Original Research Article

# Analysis of Haematological Parameters and Peripheral Smear findings of COVID- 19 Patient's Blood Sample

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## Abstract

**Introduction:** Lymphopenia is an important finding in COVID-19 is a systemic infection which can help to predict the prognosis. The ratio of neutrophils to lymphocytes ratio,lymphopenia andthrombocytopenia can help to determine the severity of an incident. Severe thrombocytopenia can lead to potentially fatal consequences in the form of DIC. **Objectives:** To know the haematological parameters and peripheral smear findings in blood samples of COVID -19 patients and their relation with severity of infection. **Materials and Methods:** This prospective study was conducted at Department of Pathology, Hassan Institute of Medical Sciences, Hassan, Karnataka. 12288 confirmed cases from Covid -19 infection from June 2020 to May 2021, were evaluated in detail for complete blood count analysis by Cell Dyn Rubyand peripheral smear study.Findings were noted and correlated with severity of infection. **Results:** In the present study from asymptomatic cases to critical cases, there was gradual increase in leucocytes count, along with neutrophilia,absolute neutrophils count, neutrophil to lymphocyte ratio and gradual decrease in lymphocytes, absolute lymphocyte count andplatelets. In our study, peripheral smear findings showed toxic granules along with other reactive changes in neutrophils. Lymphopenia worsens as the disease progresses to a more advanced state.Individual proper managementand the disease's outcome can be monitored effectively by noting above discussed haematological parameters in COVID-19 infection.

**Keywords:** COVID-19 infection, Lymphopenia, Neutrophils to lymphocytes ratio, Thrombocytopenia,



## Introduction

COVID-19 is a systemic infection that has a significant impact on the hematopoietic system and hemostasis. Lymphopenia is an important test finding that can help to predict the prognosis. The ratio of neutrophils to lymphocytes, as well as the peak platelet/lymphocyte ratio, can help to determine the severity of an incident.

Longitudinal monitoring of lymphocyte count dynamics, inflammatory indices, and other biochemical markers over the course of the disease may help in the identification of patients with poor prognoses and prompt action to improve outcomes.

Severe thrombocytopenia can lead to potentially fatal consequences. The disorder known as disseminated intravascular coagulation (DIC) needs ongoing monitoring and therapy.

### Aims and objectives of the study

To know the haematological parameters and peripheral smear findings in blood samples of COVID -19 patients and to correlate findings with severity of infection

### Materials

### Source of data

Blood samples received from the confirmed cases of Covid-19 patients.

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### Estimation of sample size

Covid blood samples received from June 2020 to May 2021

### Inclusion criteria

Blood samples of Covid patients.

### Exclusion criteria

Blood samples of Non Covid patients

### Methodology

Above prospective study was conducted from June 2020 to May 2021 at Department of Pathology, Hassan Institute of Medical Sciences, Hassan, Karnataka. Blood samples were collected from confirmed cases of Covid-19 patients. Samples were subjected for complete blood count analysis by Cell Dyn Ruby five-part automated blood sample analyser and peripheral smear study. Findings were noted and correlated with clinical symptoms and signs.

### Results

Our prospective study period was from June 01, 2020 to May 2021. The age group ranged from 01 year to 90 years. The total number of blood samples subjected for investigations for coagulation abnormalities in the Department of Pathology laboratory of Hassan Institute of Medical Sciences, Hassan were 12288. The maximum number of samples were seen in age group of 41 to 50 years (19.31%). For male it was in 41 to 50 years (20.38%) and for female it was in 31 to 40 years (19.51%). [Table 01]

**Table 1: Showing distribution of COVID-19blood sample with respect to age and sex.**

| Age (In Years) | Male - Number of COVID-19 blood sample | Female - Number of COVID-19 blood sample | Total |
|---|---|---|---|
| 01-10 | 237 | 204 | 441 |
| 11-20 | 618 | 579 | 1197 |
| 21-30 | 1296 | 894 | 2190 |
| 31-40 | 1326 | 1008 | 2334 |
| 41-50 | 1452 | 921 | 2373 |
| 51-60 | 1311 | 825 | 2136 |
| 61-70 | 642 | 522 | 1164 |
| 71-80 | 222 | 204 | 426 |
| 81-90 | 18 | 9 | 27 |
| Total Number | 7122 | 5166 | 12288 |

On receiving fresh, adequate COVID-19 blood samples to the Department of Pathology laboratory, samples were classified as below based on patient history, and categorization by clinicians.

1. Asymptomatic cases (AS): Patients' blood samples without any symptoms and signs where they had history of contacts with primary COVID-19 cases or coming on their own.
2. Influenza like illness (ILI)- Mild cases: Patient with history of fever, running nose, sore throat, cough with or without sputum production and other mild symptoms related to corona illness
3. Severe acute respiratory illness (SARI)- Moderate cases: Patient with high grade fever, chills, dyspnea, severe cough with expectoration and other symptoms of corona which impaired the daily routine life activities.
4. Critical cases (CC)- Severe cases: Critical illness was defined throughout the study as a requirement for endotracheal intubation, mechanical ventilation and careful monitoring and supervision by attending doctors.

