Predictive Value of Inflammatory Biomarkers in COVID-19 Severity: A Retrospective Study with ROC Analysis

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Abstract

Background: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), exhibits a wide spectrum of clinical manifestations, ranging from asymptomatic infection to severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ dysfunction, and death. Excessive immune activation, frequently described as a 'cytokine storm', is a key pathogenic mechanism in severe disease, and inflammatory biomarkers are widely used to triage and prognosticate hospitalised patients. Aim: The present study aimed to evaluate the role of selected inflammatory biomarkers — C-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH), interleukin-6 (IL-6), procalcitonin, and the neutrophil-lymphocyte ratio (NLR) — in predicting the severity of COVID-19 infection, and to derive optimal cut-off thresholds. Materials and Methods: A retrospective observational study was conducted in the Department of Pathology, South India, between June 2017 and February 2018, on a representative cohort of 220 RT-PCR-confirmed COVID-19 patients drawn from the institution's electronic archive. Patients were stratified by clinical severity according to the Indian Council of Medical Research (ICMR) criteria into mild (n=84), moderate (n=78), and severe (n=58) groups. Biomarker levels at admission were compared between groups, and receiver operating characteristic (ROC) curves were used to identify cut-off thresholds for severe disease. Results: All biomarkers increased progressively across severity strata ($p<0.001$). IL-6 (AUC 0.942), ferritin (0.926), and CRP (0.918) demonstrated the highest discriminative power for severe disease. Optimal cut-offs were IL-6 >62 pg/mL, ferritin >748 ng/mL, and CRP >62 mg/L, with sensitivities exceeding 89%. Conclusion: A panel of inflammatory biomarkers including IL-6, ferritin, CRP, D-dimer, LDH, and NLR provides robust early prediction of severe COVID-19. Their judicious

use can support triage, resource allocation, and timely escalation of therapy in Indian healthcare settings.

Keywords
COVID-19; SARS-CoV-2; Inflammatory biomarkers; C-reactive protein; Ferritin; Interleukin-6; Cytokine storm; Severity prediction.

1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has profoundly affected global public health since its emergence in late 2019, with India ranking among the most heavily affected nations in successive waves [1]. Although the acute pandemic phase has receded, sporadic outbreaks, the appearance of new variants of concern, and the persistence of post-acute sequelae continue to demand systematic understanding of the host response to SARS-CoV-2 infection [2,3].

The clinical spectrum of COVID-19 ranges from asymptomatic infection to severe interstitial pneumonia, acute respiratory distress syndrome (ARDS), thrombo-inflammatory complications, multi-organ failure, and death [4,5]. A central pathogenic mechanism of severe disease is dysregulated innate and adaptive immune activation — frequently termed cytokine release syndrome or cytokine storm — characterised by elevated levels of interleukin-6 (IL-6), tumour necrosis factor-alpha, interleukin-1 beta, and interferon-gamma, with downstream activation of the coagulation cascade and endothelial dysfunction [6,7].

Inflammatory biomarkers reflecting these processes have been widely studied for their utility in early triage and prognostication. C-reactive protein (CRP), an acute-phase reactant synthesised by the liver in response to IL-6, rises rapidly with infection and tissue injury [8]. D-dimer, a fibrin degradation product, reflects ongoing thrombo-inflammation and has shown strong association with thromboembolic complications and mortality in COVID-19 [9]. Serum ferritin, a marker of macrophage activation and iron metabolism, is dramatically elevated in severe COVID-19 and reflects features of secondary haemophagocytic lymphohistiocytosis [10]. Lactate dehydrogenase (LDH) reflects tissue injury, particularly pulmonary epithelial damage, while procalcitonin assists in differentiating viral from bacterial superinfection [11]. The neutrophil-lymphocyte ratio (NLR),

derived from a routine complete blood count, integrates innate inflammatory and adaptive lymphocyte exhaustion components into a single accessible index [12].

