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Evaluation and correlation of laboratory, radiological markers, and cytokine inflammation in patients with severe acute respiratory syndrome coronavirus disease 2019

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Evaluation and correlation of laboratory, radiological markers, and cytokine inflammation in patients with severe acute respiratory syndrome coronavirus disease 2019

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Abstract

Aim and objective

Fever and lymphocyte count to start the interventions and not wait for the upregulation of IL-6 and down regulation of SpO2 levels amongst the patients.

Background

In SARS-CoV-2 host immune response play a critical role in disease pathogenesis. The coronavirus not only activates antiviral immune responses, but it can also cause uncontrolled inflammatory responses categorized by marked proinflammatory cytokine release in patients with severe COVID-19, leading to lymphopenia. In this present article we have studied the basic parameters of COVID-19 that includes fever and lymphocyte count and their deep association with markers of disease prognosis and cytokine storm such as IL-6, HRCT score and SpO2 levels. With this basic routine clinical and laboratory parameters we can consider recurrent/prolonged fever and low count of lymphocytes are the earliest biomarkers of the upcoming cytokine storm.

Method

We conducted a hospital-based case-control study of patients who visited to the tertiary healthcare centre in Amravati (Maharashtra), India with a positive SARS-CoV-2 polymerase chain reaction (PCR) assay, from August 01, 2020 to July 03, 2021. Upon hospital visit, all patients underwent a HRCT chest score, complete blood count (CBCs), renal and liver panel, IL-6. Patients with fever and other symptoms which were not responsive to the treatment were admitted to the hospital. Patients who tested positive and admitted to the hospital were not discharged until they had 2 negative RTPCR tests 24 hours apart. Patient details including demographics, history of comorbidities associated with every patient, the records of the vital signs, symptoms and all the reports of radiological and blood assays were obtained from computerized medical records of the hospital.

Results

We screened total 1324 patients admitted to Tertiary health care center Amravati (Maharashtra), India, from 01 August 2020 to 03 July 2021. From these study, total 156 patients are excluded because of the number of patients, i.e. 106/156 patients founded with no prolonged fever but having same common symptoms. And remaining 50 patients excluded because of they were found with no short duration of fever and asymptomatic. Data was collected from the remaining 1168 (88.21%) as a cohort control group. The cases with recurrent/prolonged fever and short duration had median age [52 (21-83) v/s 52 (17-92) p=0.42] it shows that age is not statistically significant. On the other hand, the cases with recurrent/prolonged fever were more likely to have hypoxia were SpO2 level [90 (50-98)] v/s in short duration of fever were observed.[95 (45-98) p=0.02] shows statistically associated. Similarly, the HRCT chest score in recurrent/prolonged fever were found to be around [8 (0-24)] v/s [6 (0-22) p=0.027] it represents the significant difference.

Conclusion

Fever and lymphocyte count and their deep association with markers of disease prognosis and cytokine storm such as IL-6, HRCT score and SpO2 level. With this basic routine clinical and labortory parameters we can consider recurrent/prolonged fever and low count of lymphocytes are the earliest biomarkers of the upcoming cytokine storm. Therefore, study recommends the use of fever and lymphocyte count to start the interventions and not wait for the upregulation of IL-6 and down regulation of SpO2 which occurs in late stage.

Keywords: Coronavirus disease 2019, high-resolution computed tomography, interleukin 6, lymphopenia, oxygen saturation

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INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), initially identified in Wuhan China in December 2019, has led to a global pandemic that has not been witnessed for more than a century [1]. The pandemic, which within a year was declared as a health emergency, has caused huge devastation in terms of mortality and morbidity throughout the entire globe [2]. However, with the pandemic approaching a stable state, with the vaccination drives on full swing across all the countries, there is still a lot of concern about new emerging variants and the complications that would be associated with them, as the whole world is into relaxation of the norms and reopening of the schools and other outlets [3]. The lessons learnt in the second outbreak of the disease should be emphasized to get prepared for future outbreaks, which can save many lives.

