

Hematological Parameters as Diagnostic Factors: Correlation with Severity of COVID-19

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Abstract. *Background and aim:* Coronavirus disease 2019 (COVID-19), which is the pandemic of 21st century, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Prognostic factors play an essential role in predicting the patients who need more care. Therefore, the current study aimed to investigate the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) ratio as discriminated indexes in prognosis of patients with COVID-19. *Methods:* Age, NLR, PLR, white blood cell (WBC), neutrophil count, lymphocyte count and platelet from 1007 hospitalized patients with COVID-19, who were admitted to two referring hospitals in Ardabil, North Western Iran. All confirmed cases divided into non-severe and severe groups. *Results:* 534 (53.4%) males and 473 (47.3 %) females with mean age of 52 years were enrolled in this study. Patients with severe COVID-19 have lower counts of lymphocyte, but have higher NLR, comparing to non-severe patients ($P = 0.001$). *Conclusion:* Elevated NLR can be assumed as an independent biomarker, which could provide a crucial indicator in the monitoring patients with COVID-19 on admission. Increased NLR was correlated with the severity of COVID-19. Assessment of NLR could be proposed to identify high risk individuals with COVID-19. (www.actabiomedica.it)

Key words: COVID-19, Hematological parameters, Prognosis, Severity

Introduction

An ongoing outbreak, a novel infectious disease was first recognized in Wuhan, China, in December 2019 and fast spread worldwide (1). Based on

phylogenetic findings, the Coronavirus Study Group (CSG) designated the novel virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which leads to coronavirus disease 2019 (COVID-19) (2). Giving the fast outbreak in China and rapidly

worldwide spread of COVID-19, this virus-triggered infection has aroused as emerging global public health concern(3).Regardless of most COVID-19 patients are not severe, patients with at least one of the main criteria for severe type of pneumonia, may quickly develop to acute respiratory distress syndrome (ARDS), and even death (4). Given lack of standardized treatments and medications, it is critical to recognize risk factors of severe prognosis for COVID-19 patients. Inflammation is a well-known symptom of large number of infectious diseases, and emerging evidence supposed its critical role in the development of various viral pneumonias, including COVID-19. Indeed, the topic of inflammation and disease has reached a point, where large scale of studies are required to distinguish specific targets as a therapeutic intervention tool (5).

The neutrophil to lymphocyte ratio (NLR), according to blood test which are performed routinely, easily obtained by dividing absolute count of neutrophil and lymphocyte, has been reported given to have great value in indicating overall inflammatory status of patients (6).Increasing NLR is a crucial risk factor of mortality in some cases, including infectious diseases malignancy, acute coronary syndrome, intracerebral hemorrhage, polymyositis, and also dermatomyositis (7). Recently, it has been established that severe cases of COVID-19 tended to have higher NLR (8, 9), suggesting that NLR could be introduced as an independent factor in mortality of hospitalized COVID-19 cases.

Although many researches are focused on COVID-19 and clinical features of the patients, the valuable NLR and platelet-lymphocyte ratio (PLR) markers, which may have prognostic effective markers and can be a piovital therapeutic targets in severe and non-severe cases, were not fully studied in patients with digestive and respiratory symptoms.

The hematological biomarkers such as complete blood count (CBC), NLR, PLR, C - reactive protein, and CLR can play a vital role in prediction of disease severity in early stage and can provide a better guide for deceasing the disease morbidity, mortality, and management of patients. Furthermore, NLR and PLR markers are prognostic effective markers, thus, can be a piovital therapeutic targets in severe and non-severe

cases were not fully reported. In this study, we aim to investigate the association of NLR, PLR, LCR parameters with COVID-19 disease, and to evaluate the role of hematological parameters in diagnosis of COVID-19 disease severity.

Methods

Participants

One thousand and seven patients who were confirmed with COVID-19 admitted to the Imam Khomeini and the Imam Reza Hospitals, located in Ardabil Province in the North Western Iran, defined as the first major center responsible for the COVID-19 treatment, were enrolled from March 2020 to May 2020. COVID-19 patients were diagnosed according to National Health Committee guidance and then confirmed by real-time reverse transcriptase polymerase-chain reaction (RT-PCR) assay of the SARS-CoV-2 in the Ardabil Medical Educational Hospital. Hospitalized cases with confirmed COVID-19 were enrolled into the analysis. Patients who were died on admission, or having missed data, were excluded. Finally, 1007 patients were participated in the final analyses.

