

Identification of hematological and inflammatory parameters associated with disease severity in hospitalized patients of COVID-19

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ABSTRACT

Background and Objectives: India has emerged as the second worst hit nation by the Coronavirus pandemic in terms of total number of cases. Our data on hematological and inflammatory markers associated with COVID-19 is very limited. This study was conducted to assess the utility of various biomarkers in identifying severe disease. **Materials and Methods:** All confirmed cases of COVID-19 admitted in our tertiary care centre from 1st March 2021 to 31st March 2021 were enrolled in the study. They were categorized into severe and non-severe disease categories based on pre-decided criteria. Their complete blood count parameters, D-dimer levels, serum C-reactive protein (CRP), ferritin and Lactate dehydrogenase (LDH) values were retrieved. **Statistical Analysis:** All parameters were expressed as Mean \pm Standard deviation for the two groups of patients. Student's *t*-test was used to test significance of the above markers between severe and non-severe disease. (*P* value < 0.05 was taken as statistically significant). **Results:** A total of 150 COVID RT-PCR positive patients were evaluated. The patients with higher Absolute Neutrophil Count (ANC), Neutrophil to lymphocyte Ratio (NLR), Platelet to lymphocyte ratio (PLR), D-dimer levels and raised serum CRP, LDH, ferritin along with lymphocytopenia were associated with severe disease (*P* < 0.05). Hemoglobin, total leucocyte count and platelet count showed no correlation with disease severity. **Conclusion:** These biomarkers associated with disease severity especially NLR, PLR, D-dimer and serum CRP levels could be used to triage patients at the time of admission thereby identifying those requiring intensive care and enabling optimal resource utilization.

Keywords: COVID-19, C-reactive protein, D-dimer, ferritin, lactate dehydrogenase, neutrophil to lymphocyte ratio

Introduction

The first case of human transmission of COVID-19 was discovered in the city of Wuhan in Hubei province of China.^[1] An explosive increase in the number of cases led World Health Organization (WHO) to declare this outbreak as a public health emergency of international concern on 30th January, 2020. Subsequently, on March 11, 2020 it was declared as a pandemic. According to WHO, the global confirmed cases as on October 8, 2021 were 236,599,025 with a mortality of 4,831,486. The worst

affected regions in descending order were America, Europe, South East Asia, Eastern Mediterranean, Africa and West Pacific. Among countries, USA, India and Brazil were among the worst hit countries. India has reported 33,915,569 confirmed cases and 450,127 deaths as on 8th October 2021.^[2]

In India, Maharashtra, Chhattisgarh, Uttar Pradesh, Karnataka, Kerala, Tamil Nadu and Delhi were the states most affected by COVID-19.^[2]

Coronavirus disease is transmitted through airborne droplets and it targets lower respiratory tract and other organs expressing ACE2 receptor. The mean incubation period of COVID-19 is 2-14 days. The initial symptoms are cough, fever, myalgia, fatigue

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and shortness of breath. However, it could progress to acute respiratory distress syndrome, metabolic acidosis, disseminated intravascular coagulation (DIC), multi-organ dysfunction and shock.^[3] In severely ill cases, thrombotic complications like venous thromboembolism and stroke have also been observed.^[4] However, higher mortality is seen in patients with other pre-existing comorbidities like diabetes mellitus, chronic kidney disease, heart disease, hypertension, chronic obstructive pulmonary disease, immunosuppression, metabolic syndrome and obesity.^[5,6] There has been an overwhelming inflow of COVID-19 patients, which exceeds the capacity of the health care system. In this war against COVID-19, it is very crucial to optimize the use of limited resources at our disposal. Primary care physicians are the first link to healthcare for patients from less accessible or low socioeconomic status. It is therefore very essential for them to recognize hematological and biochemical parameters linked to disease severity to guide triage and referral of serious cases to tertiary care centre for intensive care. This will ensure optimum utilization of resources without compromising on patient care.

The present study was aimed at identification of various markers that would aid in predicting severity of disease.

Materials and Methods

The current study retrospectively enrolled all confirmed cases of COVID-19 admitted in our tertiary care centre from 1st March 2021 to 31st March 2021 after approval from Institutional Ethics committee. Written informed consent was taken from patients at the time of admission.

Inclusion criteria

All COVID-19 patients 18 years of age or above diagnosed by RT-PCR were included.

