RESEARCH

Correlation between lymphopenia and other hematological parameters with duration of hospital stay as a measure of severity in Covid-19: a retrospective study

Ila Singh[1*] , Vaishali D. Kotasthane[1] and Dhananjay S. Kotasthane[1]

Abstract

Purpose The SARS-CoV-2 (corona) virus is evolving fast because of the scale at which it has infected people around the world. Updated data is needed to manage Covid-19 efficiently with limited resources. After thorough literature review, there were very few studies that correlated variation of hematological parameters with duration of hospital stay.

Here, the aim of the study was to determine correlation between variation in hematological parameters and duration of hospital stay as a predictor of severity of disease.

Methodology The study was conducted at central laboratory of HIMS, Varanasi. The present study was an observational study done retrospectively. The study duration was from 1st July, 2020 to 30th October, 2020. RT-PCR-positive confirmed cases admitted in the institute whose CBC data was available over a period of multiple days were included in the study to find out the correlation.

Study design Patients were categorized into two study groups. Group I (A and B): duration of hospital stay from 2 to 10 days and Group II (A, B, and C): duration of hospital stay more than 11 days. Chi-square test was applied to both the groups to see whether correlation between variation in hematological parameters and duration of hospital stay was significant or not.

Result A total of 276 Covid-19 patients were included in the study. Patients were divided into groups and subgroups according to the duration of hospital stay. A total of 50% (103) of patients in Group I (hospital stay from 2 to 10 days) had lymphopenia as compared to 72.86% (51) of patients with lymphopenia in Group II (hospital stay from 11 to 40 days). Similarly, 44.17% (91) of patients in Group I had leukocytosis as compared to 72.86% (51) of patients with leukocytosis in Group II. The percentage increase in lymphopenia and leukocytosis in Group II was same, and this increase was found to be statistically significant (*p*-value < 0.05). Both these variables were increasing with increase in days of hospital stay.

Conclusion Hence, to conclude, complete blood count parameters especially lymphopenia and leukocytosis are most significant and economical in guiding the management protocol of Covid-19 patients as well as in predicting the number of days of hospital stay.

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Introduction

The original Covid-19 pandemic virus (founder variant) was Wu.Hu.1 (Wuhan virus). It originated in Wuhan, Hubei Province, China, in December 2019. Zhu et al. isolated and characterized the virus (preliminarily called 2019-nCoV) and renamed it as SARS-CoV-2 [1]. In a few months, variant D614G emerged and became globally dominant [2, 3].

The SARS-CoV-2 (corona) virus is evolving fast because of the scale at which it has infected people around the world. High levels of circulation mean it is easier for the virus to change as it is able to replicate faster, and because of the appearance of mutant strains, virus is reinfecting and reappearing in the population as a disease. This necessitates that ongoing researches and study should be continued. Several variant strains such as B.1.1.7 UK variant, P1 Brazil variant, B.1.351 South Africa variant, or B.1.617 double mutant variant strains are responsible for India's exploding second wave [2, 3].

Estimation of blood biochemistry and routine cell counts are good indicators for treatment assessment and predicting disease progression and severity. In the present study, we have focused on complete blood count as it is the most basic, efficient, and economic investigation and have tried to find out correlation between lymphopenia and other hematological parameters with duration of hospital stay in Covid-19 patients by retrospectively analyzing and interpreting the complete blood count (CBC) findings at our institute HIMS, Varanasi, which was designated as "Dedicated Covid Hospital" for treatment of Covid-19 patients.

After thorough literature review, there were very few studies that correlated variation of hematological parameters with duration of disease. One of those studies was conducted by Tan L. et al. The study was conducted over 90 Covid-19 patients, and their disease course was followed by a time-lymphocyte% model (TLM) with assessment of lymphocyte percentage at two time points, one at 10–12 days and other at 17–19 days. Patients were categorized separately as moderate, severe, and critically ill cases. Patients in each category were assessed for lymphopenia at two time points to determine the predictive ability of lymphopenia [4].

Aims and objectives

Here, the aim of the study was to determine correlation between variation in hematological parameters and duration of hospital stay as a predictor of severity of disease.

