Research article
Comparison of hematological parameters in mild and severe COVID-19 infected patients: A retrospective observational study

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(Received: August 2021 Revised: November 2021 Accepted: December 2021)

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ABSTRACT

Introduction and Aim: With the coronavirus disease 2019 (COVID-19) pandemic raging on, there is a need to identify clinical and laboratory predictors which predict progression towards severe and fatal forms of this illness. Our study aims to evaluate the ability of hematologic and biochemical biomarkers to discriminate between patients with and without severe or fatal forms of COVID-19.

Materials and Methods: A retrospective study was conducted on 200 Covid positive patients;100 with mild disease and 100 with severe disease. Medical records were reviewed to collect demographic data and results of the following blood investigations were noted at admission: Hb, Platelet count, Total and Differential leukocyte count, CRP, AST, ALT, LDH, Ferritin and D-Dimer. Comparative analysis was performed between the 2 groups.

Results: A significant difference in the basophil count (mean 2.35 and 5.92) among those with mild and severe disease respectively was noted as also with the eosinophil count (mean 6.88 and 1.79). The levels of CRP were higher in those with severe disease as compared to the mild disease group (mean 276.29 and 65.23). Ferritin levels were markedly increased severe disease patients (mean 1275.66 and 533.94). D-dimer was markedly increased in COVID-19 patients with severe disease (mean 3813.91 ng/ml) compared to those with mild disease group (mean 521.78 ng/ml).

Conclusion: Hematological and biochemical markers positively correlate to the severity of covid infection, thus highlighting their role in the early diagnosis of the disease and can act as independent markers in predicting severity and prognosis of disease.

Keywords: COVID-19; hematological parameters; severity.

INTRODUCTION

COVID 19 has been declared as a pandemic by WHO. Since it has the potential to spread during the incubation period, early diagnosis and management is the key (1). This virus affects people of all age groups. However, data from around the world has shown a higher affection of the middle and older age groups with an age range of 65-85 years (2). Coronavirus has slightly increased gender affinity towards males as compared to females globally (3-5). Patients with SARS-CoV-2 infection can experience a range of clinical manifestations, from no symptoms to critical illness. Based on the severity of illness, adults with SARS-CoV-2 infection can be grouped into the following categories (6).

- Asymptomatic or Pre-symptomatic Infection: Individuals with no symptoms of COVID-19 but who test positive for SARS-CoV-2 using a virologic test.
- Mild Illness: Patients presenting with signs and symptoms of COVID-19 like fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
- Moderate Illness: Patients with evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO2) ≥94% on room air at sea level.
- Severe Illness: Patients who have SpO2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300, respiratory frequency ≥30 breaths/min, or lung infiltrates >50%.
- Critical Illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Patients present with varied symptoms present at the onset of infection; fever (98.6%), fatigue (69.6%), dry cough (59.4%), muscle pain (34.8%), dyspnea (31.2%), and least frequently with headache (6.5%), dizziness (9.4%), abdominal pain (2.2%), diarrhea (10.1%), nausea (10.1%) and vomiting (3.6%; (5). Laboratory abnormalities in Covid affected individuals include lymphocytopenia (82.3%), thrombocytopenia (36.2%), leukopenia (33.7%). Majority of patients reported high levels of C-
reactive protein (CRP), less frequent were high levels of alanine transaminase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and D-dimer (7). Due to the lack of evidence on the relationship between hematological parameters and the severity of COVID-19, this study was designed aiming to investigate hematological parameters associated with the severity of the disease in patients with COVID-19 and to determine if these hematological parameters could potentially act as an independent factor and affect the disease prognosis. Objective of the study was to evaluate hematological parameters with respect to severity of illness and identify prognostic significance of these hematological parameters.

METHODOLOGY
This retrospective observational study was conducted at a tertiary care teaching hospital over a period of 4 months after prior ethical clearance. Patients were divided into 2 groups: mild group comprised those who were RT-PCR COVID positive but with mild symptoms, admitted in the ward and not requiring ICU admission or invasive/noninvasive ventilation. The other group was the severe group comprising those who were RT-PCR COVID positive but with symptoms warranting ICU admission. The categorization of patients was based on the New Coronavirus Pneumonia Prevention and Control Program in China (6), which classified COVID-19 affected patient into four types including mild, moderate, severe, and critical. In our study, severe or critical COVID-19 patients were grouped as severe group, and mild or moderate COVID-19 patients were grouped as the non-severe/mild group.