On detailed analysis, of 4096 blood samples [Table 02] the contribution of Asymptomatic cases (AS), Influenza like illness (ILI), Severe acute respiratory illness (SARI) and Critical cases (CC) were 3252 (26.46%), 5286 (43.01%), 2973 (24.19%) and 777 (6.32%) respectively. Maximum number of asymptomatic cases (22.69%) were seen in 31 to 40 years and no cases in 81 to 90 years of age group. ILI cases were more commonly seen in 31 to 40 years (19.46%) and very minimal case (03 case) in 81 to 90 years of age group. SARI cases were more common in 41 to 50 years (21.08%) and only six cases from 81 to 90 years of age group. Maximum number of CC (28.57%) were seen in 61 to 70 years and no cases in 01 to 20 years of age group. Most of the critical cases were having history of chronic lung disease, cardiovascular disease, immunocompromised status, diabetes mellitus, chronic kidney disease requiring dialysis, chronic liver disease, and residence in a long-term care facility.

**Table 02: Classification and typing of blood samples with respect to age.**

| Age (In Years) | Asymptomatic | Influenza like illness | Severe acute respiratory illness | Critical cases | Total |
|---|---|---|---|---|---|
| 01-10 | 159 | 219 | 63 | 00 | 441 |
| 11-20 | 366 | 687 | 144 | 00 | 1197 |
| 21-30 | 711 | 984 | 486 | 09 | 2190 |
| 31-40 | 738 | 1029 | 519 | 48 | 2334 |
| 41-50 | 678 | 891 | 627 | 177 | 2373 |
| 51-60 | 423 | 915 | 603 | 195 | 2136 |
| 61-70 | 171 | 444 | 327 | 222 | 1164 |
| 71-80 | 06 | 114 | 198 | 108 | 426 |
| 81-90 | 00 | 3 | 6 | 18 | 27 |
| Total Number | 3252 | 5286 | 2973 | 777 | 12288 |

For all the received fresh COVID-19 blood samples in Pathology laboratory the following tests were performed according to standard procedure for coagulation abnormality. For each tests, control samples results were also noted.

1. Total Leukocyte count (TLC)
2. Absolute Neutrophil count (ANC)
3. Absolute Lymphocyte count (ALC)
4. Neutrophil count to Lymphocyte count ratio (NLR)
5. Platelet count (PC)

After subjecting the blood samples of 12288 COVID-19 positive cases, results were correlated with the type of blood samples and correlated with severity of infection.

**Table 03: Showing the average values of TLC, ANC, ALC, NLR& PC of 12288 blood samples.**

| Sl No | Type of sample | TLC (Cells/cumm) | ANC (Cells/cumm) | ALC (Cells/cumm) | NLR | PC (lakhs Cells/cumm) |
|---|---|---|---|---|---|---|
| 1 | Control | 7.42 | 5.60 | 2.73 | 2.05 | 2.93 |
| 2 | AS | 7.02 | 5.18 | 2.21 | 2.34 | 1.85 |
| 3 | ILI | 10.01 | 7.13 | 1.76 | 4.05 | 1.13 |
| 4 | SARI | 13.69 | 8.26 | 1.05 | 7.86 | 0.74 |
| 5 | CC | 21.85 | 9.04 | 0.74 | 12.21 | 0.39 |

**Discussions**

Coronavirus illness 2019 (COVID-19) is a respiratory tract infection caused by SARS-CoV-2, a newly emerged coronavirus initially identified in Wuhan, China, in December 2019. The virus's genetic sequence implies that SARS-CoV-2 is a beta-coronavirus closely related to SARS[1].

While the majority of COVID-19 patients have a mild or uncomplicated sickness, about 14% develop severe disease that need hospitalisation and oxygen assistance, and 5% require admission to an

intensive care unit. SARS-CoV-2 infection has been recorded in individuals who never develop symptoms (asymptomatic) and in patients who are not yet symptomatic in several studies (pre-symptomatic)[2-6].

Males were affected more frequently than females, according to the demographic parameters of our study. This is comparable to studies by Guan WJ et al[7], and Nanshan Chen et al[8], in which disease incidence was higher in men than in women. Immunological, cultural, or racial differences in the Indian population might explain the shift in gender patterns. More research is needed in this area, and a bigger cohort study may be recommended to prove the point[1].