Exclusion criteria

Patients already diagnosed with anemia, thalassemia and liver disease were excluded from the study.

All patients were categorized into two groups severe and non-severe. The cases meeting any of the following criteria were diagnosed with severe disease-

1. Dyspnea with respiratory rate ≥ 30 breaths/minute
2. Finger oxygen saturation $\leq 93\%$ in resting state
3. $\text{PaO}_2/\text{FiO}_2 \leq 300$ mm Hg
4. Respiratory failure requiring medical ventilation

The basic demographic details (patient's age, gender, clinical symptoms and systemic complications) were collected from admission records. Whole blood EDTA samples sent on day of admission were run on DxH 900 (Beckman Coulter). Citrated sample was run on ACL Elite Pro (Instrumentation Laboratory) to obtain D-dimer values.

The sample collected in the clot activator without anticoagulant was centrifuged at 1500 rpm for 15 minutes to allow for

separation of serum. Subsequently CRP, LDH and ferritin were performed on Vitros 5600 (Ortho Clinical Diagnostics).

Statistical analysis

Statistical analysis was performed with SPSS software version 23.0. The quantized variables of hematological and biochemical parameters were expressed as Mean \pm Standard deviation for the two groups of patients. The significance of these biomarkers between the severe and non-severe group was tested using Student's *t*-test. *P* value < 0.05 was considered statistically significant for all parameters.

Results

A total of 150 COVID-19 patients were included in the study. Out of these, 81 belonged to the non-severe group while 69 had severe disease. The age of patients ranged from 25-80 years with median age of 53 years. Of these, 105 (70%) were males and 45 (30%) were females.

The hematological and coagulation parameters of the two groups along with their *P* value have been compared in Table 1. The patients with severe disease had significantly lower Absolute lymphocyte count (ALC) while Absolute neutrophil count (ANC), Neutrophil to lymphocyte ratio (NLR), Platelet to lymphocyte ratio (PLR) and D-dimer levels were raised. There was not much difference between hemoglobin, total leucocyte count and platelet counts of the two groups ($p \geq 0.05$).

The biochemical parameters of both the groups along with their *P* values have been tabulated in Table 2. Serum CRP, ferritin and

Table 1: Comparison of hematological and coagulation parameters of COVID-19 patients in the two groups

| Parameter | Non severe Mean \pm Standard deviation | Severe Mean \pm Standard deviation | <i>P</i> |
|----------------------------------|--|--|-------------|
| Hb, g/dl | 14.41 \pm 1.77 | 13.91 \pm 1.53 | 0.07 |
| TLC, per cumm | 12962.35 \pm 2406.45 | 13737.97 \pm 2482.63 | 0.05 |
| ANC, per cumm | 6586.79 \pm 1427.01 | 7347.39 \pm 1892.00 | 0.005 |
| ALC, per cumm | 1802.35 \pm 215.77 | 1657.97 \pm 249.70 | 0.0002 |
| Platelet count, lakh per cumm | 2.65 \pm 0.67 | 3.23 \pm 0.79 | 0.05 |
| NLR | 3.32 \pm 0.62 | 4.80 \pm 0.64 | < 0.00001 |
| D-dimer (ng/ml) | 557.78 \pm 213.86 | 8353.14 \pm 957.63 | < 0.00001 |
| PLR | 191.95 \pm 14.70 | 201.38 \pm 16.24 | 0.0003 |

Table 2: Comparison of biochemical parameters of COVID-19 patients in the two groups

| Parameter | Non severe Mean \pm Standard deviation | Severe Mean \pm Standard deviation | <i>P</i> |
|------------------|--|--|----------|
| CRP (mg/l) | 31.79 \pm 19.19 | 42.16 \pm 12.26 | 0.0002 |
| Ferritin (ng/ml) | 419.62 \pm 408.09 | 596.02 \pm 661.67 | 0.04 |
| LDH (IU/L) | 351.15 \pm 118.85 | 395.70 \pm 116.17 | 0.04 |

LDH were significantly higher in the patients with severe disease profile (P value <0.05).