1. To determine correlation between lymphopenia and duration of hospital stay as a predictor of severity of disease
2. To determine correlation between leukocytosis and duration of hospital stay as a predictor of severity of disease
3. To determine correlation between thrombocytopenia and duration of hospital stay as a predictor of severity of disease

Material and methods

Place of study

The study was conducted at central laboratory of HIMS, Varanasi, after taking ethical clearance from institution.

Type of study

The present study is an observational study done retrospectively.

Ethical approval number

HIMS/IEC/98/2022. The study is approved and cleared by Institutional Ethical Committee.

Study duration

The study duration was from 1st July, 2020, to 30th October, 2020.

Inclusion criteria

RT-PCR-positive confirmed cases admitted in the institute whose CBC data was available over a period of multiple days were included in the study to find out the correlation between lymphopenia and other hematological parameters with duration of illness in Covid-19 disease.

Exclusion criteria

Covid-19-positive patients whose CBC data was available for one time only such as patients who were referred or discharged to other hospitals or died without follow-up CBC were excluded from the study.

Study design and methodology

Study groups were divided as shown in flowchart (Fig. 1) below. A total of 276 patients were included within the study whose CBC data was available and who fulfilled
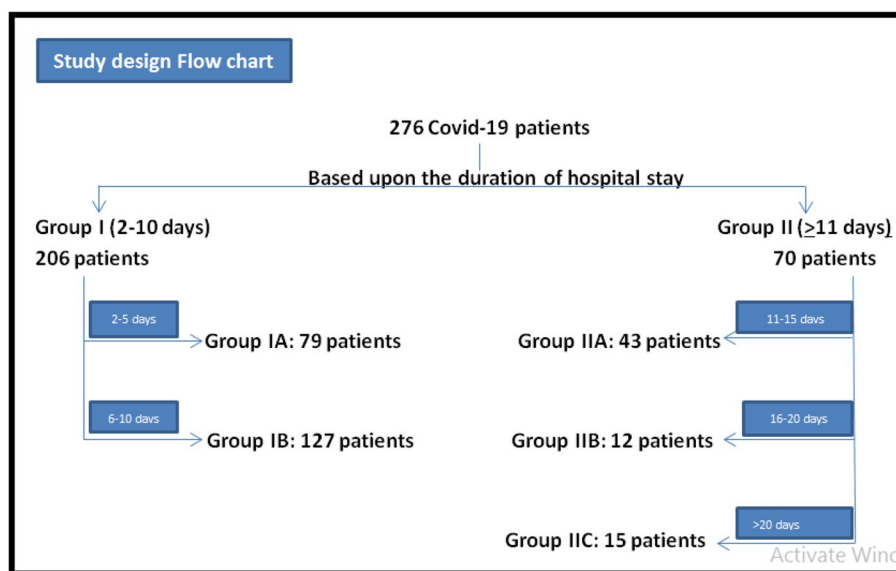
Fig. 1 Study design flow chart of Covid-19 patients. Patients were categorized into two study groups. Group I (**A** and **B**) and Group II (**A**, **B**, and **C**) based on the duration of hospital stay

inclusion and exclusion criteria of the study. Patients were categorized into two study groups — Group I and Group II: Group I (A and B): duration of hospital stay from 2 to 10 days comprised of 206 patients and Group II (A, B, and C): duration of hospital stay more than 11 days comprised of 70 patients. Group I patients were further divided into Group IA and Group IB. Group IA: duration of hospital stay from 2 to 5 days comprised of 79 patients and Group IB: duration of hospital stay from 5 to 10 days comprised of 127 patients. Group II was further divided into Group IIA, IIB, and IIC. Group IIA: duration of hospital stay from 11 to 15 days comprised of 43 patients. Group IIB: duration of hospital stay from 16 to 20 days comprised of 12 patients. Group IIC: duration of hospital stay more than 20 days comprised of 15 patients. Longest duration of hospital stay was found to be 40 days.

