

# Role of cystatin C and calprotectin as potential early prognostic biomarkers in COVID-19 patients admitted to a dedicated COVID care facility

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## ABSTRACT

**Background:** The COVID-19 pandemic has claimed millions of lives. A tool for early prediction of severity and mortality risk is desirable for better utilization of health care facilities. Several biomarkers like D-dimer, lactate dehydrogenase (LDH), C-reactive protein (CRP) and some recently explored biomarkers like serum cystatin C and serum calprotectin have been proposed as prognostic markers of COVID-19, but their role as prognostic markers is so far undefined. The present work attempted to investigate the possible role of serum cystatin C and serum calprotectin as prognostic tools to predict severity and outcome ahead of time. **Material and Methods:** This observational cohort study was carried out on 95 COVID-19 patients admitted to a dedicated COVID care facility from mid-October 2020 to January 2021. Serial estimations of serum cystatin C and serum calprotectin levels were done and assessed for significant difference between severe (NEWS 2 score  $\geq 5$ ) and non-severe (NEWS 2 score  $< 5$ ) groups, survivors and deceased and on the basis of comorbidities at each time points. Survival analysis was done based on the optimal thresholds for severity and mortality, calculated from the receiver operating characteristic (ROC). **Result:** The results showed that median cystatin C levels were significantly higher on the first day in the severe group ( $P < 0.001$ ) and in patients with cardiovascular disease ( $P < 0.05$ ), chronic lung disease ( $P = 0.009$ ) and among patients who died ( $P < 0.05$ ). It remained raised on day 3 in severe ( $P < 0.05$ ) and deceased ( $P < 0.05$ ) group. Serum calprotectin levels were significantly higher in patients with chronic lung disease ( $P = 0.008$ ) and in those who died ( $P < 0.05$ ). **Conclusion:** Serum cystatin C could be used as a tool for early prognosis and therapeutic decision-making for COVID-19 patients. Serum calprotectin seems to be a better marker of critical illness.

**Keywords:** Calprotectin, COVID-19, prognostic biomarker, serum cystatin C

## Introduction

The corona virus disease 2019 (COVID 19) pandemic has caused unprecedented morbidity and mortality worldwide affecting millions of people. It is caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>[1]</sup> The usual course of the disease is divided into three phases: the

asymptomatic phase, invasion of the upper respiratory tract and involvement of the lower respiratory tract.<sup>[2,3]</sup> The last phase is associated with invasion of type-2 and type-1 pneumocytes and release of various inflammatory biomarkers like IL1, IL6, IL8, TNF- $\alpha$  and many others.<sup>[4]</sup> Most of the COVID-19 cases are asymptomatic or show mild course. A small percentage of patients show pathological immune response often associated with cytokine storm which is a state of hyperinflammation.<sup>[5]</sup> It is characterized by extensive tissue damage leading to multiple organ failure ultimately contributing to disease severity and death.<sup>[5,6]</sup> COVID-19 patients with higher levels of

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inflammatory markers are associated with poorer outcome.<sup>[7]</sup> So, early detection of those hospitalized patients who are prone to deteriorate and pass into a state of hyperinflammation/cytokine storm and early initiation of preventive measures may improve clinical outcome.

Several biomarkers have been proposed as prognostic markers of COVID-19 like D-dimer, LDH, CRP, aspartate aminotransferase, interleukin-6, blood urea nitrogen, creatine kinase, blood bilirubin, procalcitonin and white blood cells.<sup>[8-10]</sup> However, the predictive value of these markers is yet to be established.<sup>[8]</sup> Few recent studies explored the role of other inflammatory biomarkers like serum Cystatin C and serum calprotectin as prognostic markers of severity in COVID-19 patients but their role is not much known. Both serum cystatin C and serum calprotectin are inflammatory markers. Serum cystatin C is an endogenous cysteine proteinase inhibitor produced by all nucleated cells and is an established marker of kidney dysfunction.<sup>[11]</sup> Endogenous proteinases are secreted or leaked from lysosomes of dying or diseased cells. Serum calprotectin (S100A8/A9) is a calcium binding protein present in the cytoplasm of neutrophils and also expressed on the cell membranes of monocytes.<sup>[12]</sup> It is an alarmin released when neutrophils are activated or monocytes adhere to the endothelium and act as inflammatory biomarker.<sup>[13,14]</sup> A study showed higher levels of serum calprotectin in COVID-19 patients requiring mechanical ventilation.<sup>[15]</sup>

All the earlier studies on the roles of serum cystatin C and serum calprotectin levels in prediction of severity were retrospective and based on their levels at admission. Serial measurement of these biomarkers may provide additional information and better insight. The present study aimed at serial measurement of serum cystatin C and serum calprotectin levels in admitting COVID-19 patients and assessment of their potential role as early prognostic marker. Early prediction of COVID-19 patients who are potentially at risk of developing severe disease can be utilized for their early referral to specialized centers by the primary health care providers. This will not only help in better care of those needy but also help in prevention of overwhelming of the tertiary care centers and help in better utilization of the health care resources.