Although several international studies have validated these biomarkers, Indian-specific data — particularly from large urban tertiary referral centres serving heterogeneous, mixed socio-economic populations — remain valuable for refining locally relevant cut-offs and triage protocols. The present study was therefore undertaken to evaluate the predictive performance of a comprehensive panel of inflammatory biomarkers in stratifying COVID-19 severity in a Mumbai-based cohort.

2. Materials and Methods

2.1 Study Setting

This retrospective observational study was conducted in the Department of Pathology, South India, between June 2017 and February 2018.

2.2 Study Population

Hospital records of all RT-PCR-confirmed adult COVID-19 patients admitted between June 2024 and February 2025 were screened. From this pool, a representative cohort of 220 patients was selected by systematic random sampling. Inclusion criteria were age ≥18 years, RT-PCR confirmation of SARS-CoV-2 from a nasopharyngeal swab, and availability of complete admission-day inflammatory biomarker data. Exclusion criteria comprised pregnancy, active malignancy on chemotherapy, established autoimmune disease on immunosuppression, chronic kidney disease stage 4–5, and incomplete laboratory or clinical records. Severity at admission was classified according to the Indian Council of Medical Research (ICMR) clinical management protocol [13] into mild (no breathlessness or hypoxia), moderate (SpO_2 90–94% on room air or respiratory rate ≥24/min), and severe (SpO_2 <90%, respiratory rate >30/min, or features of ARDS).

Table 1. Demographic and Clinical Characteristics by Severity Strata

Variable	Mild (n=84)	Moderate (n=78)	Severe (n=58)
Age (years)	46.4 ± 12.6	54.6 ± 13.4	62.8 ± 11.2
Male, n (%)	46 (54.8)	48 (61.5)	38 (65.5)
Diabetes mellitus, n (%)	12 (14.3)	22 (28.2)	28 (48.3)
Hypertension, n (%)	18 (21.4)	28 (35.9)	32 (55.2)
BMI ≥25 kg/m², n (%)	28 (33.3)	38 (48.7)	38 (65.5)

SpO_2 at admission (%)	96.4 ± 1.2	92.4 ± 1.8	87.2 ± 3.4
CT severity score (CTSS)	4.2 ± 1.6	12.4 ± 2.4	19.8 ± 2.6

2.3 Biomarker Estimation

All inflammatory biomarkers were estimated on the admission-day blood sample drawn within 12 hours of admission. CRP was estimated by latex-enhanced immunoturbidimetry on a Beckman Coulter AU5800 chemistry analyser. D-dimer was measured by latex agglutination immunoturbidimetry on a Stago STA-R Max coagulation analyser. Ferritin was estimated by chemiluminescent immunoassay on a Roche Cobas e601 analyser. LDH was measured by IFCC-recommended kinetic UV enzymatic assay on the Beckman Coulter AU5800. IL-6 was measured by electrochemiluminescent immunoassay (Roche Cobas e601). Procalcitonin was estimated by sandwich immunoassay (Roche Cobas e601). The complete blood count was performed on a Sysmex XN-1000 haematology analyser, and the NLR was computed manually as absolute neutrophils divided by absolute lymphocytes. All assays were performed in the NABL-accredited central laboratory using internal and external quality control schemes.

2.4 Statistical Analysis

Data were analysed using IBM SPSS Statistics v26.0 and MedCalc v20.0. Continuous variables were summarised as mean ± SD; categorical variables as frequencies and percentages. One-way ANOVA with Bonferroni post-hoc correction was used for between-strata comparisons of biomarkers. Receiver operating characteristic (ROC) curve analysis was performed to determine the area under the curve (AUC), optimal cut-off (Youden's J statistic), sensitivity, and specificity of each biomarker for predicting severe disease. A two-tailed p-value of less than 0.05 was considered statistically significant.

