



# Diagnostic Value of Novel Presepsin and Inflammatory Biomarkers in Predicting the Clinical Course of COVID-19

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## Abstract

**Aim:** The diagnostic value of inflammatory markers to determine the severity of Coronavirus disease-2019 (COVID-19) is postulated in some recent studies, but conclusions were inconsistent. Hence, we intend to examine the utility of presepsin, procalcitonin and C-reactive protein in predicting the severity of COVID-19 infection.

**Methods:** Single-center cross-sectional study was undertaken at the Intensive care unit of a university hospital. Eighty consecutive cases diagnosed with Severe Acute Respiratory syndrome-Coronavirus-2 RNA between October 2020 and July 2021 were classified according to the severity of the disease. Laboratory data related to Procalcitonin and C-reactive protein was retrieved from investigations coinciding with the day of admission. The stored plasma was subjected to an enzyme-linked immunosorbent assay to determine plasma presepsin levels. Statistical test for measures of screening was employed and a receiver operator curve was generated.

**Results:** We have determined that presepsin is the most sensitive prognostic indicator (93.3%) with a strong statistical association ( $p < 0.001$ ) for COVID-19. 15.99 ng/L could be used as a reference level to predict the progressive clinical course. Relatively lower sensitivity (88%) and positive statistical correlation ( $p = 0.049$ ) of C-reactive protein with high-risk infection were also observed. Procalcitonin showed limited diagnostic and prognostic value in our series.

**Conclusion:** Our findings seem to demonstrate the role of presepsin in providing prognostic information in COVID-19 patients. Therefore, we suggest that early monitoring of presepsin with routine marker profile might help in identifying patients suffering from a more severe disease.

**Keywords:** SARS-CoV-2 patients, biomarkers, presepsin, prognosis

## Introduction

The Coronavirus disease-2019 (COVID-19) pandemic has become a major health concern across the globe resulting in at least 4.1 million deaths to date secondary to COVID-induced pneumonia and septicemia (1). According to CDC guidelines, a vast number of COVID-19 cases exhibit mild to moderate clinical course without the need for hospitalization. However, around 19% of cases suffer from severe disease reflected by dyspnea, hypoxia with up to 50% lung involvement. Unfortunately, 5% of these severe cases may progress to a critical stage, complicated by respiratory failure, shock, or multiorgan system dysfunction (2). Therefore, early diagnosis and predicting the severity of the disease is the key to implementing appropriate therapeutic interventions that would eventually improve

outcomes in patient. In this regard, laboratory medicine lies at the core of diagnosing and monitoring the vast majority of human diseases including COVID-19 (3).

The tools presently available in laboratory medicine for diagnosing COVID-19 include molecular testing, serological testing and inflammatory biomarkers (4). Among these tools, molecular testing has certain limitations such as; anatomic variations in the patient, inadequate sample collection and storage, genetic changes in the virus and use of unsuitable reference range. Also, the biological source used to quantify the viral RNA, have a low detection rate and is not easy to obtain (5). Similarly, serological testing has the disadvantage of giving positive results after a month of the onset of symptoms (6). This leaves us with the choice of inflammatory biomarkers which are easy to

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quantify, cost-effective, have better accuracy, potential to predict disease severity and are measured in a biological material that is easy to procure such as blood (7).

In this regard, inflammatory biomarkers including procalcitonin (PCT), C-reactive protein (CRP) and recently discovered presepsin (P-SEP) has shown promising results to predict disease severity in recent studies (8). Hence, we have taken a novel initiative to conduct large-scale original research with the aim to determine the diagnostic value of P-SEP, PCT and CRP in predicting the clinical course of COVID-19 infection and also sought the association of these molecules with the progressive clinical course of infection. The outcome of this study may prove vital in identifying novel molecules that could serve as an indicator to predict rapidly progressive COVID-19 infection and also identify patients presented with advanced underlying disease.

## Methods

### Study Design and Ethical Considerations

A single-center, cross-sectional study on 80 clinically proven COVID-19 cases was undertaken at a tertiary care set-up in Karachi, Pakistan. Ethical approval was sought from Ethics Review Committee (1701219FHPAT) in February 2020 for the collection of the cases diagnosed with COVID-19 infection. Written informed consent was obtained from all participants.