Ethical approval and consent to participate

The current study was approved by the Medical Ethics Committee of Ardabil University of Medical Sciences (Approval Number IR.ARUMS.RWC.1399.006).

Diagnostic criteria

All enrolled COVID-19 patients were diagnosed by using RT-PCR assay to detection of SARS-CoV-2 virus inboth pharyngeal swab and nasal specimens with regards to the WHO guidance. The reference for the severity of disease was defined according to the Guo *et al.* (Guo *et al.*, 2020) and severe patients had at least one of the main following criteria:shortness of breath, RR \geq 30 times/min, andoxygen saturation (resting state) <90%.

Data collection

Demographic and clinical data (symptoms at onset of illness, comorbid disease, white blood cell count, neutrophil count, lymphocyte count, and platelet count) of all confirmed COVID-19 patients involved in the current study from March 2020 to May 2020 were collected with a checklist from the electronic hospital information system. Patients were classified into two severe and non-severe groups. Severe patients had one of the main following criteria as noted before(4). Moderate patients were categorized as non-severe cases, while critical cases were grouped as severe group in this study.

Laboratory examination

Throat-swab, nasopharynx-swab samples from all cases, who were suspected to SARS-CoV-2 infection patients were obtained; SARS-CoV-2 RNA was detected at central laboratory of Ardabil Medical Educational Hospital, by RT-PCR technique. Standard operative procedures were used for peripheral venous blood sampling which assessed at the central laboratory of Ardabil Medical Educational Hospital. During the hospital stay blood samples were obtained, collecting in a hematologic sample tube containing anticoagulant, blood routine tests were performed. Lymphocytes, Platelets, and Neutrophil counts according to the patients' blood routine, NLR, and also PLR were calculated. The results of baseline laboratory examinations were listed at admission.

Statistical analyses

Normal and continuously distributed data were presented as mean \pm standard deviation, and the significant differences were detected by the Student's t-test. Differences in values between experimental groups were regarded as significant at a value of $P \leq 0.05$. Correlation analysis used multifactor logistic regression analysis. A multivariate logistic regression analysis was evaluated by taking the severity of COVID-19 as dependent variable and variables which found significant during univariate analysis were diagnosed as independent variables. Data were analyzed using SPSS 32.

Results

Clinical and laboratory findings

A total of 1007 patients with COVID-19 were involved in the study. The patients were grouped into severe and non-severe COVID-19 in the final analyses, with a mean age of 52 years, consisted of 534 (53.4%) males and 473 (47.3 %) females. Giving the severity of the disease, 635(63.0%) patients were categorized as non-severe group and the other 372 cases (37.7%) were classified as severe group.

According to mean of the age, a significant difference was observed in non-severe group, compared with the severe group. No significant difference was observed in patients in the non-severe group, compared with patients in the severe group according to loss of appetite, agitation, myalgia, diarrhea, nausea, cough, rhinorrhea, dyspnea, weakness, and fever (unpublished data).

Table 1 shows the parameters of blood routine examinations in patients with severe COVID-19 and non-severe ones. Among 1007 patients who underwent blood routine tests on admission, some patients have differences in peripheral blood parameters. There were several differences in the factors of blood routine between non-severe and severe patient patients. Patients who categorized in severe group had lower lymphocyte mean count, higher mean neutrophil count, higher mean NLR, and also higher mean PLR (Table 1).

Correlation analysis of risk factors with severe COVID-19

The correlation analysis for risk factors in disease severity established that age, hypertension, lung disease, loss of appetite, dyspnea, anosmia, chest pain, WBC, PLR, and NLR, were significantly negatively correlated with severe COVID-19. Cancer, age, NLR and PLR were correlated with severe COVID19, while lymphocyte was positively correlated with severe COVID-19 (Table 2).

Finally, logistic regression analysis of variables associated with the severity of COVID-19 is listed in Table 3.

Table 1. Laboratory findings of COVID-19 patients in Ardabil North West of Iran from March 2020 to May 2020; presented as either mean \pm SD, or frequency.