## Materials and Methods

### Study design

It was an observational cohort study. The independent variables like respiration rate, oxygen saturation, pulse rate, systolic blood pressure, level of consciousness or new confusion and oxygen supplementation were used for NEWS-2 scoring and categorizing the patients into severe and non-severe groups. The dependent variables like serum cystatin C, serum calprotectin, interleukin-6, procalcitonin, serum urea, serum creatinine and plasma proteins were assessed and compared in the two groups.

### Participant

The subjects for this study were recruited from patients admitted to a dedicated COVID-19 care center from mid October 2020 to January 2021. A total of 95 patients confirming to inclusion and exclusion criteria and who gave consent for the study were recruited. All recruited patients were confirmed for SARS-CoV-2 infection by RT-PCR test of nasal and pharyngeal swabs. Prior ethical approval was taken from the institute's ethics committee. Adult patients over the age of 18 years who gave consent were included and those who had kidney disease, hepatitis, cardiac failure or any malignancy were excluded from the study. One patient with unilateral kidney and patients with myasthenia gravis and HIV infection, were also excluded from the study.

## Materials and Methods

At the time of admission routine clinical examination was done following standard COVID-19 safety protocol. Important findings of the examination including pulse rate, respiration rate, blood pressure, temperature, SPO<sub>2</sub> and significant clinical features like weakness and shortness of breath were recorded on a data sheet. These parameters were also collected on subsequent occasions of blood sampling. After admission, collection of blood samples for biomarker estimation had been planned for days 1, 3 and 5. However, due to logistic constraints related to high infectivity and loss to follow up, only two blood samples, on day 1 and day 3, could be collected from all patients and the third sample on day 5 could be collected only from 36 patients. The serum from collected samples were separated by centrifugation and was refrigerated at -20°C until tested for cystatin C and calprotectin levels. In serum samples, cystatin C was analyzed using Beckman Coulter AU480 auto-analyzer using latex enhanced immunoturbidometric assay with the kit sourced from Sekisui laboratories, Japan. Serum levels of calprotectin were determined using Human Calprotectin ELISA kit sourced from Fine Test, Wuhan Fine Biotech Co. Ltd., China. All the test procedures recommended in the kit literature were followed. The data on other investigations including CRP, procalcitonin, serum ferritin, LDH, D-dimer, total protein, albumin, globulin, serum urea, serum creatinine, electrolytes and patient outcome were collected from medical records with due permission.

The recruited patients were divided into severe (score  $\geq 5$ ) and non-severe (score  $< 5$ ) groups based on NEWS-2 score on day 1 and again on day 3, and association of biomarkers with the severity of disease was assessed. The National Early Warning Score-2 (NEWS-2) is a standardized clinical scoring system developed by the Royal College of Physicians for determination of degree of illness of a patient and initiation of early critical care intervention in severe patients.<sup>[16]</sup> The scoring is based on six physiological parameters, namely, respiration rate, oxygen saturation, pulse rate, systolic blood pressure, temperature and level of consciousness or new confusion. Two additional points are added for patients

requiring oxygen supplementation.<sup>[17]</sup> We did not consider fever for scoring, as most patients were on paracetamol at the time of admission. NEWS-2 score was recommended for use in the management of COVID-19 patients in critical care by the National Institute for Health and Care Excellence (NICE). Its use in COVID-19 patients was evaluated by Myrstad *et al.*<sup>[18]</sup> and was found to predict disease severity and in-hospital mortality.

### Statistical analysis

Data was analyzed using IBM Statistical Package for the Social Sciences (SPSS) version 22.0. Categorical data is presented as numbers and percentage. Continuous variables showing normal distribution are presented as mean ( $\pm$ SD) and those with non-normal distribution are presented as median and interquartile range. Parametric tests were used for the statistical analysis of the former and non-parametric tests for the latter.  $P < 0.05$  was considered significant for all statistical analyses and all statistical tests were 2-tailed. The sensitivity and specificity of serum cystatin C and serum calprotectin and their optimal cutoff values for predicting severity and death were analyzed using receiver operating characteristic (ROC) curve. Survival analysis was done after dividing the patients into two groups based on each cutoff value.