Among the most serious complications of coronavirus disease (COVID-2) that led to the clinician's major challenge in treating the patients were the development of an atypical upper respiratory tract pneumonia and the ensuing uncontrolled cytokine storm in COVID-19 patients. This has emerged as a cornerstone in aggravating the symptoms and disease progression, and is considered the governing factor to COVID-19 mortality [1]. This suggests that factors that lead to the development of the cytokine storm, if identified at the early stage, can prevent the deleterious effect of the same. Prolonged fever is established as one of the causative factors for immune dysregulation in many viral diseases along with the SARS-CoV-2 study by Ng *et al.* [4], which have concluded that patients with prolonged fever had a more pronounced cytokine response and thereby landed up with severe hypoxia and poorer prognosis than the control group. Along with fever, another parameter that is shown to consistently raise the cytokine response is lymphopenia. In their review article, Li *et al.* [5] have quoted that lymphopenia is one of the most remarkable predictors of severe disease and a strong indicator for the forthcoming cytokine storm. Thus, it is clearly established in the literature so far that early interventions done to prevent these factors can result in better outcomes of the disease. However, there is a dearth of actual research studies done on large sample sizes to study the exact association between prolonged fever, lymphopenia, and cytokine response in SARS-CoV-2 patients. To the best of our knowledge, this is the first study to examine the correlation between prolonged fever, lymphopenia, and raised cytokine levels in COVID-19 in the Indian population.

PATIENTS AND METHODS

We conducted a hospital-based case–control study of patients who visited the tertiary health-care center in Amravati (Maharashtra), India, with a positive SARS-CoV-2 PCR assay, from August 1, 2020 to July 3, 2021. Upon hospital visit, all patients underwent an high-resolution computed tomography (HRCT) chest score, complete blood count, renal and liver panel, and interleukin 6 (IL-6). Patients with fever and other symptoms that were not responsive to treatment were admitted to the hospital. Patients who tested positive and admitted to the hospital were not discharged until they had two negative RTPCR tests 24 h apart [6]. A standardized form was used for recording the daily signs and symptoms, vital signs, blood reports, and treatment offered to the patients. Patient details including demographics, history of comorbidities associated with every patient, the records of vital signs, symptoms, and all the reports of radiological and blood assays were obtained from computerized medical records of the hospital.

Defining the criteria for the study

The patients with prolonged fever were defined as patients with fever lasting more than 7 days. Time span of fever was calculated from the date of first rise in the body temperature noted by the patient to the date of continuous recession of the fever. Fever was only considered as a temperature of 38.0°C. Cytokines analyzed in the study were the routinely performed IL-6 and lymphocyte count from the convalescent plasma of the patients. Lymphopenia cases were considered as patients with a lymphocyte count below the normal range (20–40%) of circulating lymphocytes in adults.

Patient consent form

Approval for the data collection by retrospective charts from the hospital board was taken with a waiver of written informed consent from the patients as no additional investigation/ intervention was done for the study purpose. In addition, whole data was anonymized and the information obtained in no way revealed any personal detail of the patient.

Statistical analysis

The Mann–Whitney U test was used to evaluate the difference in medians of continuous variation. For cytokine profile, Mann–Whitney U test was applied to find out the significance difference in fever pattern. Regression analysis was used to determine the statistical significance of variables to find out the dependency of lymphocyte on a radiological marker, that is oxygen saturation (SpO$_2$), HRCT chest score. Pearson's correlation coefficient is used to measure the strength of the relationship between the two variables. A monotonic relationship between

Table 1: Laboratory markers and radiological features of cytokine inflammation in coronavirus disease 2019

	Recurrent/ prolonged fever (n=315), n (%) or median (IQR)	Short-duration fever (n=853), n (%) or median (IQR)	P
Age	52 (21-83)	52 (17-92)	0.42
SpO$_2$ level	90 (50-98)	95 (45-98)	0.02
HRCT chest score	8 (0-24)	6 (0-22)	0.027
IL-6	7.8 (0.20-517)	5.8 (0.12-444.9)	0.021
Lymphocytes	16 (3-79)	21 (4-80)	<0.01

HRCT, high-resolution computed tomography; IL-6, interleukin 6; IQR, interquartile range; SpO$_2$, oxygen saturation.

two variables is a one in which either (a) as the value of one variable increases, so does the value of the other variable or (b) as the value of one variable increases, the other variable value decreases. Statistical analyses were performed in MS excel. A *P* value of less than 0.05 indicated statistical significance.

RESULTS

We screened a total of 1324 patients admitted to the Tertiary Health-Care Center, Amravati (Maharashtra), India, from August 1, 2020 to July 3, 202. From this study, a total of 156 patients are excluded because of the number of patients, that is 106/156 patients were found with no prolonged fever but having the same common symptoms. And the remaining 50 patients were excluded because they were found with no short duration of fever and were asymptomatic. Data was collected from the remaining 1168 (88.21%) as a cohort control group. Here, we divided the control data into a recurrent/prolonged fever with 315 (23.79%) patients in group A and in group B with 853 (64.27%) patients with short duration of fever; all the study participants were male, 37.73% (475/1324) of all participants were female and the median age (interquartile range) was 52 (17–92) years.