Methods

1. Data was collected from the hospital software.
2. After considering inclusion and exclusion criteria, hematological parameters of 276 Covid-19 patients were analyzed.
3. For this, samples were collected in EDTA vial, mixed thoroughly, and labeled properly.
4. Samples were run within 2 h, and complete blood count was obtained after running EDTA vacutainers on fully Automated Cell Count Analyzer-ICount 5 part hematology Analyzer of Iris Healthcare Brand.

5. All complete blood count parameters which included hemoglobin, RBC count, hematocrit, total leucocyte count, differential leucocyte count, absolute counts, and platelet count were measured, collected, and entered in hospital software.
6. The data was retrieved from hospital central laboratory software, and an Excel sheet was prepared, and data was entered.
7. Study definitions were assigned, and data was sorted out analyzed, compared, and interpreted accordingly.

Study definitions

- Leukocytosis: Total leucocyte count > 11,000 cells/cumm
- Leukopenia: Total leucocyte count < 4000 cells/cumm
- Lymphopenia: Absolute lymphocyte count < 1500 cells/cumm [5]
- Thrombocytopenia: Platelet count < 1.5 lakh/cumm
- Thrombocytosis: Platelet count > 5 lakh/cumm
- Eosinophilia: Absolute eosinophil count > 700 cells/cumm

Statistical analysis

Statistical analysis was done using SPSS software. Chi-square test was applied to both the groups to see whether the correlation was significant or not. If the p-value was > 0.05, then the results were considered not

significant, and if *p*-value was < 0.05, then results were considered to be statistically significant.

Result

A total of 276 patients were included within the study whose CBC data was available and who fulfilled inclusion and exclusion criteria of the study (Tables 1 and 2). Patients were divided into groups and subgroups as mentioned in methodology according to the duration of hospital stay. By grouping and subgrouping, the changes in hematological parameters became much more obvious with number of days of hospital stay.

Out of 276 patients, 219 (79.35%) patients were male, and 57 (20.65%) patients were female (Fig. 2). M:F ratio was 3.84:1. So clearly, male patients are roughly four times more affected than female patients by Covid-19, and also, it was observed that four patients who had died were all males.

Patients included were of the age group 10–89 years. Within each group and subgroup, patients between

Table 1 Correlation of lymphopenia, leukocytosis, eosinophilia, and thrombocytopenia with duration of hospital stay in Group I, Group IA, and Group IB

	From 2 to 10 days (Group I)		From 6 to 10 days (Group IB)		From 2 to 5 days (Group IA)		*p*-value
Total no. of Covid-19 patients	206	(In %)	127	(In %)	79	(In %)	
Patients with lymphopenia	103	50	71	55.90	31	39.24	< 0.05
Patients with leukocytosis	91	44.17	56	44.09	36	45.57	> 0.05
Patients with eosinophilia	28	13.59	16	12.60	12	15.19	–
Patients with thrombocytopenia	56	27.18	33	25.98	21	26.58	–

p-value < 0.05 is significant

Table 2 Correlation of lymphopenia, leukocytosis, eosinophilia, and thrombocytopenia with duration of hospital stay in Group II, Group IIA, Group IIB, and Group IIC

|	From 11 to 40 days (Group II)		From 11 to 15 days (Group IIA)		From 16 to 20 days (Group IIB)		More than 21 days (Group IIC)	
Total no. of Covid-19 patients	70	(In %)	43	(In %)	12	(In %)	15	(In %)
Patients with lymphopenia	51	72.86	29	67.44	10	83.33	12	80
Patients with leukocytosis	51	72.86	28	65.11	9	75	14	93.33
Patients with eosinophilia	12	17.39	6	13.95	3	25	3	20
Patients with thrombocytopenia	16	22.86	11	25.58	1	8.33	4	27