Lippi G et al[9] conducted a systematic evaluation of the literature and found that the most important leucocytosis, neutrophilia, lymphopenia and other haematological abnormalities seen in COVID-19 patients may predict the progression to severe or critical forms of COVID-19[1].In the present study there is gradual increase in leucocytes count from asymptomatic cases to critical cases (7.02 to 21.85cells/ cumm). As per the IFCC (Information Guide on COVID-19)[1] guidelines, the CBC parameters in a COVID case show neutrophilia,leucocytosis lymphopenia, and thrombocytopenia.Chuan Qin et al[10]. found that primary dysregulation of the immune response, particularly T lymphocytes, may play a significant role in the pathogenesis of COVID-19.

In the present study there is gradual increase in absolute neutrophils count (ANC) count from asymptomatic cases to critical cases 5.18 to 9.04 cells/ cumm. A series of COVID-19 reports revealed that ICU patients were more likely to develop neutrophilia, a disease-progression sign. Various studies[11-13] have shown the median absolute neutrophil count (ANC) in ICU cases was 10.6 (5.0–11.8) 10[9]/L, substantially higher than the 4.4 (2.0–6.1) 10[9]/L in non-ICU cases. According to a retrospective study of COVID-19 patients' initial laboratory indices, 34.5 percent of them had neutrophilia, and patients with ARDS had more neutrophils than those who did not have ARDS. Furthermore, patients with ARDS who died had greater neutrophil counts than those who survived[14].In the present study there is gradual decrease in absolute lymphocyte count (ALC) count from asymptomatic cases to critical cases (5.18 to 9.04 cells/ cumm). According to study done in Zhongnan Hospital of Wuhan University, discovered that lymphocytes, the principal antiviral cells, were prone to decrease continuously and severely in ICU and dead patients[12]Lympopeniais very commonly seen in this viral infection. Lympopenia was found in 83.2 percent of patients on admission in a multicentre trial involving 1,099 patients from 552 locations in China. Many other studies in China found lymphopenia rates ranging from 26 to 80 percent[7,15].T lymphocytes and NK cells were found to be depleted in COVID-19 patients[16,17,19]. The low cycle threshold value in respiratory samples indicated that lymphopenia on presentation was associated with a high viral load[20]. Liu et al[21].looked into the relationship between dynamic variations in nasopharyngeal virus load and lymphocyte count.In our study there is significant rise in NLR ratio in critical care patients (12.21). The patient's response to inflammatory insult may be indicated by a high NLR, with neutrophils increasing in reaction to stress, which, when overwhelming, causes lymphocyte apoptosis[22-25].The Absolute Neutrophil to Lymphocyte Ratio (NLR) is a biomarker used to determine the severity of bacterial infections as well as the prognosis of pneumonia and tumour patients. NLR could be used as a surrogate marker for detecting COVID 19 infection early[1].Normal NLR values in an adult, non-geriatric population in excellent health were found to be between 0.78 and 3.53 in a research by Forget et al[26]. In contrast, Jingyuan Liu[27] determined that patients over or equal to age 50years with NRL of more than 3.53 are at risk of serious disease and should be admitted to the intensive care unit as soon as possible if necessary. A substantial percentage of cases in our study had toxic granules with other reactive changes and reactive lymphocytes, which matched the findings of Bingwen Eugene Fan et al[16].In the present study there is decrease platelet counts from asymptomatic cases to critical cases (1.85 to 0.39 Lakhs cells/ cumm). In comparison to lymphopenia, thrombocytopenia is less common in patients with COVID-19[15]. Previous SARS-CoV-1 patients' experiences suggested that coronavirus could cause thrombocytopenia through direct viral infection of bone marrow haematopoietic stem cells via CD13 or CD66a, the formation of autoantibodies and immune complexes, disseminated intravascular coagulopathy (DIC), and platelet consumption in lung epithelium[28,29]. SARS patients had higher levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), which increased vascular sequestration and caused thrombocy-topenia[30].Some illnesses, such as infectious mononucleosis, Bordetella pertussis, and hantavirus, are characterised by atypical/ reactive cells in the peripheral smear[24-26]. Infectious mononucleosis is characterised by a wide range of reactive/ pleomorphic lymphocytes, whereas hantavirus is characterised by Downey type III cells or immunoblasts. These aren't specific results, and they could appear in different numbers in other infections, autoimmune disorders, and cancers[31-34].

**Conclusions**

In conclusion, the COVID-19 infection has caused significant haematological complications.In follow-up cases with persistent symptoms, a statistically significant increase in CBC parameters, NLR, was observed; however, a larger follow-up cohort is required to reach statistical significance.

Lymphopenia worsens as the disease progresses to a more advanced state. In patients with SARS-CoV-2 infection, increased neutrophil count and neutrophil-to-lymphocyte ratio, as well as lower haemoglobin concentration, have been identified as risk factors for severe sickness.Individual treatment decisions should be made to reduce the risk of infection while not risking the disease's outcome.

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