### Results

Out of a total of 95 patients, one was critical, requiring non-invasive ventilation; 15 were very severe, requiring  $\geq 10$  L/min oxygen supplementation; 22 were severe, requiring lesser oxygen supplementation; and rest were mild to moderate. Seventy patients were males and rest females. Mean age of the patients was 53 ( $\pm 11.4$ ) years and median hospital stay was 10 days. Most common complaints were fever, cough and shortness of breath. Ten patients died and rest survived. Based on NEWS-2 score, 32 patients were severe on day 1 and 24 on day 3. Severe and non-severe groups are compared in Table 1. There was no difference in the mean age and day of illness on admission in the two groups. The patients in severe group had significantly higher days of hospital stay ( $P < 0.001$ ), higher levels of serum cystatin C ( $P < 0.001$ ), CRP ( $P = 0.002$ ), procalcitonin ( $P < 0.001$ ), serum ferritin ( $P < 0.001$ ), LDH ( $P < 0.001$ ), D-dimer ( $P < 0.001$ ), urea ( $P < 0.001$ ), creatinine ( $P = 0.016$ ) and lower levels of total protein ( $P = 0.001$ ), albumin ( $P = 0.001$ ), and serum potassium ( $P < 0.05$ ). There was no significant difference in the level of calprotectin in the two groups. Serum cystatin C levels were higher in the severe group on day 2 as well.

Based on ROC curve analysis, the optimal thresholds for predicting severity and death of COVID-19 patients for both day

**Table 1: Clinical characteristics of COVID-19 patients based on severity**

Characteristics	Non severe		Severe		P (Mann-Whitney U)
	n	Mean (SD)	n	Mean (SD)	
Age	63	52.1 (11.1)	32	54.8	0.284 <sup>a</sup>
Day of illness on admission	62	8.4 (3.6)	31	7.3	0.153 <sup>a</sup>
Hospital stays	63	9 (4) <sup>*</sup>	32 (64.4)	12.5 <sup>*</sup>	0.000
Serum Cystatin C (mg/L)					
Day 1	63	0.78 (0.2) <sup>*</sup>	32 (63.7)	0.98	0.000
Day 3	71	0.81 (0.2) <sup>*</sup>	24 (57.8)	0.91 <sup>*</sup>	0.044
Day 5	26	0.86 (0.2)	9	0.85	0.937 <sup>a</sup>
Serum Calprotectin (ng/ml)					
Day 1	63	984.2 (348.9)	32	1014.1	0.702 <sup>a</sup>
Day 3	71	794.1 (534.4) <sup>*</sup>	24 (54.7)	903.2 <sup>*</sup>	0.168
Day 5	26	686.3 (449) <sup>*</sup>	09 (16.2)	770.9 <sup>*</sup>	0.565
CRP (mg/ml)	57	22.3 (49.5) <sup>*</sup>	30 (55.6)	94.2 <sup>*</sup>	0.002
Procalcitonin (ng/ml)	56	0.1 (0.2) <sup>*</sup>	29 (56.5)	0.3 <sup>*</sup>	0.000
Serum ferritin (ng/ml)	59	327.6 (504.3) <sup>*</sup>	28 (57.8)	819.8 <sup>*</sup>	0.000
LDH (U/L)	54	664 (218)	25 (54.5)	868.5 <sup>*</sup>	0.000
D-dimer ( $\mu$ g/ml)	51	0.62 (0.63) <sup>*</sup>	25 (47.5)	0.9 <sup>*</sup>	0.013
Total protein (g/dl)	63	7.04 (0.62)	32	6.4	0.001 <sup>a</sup>
Albumin (g/dl)	63	3.8 (0.34)	32	3.6	0.001 <sup>a</sup>
Globulin (g/dl)	63	3.2 (0.46)	32	3.1	0.318 <sup>a</sup>
Albumin/globulin ratio	63	1.2 (0.2)	32	1.2	0.511 <sup>a</sup>
Serum urea (mg/dl)	63	29.9 (11.9)	32 (63.5)	48.3	0.000 <sup>a</sup>
Serum creatinine (mg/dl)	63	0.84 (0.24) <sup>*</sup>	32 (57.5)	0.95 <sup>*</sup>	0.016
Serum uric acid (mg/dl)	63	4.98 (1.9)	32 (48.4)	4.7 <sup>*</sup>	0.912
Serum calcium (mg/dl)	63	9.1 (0.67) <sup>*</sup>	32 (41.9)	8.9	0.123
Serum phosphorus (mg/dl)	63	3.5 (0.74) <sup>*</sup>	32 (44.1)	3.3	0.327
Serum sodium (meq/l)	63	135.4 (4.5)	32	135.1	0.810 <sup>a</sup>
Serum potassium (meq/l)	63	4.2 (0.6) <sup>*</sup>	32 (55.9)	4.6	0.025
Serum chlorine (meq/l)	63	101.4 (4.9)	32	100.8	0.575 <sup>a</sup>