### Sampling

All patients were receiving in-patient care at ICU and isolation wards of various tertiary care hospitals from October 2020 to July 2021. The diagnosis of COVID-19 was done using sensitive PCR and the subjects were selected using a non-probability consecutive sampling model. The cases were characterized into 2 groups: Moderate, and severe to critical, based on the clinical course according to CDC guidelines. Patients with the outcome of death were excluded from this study.

### Data Collection

The clinical data of lab investigation including blood culture, PCT and CRP at day 0 of admission and demographic data, age and gender were obtained from the hospital record, while the stored blood drawn at day 0 of admission for routine workup was also retrieved from specimen storage facility of the lab for P-SEP analysis.

### Laboratory Assessments

The frozen plasma was allowed to melt and 3 cc was subjected to an enzyme-linked immunosorbent assay (ELISA) for P-SEP, using a commercially available kit (Bioassay technology; cat. no E3754Hu). The reaction was performed according to manufacturer protocol in

a multidisciplinary BSL-2 category lab following WHO prescribed standard operating procedures (SOP) (9). Briefly, 3cc plasma was centrifugated at 3000 x g for 10 min. ELISA assay was done at room temperature by adding standard, streptavidin-HRP, sample, anti-IgA antibodies into the standard well. The well was then covered with sealer and the sample was incubated for 60 mins at 37 °C. Then, the sealer was removed and the plate was washed with wash buffer. The plate was then blotted onto paper towels and substrate solutions A&B were then added to each well. Finally, for color change stop solution was added and the optical density at 450 nm was determined by a microplate reader.

### Statistical Analysis

For statistical analysis, SPSS version 25.0 was used. The data were analyzed for normality by Kolmogorov-Smirnov test and the Shapiro-Wilk test and median was taken as the determinant of central tendency. Crosstabulation was performed for measures of screening i.e; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Receiver operator curve (ROC) was generated and the area under the curve and cut-off levels which corresponds to severe infection were estimated. The association of biomarkers with COVID-19 was assessed and  $p < 0.05$  was considered statistically significant. 95% confidence interval (CI) was used for all statistical calculations.

### Results

Overall, 60 participants were in a state of severe to a critical disease whereas the remaining 20 reflected a moderate pattern. Fever and dry cough were universally present in all subjects as the chief complaint. Severe to critically ill patients showed extensive lung damage on radiology and requires intubation or mechanical ventilation to deal with hypoxia while those suffering from moderate-intensity required minimal oxygen support to maintain saturation.

Table 1 presents statistical estimates of all participants included in this research. Among all patients, most subjects were men and aged above 60 years. The median age was recorded as 67.5 years. The median levels for P-SEP, PCT and CRP among patients within the severe disease group were observed as; 55.05 ng/L, 1.25 ng/mL and 136.95 mg/L respectively. On descriptive statistics, P-SEP proved to be the most sensitive marker (93.3%) for advanced COVID infection and showed the highest rate of identifying patients with underlying severe disease (76.7%), compared to CRP and PCT. The highest specificity rate was accounted for PCT (30%) in the current study.

On ROC analysis, a strong statistical association ( $p < 0.001$ ) of P-SEP with severe COVID infection was seen,

**Table 1. Distribution of all participants with respect to clinicopathological characteristics and statistical estimates**

Clinical characteristics	Distribution (n=80)							
Sex (male/female)	54/26							
Median age (years)	67.5 (33-90)							
Investigations (reference level) †	The severity of COVID-19		p (p=0.5) †	Sensitivity (%)	Specificity (%)	PPV <sup>§</sup> (%)	NPV <sup>‡</sup> (%)	AUC †
	Moderate † (n=20)	Severe-to-critical † (n=60)						
CRP (6 mg/L)	19	54	0.047 *	90	5	74	14.3	0.649
PCT (0.15 ng/mL)	14	47	0.549	78.3	30	77	31.6	0.455
P-SEP (5.5 ng/L)	17	56	0.001 *	93.3	15	76.7	42.9	0.778

