

## Original Research Article

### A Comparative study in hematological parameters between first wave vs second wave in ICU and non- ICU COVID-19 patients in India.

#### **ABSTRACT :**

**Aim:** This study aims to determine similarities and differences in hematological parameters between the patients from two waves of severe acute respiratory syndrome coronavirus-2 infection at the time of hospital admission, as well as to identify risk biomarkers of COVID19 severity.

**Study design :** A retrospective study

**Place of study :** The study was carried out in Saveetha Medical College and Hospital , Tamil Nadu ,India.

**Methodology:** Total of 300 patients with COVID-19 infection from both 1<sup>st</sup> and 2<sup>nd</sup> wave was taken for this study. Haematological parameters of 150 patients affected in first wave , out off which 131 patient were admitted in non intensive care unit and 19 patients were admitted in intensive care unit during the period of april to june 2020. 150 patients affected in second wave, out off which 101 patients were admitted in non intensive care unit and 49 patients were admitted in intentional care unit during the period of March to May 2021. Statistical analysis of the data for both waves was carried out, and relevant findings were presented.

**Results:** Haematological parameters data were compared between the COVID19 positive patients admitted ICU and non- ICU across both infection waves. On gender distribution males ratio was predominant than females in both the wave. Degree of freedom analyses revealed an association of haematological parameters with the subsequent illness progression and severity in both the waves.

**Conclusion:** Our study showed , severity of the disease was more in the second wave, especially among ICU patients than first wave. Majority of the infected patients were male predominance in both waves. However, presence of comorbidities , immune response, severity of the infection and other risk factors determines the progression of disease.

**Keywords:** COVID-19 first wave, second wave, hematological parameters , neutrophilia, lymphopenia, -and neutrophil- lymphocytes ratio.

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**Methodology:** A total of 300 patients with COVID-19 infection, consisting of 150 patients from the first wave (April to June 2020) and 150 patients from the second wave (March to May, 20) were considered for this study. The hematological parameters of the patients were examined. Of the sample population from the first wave, 131 were admitted to the non-intensive care unit, and 19 were admitted to the intensive care unit (ICU). Of the sample population selected from the second wave, 101 were admitted to the non-intensive care unit and 49 were admitted to the intensive care unit. Statistical analysis of the data for both waves was carried out, and relevant findings were presented.

**Results:** Haematological parameters data were compared between the COVID19 positive patients admitted to ICU and non- ICU across both infection waves. On gender distribution, males were more than females in both waves. Degree of freedom analyses revealed an association of hematological parameters with the subsequent illness progression and severity in both the waves.

**Conclusion:** Our study showed that the severity of the disease was more in the second wave, especially among ICU patients than in the first wave. The majority of the infected patients were males in both waves. However, the presence of comorbidities, immune response, the severity of the infection, and other risk factors determines the progression of the disease.

**Keywords:** COVID-19 first wave, second wave, hematological parameters , neutrophilia, lymphopenia, neutrophil- lymphocytes ratio.

## INTRODUCTION:

COVID-19 is caused by the virus SARS-CoV-2 and ~~is~~ **was** declared ~~as~~ a global pandemic by the World Health Organization. It was first recognized in Wuhan city, China, in December 2019 [1]. In India, approximately 31,998,158 got infected, **and** 428,715 died due **to** COVID-19 infection till August 10, 2021. Genetic sequencing of the virus suggests that it is a beta coronavirus closely linked to the SARS virus. The exact mode of transmission of the disease is not well known, and while the current information is limited, it supports person-to-person transmission. The most possible routes of transmission are thought to be droplet and contact-based [1,2]. The clinicopathological spectrum of SARS-CoV-2 infection is broad ranging from asymptomatic infection to fatal condition. Patients **with** mainly an upper respiratory tract infection are over 90% more likely to have more severe conditions and death, usually in older adults and people with certain pre-existing medical conditions [3]. Some common clinical manifestations in patients suffering from critical COVID-19 disease includes pneumonia, respiratory failure, acute respiratory distress syndrome (ARDS), venous thrombosis, lung thromboembolism, lung fibrosis, septic shock, systemic inflammatory response, renal damage, cardiovascular damage, **blood vessel** damage, and multiple organ failure[3]. Early detection of patients prevents **the** development of critical illness and optimizes the use of hospital resources[2,3]. COVID-19 diseases are closely associated with haematological parameter changes in **the** complete blood picture. Haematological parameters have an important role in **the** early detection of the disease, considering the information they provide to clinicians regarding the inflammatory process [22?? **is this reference correct? Kindly check and ensure that it is not 22 (twenty two)**]. (**please, where are the references 4 and 5? You can't jump from 3 to 6**) This information includes Hemoglobin, red blood cells indices, Total Leukocyte counts, Differential leukocyte counts, Platelet counts, neutrophil-lymphocyte ratio, **and** platelet- lymphocytes ratio to determine the severity of the infection [6]. Countries like India have faced a two-wave pattern in reported cases of ~~coronavirus disease-19~~ **COVID-19**. In India, the first period of **the** pandemic was between the end of January to November of 2020, corresponding to the entire first wave, and the second period, was between February to June of 2021, corresponding to part of the second wave[2]. Alterations in various haematological parameters between both waves have been recently documented in the world literature—~~in~~ **on** SARS-Cov-2 infection. Hence, this study is an attempt to evaluate the similarities and differences of haematological parameters in COVID-19 patients in the Indian population in a single tertiary care centre.