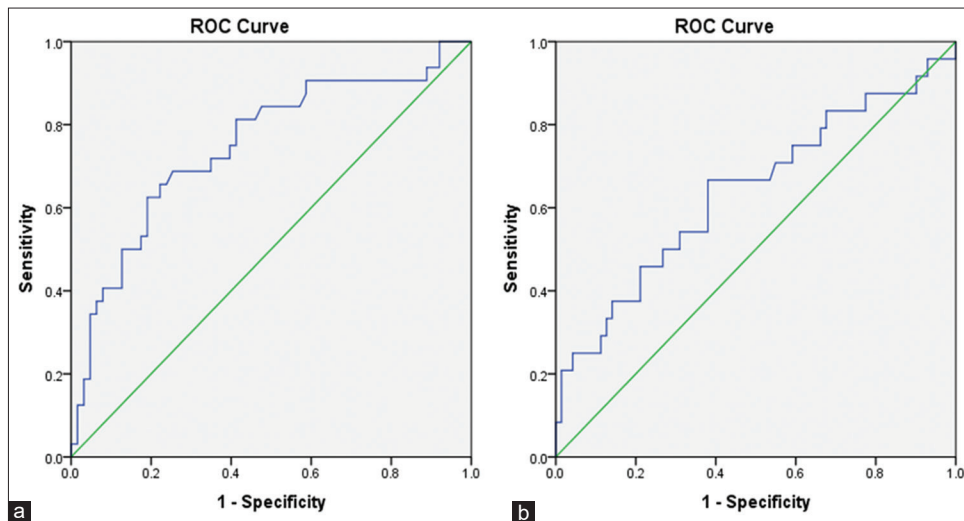
<sup>\*</sup>Median (IQR), <sup>a</sup>Independent sample t-test

1's and day 3's serum cystatin C values were obtained separately. For prediction of severity, only day 1's serum cystatin C values gave significant result [Figure 1]. The area under the curve (AUC), optimal threshold, specificity and sensitivity were 0.749, 0.888, 0.746 and 0.688, respectively. For prediction of mortality, both days' values gave significant result [Figure 2]. AUC, optimal threshold, specificity and sensitivity for day 1 were 0.695, 1.04, 0.859, and 0.60, respectively, and for day 3 were 0.719, 0.916, 0.718 and 0.70, respectively. Comparison of cystatin C and calprotectin levels based on outcome is summarized in Table 2. Only day 1's serum calprotectin values were significantly different in the outcome groups, so it was used for obtaining optimal cutoff for predicting risk of mortality. On ROC analysis, day 1 calprotectin levels gave significant result for prediction of mortality [Figure 3a]. AUC, optimal threshold, specificity and sensitivity were 0.692, 1202.5, 0.776 and 0.60, respectively.