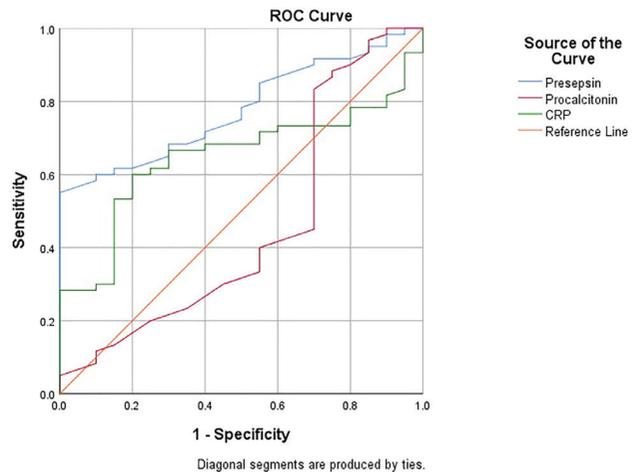
†Prescribed laboratory reference levels for positivity ‡CDC Guideline for COVID-19 † ROC indicator for null-hypothesis, \*Statistically significant ROC values †Positive predictive value ‡Negative predictive value †Area under the curve, PCT: Procalcitonin, CRP: C-reactive protein, COVID-19: Coronavirus disease-2019

reflected by the area under the curve (AUC) of 0.778. Also, CRP levels were significantly higher (p<0.05) among critically ill patients accompanied by the ROC curve (AUC) of 0.649 (Figure 1). No significant difference was recorded for PCT. When 15.99 ng/L was used as a cut-off for P-SEP, we observed 93.3% sensitivity and 85% specificity for severe infection whereas when cut-off levels for PCT; 0.156 ng/mL and CRP; 7.95 mg/L was considered, 78% sensitivity and 70% specificity for PCT while 88% sensitivity and 95% specificity for CRP was recorded.

**Discussion**

Recent CDC guidelines describe that the core principle behind the management of Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection (COVID-19) relies on monitoring respiratory rate, oxygen saturation & indicators of oxygenation such as; PaO<sub>2</sub>/FiO<sub>2</sub>. Unfortunately, the prescribed parameters are unreliable & subjective resulting in misdiagnosis, false interpretations & late diagnosis especially in cases with high-risk COVID-19 infection (10). So, there is an urgent need for novel bioassays that could identify the complex case or predict the clinical course of disease in order to improve outcomes among patients' by implementing appropriate treatment plans.

Measuring proinflammatory markers is considered as a cornerstone in risk assessment of patients with a wide variety of infectious diseases, due to their potential for predicting the clinical progression as well as guiding therapeutic decisions (8). Primarily, cytokine storm is the major factor behind the worst outcome among COVID-19 patients'. It is hypothesized that the interaction of macrophage and activated viral products triggers this release of cytokine and some immune mediators such as P-SEP (8). Therefore, measuring the concentration of P-SEP may provide valuable clinical information for



**Figure 1.** Receiver operating characteristic curve comparing levels of P-SEP (blue) and procalcitonin (red) and C-reactive protein (green) in severe to critical patients of COVID-19 COVID-19: Coronavirus disease-2019

risk stratification of COVID-19 patients'. In this context, recently, Domi et al. (11) and Ducastel et al. (12) showed the benefits of monitoring P-SEP in risk stratification of COVID-19 patients'. These findings are further strengthened by the results of a recent pooled analysis (8). Previously, Zaninotto et al. (3) and Fukada et al. (10) found a positive relationship of inflammatory biomarkers with COVID-19 and its clinical course in a limited set of cases and recommended further research. We, therefore, took a lead to investigate this association in a large group of patients with proven COVID-19 infection. To the best of our knowledge, this is the first original research conducted with the intent to determine the independent prognostic value of P-SEP, PCT and CRP for COVID-19 infection. Our findings may have long-lasting clinical implications. P-SEP is a recently identified biomarker which may facilitate in the

diagnosis of sepsis. Structurally, it is a truncated N-terminal soluble CD14 subtype (10). Whereas, PCT is a derivative of calcitonin, which is released primarily from C cells of the thyroid gland, monocytes as well as hepatocytes. PCT is a well-established molecule for identifying inflammatory response of infectious origin (13). Compared to P-SEP and PCT, CRP is produced by the liver in response to an ongoing inflammation anywhere in the body (14).