## MATERIALS AND METHODS:

A retrospective study was carried out in line with research regulations, including the approval of the Ethical Committee. **A total** of 300 patients with COVID-19 infection from both 1<sup>st</sup> and 2<sup>nd</sup> waves **were** taken for this study. The diagnosis was confirmed by detecting SARS-CoV-2 RNA in oronasopharyngeal swab samples and Radiological images findings. Haematological parameters of 150 patients affected in the first wave, out of which 131 patients were admitted in non-intensive care units and 19 patients were admitted in intensive care units during the period of April to June 2020. 150 patients were affected in the second wave, out of which 101 patients were admitted in non-intensive care units and 49 patients were admitted in intensive care units during the period of March to May 2021. Analysis of haematological parameters was done by Sysmex Automated Haematology Analyser XN-1000 ~~from~~ **at** the department of Haematology, **Saveetha Medical College and Hospital, India**, ~~obtained during the time of admission.~~

## RESULTS:

~~Among~~ **Of** the 150 cases admitted ~~in~~ **during** the first wave, ~~showed 69% of male and 31% female~~ **there were more males (69%) than females (31%)** with a male to female ratio of 2.1:1. Among the 150 cases admitted in the second wave, ~~showed 63% of male and 37% female~~ **there were more males (69%) than females (31%)** with a male to female ratio of 1.7:1. **The** gender distribution among the

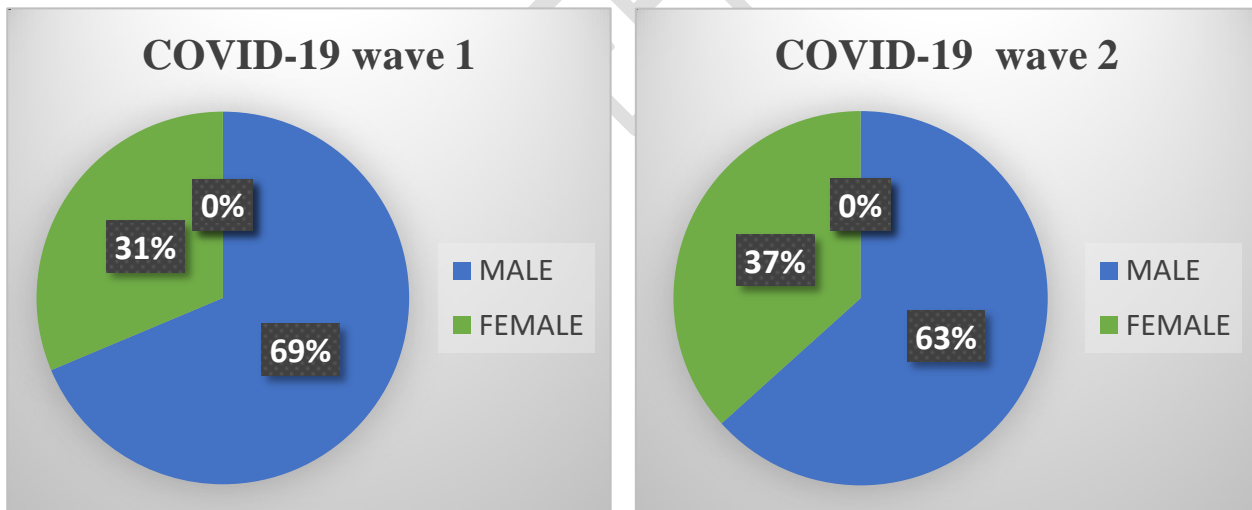
admitted cases in both waves showed a male predominance (Table 1) (fig 1). The most common changes seen in both the waves include Anaemia, Leukopenia, Leukocytosis, Neutrophilia, lymphopenia, thrombocytopenia and a few cases of thrombocytosis. Furthermore, the need for intensive care unit admission, length of hospitalization and oxygen saturation-demand at the time of hospital admission for the patients were more in the second wave than in the first infection wave.

**Table 1. Gender distribution comparison of 1<sup>st</sup> and 2<sup>nd</sup> wave.**

Parameters	COVID-19 patients of in the 1 <sup>st</sup> wave ( Mean ± SD )	COVID-19 patients of in the 2 <sup>nd</sup> wave ( Mean ± SD )
Total no. of cases	150	150
Sex	Male - 103 Female - 47	Male - 95 Female - 55

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**Fig 1 : Gender distribution showing male predominance in both waves.**



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**Table 2 : Hematological parameters—comparison between 1<sup>st</sup> and 2<sup>nd</sup> wave in COVID19 patients admitted in the intensive care unit .**

