteins; C-reactive protein, ferritin, and fibrinogen. In the majority of the cases these factors were increased. Analysis of all patients revealed that CRP was above 0.5 mg/dL in 64.6% of patients, ferritin was above 300mg/dl in 63.9% patients, and fibrinogen levels were over 400 mg/dl in 81.1% of the subjects examined. D-dimers were above the threshold of 500μg/dl in 69.2% of the cases. Similar results were observed for LDH, which was above the normal threshold of 230 U/l in 73.6% of cases (normal limits 120-220 U/l). Then we analyzed these factors for the two patient groups separately (SOFA score>2; group 1, SOFA score <2; group 2). All factors analyzed were statistically significantly increased in group 1 compared to group 2; ferritin (1572±3512 vs 266±426, p=0.03), LDH (323±134 vs 211±55, p=0.019), d-dimers (1498±1613 vs 481±338, p:0.024), CRP (4.78±4.5 vs 2.64±4.8, p=0.01) and fibrinogen (556.5±151 vs 402.5±83, p=0.004) were increased among patients who were admitted to hospital compared to those treated at home. (Figure 1)

Further analysis was performed on complete blood counts in all patients. Lymphocyte and monocyte count, and neutrophil to lymphocyte ratio (NLR) was examined. Moderate monocytosis (500-1000 absolute monocyte count) was observed in 43.75% of the cases, and severe monocytosis (>1000 absolute monocyte count) was found in 3.1 % cases. Analysis of the absolute monocyte count between the two groups did not reveal statistically significant differences between them (group 1 vs group 2: 580±185 vs 540±231, p=0.54). It is notable that examination of peripheral blood smears of patients revealed activated monocytes in few cases, mainly in patients from group 1. NLR >3 was observed in 43.1% of patients. The NLR in group 1 was statistically significant increased compared to group 2 (4.09±2.9 vs 2.9±0.99, p:0.001). Lymphopenia (<1100 K/μL) occurred in 46.3% of patients. Absolute lymphocyte count (ALC) in both groups were as follows: group 1 vs group 2: 1792±3404 vs 1662±395 (p:0.809). Platelet count was also examined. Patients with SOFA score above 2, had slightly lower platelet count compared to group 2 (242.3±105 vs 249.3±84.2, p:0.619). All measurements are presented in Figure 2. Mean platelet volume (MPV) and platelet distribution width (PDW) were examined in all patients. There were no statistical significant differences in both MPV and PDW between the two groups. Absolute lymphocyte count <1100/μL was observed as a negative predictive factor in SARS-CoV 2 infection.

Eight patients (12.5%) had beta-thalasaemia trait,

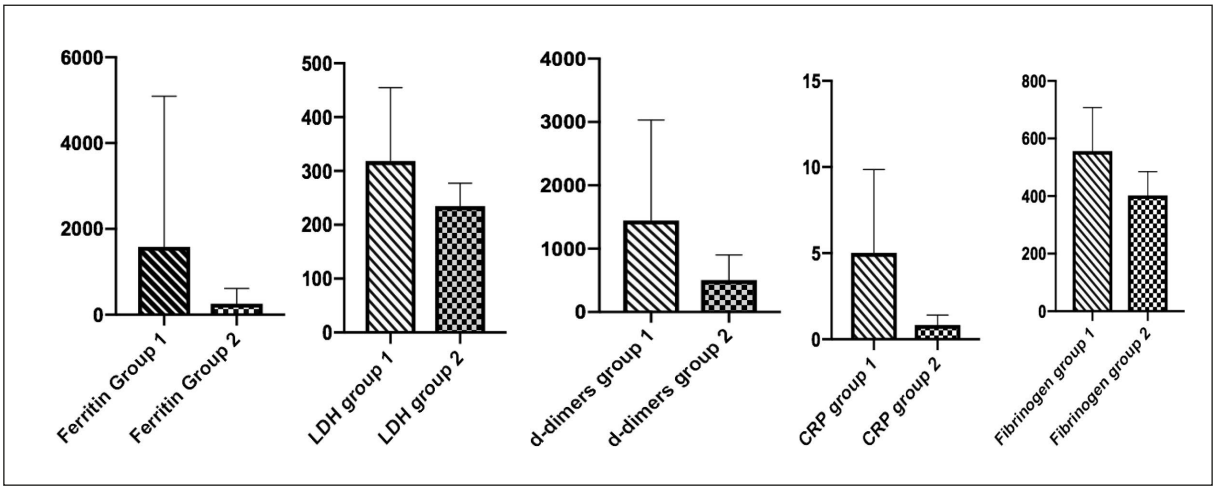

Figure 1 - Measurements of ferritin, LDH, d-dimers, CRP and fibrinogen represented per group. The box plots show the data from all analyzed patients. In every box plot, we show the mean values and the standard error of the means. In every box plot, the upper line is the highest measurement detected. All measurements are grouped as 1, 2: group 1: patients with SOFA score >2 and were admitted to hospital, group 2: patients with SOFA score < 2, and were treated at home).

