

Letter to the Editor

Inflammatory Markers in COVID-19

Dear Editor

As the cataclysmic repercussion of COVID-19 continues to spread across the globe, it is imperative to explore for markers that can be used to monitor disease severity in COVID-19 patients. Previous studies have suggested that Interleukin-6 (IL-6) and acute phase reactants such as C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, procalcitonin (PCT), fibrin degradation product (FDP), D-dimer were elevated in patients more severely affected by COVID-19¹ and H1N1.² These markers can be explored to predict the prognosis in COVID-19 patients in the initial stages so that aggressive and specific therapeutic interventions can be adopted timely.

A novel coronavirus disease COVID-19 emerged from Wuhan, China in 2019. Since then it has caused massive loss of lives. More than 5.86 million people have been affected and more than 360,000 patients have succumbed to the illness.³ SARS-CoV-2, the virus that causes COVID-19, belongs to the family of beta coronavirus and is postulated to have been spread from bats to humans, and later mutations have enabled it to transmit from human to human.^{4,5} In the past decade, 2 other zoonotic coronaviruses, the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), have been reported to damage the respiratory tract and cause severe outbreaks and deaths.^{4,5}

The clinical spectrum of the disease ranges from being asymptomatic to full blown acute respiratory distress syndrome (ARDS).^{6,7} The body's immune system, age and inherent comorbidities determine the varied presentation of the disease. Though the exact pathogenesis of COVID-19 remains elusive to mankind, the studies previously done on other coronavirus such as SARS-CoV have demonstrated release of a considerable amount of pro-inflammatory cytokines resulting in a cytokine storm syndrome.⁸ Cytokine storm syndrome is a diverse set of conditions of pleotropic causation associated with exuberant systemic inflammation, multi-organ failure, hyperferritinemia and high mortality.⁹ At present,

due to a lack of reliable markers, the monitoring of COVID-19 patients relies mainly on the observation of clinical parameters. The aim of this study is to evaluate the role of various inflammatory markers which have been used earlier in lung injury assessment in SARS-CoV and MERS-CoV.¹⁰

Method

All consecutive patients with confirmed COVID-19 infection admitted to Sawai Mansingh (SMS) Hospital, Rajasthan, India, from 1 March to 1 April 2020 were enrolled. Their clinical profile, management and outcomes were monitored. Patients were divided into severe and non-severe categories. Severe category was defined when any one of the following criteria was met:

- 1) Dyspnea, respiratory rate ≥ 30 /min
- 2) Oxygen saturation by pulse oximeter $\leq 93\%$ in resting state
- 3) Partial pressure of arterial oxygen (PaO₂) to fraction inspired oxygen (FiO₂) ratio ≤ 300 mm Hg

The medical records of patients were analysed for epidemiological (demographics and exposure history), clinical (signs, symptoms and underlying comorbidities), and laboratory parameters, besides radiological characteristics (CT scan and X-ray findings), treatment (antiviral therapy, antibiotics, respiratory support) and outcome data. This data was obtained from history given by patients and with data collection forms from electronic medical records.

Besides baseline investigations like blood cell count, liver function tests, renal function tests, quantitative assessment of LDH, serum ferritin, procalcitonin, CRP, FDP, D-dimer and IL-6 was also done and values >250 U/L for LDH, >270 ng/ml for serum ferritin, >0.5 ng/ml for procalcitonin, >5 mg/dl for CRP, >5 ug/ml for FDP, >0.5 ug/dl for D-dimer and >7 pg/ml for IL-6 were considered abnormal. These investigations were done on the day when the patient was admitted as a case of COVID-19 (Day 0) and on Day 7.

Oropharyngeal swab specimens were collected for extracting SARS-CoV-2 RNA from suspected

COVID-19 patients and placed into a collection tube containing virus transport medium (VTM) for extraction of total RNA. RT-PCR assay for SARS-CoV-2 was conducted by viral nucleic acid detection kit based on the recommendation by National Institute of Virology, Pune, India. Proportions for categorical variables were compared by the Fisher's Exact test.

Discussion

Forty-eight COVID-19 patients were included in this study. The mean age of patients in the severe category was 57.5 years, and in non-severe category 37.9 years. Male patients comprised 66.7% of total patients. Of the 48 patients, 32 (66.7%) were asymptomatic. In patients

who were symptomatic, cough (87.5%) was the most common symptom followed by fever (81.3%), myalgia (50%), headache (43.8%) and 6 patients (37.5%) had dyspnea. Four out of 6 patients requiring oxygen support had underlying comorbidities such as COPD (chronic obstructive pulmonary disease), diabetes mellitus, hypertension, hypothyroidism, CAD (coronary artery disease), CKD (chronic kidney disease).