Parameters	COVID-19 patients in the intensive care unit of during the first wave ( Mean $\pm$ SD )	COVID-19 patients in the intensive care unit of during the second wave ( Mean $\pm$ SD )	P- value
Haemoglobin (g/dl)	11.83 $\pm$ 2.2 Minimum- 8.3 Maximum- 16.5	11.94 $\pm$ 2.3 Minimum- 6.6 Maximum- 15.7	0.87
RBC (x 10 <sup>6</sup> / $\mu$ l)	4.27 $\pm$ 0.5 Minimum- 3.4 Maximum- 5.3	4.35 $\pm$ 0.8 Minimum- 2.2 Maximum- 5.4	0.06
Haematocrit (%)	36.4 $\pm$ 6.3 Minimum- 26.8 Maximum- 49.1	39.27 $\pm$ 8.1 Minimum- 20.6 Maximum- 66.8	0.18
MCV (fL)	85.4 $\pm$ 9.1 Minimum- 62.9 Maximum-95.9	88.6 $\pm$ 7.5 Minimum- 66.1 Maximum- 102.3	0.14
MCH (pg)	27.25 $\pm$ 3.4 Minimum- 19.6 Maximum- 31.3	27.40 $\pm$ 2.8 Minimum- 18.0 Maximum- 35.0	0.85
MCHC (g/dl)	31.86 $\pm$ 1.3 Minimum- 29.1 Maximum- 33.6	30.73 $\pm$ 1.8 Minimum- 27.0 Maximum- 34.7	0.02
RBC distribution width(%)	14.95 $\pm$ 1.6 Minimum- 12.3 Maximum- 19.4	15.01 $\pm$ 2.0 Minimum- 12.3 Maximum- 19.4	0.90
WBC (x 10 <sup>3</sup> / $\mu$ l)	12.7 $\pm$ 5.6 Minimum- 5.58 Maximum- 28.02	18.1 $\pm$ 11.8 Minimum- 3.52 Maximum- 59.37	0.06
Neutrophil count (%)	84.4 $\pm$ 6.8 Minimum- 61.4 Maximum- 91.0	90.3 $\pm$ 7.0 Minimum- 64.7 Maximum- 97.4	0.003
Lymphocyte count (%)	10.9 $\pm$ 5.2 Minimum- 4.9 Maximum- 25.8	6.6 $\pm$ 1.2 Minimum- 1.2 Maximum- 31.0	0.01
Eosinophil count (%)	0.5 $\pm$ 1.5 Minimum- 0 Maximum- 5.6	0.3 $\pm$ 0.9 Minimum- 0 Maximum- 5.8	0.40
Monocyte count (%)	3.8 $\pm$ 1.7 Minimum- 0 Maximum- 7.1	2.5 $\pm$ 1.3 Minimum- 0.6 Maximum- 6.2	0.001
Basophil count (%)	0.1 $\pm$ 0.07 Minimum- 0.1 Maximum- 0.4	0.1 $\pm$ 0.13 Minimum- 0 Maximum- 0.6	0.70
Absolute Neutrophil count	10,918 $\pm$ 5155 Minimum- 3426 Maximum- 24,601	16,923 $\pm$ 11,517 Minimum- 2478 Maximum- 56,579	0.03
Absolute Lymphocyte count	1255 $\pm$ 427 Minimum- 591 Maximum- 2297	778 $\pm$ 414 Minimum- 145 Maximum- 2648	0.001
Absolute Eosinophil count	46 $\pm$ 125 Minimum- 0 Maximum- 456	27 $\pm$ 77 Minimum- 0 Maximum- 459	0.45
Absolute Monocyte count	489 $\pm$ 415 Minimum- 0 Maximum- 1837	438 $\pm$ 372 Minimum- 40.9 Maximum- 1781	0.63
Absolute Basophil count	18 $\pm$ 11 Minimum- 1 Maximum- 56	23 $\pm$ 24 Minimum- 0 Maximum- 118	0.44
Platelet count (x 10 <sup>3</sup> / $\mu$ l)	3.01 $\pm$ 1.4 Minimum- 0.93 Maximum- 6.06	1.77 $\pm$ 1.1 Minimum- 0.21 Maximum- 5.41	0.001
Neutrophil lymphocyte ratio	9.32 $\pm$ 4.08 Minimum- 2.38 Maximum- 17.92	27.13 $\pm$ 22.15 Minimum- 2.08 Maximum- 80.91	0.001
Lymphocyte monocyte ratio	2.69 $\pm$ 1.2 Minimum- 0 Maximum- 4.7	3.3 $\pm$ 3.7 Minimum- 0.3 Maximum- 21.0	0.45

**Table 3 : Hematological parameters comparison between 1<sup>st</sup> and 2<sup>nd</sup> wave in COVID19 patients admitted in non-intensive care units.**