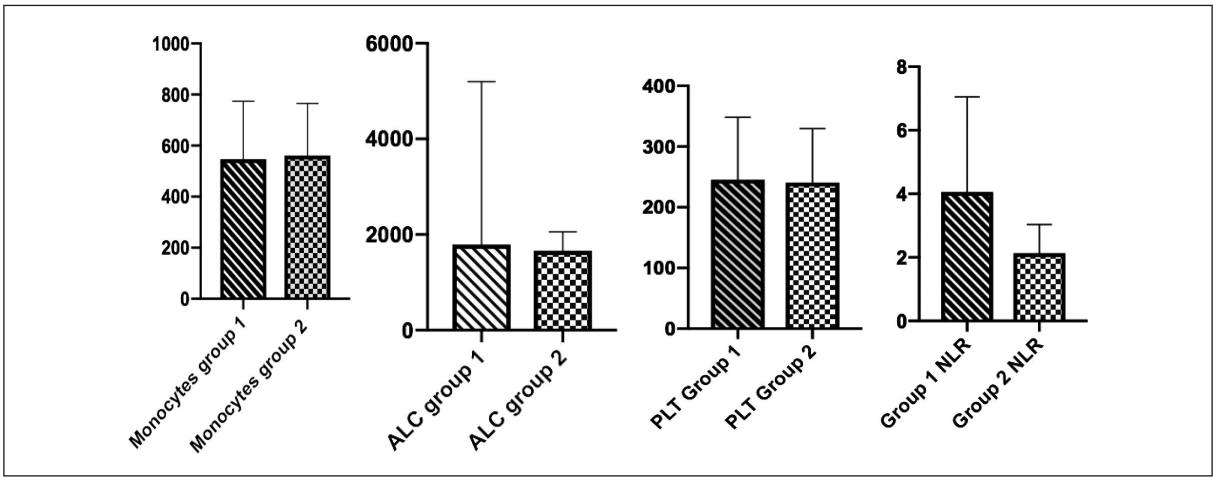

Figure 2 - Measurements of monocytes, lymphocytes absolute count, platelets and NLR represented per group. The box plots show the data from all the patients analyzed. In every box plot, we show the mean values and the standard error of the means. In every box plot, the upper line is the highest measurement detected. All measurements are grouped as 1, 2: group 1: patients with SOFA score >2 and were admitted to hospital, group 2: patients with SOFA score <2, and were treated at home).

and 6 of them (75%) were included in the admitted to Hospital group. Five of 43 severe cases (11.63%), died during the study period. The median age of deceased cases was 61.4 years old. Four were male and one female. Remarkably, in all patients who have died, absolute lymphocyte count upon admission was lower than the cut off of 1100K/μL and NLR ratio as well as LDH were very high.

We further compared NLR, CRP, and LDH with radiological findings (lung infiltrations) at first visit of all patients included in the study. These three factors were statistically significant higher in all patients with radiological findings during their first visit at the hospital compared to those with no findings (p=0.002, p=0.048 and p=0.002 respectively).

DISCUSSION

The pandemic of SARS-CoV 2 caused by the Severe Acute Respiratory Syndrome -Corona Virus 2, has led to numerous cases and deaths around the world, as the new virus is spreading far more quickly and is very contagious [5]. Although a good knowledge has been gained on clinical

Table 2 - Laboratory blood tests of all examined patients. The minimum value of the level of statistical significance, p-value, in all statistical tests was set at 0.05.

Parameters	*All patients*	*Admitted (Group 1)*	*Outpatients (Group 2)*	*P value*
CRP (mg/dL)	4.38±4	4.78±4.5	2.64±4.8	*0.01*
Ferritin (mg/dl)	1457±3327	1572±3512	266±426	*0.03*
LDH (mg/dl)	302±130	323±134	211±55	*0.019*
NLR	1.5±0.5	3.98±2.9	3.09±2.38	*0.001*
Monocytes (K/μL)	550±220	580±185	540±231	0.54
Lymphocytes	2457±8496	2677±9438	1511±938	0.809
Platelets (K/μL)	244±99	242.3±105	249.3±84.2	0.619
d-dimers (μg/dl)	1227±1464	1498±1613	481±338	*0.024*
Fibrinogen(mg/dl)	532±153	556±151	402±83	*0.004*

features of SARS-CoV 2 infection, less clear information has been provided on laboratory abnormalities [6]. In the present study we aimed to identify any possible relation between laboratory tests and the disease severity, additionally any tests that could work as potential risk factors for prediction of disease progression and severity [5].

In consistence to previous reports, the present study showed that male are more susceptible in developing severe disease [7]. It is already known that older males (>50 years), particularly those with underlying co-morbidities, may be more likely to develop severe SARS-CoV 2. Moreover, although SARS-CoV 2 infection has a relatively low mortality rate, it can be highly deadly and lethal, especially in high risk patients with co-morbidities. The reported incidence of SARS-CoV 2 infection accompanied to underlying co-morbidities in the literature were up to 26%. We reported a similar incidence rate of 30.8%, with malignancy being the most common co-morbidity (30%).