■ Male (79.35%)
■ Female (20.65%)
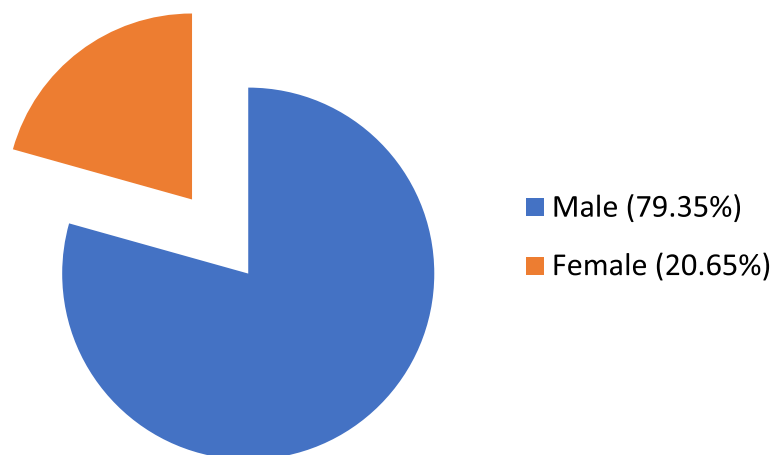
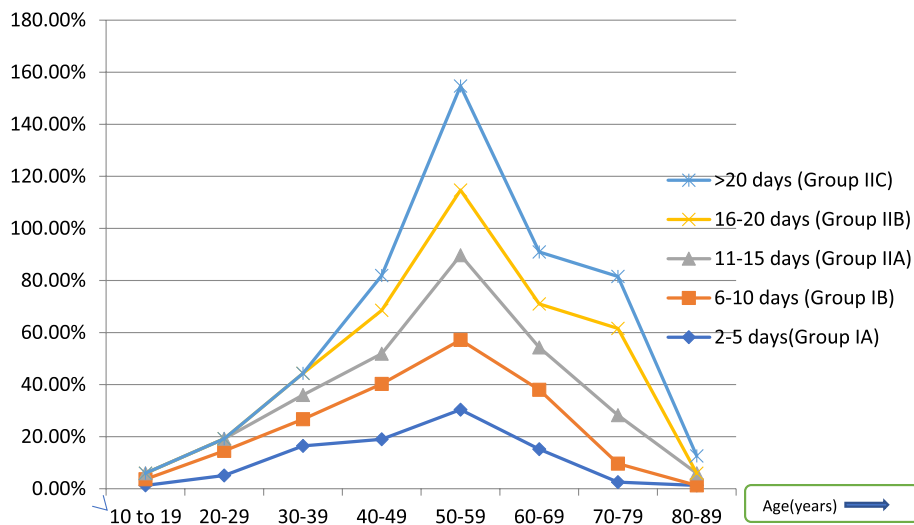**Fig. 2** Relative percentage of male and female affected by Covid-19

Fig. 3 In all the subgroups, patients within age group 50–59 were in maximum number when expressed as percentage of total number of patients in that subgroup, and also, there is no correlation between age that could be affected and number of days of hospital stay of Covid-19 patients

the ages 50–59 were mostly affected (Fig. 3). Only five patients were there between the ages 10 and 19 years and four patients in Group I (hospital stay 2–10 days). There was no patient < 10 years of age, only one patient of age 10 years, and four patients in their teens or adolescence. So, it is possible that even though children might be infected, but they seldom develop the disease, and if they develop disease, they recover fast.

A total of 50% (103) of patients in Group I (hospital stay from 2 to 10 days) had lymphopenia as compared to 72.86% (51) of patients with lymphopenia in Group II (hospital stay from 11 to 40 days). Similarly, 44.17% (91) of patients in Group I had leukocytosis as compared to 72.86% (51) of patients with leukocytosis in Group II. The percentage increase in lymphopenia and leukocytosis in Group II was same, and this increase was found to be statistically significant (p-value < 0.05). Both these variables were increasing with increase in days of hospital stay.

Percentage of patients with eosinophilia and thrombocytopenia in Group I was 13.59% and 27.18%, respectively, and in Group II, it was 17.39% and 22.86% which is almost similar, and it was found to be statistically not significant (p-value > 0.05).

Group IA (hospital stay 2–5 days) had 39.24% of patients with lymphopenia and 45.57% of patients with leukocytosis, whereas Group IB had 55.90% of patients with lymphopenia and 44.09% of patients with leukocytosis. In intragroup comparison, it was observed that as the duration of stay in hospital increased, there was statistically significant increase in the percentage of lymphopenia (p-value < 0.05), whereas effect of increase in leucocyte percentage was not proportionate to the

duration of stay in hospital (Fig. 4). From this, it could be inferred that lymphopenia is a better predictor of duration of stay in hospital or in other words severity of disease as compared to leukocytosis in the initial 10 days.