Parameters	COVID-19 patients in non- ICU of in the first wave ( Mean ± SD )	COVID-19 patients in non- ICU of in the second wave ( Mean ± SD )	P- value
Haemoglobin (g/dl)	13.0 ± 2.0 Minimum- 4.2 Maximum- 17.2	12.5 ± 2.1 Minimum- 5.4 Maximum- 16.1	0.05
RBC (x 10 <sup>9</sup> / μl)	4.5 ± 0.7 Minimum- 1.3 Maximum- 6.8	4.5 ± 1.1 Minimum- 1.6 Maximum- 13.7	0.93
Haematocrit (%)	39.6 ± 9.6 Minimum- 4.8 Maximum- 91.3	39.6 ± 8.0 Minimum- 20.6 Maximum- 94.5	1.00
MCV (fL)	87.2 ± 6.2 Minimum- 67.4 Maximum- 114	87.0 ± 9.8 Minimum- 30.8 Maximum- 107.8	0.82
MCH (pg)	28.5 ± 3.1 Minimum- 13.3 Maximum- 39.7	28.0 ± 3.6 Minimum- 14.3 Maximum- 40.4	0.26
MCHC (g/dl)	32.8 ± 1.4 Minimum- 28.0 Maximum- 36.4	31.7 ± 2.0 Minimum- 24.4 Maximum- 41.7	0.001
RBC distribution width(%)	13.6 ± 2.0 Minimum- 2.8 Maximum- 25.5	15.4 ± 11.1 Minimum- 11.5 Maximum- 123.0	0.07
WBC (x 10 <sup>3</sup> /μl)	6.78 ± 2.4 Minimum- 1.54 Maximum- 14.8	7.4 ± 4.3 Minimum- 2.03 Maximum- 31.26	0.17
Neutrophil count (%)	62.5 ± 12.7 Minimum- 34.7 Maximum- 91.5	71.8 ± 14.0 Minimum- 36.2 Maximum- 95.7	0.001
Lymphocyte count (%)	29.4 ± 11.2 Minimum- 5.7 Maximum- 56.5	22.2 ± 12.0 Minimum- 1.8 Maximum- 52.4	0.001
Eosinophil count (%)	1.5 ± 2.1 Minimum- 0 Maximum- 12.1	0.7 ± 1.4 Minimum- 0 Maximum- 9.7	0.001
Monocyte count (%)	5.6 ± 2.2 Minimum- 1.1 Maximum- 13.1	4.9 ± 2.5 Minimum- 0.3 Maximum- 13.3	0.002
Basophil count (%)	0.3 ± 0.3 Minimum- 0 Maximum- 2.0	0.1 ± 0.1 Minimum- 0 Maximum- 0.7	0.001
Absolute Neutrophil count	4308 ± 2025 Minimum- 900 Maximum- 11,471	5628 ± 4112 Minimum- 1129 Maximum- 28,978	0.002
Absolute Lymphocyte count	1916 ± 948 Minimum- 449 Maximum- 5717	1369 ± 680 Minimum- 183 Maximum- 3513	0.001
Absolute Eosinophil count	126 ± 219 Minimum- 0 Maximum- 1637	46 ± 83 Minimum- 0 Maximum- 428	0.001
Absolute Monocyte count	368 ± 152 Minimum- 39 Maximum- 767	340 ± 220 Minimum- 22 Maximum- 1250	0.26
Absolute Basophil count	22 ± 23 Minimum- 0 Maximum- 183	13 ± 13 Minimum- 0 Maximum- 58	0.001
Platelet count (x 10 <sup>3</sup> / μl)	2.25 ± 0.8 Minimum- 0.14 Maximum- 4.70	2.05 ± 0.8 Minimum- 0.41 Maximum- 4.71	0.06
Neutrophil lymphocyte ratio	2.8 ± 2.2 Minimum- 0.6 Maximum- 16.0	5.6 ± 6.8 Minimum- 0.7 Maximum- 53.1	0.001
Lymphocyte monocyte ratio	5.8 ± 2.9 Minimum- 1.5	5.6 ± 5.5 Minimum- 0.7	0.74

	Maximum- 19.1	Maximum- 46.8	
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### **Hb concentration changes among both the waves in the ICU**

Among the 19 cases of ICU admitted in the first wave, 5 cases were anemic and 14 cases were normal. Among the 49 cases in the second wave, 13 cases were anaemic. Hence, anaemia ~~were was~~ observed among the ICU cases of both waves of covid-19. The Minimum haemoglobin observed in the first wave was 8.3 g% and the second wave showed a minimum of 6.6g% .

### **WBC changes among both the waves in the ICU**

In the first wave, no one ~~were was~~ leukopenia, 7 cases ~~were with~~ had a normal WBC count, and 12 cases ~~showed had~~ leucocytosis. ~~while In~~ the second wave, 14 cases had normal WBC count, 2 cases ~~were had~~ leukopenia, 14 cases ~~were normal~~ and 33 cases ~~showed had~~ leukocytosis. The minimum WBC count observed was 5580 cells/cu.mm and the maximum WBC count was 28000cells/cumm in the first wave, ~~while whereas~~ in the second wave, the minimum WBC count observed was 3520 cells/cu.mm and the maximum WBC count was 59370 cells/cumm.