Inclusion criteria
- All patients who were admitted with positive real time reverse transcriptase polymerase chain reaction.
- All severe covid positive patients admitted to the ICU.

Table 1: Age distribution of cases

| Age Group | Count | Severe | Mild | Total |
|-----------|-------|--------|------|-------|
| 20 and below | | | | |
| % Within Group | 2.0% | 3.0% | 2.5% |
| 21 - 30 | | | | |
| Count | 5 | 12 | 17 |
| % Within Group | 5.0% | 12.0% | 8.5% |
| 31 - 40 | | | | |
| Count | 5 | 7 | 12 |
| % Within Group | 5.0% | 7.0% | 6.0% |
| 41 - 50 | | | | |
| Count | 18 | 17 | 35 |
| % Within Group | 18.0% | 17.0% | 17.5% |
| 51 - 60 | | | | |
| Count | 23 | 29 | 52 |
| % Within Group | 23.0% | 29.0% | 26.0% |
| 61 - 70 | | | | |
| Count | 24 | 20 | 44 |
| % Within Group | 24.0% | 20.0% | 22.0% |
| Above 70 | | | | |
| Count | 23 | 12 | 35 |
| % Within Group | 23.0% | 12.0% | 17.5% |
| Total | 100 | 100 | 200 |
| % Within Group | 100.0% | 100.0% | 100.0% |

Exclusion criteria
- All covid positive patients with mild disease who were admitted to the ward
- Age group of 18-80 years with or without comorbidities

Results
- Patients with mild covid disease admitted in the ward if shifted to ICU during hospital stay due to worsening of symptoms. Age less than 18 years and more than 80 years.
- Medical records were reviewed to collect the information on patient’s demographics, symptoms and presence or absence of any comorbidities.
- The results of the blood samples which were collected at admission were analyzed. Laboratory tests done subsequently were not considered. The following bio markers were analyzed: white blood cell count, platelet count, lymphocyte count (×109/L), neutrophil granulocyte count (×109/L), C-reactive protein level (in milligrams per liter), lactate dehydrogenase level (LDH) (in international units per liter), ferritin, D-dimer, and liver enzymes.
- The hematological parameters of both mild and severe covid positive were compared to determine its association with severity of disease.

Statistical analysis
The collected data was summarized by frequency, percentage, mean, standard deviation, median and IQR. Comparison of blood parameters between severe and non-severe patients was performed by Chi-square test and Mann Whitney test. Optimal cut off was obtained by ROC.

RESULTS
This retrospective, observational study was conducted on 200 COVID positive patients admitted in Father Muller Medical College, Mangalore. One hundred patients had mild disease and other 100 had severe disease and comparison was done between the 2 groups.
Chi square test p=0.241, NS
The younger population has milder disease and the older population had severe disease. The difference, however, was not statistically significant (Table 1).

**Table 2: Gender distribution of cases**

|       | Group | Total |
|-------|-------|-------|
|       | severe | Mild  |     |
| sex   | F      | 33    | 39  | 72  |
|       | % Within Group | 33.0% | 39.0% | 36.0% |
| M     | Count  | 67    | 61  | 128 |
|       | % Within Group | 67.0% | 61.0% | 64.0% |
| Total | Count  | 100   | 100 | 200 |
|       | % Within Group | 100.0% | 100.0% | 100.0% |

Chi square test p=0.376, NS
There was no statistical significance between males and females in terms of severity of the disease (Table 2).