Discussion

In the present study, we evaluated 150 COVID positive patients out of which 69 had severe disease. Lymphocytopenia was a consistent feature seen in all patients, however it showed a significant association with disease severity. The decrease in CD4 and CD8 counts may be attributed to the role of these T lymphocytes in eliminating the virus-infected cells. This also may be the reason for low lymphocyte count being associated with poor prognosis and increased complication rate.^[7,8] An overwhelming rise in the neutrophil count may also induce lymphocyte apoptosis. Additionally, lymphocytes have ACE receptors that may be responsible for direct cytopathic effect by the virus.^[9]

Elevated TNF- α and lactic acid seen in patients of COVID-19 may lead to lymphocytopenia by inducing lymphocyte apoptosis and suppressing lymphocyte proliferation.^[10-12]

We also demonstrated a higher ANC and thus a significantly higher NLR in the severely affected group. These results are in concordance with the findings of other researchers.^[13-25] Waris *et al.*^[26] also demonstrated similar results except that they did not find a significant association between disease severity and lymphocytopenia (P value = 0.28). The raised ANC may be attributed to the cytokine storm leading to elevated levels of IL-1 and TNF- α .^[27,28]

Platelet to lymphocyte ratio was also raised in the patients with severe disease. This was similar to the results by Zhao *et al.*^[16] and Singh *et al.*^[18,20,23,24,26]

The neutrophils release Neutrophil extracellular traps, which lead to organ dysfunction, acute respiratory distress syndrome and death thus correlating with disease progression.^[29]

In the current study, no association was found between thrombocytopenia and disease severity. Similar to our results, Asan *et al.*,^[20] Suryavanshi *et al.*,^[21] Nizami *et al.*,^[22] Asghar *et al.*^[23] and Lin *et al.*^[24] did not find any significant difference between platelet counts of the severe and non severe group of COVID-19 patients. However, Shang *et al.*^[13] and Ding *et al.*^[14] found a significant correlation with low platelet count in the Chinese population. These studies attributed the low platelet count in the severe group to reduced megakaryopoiesis, immunological destruction of platelets, thrombin generation and consumption coagulopathy due to microthrombi formation.^[30]

Significantly high D- dimer values were observed in the severe group that was in concordance with results of other studies, which evaluated D-dimer levels.^[13,21,22,25] This was possibly due to a hypercoagulable state caused by cytokine storm and viremia, which leads to fibrin polymerization, thrombus formation and eventually adverse outcome.^[31]

In the current study, serum CRP, ferritin and LDH were significantly high in patients with severe disease. Our findings were in concordance with the results of Shang *et al.*,^[13] Ding *et al.*,^[14] Keski *et al.*,^[15] Kantri *et al.*,^[17] Bhandari *et al.*,^[19] Asan *et al.*,^[20] Nizami *et al.*,^[22] Asghar *et al.*^[23] and Rahman *et al.*^[25]

Singh *et al.*^[18] observed CRP and LDH to be higher in severely ill patients but no correlation between serum ferritin and disease progression. CRP is an inflammatory marker, which was highly correlated with cardiac injury, acute respiratory distress syndrome and even death.^[32] The raised ferritin levels could possibly be due to cytokine storm leading to increased synthesis and release from macrophages in the lung parenchyma.^[33] The proinflammatory action of ferritin is responsible for ARDS thus associated with disease severity and secondary hemophagocytic lymphohistiocytosis in COVID-19 patients.^[34]

Zhao *et al.*^[16] evaluated CRP and IL-6 among the inflammatory markers and found a significant association with both.

It has been postulated that SARS-CoV-2 targets alveolar macrophages via ACE2 receptor leading to increased cytokines (IL-6, TNF- α) and subsequently elevation of CRP and serum amyloid A (SAA) which are markedly raised in patients with severe disease.^[35,36]

The limitation of the current study is that it was a retrospective study design with a small sample size which impacts generalizability of data.

Conclusion

A high ANC, NLR and PLR along with lymphocytopenia and raised serum CRP, LDH, ferritin and D-dimer levels were associated with disease severity in patients of COVID-19. Out of these markers, NLR, D-dimer and CRP were among the best predictors of disease progression.

These biomarkers may be used to segregate patients requiring intensive care at the time of admission enabling risk stratification and thereby guiding further patient management. This will also help in reducing patient mortality by early identification of markers of disease progression. Primary care physicians are the first point of contact for most of the patients with healthcare system. So awareness about these hematological and biochemical parameters associated with disease severity can help in optimal utilization of already overburdened healthcare facilities.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

Key messages

These biomarkers can be utilized to triage patients into severe and non-severe disease and accordingly guide patient management.

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Conflicts of interest

There are no conflicts of interest.

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