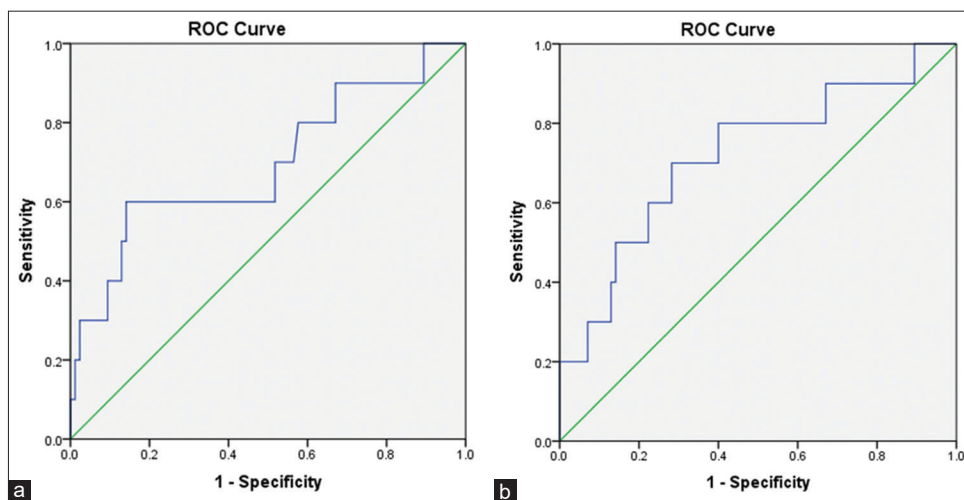
Based on the optimal thresholds obtained for groups giving significant result on ROC analysis, the patients were divided into two groups for each cutoff and Kaplan–Meier survival curves were obtained for each [Figures 3b and 4]. The results are summarized in Table 3. None of the cutoffs were effective in predicting severe groups. Cystatin C cutoff of 1.04 was effective in predicting death from day 1 values (Log rank = 4.67,  $P < 0.05$ ) and its cutoff of 0.916 from day 3 values (Log rank = 5.065,  $P < 0.05$ ). Serum calprotectin cutoff of 1202.5 was also effective in predicting mortality from day one values (Log rank = 6.984,  $P = 0.008$ ).

## Discussion

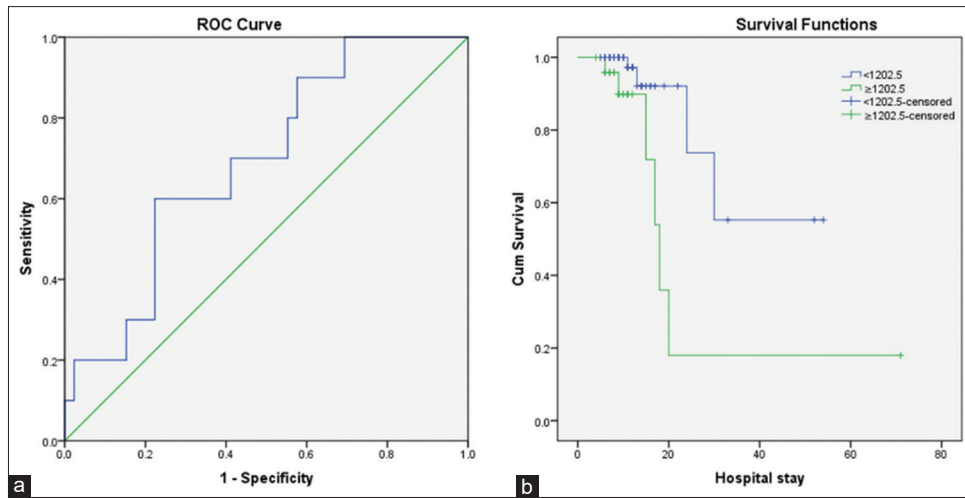
In this observational cohort study, serum cystatin C and serum calprotectin levels were assessed for any statistically significant difference between severe and non-severe groups, surviving and



**Figure 1:** Receiver operating characteristic curves obtained from serum cystatin C levels of day 1 (a) and day 3 (b) for predicting severity (based on NEWS 2 score) in moderate to severe COVID19 patients. Area under the curve, optimal threshold, specificity and sensitivity for day 1 were 0.749, 0.888, 0.746 and 0.688 and for day 3 were 0.638, 0.864, 0.624 and 0.667 respectively



**Figure 2:** Receiver operating characteristic curves obtained from serum cystatin C levels of day 1 (a) and day 3 (b) for predicting mortality risk in moderate to severe COVID19 patients. Area under the curve, optimal threshold, specificity and sensitivity for day 1 were 0.695, 1.04, 0.859 and 0.60 and for day 3 were 0.719, 0.916, 0.718 and 0.70 respectively



**Figure 3:** (a) Receiver operating characteristic curve of serum Calprotectin values of day 1 for predicting mortality risk in covid 19 patients. The area under the curve, optimal threshold, specificity and sensitivity were 0.692, 1202.5, 0.776 and 0.60 respectively. (b) Effect of day 1 Calprotectin value on survival. Kaplan-Meier survival curve of covid 19 patients with a cut-off value of 1202.5 ng/l