Group II was divided into Group IIA (hospital stay from 11 to 15 days), Group IIB (hospital stay from 16 to 20 days), and Group IIC (hospital stay for more than 21 days) (Tables 1 and 2). Group IIA, Group IIB, and Group IIC had 67.44%, 83%, and 80% patients respectively with lymphopenia and 65%, 75%, and 93% patients respectively with leukocytosis. As the duration of stay became more than 10 days, it was observed that lymphopenia and leukocytosis both became predictor of severity and stay for the number of days in hospital. So, if there is persistent lymphopenia and leukocytosis, it is predictable that the patient has chances of becoming severely ill with more number of days staying in hospital.

Out of 276 Covid-19 patients, 26.09% patients had thrombocytopenia. However, the number of patients with thrombocytopenia in both the groups, Group I and Group II, was similar, 27.18% and 22.86%, respectively, and statistically not significant (p-value < 0.05), and thus, it did not correlate with number of admission days in hospital as well as severity. Also, in both the groups, majority of the patients had mild thrombocytopenia.

Out of 276 Covid-19 patients, 14.49% patients had eosinophilia. However, the number of patients with eosinophilia in both the groups, Group I and Group II, was similar, 13.46% and 17.39%, and no correlation could be found with the number of days.

There was no significant fluctuation seen in monocyte and basophil count with number of days.
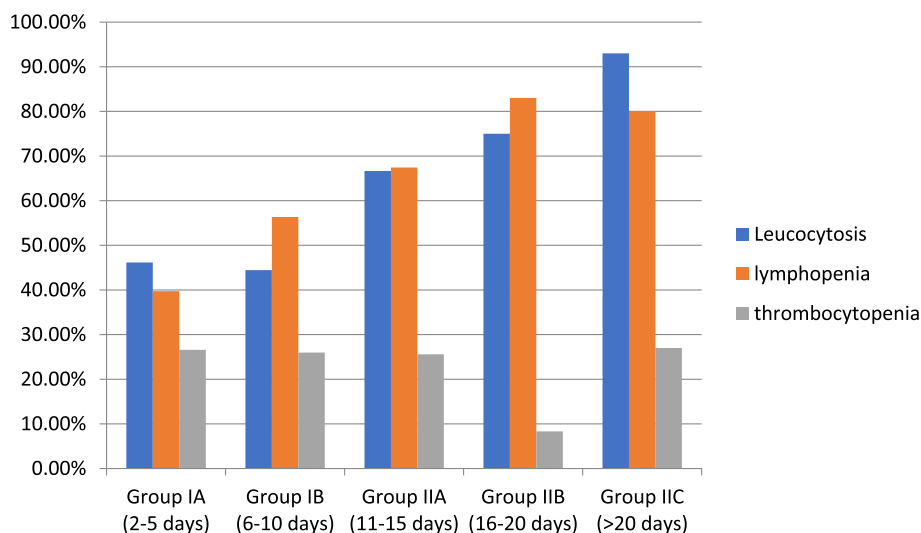
Fig. 4 Bar diagram presentation of variation of leukocytosis, lymphopenia, and thrombocytopenia with duration of hospital stay of Covid-19 patients

Hemoglobin assessment and its correlation have many confounding factors. In India, females generally have low hemoglobin level; the presence of other chronic diseases may also lower down the hemoglobin level. Baseline assessment of hemoglobin for which Covid-19 could be the obvious cause could not be assessed. So, change in hemoglobin level with number of days was not assessed.

Four patients who died of corona were included in the study. All four patients had lymphopenia; 3 patients (75%) had lymphopenia < 10%. Three patients (75%) had leukocytosis, and two patients (50%) had mild thrombocytopenia. So, patients with lymphopenia < 10% needs more supervision as these patients have higher mortality.