In terms of laboratory results, there were obvious differences between severe and non-severe cases in LDH, d-dimers and inflammatory markers including CRP, ferritin, fibrinogen and NLR. Our analysis revealed statistically significant elevated CRP, ferritin and NLR in the group of patients who were admitted to hospital, suggesting the close relation of SARS-CoV 2 infection and inflammation [8]. Although lymphocytopenia has been well described in a retrospective analysis of patients in Hong Kong and Singapore afflicted with SARS in 2003 and was associated with adverse outcomes and ICU admission, we did not find statistically significant differences between the two groups of patients we studied [9]. It is thought that SARS-CoV 2 infection is associated with coagulation as well. Regarding d-dimers and fibrinogen, the statistically significant elevation that was found in more severe cases, may be related to the activated and accelerated response to infection. Coagulation has also an immune function which can be hence considered another line of defense against severe infections [6]. All these findings would imply that routine blood tests may be additional useful tools for improving early prognosis and provide more intensive treatment.

In addition, platelets are important immune cells in hemostasis, coagulation, vascular integrity maintenance, angiogenesis, anti-inflammatory response Changes in their number and activity are closely related to a variety of diseases [10]. Previous studies have shown that severe infections can cause thrombocytopenia, which is characterized by a rapid platelet decline. It has also been suggested that a consistently present low grade disseminated intravascular coagulation (DIC) may cause a low platelet count in SARS. Furthermore, low platelet count is associated with increased risk of severe disease and mortality in patients with SARS-CoV 2 infection and has been suggested to serve as clinical indicator of worsening illness during hospitalization [11]. In our study, we found that platelets were slightly lower in severe ill patients compared to the moderate ones, but there were not statistically significant differences, and this can be attributed to small sample. Nevertheless, six patients had thrombocytopenia (PLT<150.000K/μL). All of them developed severe respiratory failure during hospitalization needing mechanical ventilation support. A possible explanation could be that the lung may be one of the organs in which mature megakaryocytes release platelets and that thrombocytopenia in patients with SARS-CoV 2 infection may be associated with lung damage observed in that type of infection [10]. Moreover, injury of lung tissue and pulmonary endothelial cells can lead to activation, aggregation and retention of platelets in the lung and the formation of thrombus at the injured site, which may lead to the depletion of platelets and megakaryocytes, resulting in decreased platelet production and increased consumption [10].

Due to alterations in coagulation, not only the platelet count but also the platelet function should be carefully assessed. In some studies it has been suggested that platelets distribution width (PDW) and mean platelet volume (MPV) could be useful tools to evaluate the activation of coagulation or thrombocytosis-related disease [12]. In our cases, no statistically significant differences in MPV and the PDW between severe and no-severe ill patients was found.

It is remarkable that we found higher NLR in severe ill patients and that was statistically significant related to the disease severity [8]. NLR, a well-known marker of systemic inflammation and infection, has been studied as a predictor fac-

tor of infections. The increase of NLR in our study, is consistent with the findings from another study [13] where several patients with SARS-CoV 2 infection had a rising neutrophil count and a falling lymphocyte count during the severe phase [1, 13]. In regards to hemoglobulin disorders, so far very little clinical experience of infected patients with such disorders, especially beta-thalassaemia trait, has been recorded. We reported that 8 patients with beta-thalassaemia trait were included in our study subjects, and six of them (75%) were severely ill and required hospitalization. Haemoglobin disorders are generally not associated with respiratory conditions. However, complications involving the heart, lungs and the immune system, can be present in these patients and in a SARS-CoV-2 positive patient may trigger very serious complications [14].

Our study provides a list of potential predictor markers for SARS-CoV 2 severity. We found a statistically significant correlation between SOFA score and LDH, NLR and ferritin. Especially for LDH, Tsui et al reported that elevated LDH level on admission of SARS-CoV 2 patients, was independent predictor factor of an adverse clinical outcome [15, 16]. Moreover, NLR, LDH, ferritin, d-dimers, fibrinogen and CRP were statistically significant higher in the group of patients who were hospitalized. Therefore, the combination of the above easily measured markers, in SARS-CoV 2 infected patients, even in the Emergency Department may predict more serious disease progression.

In an earlier published study, of 41 patients with laboratory confirmed SARS-CoV 2 infection, Huang et al reported a mortality rate of 15% (6 deaths among 41 patients) [7]. We reported comparable results with a mortality rate up to 11.63% (5 deaths among 43 severe ill patients).

In this study, we provide evidence for the first time to our knowledge that some very common laboratory values can be used as independent predictive factors in SARS-CoV 2 infection. Despite the retrospective nature of this study and the small number of subjects analyzed, we showed that NLR, LDH, d-dimers, CRP, fibrinogen and ferritin can be used early at the patient's first visit for SARS-CoV 2 infection symptoms and can predict the severity of infection. These markers can be used in every hospital setting while expecting confirmation of SARS-CoV 2 PCR results and guide clinicians to provide the best treatment options to these patients. More studies are warranted to further objectively confirm the clinical value of prognostic factors related to SARS-CoV 2 and establish an easy-to-get panel of laboratory findings for evaluating the disease severity.

Conflicts of interest

The authors declare no conflicts of interest.

Funding sources

None

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