### **Platelet changes among both the waves in the ICU**

In the first wave, 12 cases had normal platelet count, 4 cases ~~were with~~ had low platelet count and 12 cases ~~were normal~~ and 3 cases ~~showed had~~ thrombocytosis. In the second wave, 22 cases had normal platelet count, 25 cases ~~were with~~ had low platelet count and 22 cases ~~were normal~~ and 2 cases ~~showed had~~ thrombocytosis. The minimum platelet count observed was 93000 cells/cu.mm and the maximum platelet count was 6.06 lakhs/cumm in the first wave while in the second wave the minimum platelet count observed was 21000 cells/cu.mm and the maximum platelet count was 5.41lakhs/cumm .

### **NLR changes among both the waves in ICU**

The minimum NLR observed was 2.38 and the maximum NLR was 17.92 in the first wave, while in the second wave, the minimum NLR observed was 2.08 and the maximum NLR was 80.91 .

### **Hb concentration changes among both the waves in non-ICU**

Among the 131 cases of non-ICU admitted in the first covid, 16 cases were anemic and 115 cases were normal. Among 101 cases in the second wave 18 cases were anaemic and the remaining were normal. Hence, anemia ~~were was~~ observed among the non- ICU cases of both waves of covid-19 .The Minimum haemoglobin observed in the first wave was 4.2 g% and while the minimum for the second wave was 5.4g%. ~~second wave showed minimum of 5.4 g% .~~

### **WBC changes among both the waves in non-ICU**

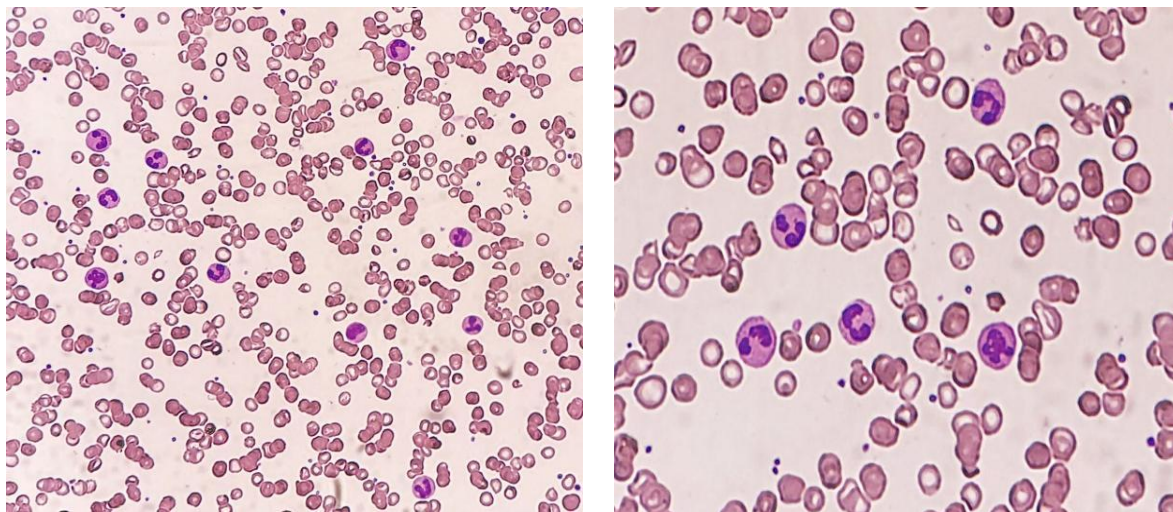
In the first wave, 13 cases ~~showed had~~ neutropenia, 110 cases ~~were with~~ had normal WBC count and 8 cases ~~showed had~~ leukocytosis. ~~While In~~ the second wave, 19 cases ~~were had~~ leukopenia, 69 cases ~~were had~~ normal counts, and 13 cases ~~showed had~~ leukocytosis. The minimum WBC count observed was 5580 cells/cu.mm and the maximum WBC count was 28000cells/cumm in the first wave while in the second wave, the minimum WBC count observed was 3520 cells/cu.mm and the maximum WBC count was 59370 cells/cumm .

### **Platelet changes among both the waves in non- ICU**

In the first wave, 20 cases ~~were with~~ had low platelet count, ~~and~~ 110 cases ~~were had~~ normal counts, and 1 cases ~~case showed had~~ thrombocytosis. In the second wave, 27 cases ~~were with~~ had low platelet count, and 74 cases ~~were had~~ normal counts, and 1 cases ~~showed case had~~ thrombocytosis. The minimum platelet count observed was 14,000 cells/cu.mm and the maximum ~~platelet~~ count was 4.7 lakhs/cumm in the first wave while in the second wave, the minimum platelet count observed was 41,000 cells/cu.mm and the maximum ~~platelet~~ count was 4.7 lakhs/cumm .

### NLR changes among both the waves in non- ICU

The minimum NLR observed was 0.6 and the maximum NLR was 16.0 in the first wave while in the second wave the minimum NLR observed was 0.7 and the maximum NLR was 53.1.