Discussion

Estimation of routine cell counts and blood biochemistry is good indicators for treatment assessment and predicting disease progression, severity, and duration of hospital stay. In the present study, we focused on complete blood count as it is the most basic, efficient, and economic examination. The aim of the present retrospective study is to assess the predictability of variation of cellular components of blood with time courses (disease severity) and hospital stay. In this study, assessment of Covid-19 dead patients' blood parameters was done separately, and duration of hospital stay was taken synonymous with disease severity. That implies that patients staying for greater number of days in hospital would obviously be more sick. Therefore, patients were solely categorized based upon the duration of hospital stay to analyze the variation in blood parameters with disease duration. According to Liu H. et al., patients who were more seriously ill stayed

longer [6]. The present study gives insights about key indicators of disease progression and outcome and provides clinical guidance.

Out of 276 patients, 219 patients (79.35%) were male, and 57 patients (20.65%) were female (Fig. 2). M:F ratio was 3.84:1. So clearly, male patients are roughly four times more affected than female patients by Covid-19, and also, it was observed that four patients who had died were all males. Now, there are various studies that show gender predilection affected by Covid-19, but one study worth's mention is by Kopel J. et al. who has examined various current literature on the gender differences among COVID-19 patients concludes that the current literature suggests that men tend to have a higher risk of severe infection and mortality related to COVID-19 [7]. The author further examined the possible biological mechanisms underlying these differences and believed that elevated estrogen levels in female COVID-19 patients might have reduced the severity and mortality of COVID-19 deaths through an elevation in the innate and humoral response [7].

Age group of Covid-19 admitted in our institute ranged from 10 to 89 years. In all the subgroups, patients within age group 50–59 were in maximum number when expressed as percentage of total number of patients in that subgroup, and also, there is no correlation between age that could be affected and number of days of hospital stay. This indicates that this age group was more susceptible to hospital admissions as compared to other age group. This correlated with the study conducted by Bakshi A. S. et al. where the median age was 52 years for the hospitalized patients

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who recovered from Covid-19 [8]. In the present study, median age for the patients who died of Covid-19 was 66 years which is similar to the median age in the study done by Bakshi A. S. et al. which was 60 years [8].

Only 5 patients were there between the ages 10 and 19 years and 4 patients in Group I (hospital stay 2–10 days). There was no patient below 10 years of age, only one patient of age 10 years, and 4 patients in their teens or adolescence. So, it is possible that even though children might be infected, but they seldom develop the disease, and if they develop disease, they recover fast [four patients in Group I (hospital stay 2–10 days)]. Study done by Lingappan K. et al. as well as current literature review suggests that children are less likely to have symptoms and had a lower rate of hospitalization, so there is significantly reduced prevalence, severity, and mortality among pediatric patients [9].

Routine hematological parameters observed in the present study included white blood cells and platelets, i.e., total white blood cell count and differential as well as absolute counts of different types of white blood cells and platelets. However, results obtained showed significant observation in total leucocyte count, lymphocyte count, neutrophil count, eosinophil count, and platelets.

Until now, there are few studies showing variation of cells in blood and duration of stay in hospital. Fan et al. critically studied the difference in hematological parameters between the ICU vs. non-ICU COVID-19 cases [10]. Liu X. et al., Guang Chen et al., Huang et al., Wan et al., and Zhang et al. all analyzed patients with different severity based upon criteria such as tachypnea, oxygen saturation, requirement of mechanical ventilation, and development of lung complications, whereas the present study mainly focuses on the variation of cell counts with duration of stay in hospital.

A very informative study was conducted by Tan L. et al. The study was conducted over 90 Covid-19 patients, and their disease course was followed by a time-lymphocyte% model (TLM) with assessment of lymphocyte percentage at two time points, 1 at 10–12 days, and other at 17–19 days. Patients were categorized separately as moderate, severe, and critically ill cases. Patients in each category were assessed for lymphopenia at two time points to determine the predictive ability of lymphopenia. A total of 61% of patients (55 patients out of 90) had lymphopenia at first time point (10–12 days) that included all patients in critically ill category. At second time point (17–19 days), it was observed that maximum patients who were from critically ill category had lymphopenia < 5%. In this way, lymphopenia was helpful in predicting the disease course and prognosis of Covid-19 patients [4].