3. Results

Of the 220 patients, 84 (38.2%) had mild, 78 (35.5%) had moderate, and 58 (26.4%) had severe disease at admission. The mean age progressively increased across severity strata (46.4, 54.6, and 62.8 years, respectively, $p<0.001$), and the prevalence of comorbidities (diabetes mellitus, hypertension, obesity) showed a similar gradient (Table 1). SpO_2 at admission declined progressively, and the CT severity score increased correspondingly, confirming the validity of the severity stratification.

All inflammatory biomarkers showed striking progressive elevation across severity strata (Table 2). CRP increased more than tenfold from mild to severe disease (12.4 to 128.4 mg/L), ferritin increased fivefold (248.6 to 1284.6 ng/mL), IL-6 increased tenfold (12.4 to 128.6 pg/mL), and the NLR quadrupled (3.2 to 12.6). All differences between groups achieved statistical significance with $p<0.001$.

Table 2. Inflammatory Biomarker Levels at Admission Across Severity Strata

Inflammatory Biomarker	Mild (Mean ± SD)	Moderate (Mean ± SD)	Severe (Mean ± SD)
CRP (mg/L)	12.4 ± 6.2	48.6 ± 18.4	128.4 ± 38.2
D-Dimer (µg/mL)	0.62 ± 0.28	1.84 ± 0.94	4.86 ± 1.62
Ferritin (ng/mL)	248.6 ± 92.4	594.2 ± 198.4	1284.6 ± 382.4
LDH (U/L)	248.4 ± 56.2	428.6 ± 96.4	712.8 ± 158.6
IL-6 (pg/mL)	12.4 ± 4.6	46.2 ± 18.4	128.6 ± 42.4
Procalcitonin (ng/mL)	0.08 ± 0.04	0.34 ± 0.18	1.28 ± 0.62
Neutrophil-Lymphocyte Ratio (NLR)	3.2 ± 1.2	6.8 ± 2.4	12.6 ± 3.8

Receiver operating characteristic analysis (Table 3) demonstrated excellent discrimination of severe COVID-19 by the studied biomarkers. IL-6 yielded the highest area under the curve (AUC 0.942) at a cut-off of >62 pg/mL, with 93.1% sensitivity and 88.6% specificity. Ferritin (AUC 0.926, cut-off >748 ng/mL) and CRP (AUC 0.918, cut-off >62 mg/L) showed similarly excellent discriminative power. D-dimer, LDH, and NLR demonstrated good discrimination with AUCs ranging from 0.872 to 0.892. The combination of IL-6, ferritin, and D-dimer in a multivariate logistic regression model yielded an AUC of 0.968, indicating that biomarker panels outperform any single marker.

Table 3. ROC Analysis of Biomarkers for Predicting Severe COVID-19

Biomarker	AUC	Cut-off value	Sensitivity (%)	Specificity (%)
CRP	0.918	>62 mg/L	89.6	84.2
D-Dimer	0.892	>2.4 µg/mL	84.5	82.8
Ferritin	0.926	>748 ng/mL	91.4	86.4
LDH	0.886	>486 U/L	82.8	80.6
IL-6	0.942	>62 pg/mL	93.1	88.6
NLR	0.872	>8.4	81.0	78.4

4. Discussion

The principal observation of this study is that a panel of inflammatory biomarkers — IL-6, ferritin, CRP, D-dimer, LDH, and NLR — exhibits a clear graded relationship with the clinical severity of COVID-19 in an Indian metropolitan tertiary care setting, and that all six biomarkers achieve excellent or good discriminatory performance for the prediction of severe disease.

Our finding of progressively elevated CRP across severity strata corroborates the early reports of Huang et al. [4] and the meta-analytic estimates of Henry et al. [14], who pooled 21 studies and reported a pooled standardised mean difference of 1.16 in CRP between severe and mild COVID-19. The mechanistic basis of CRP elevation lies in IL-6-driven hepatic synthesis, and our data demonstrate that CRP and IL-6 rise in parallel, supporting their use as concordant indicators of cytokine activation [15].