**Fig 2a & b : Peripheral blood smear of COVID-19 patient showing microcytic hypochromic anemia- red blood cells and increased neutrophils counts**

### DISCUSSION:

The present study showed that most of the haematological parameters behaved similarly across both 1<sup>st</sup> and 2<sup>nd</sup> waves of COVID19 infection with regard to disease severity, but however, remarkable differences were found in Hemoglobin concentration, Leukocyte counts, Neutrophil counts, Lymphocytes counts, Absolute neutrophil count, absolute lymphocyte count, platelet count and Neutrophil lymphocyte ratio (NLR) in critically ill patients admitted in ICU. and in the differential counts of Neutrophils, Lymphocytes, and Monocytes. Statistically significant differences were also seen in the absolute counts of Neutrophils and Lymphocytes, Platelet count, and the Neutrophil Lymphocyte ratio (NLR) in critically ill patients admitted to the ICU. Whereas in non- ICU patients, Hemoglobin concentration, Mean corpuscular hemoglobin concentration, Red cells distribution width, Leukocyte counts, Neutrophil counts, lymphocytes counts, eosinophils count monocytes counts, Absolute neutrophil counts, Absolute lymphocyte counts, Absolute eosinophils counts, Absolute basophil counts, platelet counts and Neutrophil lymphocyte ratio (NLR) showed significant differences between both waves. On combined analysis of both ICU and non-ICU patients showed a decrease in haemoglobin concentration and MCHC in the second wave rather than the first wave. Neutrophilia and Lymphopenia were observed in second wave patients but not in first wave patients. There was an increase in Neutrophil-lymphocyte ratio NLR in both the waves but more severe in second wave patients. Here, by using a single tertiary care centre study and keeping several variables identical, we found that a predictive model developed with data from the first wave of infection could be validated in a second wave, but some risk biomarkers lost their independent significance. Our study, from a multivariate analysis, could be affected by a series of confounding and collider biases. Therefore, it should be considered as a descriptive analysis that cannot be interpreted in causal terms, but that

suggests candidate predictors of poor prognosis at the time of hospital admission. Blood cell interactions are essential in the pathogenesis of inflammation, immune responses, hemostasis, and oncogenesis [8]. Several observational studies have suggested that the neutrophil-to-lymphocyte ratio (NLR), lymphocyte proportion, and the platelet-to-lymphocyte ratio (PLR) are inflammatory markers of immune-mediated, metabolic, prothrombotic, neoplastic diseases, and are widely investigated as useful predictors for prognosis in various diseases caused by infection [10]. Many recent researches of Researches done recently on COVID-19 indicated that severe patients tended to have higher NLR. (insert references, please)

Our pooled analysis showed a significant association of decreased hemoglobin and RBC indices. On Peripheral blood smear examination of some patients showed microcytic hypochromic red blood cells in both waves but more in ICU patients (Figure 2 a&b). Based on a meta-analysis, anemia seems to be associated with an enhanced risk of severe COVID-19 infection. The possible pathophysiological link between anemia and severe COVID-19 may be multifactorial [6]. In the circulation system, hemoglobin acts as a carrier for oxygen to target organs in the body. When the concentration of the Hb hemoglobin in the circulation is low, the transport of oxygen to several organs in the body will be disrupted, therefore causing hypoxia that will eventually result in multiple organ dysfunction, especially the respiratory system dysfunction. SARS-CoV-2 can interact with hemoglobin on the erythrocyte through ACE2, CD147, and CD26 receptors. Both viral-hemoglobin virus-hemoglobin interactions will cause the virus to attack the heme on the 1-beta chain of hemoglobin and causing cause hemolysis [16]. Other recent studies suggest SARS-CoV-2 may mimic the action of hepcidin which increases circulating and tissue ferritin while inducing serum iron deficiency and lack of hemoglobin, by consequence. The resulting hyperferritinemia will give rise to ferroptosis, with highly high oxidative stress and lipoperoxidation that can precipitate the inflammatory/immune over-response 'cytokine storm', and cause a severe outcome of the disease. In the studies done by authors Guan et al., Huang et al., Young et al., 14, Fan et al, Zhou et al, Liu et al and Chen et al, hb concentration were 13.4, 12.6, 13.5, 14.2, 12.8 gm and 14.8 respectively [16]. (Please, rearrange the authors according to the cited reference and cite each. All et al. must be followed by a full stop. I did not see Young et al., Chen et al., cited in your reference but yet, it appeared in the discussion. Kindly include Young et al. and Chen et al. in the reference section) A study by Agrawal A et al. found hemoglobin concentration was 13.85g/l & 13.12g/l (Please, include the standard unit for hemoglobin in the entire manuscript and remove the g/% in all) in asymptomatic and symptomatic patients, respectively. (a comma should come before the word 'respectively' in all your writing) Patients with decreased RBC indices should be advised to take extra precautions to minimize risk exposure. A high white blood cell count is common in critically ill patients because damaged cells induce innate inflammation in the pulmonary parenchyma, which is largely mediated by pro-inflammatory macrophages and granulocytes [20]. Increased neutrophil differential counts and absolute neutrophil counts have also been detected reported as a feature of COVID-19, with and neutrophil activation are being a prominent feature of blood transcriptomes of in severe cases. Further analysis of granulocytes samples reveals alteration to in gene expression changes in these cells, as opposed to a mere change in abundance number in the circulation. with prominent feature Some markers of immature neutrophil markers are also upregulated in severe vs mild COVID-19 patients. In addition, plasma levels of LCN2, RETN and HGF produced by neutrophils, were recently proposed as predictive predictors of for the clinical severity range of in critically ill patients. (insert reference) The median of absolute neutrophil count in a study by Huang et al. 11 was 5.0 x10<sup>9</sup>/L, 5.0 x10<sup>9</sup>/L in Wang et al. 12, 4.47 x10<sup>9</sup>/L in Wu et al. 13, 2.7 x10<sup>9</sup>/L in Young et al. 14 and 2.6 x10<sup>9</sup>/L in a study by Fan et al. Chen et al. 22 study represented a median absolute neutrophil count of 5.0 x10<sup>9</sup>/L and 38 cases (38%) showed neutrophilia. In the present this study, we analyzed found that lymphopenia was a good predictor for of the clinical severity and slow recovery of patients with COVID-19 disease. It was associated with inflammatory markers, grades of pneumonia severity and prolonged duration hospitalization. Normalization of lymphocyte count denotes recovery of COVID-19. SARS-CoV directly infects primary T cells and induces massive apoptosis leading to lymphopenia, while aborting the viral expansion in these cells is abortive. Coronavirus, also infects and destroys lymphocytes, which facilitate viral replication and persistence. Many previous studies, showed that the pathogenesis of COVID-19 patients have been linked to the virus's ability to infect T cells through the