Laboratory Findings	Total (%) <i>n</i> = 1007	Non-Severe (<i>n</i> =635)	Severe (<i>n</i> =372)	<i>P</i> .value
White blood cell count	7.84 \pm 6.9	7.60 \pm 7.85	8.30 \pm 4.7	0.17
Neutrophil count	73.05 \pm 28.86	72.08 \pm 34.36	47.90 \pm 13.16	0.20
Lymphocyte count	21.80 \pm 11.32	23.26 \pm 11.2	19.03 \pm 11.4	***0.001
Hemoglobin (g/L)	13.44 \pm 4.00	13.58 \pm 4.54	13.17 \pm 2.4	0.178
Platelet count	200.177 \pm 113.35	200.2 \pm 117.57	200.12 \pm 104.99	0.38
C reactive Protein (mg/L)	1.02 \pm 1.01	1.08 \pm 0.99	1.196 \pm 0.71	*0.05
NLR	4.993 \pm 4.7197	4.336 \pm 4.017	5.944 \pm 5.3968	***0.001
PLR	15.151 \pm 43.4818	14.762 \pm 55.138	15.715 \pm 15.0174	0.814
LCR	15.22 \pm 9.57	9.900 \pm 0.7	8.830 \pm 0.78	*0.012

*,*** Indicator of significant correlation

Data are median (IQR), *n* (%), or *n*, where *N* is the total number of patients with available data. *P* values comparing non-severe patients and severe group from *t test*. NLR: Neutrophils-to-lymphocytes ratio; PLR: Platelet-to-lymphocytes ratio.

Table 2. Correlation analysis of risk factors with severe COVID-19.

Variable	<i>r</i>	<i>P</i> value	Variable	<i>r</i>	<i>P</i> value
Age, Years	-0.138	0.001	NLR	-0.202	0.01
Hypertension	0.062-	0.050	PLR	-0.076	0.019
Lung Disease	-0.056	0.058	lymphocyte	0.130	0.01
Loss of Appetite	-0.105	0.002	Neutc	0.111-	0.01
Dyspnea	0.143	0.001	WBCt	0.072-	0.025
Anosmia	0.066	0.058	Chest pain	-0.100	0.005

*** Correlation is significant at the 0.001 level, ** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

Discussion

COVID-19 has spread in almost all countries and territories all over the world and its mortality rate is increasing day by day (10). It seems that NLR and PLR can help determine the severity of this disease earlier. The results obtained from the present study, which was conducted on the peripheral blood changes of 1007 hospitalized COVID-19 patients, indicated that the increase in NLR was correlated with the severity of COVID-19. With the increase of NLR, the severity of the related clinical symptoms also increased and the progression of the disease became more aggressive.

Various studies have shown that COVID-19 patients have different numbers of leukocytes in different stages of the disease. Therefore, detecting the primary

warning signs of the severity of the disease is crucially important in order to begin therapeutic intervention before the exacerbation of the disease. Neutrophils, lymphocytes, and platelets are cells which are quite effective in the immune response of the human body. Changes in their number or their activity are associated with various sorts of diseases (11). NLR is an important indicator of systemic inflammatory response and is considered as a precise criterion in the determination of the balance between systemic inflammation and immune response (12). Our findings are consistent with the findings reported in other studies regarding the relationship between NLR and the prognosis of various types of other infections (11, 13). In another study, Wang *et al.*(4) investigated 90 COVID-19 patients, 60 cases with mild symptoms and 30 cases with severe symptoms, in order to recognize the warning

Table 3. Logistic regression analysis of variables associated with the severity of COVID-19.