**Table 2: Serial changes in serum cystatin C and serum calprotectin levels in COVID-19 patients based on outcome**

Parameters	Day 1	P	Day 3	P	Day 5	P
	(n=95)		(n=95)		(n=36)	
	Survived: 85		Survived: 85		Survived: 32	
	Death: 10		Death: 10		Death: 04	
	Mean (SD)		Mean (SD)		Mean (SD)	
Serum cystatin C						
Survived	0.82 (0.23)*	0.045	0.82 (0.21)*	0.044	0.83 (0.25)	0.173
Death	1.17 (0.55)		1.1 (1.6)*		1.02 (0.4)	
Serum calprotectin						
Survived	966 (339.8)	0.024	824.3 (476.8)*	0.168	700.2 (511)*	0.513
Death	1234.52 (427)		929.9 (926.4)*		736.1 (344.6)	

\*Median (IQR)

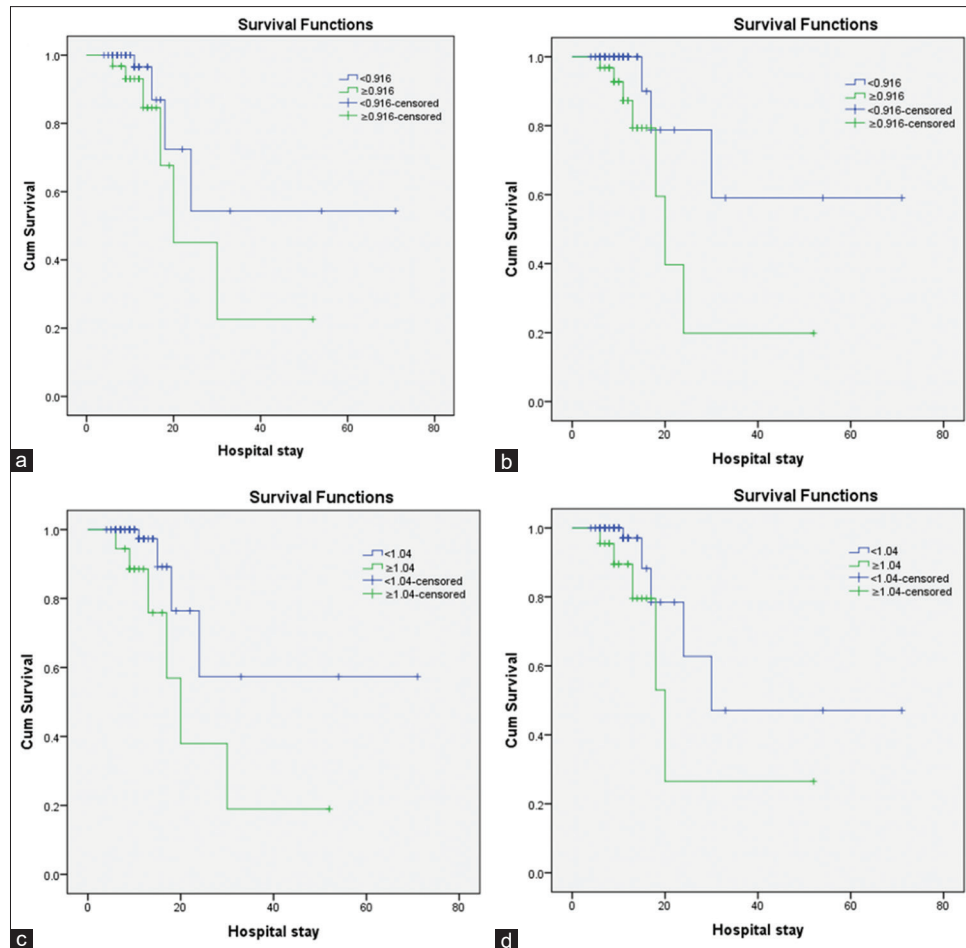
**Table 3: Survival characteristics of study population based on optimal cutoffs obtained from ROC curves for predicting mortality**