angiotensin-converting enzyme 2 receptors and cluster of differentiation, CD147-spike proteins. (provide references, please) The final results were decreased levels of CD3+, CD4+, CD8+ T lymphocytes, and increased regulatory T cells(22). The elevation of pro-inflammatory cytokines with T cell lymphopenia predisposes to cytokine storm, thus resulting in more lymphocytic apoptosis and multi-organ failure in COVID-19 patients(10). This mechanism is due to the activation of caspase-1 as an effector element of inflammasome promoting IL-1 $\beta$  production and inducing pyroptosis of lymphocytes. It was reported that SARS-CoV-related Viro-porin 3a activates the NLRP3 inflammasome and induces the secretion of IL-1 $\beta$ , which indicates that the SARS-CoV infection can cause cell pyroptosis. The Viro-porin 3a has also been identified on the genome of SARS-CoV-2, which indicates that SARS-CoV-2 may cause NLRP3 inflammasome activation. SARS-CoV-2 can induce pyroptosis, particularly in lymphocytes, by induction of NLRP3 inflammasome. Elevated serum levels of IL-1 $\beta$  in COVID-19 patients also indicate the occurrence of pyroptosis, because IL-1 $\beta$  release is a downstream process of lymphocytes pyroptosis(22). In the study by Guan et al.10?? there were 914 patients out of 1099 with lymphopenia on admission while 370 cases (33.7%) had leukopenia. Huang et al.11?? (Your references do not correspond) highlighted an association between lymphopenia and the need for ICU care. They had 11 out of 13 cases (85%) in ICU-patients and 15 out of 28 cases (54%) in non-ICU patients with lymphopenia[22]. Wang et al.12?? found, 97 out of 138 cases (70.3%) showed lymphopenia. Wu et al.13 showed an association between lymphopenia and the development of acute respiratory distress syndrome (ARDS). A study done by Agrawal A et al in India highlighted a comparison of hematological parameters among asymptomatic and symptomatic COVID-19 patients. They had 9 out of 17 cases (52.94%) that were symptomatic and 10 out of 85 cases (11.76%) of asymptomatic patients with lymphopenia. A total of 19 out of 102 (18.63%) patients showed lymphopenia.

In our study, haematological biomarker showed of increased neutrophil-to-lymphocyte ratio at admission was found to be an independent risk factor for severe disease and mortality in COVID-19 patients. Neutrophil-to-lymphocyte ratio is stress and immune parameter [15]. In COVID-19, the elevated neutrophils indicate the degree of the inflammatory response, and the decreased lymphocytes indicate the degree of immune imbalance [11]. These associations are amplified by the concept of NLR [16]. The normal values of NLR in adults are in the range between ranges from 1 to 2.3. The cut-off value of NLR could predict poor COVID-19 infection and it varies widely (19). Liu et al. studied 115 COVID-19 infected patients and found the risk of developing severe disease is >50% in those with age  $\geq$  50 and NLR  $\geq$  3,13 at the time of first admission[12]. The study with 161 COVID-19 patients done by Wang et al found that NLR was statistically significantly higher in those who had severe infection than those who had more moderate infection. Lagadinou et al. Stated in their study with 64 adult patients in Greece that neutrophil lymphocyte ratio could predict the severity of SARS-cov2 infection. The presence of dyspnea, increased NLR were determined as independent risk factors for ICU admission, and NLR > 5.3 were found as optimal cut-off values for predicting admission to ICU. Several cohort studies on similar population found a statistically significant strong association of in-hospital mortality with neutrophil-lymphocyte ratio > 3.1. Due to some lack of a single universal definition for severe COVID-19 infection and the variable outcome measures used in different studies could explain this wide range and variances. However, there is an idea about the value of elevated neutrophil lymphocyte ratio in predicting severe COVID infection.