Evaluation of the haemogram in severe category patients revealed that 83.3% patients had lymphopenia, 16.7% had thrombocytopenia and 16.7% had eosinopenia. In the non-severe category, there were 19% patients who had lymphopenia, 9.5% thrombocytopenia, 16.7% eosinopenia (Table 1).

Table 1. Laboratory Findings of COVID-19 Patients

Laboratory findings	All patients (n=48)	Non-severe (n=42)	Severe (n=6)	P value
Blood cell count				
Lymphopenia				
WBC <1.5*10 ⁹ /L, n (%)	13 (27.0)	8 (19.0)	5 (83.3)	<0.05
Thrombocytopenia				
Platelets <1.4*10 ⁹ /L, n (%)	5 (10.4)	4 (9.5)	1 (16.7)	0.503
Eosinopenia				
Eosinophils <1% of total WBC, n (%)	8 (16.7)	7 (16.7)	1 (16.7)	>0.05
CRP (mg/dl), median (range)				
>5 mg/L, n (%)	7 (14.6)	2 (4.7)	5 (83.3)	<0.05
Procalcitonin (ng/ml), median (range)				
>0.5 ng/L, n (%)	6 (12.5)	1 (2.4)	5 (83.3)	<0.05
LDH (U/L), median (range)				
>460 U/L, n (%)	8 (16.7)	3 (7.1)	5 (83.3)	<0.05
SGOT (U/L), median (range)				
>40 U/L, n (%)	11 (22.9)	6 (14.3)	5 (83.3)	<0.05
SGPT (U/L), median (range)				
>40 U/L, n (%)	11 (22.9)	6 (14.3)	5 (83.3)	<0.05
FDP (ug/ml), median (range)				
>5 ug/ml, n (%)	6 (12.5)	1 (2.4)	5 (83.3)	<0.05
D-dimer (ug/ml), median (range)				
>0.5 ug/ml, n (%)	6 (12.5)	1 (2.4)	5 (83.3)	<0.05
Ferritin (ng/ml), median (range)				
>270 ng/ml, n (%)	8	3 (7.1)	5 (83.3)	<0.05
IL-6 (pg/ml), median (range)				
>7 pg/ml, n (%)	6 (12.5)	0 (0)	6 (100.0)	<0.05

Overall, 22.9% patients had deranged liver function tests while 83.3% patients in the severe category had elevated SGOT/SGPT levels.

Inflammatory markers were predominantly increased in patients in the severe category namely CRP (83.3%), procalcitonin (83.3%), LDH (83.3%), serum ferritin (83.3%), FDP (83.3%), D-dimer levels (83.3%) and IL-6 (100%) (Table 1). The levels of inflammatory markers were significantly increased at the time of admission in patients in severe category. The median duration of performing these tests from the onset of symptoms was 7.5 days (range 6–14 days). Patients in the non-severe category had lower IL-6 levels and other inflammatory markers at the time of admission and took lesser time to get a first sample negative and had lesser symptoms.

In comparison, the mean duration of getting first and second COVID-19 sample negative from positive was 8 days and 9.2 days respectively in severe category. In patients who could maintain saturation at room air it was 7.7 days and 9.2 days respectively (Table 2). Only 1 patient who could not maintain saturation at room air was first given Bilevel Positive Airway Pressure (BiPaP) and later required a ventilator while others were able to maintain oxygen saturation with the help of nasal prongs or oxygen masks. One patient who eventually needed intubation had a very high spike (>500pg/ml) of IL-6.