In the present study, patients were not categorized into mild, moderate, or severe cases. Patients were categorized into two groups based upon the number of days of hospital stay − 1 to 10 days and beyond 10 days. They were further subdivided to make the observations more obvious. Patients with longer duration of hospital stay (more than 10 days) were assumed to be more sick. A total of 50.00% patients had lymphopenia in Group I, whereas number of patients with lymphopenia increased significantly to 72.86% in Group II with longer stay indicating that persistent lymphopenia increased hospital stay due to disease severity. Also, it was observed that except two patients, all patients with lymphopenia in Group II (49 out of 51 patients with lymphopenia) had lymphocyte percentage < 15%. Hence, firstly, Covid-19 patients show lymphopenia; secondly, persistent lymphopenia is a marker of longer hospital stay; and thirdly, patients with probability of longer hospital stay (> 10 days) will have lymphopenia percentage < 15%, so lymphopenia is also helpful in determining the disease course. There was a gradual increase in percentage of lymphopenic patients when they were further subdivided 39.24% (2–5 days), 55.90% (6–10 days), 67.44% (11–15 days), 83.33% (16–20 days), 80% (> 21 days) that makes the observation more evident. Thus, total number of patients from both the groups who had lymphopenia was 56%. This finding was comparable with Tan L. et al. [4].

Group IA had 39.24% of patients with lymphopenia and 45.57% of patients with leukocytosis, whereas Group IB had 55.90% of patients with lymphopenia and 44.09% of patients with leukocytosis. It was observed that as the duration of stay in hospital increased, there was significant increase in the percentage of lymphopenia, whereas effect of increase in leucocyte percentage was not proportionate to the duration of stay in hospital. From this, it could be inferred that lymphopenia is a better predictor of duration of stay in hospital or in other words severity of disease as compared to leukocytosis in the initial 10 days.

Further, it was observed that with increased duration of stay in hospital, more and more patients developed leukocytosis. Leukocytosis, irrespective of whether it represents a neutrophilia, lymphocytosis, or both, appears to herald bacterial infection or superinfection [1]. In Group I (2–10 days), it was 44.17%, and in Group II (11–40 days), it increased significantly to 72.86%. Also, the proportion of patients having leukocytosis increased gradually with time indicating increased severity of disease; 45.57% (2–5 days), 44.09% (6–10 days), 65.11% (11–15 days), 75% (16–20 days), and 93% (> 21 days). The available data suggest that neutrophilia is an expression of the cytokine storm and hyperinflammatory state or

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may also indicate superimposed bacterial infection [1]. In total, out of 276 patients, 51.45% developed leukocytosis.

Out of 276 Covid-19 patients, 26.09% patients had thrombocytopenia, but it did not correlate with the duration of hospital stay. Mostly, thrombocytopenia in Covid-19 patients was of mild degree. Similar to the present study, Fan et al. mentioned that none of their patients was moderately or severely thrombocytopenic [10].

Four patients who died of corona were included in the study. All four patients had lymphopenia, and three patients (75%) had lymphopenia < 10%. Three patients (75%) had leukocytosis, and two patients (50%) had mild thrombocytopenia. So, patients with lymphopenia < 10% needs more supervision as these patients have more severe disease and higher mortality.

Conclusion

Hence, to conclude complete blood count parameters, especially lymphopenia and leukocytosis are most significant and economical in guiding the management protocol of Covid patients as well as in predicting the number of days of hospital stay.

Lymphopenia is a better predictor of duration of stay in hospital or in other words severity of disease as compared to leukocytosis in the initial 10 days. Also, if the patient has persistent lymphopenia and leukocytosis, it is predictable that the patient has chances of becoming severely ill with more number of days staying in hospital.

Authors' contributions
On behalf of all the co-authors, I shall bear full responsibility for the submission. I confirm that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission.

Availability of data and materials
The datasets generated and/or analyzed during the current study are not publicly available as permission has to be taken from the institution in which the study was done, but the data is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Institutional ethical committee reference number is as follows: HIMS/IEC/98/2022. This is an observational study. The HIMS Research Ethics Committee has confirmed that no ethical approval is required. Does the manuscript report on or involve any animals, humans, human data, human tissue, or plants? Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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