**Table 3: Hematological parameters**

| Group   | n | Mean | Standard Deviation | Median | IQR | Mann Whitney test |
|---------|---|------|--------------------|--------|-----|-------------------|
|         |   |      |                    |        |     | P value           |
| HB      | 100 | 12.01 | 2.33              | 12.00  | 11-14 | 0.303 NS          |
| Mild    | 100 | 11.79 | 2.29              | 12.00  | 10-13 |                   |
| TC      | 100 | 8970.20 | 3936.01        | 9000.00 | 6225-11450 | 0.207 NS          |
| Mild    | 100 | 8741.49 | 5137.86        | 7650.00 | 4800-11700 |                   |
| PC      | 100 | 239900.00 | 113764.55     | 220000.00 | 170000-290000 | 0.728 NS          |
| Mild    | 100 | 247190.00 | 119960.83     | 230000.00 | 177250-294000 |                   |
| N       | 100 | 74.89 | 14.00             | 76.50  | 68.25-85 | 0.143 NS          |
| Mild    | 100 | 72.59 | 12.90             | 75.00  | 65.25-80.75 |                   |
| L       | 100 | 15.72 | 9.15              | 14.00  | 8-21.75 | 0.263 NS          |
| Mild    | 100 | 17.84 | 10.97             | 14.50  | 10-25  |                   |
| B       | 100 | 5.92  | 2.98              | 6.00   | 4.8-7.5 | 0.000 HS          |
| Mild    | 100 | 2.35  | 3.41              | 1.00   | 1-2    |                   |
| E       | 100 | 1.79  | 2.11              | 1.00   | 1-1    | 0.000 HS          |
| Mild    | 100 | 6.88  | 3.41              | 7.00   | 5.9-75 |                   |
| D DIMER | 99  | 3813.91 | 10046.80       | 1008.00 | 445-2601 | 0.000 HS          |
| CRP     | 100 | 521.78 | 637.04         | 319.00 | 188.25-563.75 | 0.000 HS          |
| FERRITIN | 100 | 1275.66 | 2107.95      | 657.50 | 456.75-977 | 0.000 HS          |
| LDH     | 100 | 533.94 | 769.67         | 319.00 | 154.5-615 | 0.058 NS          |
| AST     | 100 | 399.93 | 293.84         | 377.50 | 187-563.75 | 0.031 Sig         |
| ALT     | 100 | 319.41 | 143.31         | 285.50 | 234.5-375 | 0.190 NS          |

a) **Hemogram**

Hemoglobin levels were found to be lower in those with mild disease (mean of 11.79g/dl) as against those with severe disease (mean 12.01%) p value 0.303 which was not found to be significant.

Platelet counts did not show much difference between 2 groups (2.47 vs 2.39) and was not significant.

The mean total counts were 8741 cells/mm³ for those with mild disease as against 8970 cells/mm³ for those with severe disease (p value 0.207) which was not found to be significant.

Neutrophilia was noted in both groups with a mean of 72.59% among those with mild disease and 74.89% among those with severe disease, which was not found to be significant.

Lymphocytopenia was noted in both groups with a mean of 17.84% and 15.72% respectively, which was not significant.

There was a significant difference in the basophil count (mean 2.35 and 5.92) among those with mild and severe disease respectively; as also with the eosinophil count (mean 6.88 and 1.79) for those with mild and severe disease; both were highly significant (p value 0.000).
ROC Curve demonstrated a sensitivity 92%, specificity 37% (AUC- 0.854, p value 0.000) with a cut off of 0-1% for basophils and sensitivity 92%, specificity 45%. (AUC- 0.880, p value 0.000) with a cut off of 1-6% for eosinophils (Table 3).

b) Liver enzymes

Transaminases (AST/ALT) were elevated in COVID positive patients; however, AST levels showed a significant difference between the 2 groups (mean 43.06 and 60.22; p value 0.031). ROC Curve demonstrated a sensitivity 64%, specificity 45%, (AUC- 0.588, p value- 0.031) with a cut off of 0-35IU/L for AST levels (Table 3).

c) Inflammatory and biochemical markers

Coagulation tests suggested that D-dimer were increased in COVID-19 patients, and the increase was more pronounced in those with severe disease (mean 521.78 ng/ml) than in the mild disease group (mean 521.78 ng/ml) P<0.000 was found to be highly significant. An ROC curve demonstrated sensitivity 96%, specificity 65%, (AUC-0.81, p value-0.000) with a cut off of 0-243 ng/ml for D-dimer levels (Table 3).