In our study, more males were infected (66.7%) by SARS-CoV-2 probably due to more frequent foreign travels in pursuit of education and employment. This was lower than that reported by Chen et al¹¹ and Huang et al¹² where male patients comprised 73% of the enrolled cases, and higher than that reported by Wang et al (54.3%).¹³ The mean age of patients in the study was 40.4 years, which is a decade younger than that reported by Wang et al¹³ (56.0 years), Chen et al¹¹ (55.5 years) and closest to that reported by Huang et al¹² (49.0 years). The mean age of patients in the severe category was 57.5 years as compared to 37.9 years in the non-severe category, thus demonstrating that older patients were at greater risk of lung injury requiring ventilator support. Previous studies have demonstrated that lymphopenia and cytokine storm is associated with more severe infection by coronavirus.^{14, 15}

In our study, 83.3% patients belonging to the severe category had elevated CRP, LDH, ferritin, PCT, FDP and D-dimer while they were elevated in <5% patients belonging to the non-severe category. These laboratory parameters were elevated in most of the patients in the severe category and showed an overlap with each

Table 2. Management and Outcome of Patients Requiring Oxygen Support

Age, Sex	Treatment			Oxygen support				COVID-19 Tests		
	Lopinavir/Ritonavir	Chloroquine	Oseltamivir	Low flow O ₂ mask	High flow O ₂ mask	Non-invasive ventilation	Invasive ventilation	1st Positive	1st Negative	2nd Negative
67, M	✓	✓	✓	✗	✗	✓	✓	Mar 2	Mar 14	Mar 15
85, M	✓	✓	✓	✓	✗	✗	✗	Mar 10	Mar 14	Mar 15
38, M	✓	✓	✓	✓	✗	✗	✗	Mar 20	Mar 28	Mar 29
37, M	✓	✓	✓	✗	✓	✗	✗	Mar 18	Mar 28	Mar 29
58, M	✓	✓	✓	✓	✗	✗	✗	Mar 25	Mar 30	Mar 31
60, M	✗	✗	✗	✗	✓	✗	✗	Mar 30	-	-

Table 3. Laboratory Parameters of Patients at Day 0 and Day 7

Age, Sex	Days from onset of symptoms to admission (Day 0)	LDH (230-460 U/L)		CRP (0.0-5 mg/L)		Ferritin (12-270 ng/ml)		PCT (0.0-0.5 ng/ml)		FDP (0-5 ug/ml)		D-dimer (0-0.5 ug/ml)		IL-6 (0-7 pg/ml)	
		Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
67, M	13	1221	1453	8.9	10.2	1050	1500	1.2	2.2	9.1	15	1.5	2.2	512	700
85, M	14	1098	687	7.4	5.1	898	787	0.98	0.5	7.2	4.1	1.2	0.7	34	50
38, M	7	982	788	6.8	3.4	888	563	0.78	0.21	6.9	3.2	0.8	0.3	12	8
37, M	6	780	800	7	3.5	1120	232	0.77	0.09	7	3.2	1.4	0.2	72	11
58, M	8	439	231	4.4	2.2	800	321	0.4	0.12	4.2	2.1	0.2	0.25	75	10
60, M	7	515	333	8.3	3.9	112	70	0.72	0.49	6.3	4.8	1.7	0.3	65	70

other, indicating that if one single parameter could be developed to identify the patients at highest risk of mortality, then other parameters may not be done so as to conserve resources and decrease the costs of conducting these tests. The elevation in inflammatory markers in our study was more than that reported by Liu et al¹⁶ where CRP, LDH, D-dimer were elevated in 85.5%, 65.2%, 65.2% patients respectively in the severe category.

Paquette et al² reported that IL-6 concentrations were found to be significantly higher in H1N1 patients who required critical care support compared to patients who did not. Also, IL-6 levels were higher in patients who died (22.2%) compared to survivors (77.8%). Similarly, Liu et al¹⁶ reported that among the COVID-19 patients in whom IL-6 was assessed before and after treatment, significant decrease in IL-6 and improved CT assessment was found in 81.3% patients after treatment. In our study, we found that IL-6 was elevated in all patients belonging to the severe category while none of the patients belonging to the non-severe category had increased IL-6. These results indicate that IL-6 and other inflammatory markers may be valuable parameters in monitoring disease severity in COVID-19 patients.

Conclusion

The clinical spectrum of COVID-19 ranges from being asymptomatic to ARDS. Severely affected patients are characterised by cytokine release syndrome. Laboratory parameters such as IL-6 and other markers of inflammation such as CRP, PCT, ferritin, LDH, FDP and D-dimer can be used in monitoring severity of disease in COVID-19 patients instead of COVID-19 affected patients. Targeting IL-6 may be useful in treating the cytokine storm in severely affected individuals. However, more research is needed for the suitability of IL-6 as a therapeutic target and disease severity biomarker. Our study covered only the early COVID-19 cases of North India, but a larger sample size would be needed to conclusively establish the role of dynamic changes to the markers of the disease.

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