Group	No. of cases	No. of deaths	No. censored	Mean survival time in days (95% CI)	Log rank (P-value)
Based on day 1 cystatin C values					
≥0.916	31	6	25 (80.6%)	28 (16, 39)	1.980 (0.159)
<0.916	64	4	60 (93.8%)	47 (28, 67)	
≥1.04	18	6	12 (66.7%)	25 (14, 37)	4.670 (0.031)
<1.04	76	4	72 (94.7%)	49 (30, 68)	
Based on day 3 cystatin C values					
≥0.916	31	7	24 (77.4%)	25 (14, 36)	5.065 (0.024)
<0.916	64	3	61 (95.3%)	51 (33, 69)	
≥1.04	22	5	17 (77.3%)	26 (11, 41)	2.784 (0.095)
<1.04	72	5	67 (93.1%)	45 (28, 63)	
Based on day 1 calprotectin values					
≥1202.5	25	6	19 (76.3%)	26 (9, 43)	6.984 (0.008)
<1202.5	70	4	66 (94.3%)	41 (30, 52)	

non-surviving groups and also between patients with and without any of the comorbidities like chronic lung disease, cardiovascular disease, diabetes or hypertension. The results from 95 patients showed serum cystatin C levels to be significantly higher in severe group and in patients with cardiovascular disease, chronic lung disease and among patients who died [Table 4]. In severe and deceased group, it was consistently raised on day 1 and day 3 [Tables 1 and 2]. Serum calprotectin levels were significantly higher in patients with chronic lung disease and in those who died.

Higher cystatin C levels in severe and deceased patients are in line with earlier studies, despite of the fact that other factors that may increase serum cystatin C levels such as renal dysfunction, hepatitis, carcinomas or autoimmune conditions were excluded from the study.<sup>[19–21]</sup> These findings are consistent with earlier studies. Chen *et al.*<sup>[19]</sup> assessed the relationship between baseline serum cystatin C and COVID-19. They divided the patients into quartiles based on serum cystatin C levels and observed significantly more severe inflammation and worse outcome in highest quartile. In this study, highest cystatin C quartile was also an independent risk factor for critical illness and all-cause mortality, even after exclusion of patients with chronic kidney disease. Serum cystatin C was found to be an





**Figure 4:** Effect of day 1 and day 3 serum cystatin C levels on survival. Kaplan-Meier survival curves for COVID-19 patients with a cut-off value of 0.916mg/L (a) for day 1, (b) for day 3 and a cut-off value of 1.04 mg/L (c) for day 1 and (d) for day 3

independent risk factor in another retrospective study in which prognostic factors were studied in the discharge and death groups. However, the significantly different mean age of the two groups was a significant confounder which may have affected the result.<sup>[21]</sup> Our study is also in line with a meta-analysis which observed association of higher serum cystatin C concentrations with disease severity and increased mortality in hospitalized COVID-19 patients.<sup>[22]</sup> Thus, the results of our study support the possible role of serum cystatin C as an early prognostic marker of COVID-19. This is also supported by the fact that cystatin C is a major secretory product of alveolar macrophages and a potent regulator of inflammation.<sup>[23]</sup> This may be one of the reasons for its rise in patients with chronic lung disease. A recent study has also demonstrated that gene expression of cathepsin L is promoted by SARS-CoV-2 infection both *in vivo* and *in vitro*.<sup>[24]</sup> Cystatin C, being the main inhibitor of cathepsin L, may be one of the reasons for its rise in severe COVID-19 patients.<sup>[25]</sup>

There is a common perception that presence of comorbidities like diabetes or hypertension in COVID-19 patients is associated with severity and adverse outcome. Earlier studies have shown conflicting results. We did not observe any significant difference

in either mean serum cystatin C or mean calprotectin levels in patients with and without these conditions. This is in line with study by Li *et al.*<sup>[21]</sup> who also observed no significant difference in mean cystatin C levels in patients with and without these conditions. A systematic review and meta-analysis also observed no association between standardized mean difference and hypertension.<sup>[22]</sup> In contrast highly significant difference in serum cystatin C levels in patients with and without these conditions were observed by Chen *et al.*<sup>[19]</sup>

We observed significant difference in the value of calprotectin between deceased and survived groups but not between severe and non-severe groups. In line with our results, Chen *et al.* observed significantly raised serum calprotectin levels in COVID-19 patients admitted to the ICU.<sup>[26]</sup> Bauer *et al.*<sup>[27]</sup> also observed significant difference in serum calprotectin levels between with and without 90-day mortality, but, in contrast, they did not observe any significant difference in COVID-19 patients treated in the ICU. However, its small sample size was a major limitation. Calprotectin is an inflammatory biomarker that is released when neutrophils get activated.<sup>[13,14]</sup> Conditions other than COVID-19 have also showed elevated serum calprotectin levels like critically

**Table 4: Association of day 1 serum cystatin C/calprotectin with gender, outcome and comorbidities in COVID-19 patients**