Outcomes
The cases with recurrent/prolonged fever and short duration had a median age of [52 (21–83) vs. 52 (17–92), *P* = 0.42]. It shows that age is not statistically significant (Table 1). On the

other hand, cases with recurrent/prolonged fever were more likely to have hypoxia with an SpO_2 level of [90 (50–98)] versus in short duration of fever, which was [95 (45–98), *P* = 0.02] showing a statistically significant association. Similarly, the HRCT chest score in recurrent/prolonged fever was found to be around [8 (0–24) vs. 6 (0–22), *P* = 0.027]; it represents the significant difference between them. The cytokine and chemokine concentration in recurrent/prolonged fever denoted as IL-6 [7.8 (0.20–517) pg/ml] were significantly high as compared with the short duration of fever [5.8 (0.12–444.9), *P* = 0.021] (Fig. 1). Inflammatory marker IL-6 is a key mediator for the development of cytokine storm in COVID-19 cases. The average of lymphocyte counts in recurrent/prolonged fever patients was [16% (3–79) vs. 21% (4–80), *P* < 0.01], which demonstrates statistical significance. The study thereby establishes a strong association of recurrent/prolonged fever and low lymphocyte count with drop in SpO_2 levels and high HRCT scores.

Graphical representation (Fig. 2) shows that the average of lymphocyte count (16%) in recurrent/prolonged fever is low as compared with short duration of fever, whereas 90% of SpO_2 depletion and higher HRCT chest were found in recurrent/prolonged fever as compared with short duration of fever. These findings are closely associated with disease severity and complications (Fig. 3 and Table 1). In this study we showed that IL-6 inflammatory cytokine dramatically increased in severely ill patients, with low levels of SpO_2 and higher HRCT score, which indicates the viral load in SARS-CoV-2.

The above graphical representation shows the depletion in SpO_2 level and higher score of HRCT chest. In patients with the recurrent/prolonged fever [8 (0–24), *P* = 0.01] the results are statistically significant.

Correlation coefficient between high-resolution computed tomography chest and oxygen saturation level
Pearson's correlation coefficient is the specific measure that quantifies the strength of the linear relationship between two variables in correlation analysis, that is if one variable increases it ultimately results in an increase of the other variable. Based on the patient's correlation test in recurrent/prolonged fever, we showed that there was a significant reverse relationship between HRCT chest score and SpO_2 (Fig. 3).This result also
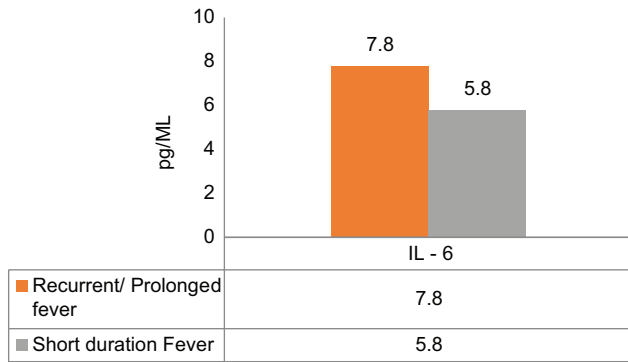

Figure 1: IL-6 cytokine levels in COVID-19 infection; comparison between two groups' recurrence/prolonged fever and short duration of fever. COVID-19, coronavirus disease 2019; IL-6, interleukin 6.
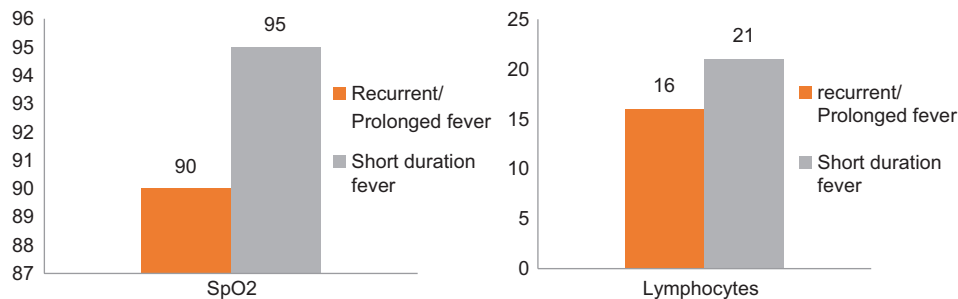