## CONCLUSIONS :

We concluded, that most of the hematological parameters analyzed behaved similarly in the two waves of COVID19 depending on the different disease stage of patients at the time of hospitalization. However, simple univariate analysis conducted in both waves revealed that hematological parameters such as anemia, neutrophilia, lymphopenia, monocytosis and increased NLR were associated with poor prognosis in patients admitted to the intensive care unit in the second compared to the first wave of covid-19. neutrophil-lymphocyte ratio associated with poor prognosis in patients admitted in intensive care unit like anemia, neutrophilia, lymphopenia, monocytosis and increased neutrophil-lymphocyte ratio seen severely in second wave than compared with first wave. Other hematological parameters did not showed show any significant changes. Also, increased hospital stay, demand for

oxygen supply, intensive care unit necessities **necessity** and increased mortality rates **rate was were** higher in COVID 19 second wave in India.

According to our knowledge many people died and children became orphaned during this COVID-19 pandemic in India. So an absolute **there is a** need of for both psychological and financial support to for the people who lost ~~there~~ **their** loved ones.

### Limitations of the study

There were some limitations in our study. Firstly, this was a retrospective study, therefore, complete information was not available for all the patients. Secondly, ~~though~~ our study was based on ~~the~~ **a** data from of the **a** single tertiary care centre in Tamil Nadu; large-scale **studies** involving other Tertiary hospitals are required.

### Statement of Ethics

This study was approved by **the** Ethics Committee of Saveetha Medical and Hospital. As this study was a retrospective study, there **were** no patient privacy data such as patient name, ID number, telephone and address ~~were~~ involved. Only demographic information and laboratory testing data of patients were collected and analyzed in this study.

### COMPETING INTERESTS DISCLAIMER:

**Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.**

### REFERENCE:

- 1).World Health Organization: Coronavirus disease (COVID-2019) weekly epidemiological update. 2021. Available at:<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>. Accessed January 5, 2021.
- 2).Jalali S.F., Ghassemzadeh M., Mouodi S., Javanian M., Akbari Kani M., Ghadimi R., Bijani A. Epidemiologic comparison of the first and second waves of coronavirus disease in Babol, North of Iran. Caspian J Intern Med. 2020 Fall;11(Suppl 1):544–550.
- 3).Second wave of COVID-19 in India: Dissection of the causes and lessons learnt <https://doi.org/10.1016/j.tmaid.2021.102126>
- 4).Huang C, Wang Y, Li X, et al: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395:497–506.
- 5).Wang D, Hu B, Hu C, et al: Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323:1061–1069.
- 6).Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020;95(6):E131-E153.

- 7).Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; 395: 1054-62.
- 8). Arentz M, Yim E, Klaff L, et al. Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA* 2020;323:1612-14.
- 9).Bhatraju PK, Ghassemieh BJ, Nichols M, et al. Covid-19 in Critically Ill Patients in the Seattle Region - Case Series. *N Engl J Med.* 2020; 382:2012-22.
- 10).Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther.* 2020; 5: 33
- 11).Naess A et al. (2017) Role of neutrophil to lymphocyte and monocyte to lymphocyte ratios in the diagnosis of bacterial infection in patients with fever. *Infection* 45, 299–307.
- 12).Azab B et al. (2010) Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. *The American Journal of Cardiology* 106, 470–476.
- 13).Henry BM, Santos de Oliveira MH, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019(COVID-19): a meta-analysis. *Clin Chem Lab Med.* 2020. Available from: <https://doi.org/10.1515/cclm-2020-0369> PMID: 32286245
- 14).Han Q et al. (2020) Role of hematological parameters in the diagnosis of influenza virus infection in patients with respiratory tract infection symptoms. *Journal of Clinical Laboratory Analysis* 34, e23191.
- 15).Yang AP, Liu J, Tao W, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int. Immunopharmacol.* 84, 106504 (2020).
- 16).Guan W-J et al. (2020) Clinical characteristics of coronavirus disease 2019 in China. *New England Journal of Medicine* 382, 1708–1720.
- 17).Kermali M et al. (2020) The role of biomarkers in diagnosis of COVID-19– a systematic review. *Life Sciences* 254, 117788.
- 18).Lagunas-Rangel FA (2020) Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *Journal of Medical Virology* 92,1733–1734.
- 19).Kong M et al. (2020) Higher level of neutrophil-to-lymphocyte is associated with severe COVID-19. *Epidemiology and Infection* 146, 1–6. Lagadinou M, Salomou EE, Zareifopoulos N, marangosm, Gogos C, Velissaris D. Prognosis of COVID-19: Changes in laboratory parameters. *Infez Med* 2020;28(1):89-95.
- 20).Mermutluoğlu Ç, Dursun R, Aktar F, et al. Evaluation of Inflammatory and Hematological Parameters in Patients Diagnosed with COVID-19. *Authorea* 2020.
- 21).Liu T, et al. (2020) The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. *EMBO Mol Med* 12, e12421.
- 22).Liu J et al. (2020) Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *E Bio Medicine* 55, 102763.
- 23). Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020;180(7):934-943.

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