Variable	Statistics	Odds ratio	95% CIs	P value
Age, Years	12.4511 ± 0.006	0.980	0.969-0.991	0.001
Hypertension	2.357 ± 0.150	0.794	0.591-1.066	0.125
Neurogenic Disease	0.090 ± 0.293	0.092	0.615-1.939	0.764
CDK	0.028 ± 0.335	0.972	0.504-1.786	0.933
Cancer	0.193 ± 0.635	0.824	0.237-2.862	0.761
Lung Disease	0.185 ± 0.173	0.831	0.592-1.166	0.284
IHD	0.954 ± 0.244	2.596	1.608-4.190	0.001
DM	0.293 ± 0.220	0.746	0.485-1.148	0.182
Fever	0.238 ± 0.193	0.788	0.540-1.150	0.217
Vomiting	0.251 ± 0.281	1.285	0.741-2.227	0.372
Rhinorrhea	0.076± 0.409	1.097	0.484-2.402	0.853
Cough	0.179± 0.196	1.196	0.815-1.756	0.360
Loss of Appetite	0.430 ± 0.194	0.651	0.444-0.953	0.027
Dyspnea	0.450± 0.195	0.638	0.435-0.935	0.021
Anosmia	0.294 ± 0.342	1.341	0.686-2.621	0.390
Sore throat	0.464± 0.232	0.629	0.399-0.990	0.045
Nausea	0.296 ± 0.244	1.345	0.833-2.170	0.225
Diarrhea	0.049 ± 0.278	1.050	0.608-1.812	0.861
Myalgia	0.537 ± 0.194	1.711	1.169-2.505	0.006
Chest Pain	0.546 ± 0.223	1.727	1.115-2.673	0.014
Weakness	0.556 ± 0.194	1.744	1.193-2.550	0.004
Headache	0.132± 0.224	1.142	0.736-1.770	0.554
White Blood Cell	0.000±0.000	1.000	1.000	0.962

signs of pneumonia resulting from COVID-19. They found that NLR can be a primary warning sign of severe COVID-19. Moreover, their study indicated that there was a positive correlation between NLR and other factors such as COVID-19, cancer, and age, which are all in line with our findings. In another study, Xia *et al.* (14) indicated that the increase of NLR can be a primary warning sign of severe COVID-19.

This finding is also consistent with our findings in the current study. Also, similar to our findings, Eid *et al.* (15) found that NLR can be particularly special and sensitive in the recognition of COVID-19 patients above 50 years of age, who need more invasive care. Neutrophil releases large quantities of reactive oxygen species which can damage the DNA of the cells and release viruses from them. In addition, neutrophil stimulates the production of vascular endothelial

growth factor (VEGF) in the blood (16). Compared to natural tissues, the levels of VEGF-A and VEGF-C in COVID-19 patients are higher, which leads to more tissue damages (17). Neutrophil is also triggered by other inflammatory factors such as interleukin 6, interleukin 8, etc (18).

Lymphocytes, on the other hand, are effective cells in the immune responses of the human body. The immune response of the human body to viral infections is largely dependent on lymphocytes. Systemic inflammation severely reduces cellular immunity and causes lymphopenia. Therefore, the inflammation, resulting from the virus, causes an increase in NLR. In another study, it was found that in the early stages of the disease, the number of white blood cells either stayed in the normal range or decreased while the number of lymphocytes generally decreased (19). A new recent

study highlighted the NLR role greater than 6.5 which may reflect the progression of the disease that results in unfavorable clinical outcome (20). In the present study, we observed significant differences between the two groups of patients as regards the number of lymphocytes, neutrophils, and platelets. We also found that the number of blood cells was correlated with the severity of the disease. Our results also indicated that the reduction of blood cells was more considerable in patients with higher ages; therefore, they experienced more severe forms of the disease. Previous studies have shown that severe infection, DIC, and TTP cause a reduction in the number of platelets. The number of platelets in patients with severe COVID-19 was lower than that of the patients who had mild forms of the disease. Therefore, the number of the platelets was found to be correlated with the severity of the disease. The reason for the changes in the number of the platelets in COVID-19 patients might be the direct inhibition of bone marrow by the coronavirus (21). Moreover, it might be that lung injury leads to the activation, accumulation, and maintenance of platelets in the lung; then, the formation of thrombus in the affected part leads to the destruction of the platelets and megakaryocytes and, as a result, the number of the platelets decreases (22, 23). Indeed it might be the result of the secretion of cytokines like MCP-1, IP-10, IFN- γ , and IL-1, since it has been demonstrated that the concentration of cytokines is higher in patients experiencing severe forms of the disease; therefore, the number of the platelets is correlated with the severity of the disease (24).

The findings of this study revealed that elevated NLR can be suggested as an independent biomarker, which could provide a crucial indicator in the monitoring in patients with COVID-19 on admission. Correlation of increased NLR with the severity of COVID-19 should be noted meanwhile. Assessment of NLR may help in identifying high risk patients with COVID-19.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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