Numerous studies have reported the utility of P-SEP in predicting the severity and mortality in some inflammatory conditions related to infections (15). In the present research, we retrospectively compared the plasma levels of inflammatory markers including P-SEP between moderate and severe to critically ill COVID-19 patients'. The diagnosis of COVID-19 infection was performed by isolating SARS-CoV-2 RNA from nasopharyngeal swab specimens while patients' clinical status was determined in accordance with CDC guidelines (2). We have determined that P-SEP were significantly higher among severe to critically ill patients on admission than those who had a moderate disease. Furthermore, P-SEP has shown better sensitivity and specificity, which suggested that this molecule could serve as a useful prognostic indicator of COVID infection. Similar findings were reported in case series by Fukada et al. (10).

Despite no established mechanism of P-SEP elevation in COVID-induced pneumonia is documented to date, several studies have shown a strong relationship of P-SEP with short-term mortality in patients of acute respiratory distress syndrome (ARDS) (16). Because the potential of P-SEP to predict the progression of ARDS is well recognized, we believe this might assist clinicians to distinguish high-risk COVID-19 patients on admission and implement appropriate treatment strategies at an early stage.

In agreement with previous studies (17-19), we came across a weak statistical association of CRP accompanied by reasonably good sensitivity. We suggest that the observed relationship of CRP could be contributed by the inflammatory environment due to its natural proinflammatory characteristic, rather than the infection itself. However, this inference is subject to confirmation.

In a recent study by Tuncer et al. (20) PCT has shown the highest odds for predicting deterioration among COVID-19 patients', however, P-SEP status was not investigated either compared with other inflammatory markers. In contrast, some previous studies had shown limited or no role of PCT in the diagnosis and prognosis of COVID-19 (3,10). In the current research, although, PCT levels were elevated in the majority of cases, no difference between moderate and severe COVID-19 groups was seen. Moreover, the lowest sensitivity and specificity values were recorded for PCT in the present study. This observation is

in agreement with Zaninotto et al. (3) and Fakuda et al. (10). We believe that steady and slow increase of PCT is the inherent property under infectious conditions and thus the values taken on day 0 of admission may have biased our findings. Therefore, further research is recommended in this regard.

Another important aspect of the present study is the proposed cut-off levels of inflammatory markers for risk stratification of COVID-19 infection. Previously, Zaninotto et al. (3) suggested a cut-off level of P-SEP for severe infection; however, the cut-off range of other inflammatory biomarkers was not evaluated and compared. Also, the study was unable to measure the sensitivity and specificity for the proposed P-SEP cut-off (3). Contrary to that, we suggest independent concentrations of P-SEP, PCT and CRP can be used to identify COVID cases with poor outcomes. Besides that, we also described sensitivity and specificity for corresponding cut-off concentration which gives further strength to our findings. However, we recommend more research using a larger sample size to establish precise cut-off limits for each of those molecules.

#### Study Limitations

Our study has some limitations. Firstly, due to consecutive sampling, the distribution of cases across two groups was not equal which might have influenced our findings. Secondly, we were not able to include cases with outcomes of death and hence were unable to assess the utility of these molecules to predict mortality. This limitation is contributed by the hospital policy of refraining the data of those who died from COVID-19. Thirdly, the study was conducted with the aim to determine the prognostic utility of inflammatory markers in COVID infection and hence only values corresponding to the day of admission were taken into account, thus preventing us to compare plasma levels with disease progression. Lastly, further studies are needed to better explain the mechanisms responsible for elevated levels of inflammatory markers especially; P-SEP in SARS-CoV-2 patients' and in particular its relation with multiorgan failure syndrome. Despite these limitations, there are certain strengths of the present research which are worth mentioning. This study is among a few studies to analyze the role of pro-inflammatory markers for the severity of COVID-19. We have conducted our research on a larger sample size compared to previous studies and performed a comprehensive comparative analysis of conventional markers and novel P-SEP. Overall, our findings may offer a useful strategy to stratify high-risk COVID-19 patients' in ICU admission who would benefit from intensive treatment.

#### Conclusion

The data obtained seems to demonstrate the role of the inflammatory biomarkers in providing prognostic

information in high-risk COVID-19 patients', as already described in several other diseases. Nevertheless, our findings showed that among all analyzed molecules; P-SEP proved to be the most useful tool in predicting the severity of COVID-19 infection. Further multicenter studies with a large number of subjects are warranted to confirm our findings.

#### Authorship Contributions

Concept: S.S., F.A.B., Design: S.S., F.A.B., Data Collection or Processing: F.H.M., F.A.B., Analysis or Interpretation: F.A.B., Literature Search: F.A.B., Writing: F.A.B.

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