The levels of CRP were higher in those with severe disease as compared to the mild disease group (mean 276.29 and 65.23). Also, Ferritin levels were markedly increased severe disease patients (mean 1275.66 and 533.94); both were statistically significant with p value 0.000.

ROC Curve demonstrated a sensitivity of 97%, specificity 75% (AUC- 0.735, p value – 0.000) with cut off of 13-150ng/ml for ferritin levels.

ROC Curve demonstrated a sensitivity of 100% specificity 84% (AUC – 0.85, p value-0.000) with a cut off of 0-5mg/l for CRP levels (Table 3).

Lactate dehydrogenase (LDH) levels were higher than those in regular patients, however, no significant difference was noted between both groups.

ROC Curve demonstrated a sensitivity 69%, specificity 66%, (AUC 0.578, p value 0.058) with a cut off of 0-250IU/L for LDH levels.

Table 4: Distribution based on Neutrophil-Lymphocyte Ratio (NLR), Platelet-Lymphocyte Ratio (PLR), Lymphocyte-Monocyte Ratio (LMR)

| Group | N  | Mean | Std. Deviation | 50th (Median) | 75th | Mann Whitney test p value |
|-------|----|------|----------------|--------------|------|-------------------------|
|       |    |      |                | 25th         |      |                         |
|       |    |      |                |              |      |                         |
|       |    |      |                |              |      |                         |
| NLR   |    |      |                |              |      |                         |
| severe| 99 | 7.53 | 6.50           | 5.3          | 9.3  | 0.404                   | NS                        |
| Mild  | 100| 6.71 | 5.75           | 5.3          | 8.3  |                         |                          |
| PLR   |    |      |                |              |      |                         |
| severe| 99 | 21599.83 | 17143.73 | 16000.0      | 9565.2 | 29000.0 | 0.674 | NS                   |
| Mild  | 100| 22132.76 | 20805.74 | 15479.2      | 7904.2 | 28562.5 | 0.894 | NS                   |
| LMR   |    |      |                |              |      |                         |
| severe| 100| 4.35 | 4.94           | 3.0          | 5.0  |                         |                          |
| Mild  | 99 | 4.42 | 4.27           | 3.0          | 5.5  |                         |                          |

In our study, none of the ratios predict the severity of disease (Table 4).

DISCUSSION

Coronavirus (COVID-19) is an emerging infectious respiratory disease. In the absence of an effective definitive treatment (8,9), it is imperative to identify the markers that can help monitor the progression of disease and in early management of patients. The present study aimed to investigate the relationship between hematological parameters and disease severity in COVID-19 patients and the results revealed that Ferritin, D-dimer, CRP levels along with basophil counts were significantly elevated among those with severe disease as against those with mild disease.

As lymphocytes are virus-fighting effector cells, most viral infections in humans cause lymphocytosis (10). The coronavirus family however caused lymphocytopenia in infected patients in some studies (11,12), the mechanism probably being direct attack of coronavirus on lymphocytes or by immune-mediated apoptosis of lymphocytes (13-15). Our study also noted lymphocytopenia in COVID infected patients.

Qin et al., reported that lymphopenia and an increased neutrophil–lymphocyte ratio were frequently observed in patients with severe COVID-19 compared with those with mild disease (16).

Henry et al., in their study about the hematological parameters of COVID-19 patients (17) suggested that the more severe and deadly cases of the disease had higher WBC count and lower lymphocyte and PLT than the mild cases. In their study, CRP had also increased as an indicator of systemic inflammation in COVID-19 patients. In this regard, routine evaluation of hematological parameters, including PLR and NLR, was suggested as a prognostic predictor of COVID-19 patients (18). In our study however, PLR and NLR could not predict severity of disease.

In a meta-analysis conducted by Zeng et al., (19) to determine the association of inflammatory markers with the severity of COVID-19,16 studies comprising 3962 patients with COVID 19 were analyzed and it was found that patients with COVID-19 in the non-severe group had lower levels for platelets, CRP, IL-
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Authors declare that there is no conflict of interest.

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