Figure 2: Graphical representation showing the severity of disease associated with recurrent/prolonged fever patients, which significantly depends on the low level of SpO_2 and lymphocyte count. SpO_2, oxygen saturation.
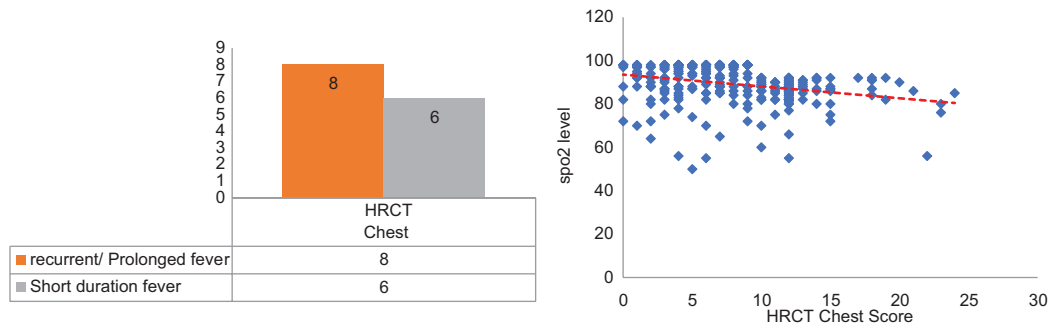
Figure 3: Correlation between HRCT chest and low level of SpO$_2$ levels demonstrated by radiological markers. HRCT, high-resolution computed tomography; SpO$_2$, oxygen saturation.

concludes that there is a weak negative correlation between SpO$_2$ level and HRCT Chest score. If HRCT chest score increases, then the SpO$_2$ level in patients decreases. Study shows that lymphocytes were significantly dependent on SpO$_2$ level decrease in lymphocyte count decreases in SpO$_2$.

DISCUSSION

The present study is the first to examine the correlation of lymphopenia, with two crucial parameters including HRCT and SpO$_2$ levels in the Indian population. In our study, we found that lymphopenia is a common feature in patients with COVID-19 and it is a clinical factor associated with disease severity and decreased level of SpO$_2$ that leads to multiorgan dysfunction and mortality. The clinical symptoms of COVID-19 infection varied from asymptomatic and symptomatic infections. Patients with mild disease may not initially require hospitalization and recommended home isolation with close monitoring of oxygenation. Patients are classified under moderate category when patients require oxygenation, respiratory rate of the patients is more than or equal to 24, and SpO$_2$ of less than 94% on room air (range, 90–94%). Although, much is already known and said about clinical parameters such as SpO$_2$ levels, fever pattern, HRCT, and inflammatory markers, these associations with each other and final outcomes on the patient are not being validated. In this paper, we have studied the basic parameters of COVID-19 that includes fever and lymphocyte count and their deep association with markers of disease prognosis and cytokine storm such as IL-6, HRCT score, and SpO$_2$ levels.

A study by Li *et al*. [5] corroborate with our findings. Patients with COVID-19 exhibit lymphopenia and high cytokine levels, which can be considered as a potential biomarker for disease progression. The host immune response to SARS-CoV-2 seems to play a critical role in disease pathogenesis and clinical manifestation. SARS-CoV-2 activates antiviral immune response in patients with severe COVID-19, leading to lymphopenia and lymphocyte dysfunction.

Another study by Rai and Thakur [7] also showed agreement with our study findings. They identified a predictor of hypoxia

in COVID-19 infection and laboratory variables, including leukocytosis, neutrophilia, lymphocytopenia, C-reactive protein, and IL-6 as predictors of hypoxia in COVID infection. These studies are a breakthrough to identify the risk factors in the early stage to reduce mortality in a resource-limited situation.

In this study, we have studied interdependent roles of all the parameters including clinical, laboratory, and radiological features. We identified the important risk factors of hypoxic condition or SpO$_2$ in prolonged fever of COVID-19 infection. We observed comorbidity such as depletion in SpO$_2$ and higher HRCT chest score, lymphopenia, and inflammatory markers like higher IL-6 associated with the development of severity of disease during prolonged fever. Hence, SARS-CoV-2-induced immune response abnormalities or overproduction may lead autoimmune diseases, which can cause septic shock and severe multiple organ dysfunction.

CONCLUSION

With the emerging fear of the Delta plus variant which is spreading throughout the globe, the lesson learnt from the first and second waves regarding diagnosis, prognosis, and treatment of COVID-19 will help us to increase preparedness for the fourthcoming complications.

Hence, in this article, we have studied the basic parameters of COVID-19 that includes fever and lymphocyte count and their deep association with markers of disease prognosis and cytokine storm such as IL-6, HRCT score, and SpO$_2$ level. With this basic routine clinical and labortory parameters we can that consider recurrent/prolonged fever and low count of lymphocytes are the earliest biomarkers of the upcoming cytokine storm. Therefore, the study recommends the use of fever and lymphocyte count to start interventions and not wait for the upregulation of IL-6 and downregulation of SpO$_2$ which occurs in the late stage.

Conflicts of interest
There are no conflicts of interest.

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