Characteristics (n)	Cystatin C (mg/L)		Calprotectin (mg/L)	
	Median (IQR)	P (Mann-Whitney U)	Mean (SD)	P (t-test)
Gender				
Male (70)	0.84 (0.2)	0.577	1010.4 (34.2)	0.464
Female (25)	0.81 (0.3)		949.1 (339.3)	
Outcome				
Survived (85)	0.83 (0.2)	0.045	966 (339.8)	0.024
Death (10)	1.1 (0.6)		1234.5 (427)	
Comorbidities				
Diabetes mellitus				
No (53)	0.82 (0.2)	0.710	1049 (367.9)	0.125
Yes (40)	0.9 (0.3)		933.4 (341)	
Hypertension				
No (49)	0.81 (0.3)	0.755	978.9 (312)	0.566
Yes (44)	0.87 (0.2)		1022 (408)	
Chronic lung disease				
No (86)	0.82 (0.2)	0.009	971.5 (335.2)	0.008
Yes (7)	1.2 (0.6)		1340.4 (490.4)	
Cardiovascular disease				
No (88)	0.82 (0.3)	0.025	1000.3 (362.7)	0.722
Yes (6)	0.97 (0.4)		946 (305.7)	

ill patients, critical patients with sepsis, and is associated with renal and respiratory dysfunction and predicts long term mortality risk.<sup>[28,29]</sup> Another study showed higher levels in COVID-19 patients requiring mechanical ventilation.<sup>[20]</sup> These observations and the fact that calprotectin was raised in deceased patients [Table 2] in our study suggest its possible role in prediction of critical illness and death.

In line with earlier studies, the levels of other inflammatory biomarkers including CRP, procalcitonin, serum ferritin, LDH, D-dimer, etc., were significantly raised in severe group in our study.<sup>[8,9,20,30-32]</sup> However, the role of these markers are reported to be complementary to serum cystatin C rather than to be separately associated with inflammation or renal function.<sup>[22]</sup> Besides, significantly lower levels of albumin and protein and higher levels of serum urea and creatinine in severe group point toward liver and kidney dysfunction. But the involvement of these organs in the severe group seems to be subclinical as median values of these biomarkers are within normal range with only marginally raised serum urea level [Table 1]. These biomarkers are significantly different in outcome group too but with values within normal range.

The optimal threshold of cystatin C for predicting mortality risk was 1.04 mg/L with sensitivity of 0.60 and specificity of 0.865 from first day's sample and 0.916 mg/L with sensitivity of 0.70 and specificity of 0.718 from third days sample. These cutoffs are higher than the optimal threshold of 0.888

obtained from an earlier study.<sup>[21]</sup> The survival analysis taking these optimal thresholds as cutoff showed that the cutoff of 1.04 mg/L gave significant result only for day 1 values and cutoff of 0.916 mg/L gave significant result only for day 3 sample [Table 3]. This inconsistency may be due to the small sample size, so we recommend further studies with larger sample size for optimization of the cutoff levels for predicting severity and mortality in COVID-19 patients. Nevertheless, our study supports the result of earlier studies that severe COVID-19 patients have raised serum cystatin C levels even in the absence of other factors known to raise its level and so, it can be reliably used in predicting severity and mortality risk in COVID-19 patients. The optimal threshold of serum calprotectin for predicting mortality risk was 1202.5 ng/ml with sensitivity of 0.60 and specificity of 0.776. On survival analysis, this cutoff gave significant result from the day 1 sample but not from day 3 sample. This again may be due to the small sample size, but it may also be due to very small number of critically ill patients.

## Conclusion

To conclude, our study gave good evidence that there is early and persistent rise in serum cystatin C levels in severe COVID-19 patients and is capable of predicting severity and mortality risk in these patients. Optimal cutoff value for prediction of severity and mortality risk needs to be optimized by larger studies. Serum calprotectin levels seem to be significantly raised in very critical patients. However, further studies with larger sample size will give better insight.

## Limitations

Owing to logistic issues related to high infectivity of COVID-19 patients and management protocols, only limited subjects could be recruited in the study. For the same reasons, follow-up sampling also suffered resulting in the limited sampling on day 5.

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## Key messages

Owing to the devastating effect of the COVID-19 pandemic across the globe and availability of limited health care infrastructure in countries like India, a sensitive and specific early prognostic biomarker for COVID prognostication is the need of the time. The present study highlights the potential role of two relatively less known serum biomarkers, that is, serum cystatin C and calprotectin, as an adjuvant in early prediction of severity and survival among moderate-to-severe COVID-19 patients.

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## Conflicts of interest

There are no conflicts of interest.

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