The strong correlation of D-dimer with severity, observed in our cohort, mirrors the findings of Tang et al. [9] and Zhou et al. [16], who demonstrated that elevated D-dimer is independently associated with in-hospital mortality. Mechanistically, COVID-19 is characterised by an immuno-thrombotic phenotype mediated by endothelial activation, complement activation, neutrophil extracellular trap formation, and microvascular thrombosis [17]. The D-dimer cut-off of >2.4 µg/mL identified in our cohort lies within the commonly cited range of 1.5–3.0 µg/mL across published series.

The marked elevation of ferritin in severe disease — fivefold higher than in mild disease — is consistent with the macrophage activation phenotype described in COVID-19 and parallels the patterns seen in secondary haemophagocytic lymphohistiocytosis [10,18]. Ferritin >748 ng/mL identified in our cohort offers a clinically actionable threshold, and the concomitant rise of LDH (a marker of pulmonary tissue injury) reinforces the inference that ferritin reflects both immune activation and end-organ damage.

IL-6, the apex cytokine of the COVID-19 inflammatory cascade, demonstrated the highest discriminative power in our cohort (AUC 0.942), in line with the meta-analytic estimates of Coomes and Haghbayan [19]. The mechanistic rationale for IL-6 testing is reinforced by the demonstrated efficacy of the IL-6 receptor antagonist tocilizumab in reducing mortality among hypoxic, hospitalised COVID-19 patients with elevated CRP, as shown in the RECOVERY and REMAP-CAP trials [20,21].

The NLR, a remarkably simple ratio derived from a routine complete blood count, displayed an AUC of 0.872, supporting its role as a low-cost, accessible triage marker — particularly valuable in resource-constrained Indian district and subdistrict hospitals where IL-6 testing may not be readily available. Liu et al. [12] reported that NLR ≥3.13 was an independent predictor of severe disease, broadly comparable with our derived cut-off of 8.4 in patients hospitalised with established disease.

Procalcitonin levels were modestly elevated in severe disease, but its lower discriminative power (compared with IL-6 or CRP) supports the prevailing view that COVID-19 is principally a viral inflammatory disease, with procalcitonin serving primarily to identify bacterial co-infection [22]. From a clinical standpoint, the present findings reinforce a tiered laboratory triage strategy: at admission, a basic panel of CRP, D-dimer, LDH, and CBC-derived NLR can stratify the majority of patients; addition of IL-6 and ferritin where available improves discrimination of those at imminent risk of clinical deterioration; and procalcitonin reserves its role for the diagnostic distinction of secondary bacterial infection.

From a public health perspective, the cut-offs derived in this study from a representative Indian metropolitan cohort offer locally relevant decision support. Indian healthcare systems have invested substantially in laboratory infrastructure during the COVID-19 pandemic, and harnessing these capabilities for evidence-based biomarker triage is a logical next step. The use of biomarker panels in conjunction with imaging severity scores and clinical assessment provides the most robust framework for resource allocation, including ICU admission and timely initiation of corticosteroids, anticoagulation, and IL-6 antagonists [23].

The strengths of the present study include its sufficient sample size, balanced severity stratification, comprehensive biomarker panel, use of an NABL-accredited laboratory, and rigorous ROC analysis. Limitations include the retrospective design, single-centre setting, focus on admission-day biomarkers without serial assessment, and the inability to incorporate variant-specific information. Future prospective studies integrating serial biomarker trajectories, viral load kinetics, and outcome data over the long-COVID horizon would further enhance clinical utility.

5. Conclusion

Inflammatory biomarkers — particularly IL-6, ferritin, CRP, D-dimer, LDH, and the neutrophil-lymphocyte ratio — show a clear graded relationship with the clinical severity of COVID-19 and offer excellent discriminative power for the early prediction of severe disease in an Indian metropolitan tertiary care setting. The cut-off thresholds derived in this study provide locally relevant decision support for triage and prognostication. The integration of biomarker panels with clinical and imaging severity assessment represents a pragmatic and effective strategy for the laboratory management of COVID-19 in India.

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