RESEARCH ARTICLE

Role of inflammatory biomarkers in the prediction of ICU admission and mortality in patients with COVID-19

Authors
Qamar Ahmad, MD[1]; Sarah E DePerrior, MPH[2]; Sunita Dodani MD, FCPS, MSc, PhD[2]; Joshua F Edwards, MPH[2]; Paul E Marik, MD, FCCP, FCCM[1]

Affiliations
[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Eastern Virginia Medical School, Norfolk, Virginia
[2] EVMS-Sentara Healthcare Analytics and Delivery Science Institute, Eastern Virginia Medical School, Norfolk, Virginia

This study was conducted at Eastern Virginia Medical School, Norfolk, Virginia

Corresponding Author Information:
Paul E Marik, MD, FCCP
Department of Internal Medicine, Chief, Pulmonary and Critical Care Medicine
825 Fairfax Ave, Rm 575, Norfolk, VA 23507

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Abbreviation List

AUC area under the receiver operating characteristic curve

COVID-19 coronavirus disease-19

CRP C-reactive protein

EMR Electronic Medical Record

EVMS Eastern Virginia Medical School

HADSI Healthcare Analytics and Delivery Science Institute

IL-1 interleukin-1

IL-6 interleukin-6

N/L ratio ratio of Neutrophils to Lymphocytes

CRP*D-Dimer*N/L ratio product of CRP, D-Dimer, and N/L ratio

CRP + D-Dimer + N/L ratio sum of CRP, D-Dimer, and N/L ratio

ROC receiver operating characteristic

SARS severe acute respiratory syndrome

SARS-CoV-2 severe acute respiratory syndrome (SARS) coronavirus 2

Abstract

Background: Inflammatory cytokines have been implicated in the pathophysiology and prognosis of severe COVID-19. Inflammatory biomarkers may guide the clinician in making treatment decisions as well as in the allocation of resources.

Objective: This study aimed to assess how levels of inflammatory biomarkers predict disease severity and mortality in patients with COVID-19 by testing a predictive scoring model developed by Zhou et al and further refining the model in a population of patients hospitalized with COVID-19.

Study Design and Methods: This retrospective study included patients with COVID-19 admitted to ten Virginia hospitals from January 1, 2020, to June 15, 2020. Inflammatory markers including CRP, D-Dimer, ferritin, N/L ratio, and procalcitonin were studied and logistic regression models were applied to ascertain the risk of ICU admission and mortality with elevated markers.

Results: Data from a total of 701 patients were analyzed. In bivariate tests age, CRP, D-Dimer, and N/L ratio were associated with in-hospital mortality as well as admission to the ICU. Procalcitonin was associated with admission to the ICU but not mortality. Males and African Americans were more likely to require ICU-level care. In final models, age and CRP were significantly associated with mortality (OR 1.06, 95% CI 1.04-1.08, and OR 1.06, 95% CI 1.03-1.10 respectively) as well as ICU admissions (OR 1.02, 95% CI 1.01-1.03 and OR 1.03, 95% CI 1.01-1.06 respectively). The previously established composite scores of CRP, D-Dimer, and N/L ratio were predictive of mortality (Area under the curve [AUC] 0.69 for multiplicative score) as well as ICU admissions (AUC 0.61 for multiplicative score). However, improved accuracy was obtained with age and CRP for predicting mortality (AUC 0.77) and ICU admission (AUC 0.62).

Conclusions: CRP and age appear to be the strongest predictors for ICU admission and mortality compared to D-Dimer, Ferritin, Procalcitonin, and N/L ratio in patients with COVID-19.

Keywords: COVID-19; SARS-CoV-2; inflammatory; C-reactive protein; predict; mortality

1. Introduction:

Coronavirus disease 2019 (COVID-19), a novel pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has currently become a severe global health problem. The COVID-19 pandemic has almost reached 19 million cases worldwide, with over 5 million cases in the US as of August 2020 and over 170,000 deaths.[1] There has been extensive research

on the pathogenesis of this novel SARS-CoV-2 virus, and many pathways including direct viral cytotoxicity, immune dysregulation, and cytokine storm, thrombogenesis, and organizing pneumonia have been implicated.[2] Markers pertaining to the immune pathway have been studied and reported to be important in disease prognosis as well as in treatment decisions.[3,4]

The levels of inflammatory markers such as C-reactive protein (CRP), D-Dimer, ferritin, and procalcitonin are increased in COVID-19 critically ill patients compared to those with less severe illness.[5] Increased levels of CRP were also reported during the severe acute respiratory syndrome (SARS) outbreak in 2002 and were associated with respiratory dysfunctions and increased mortality.[6] In patients with COVID-19 Tan et al reported that increased levels of CRP in the early stages of the disease were associated with worse findings on chest imaging and a worse prognosis.[7] Similarly, a meta-analysis by Huang et al of 25 studies showed that CRP, D-dimer, ferritin, and PCT are associated with poor outcomes in patients with COVID-19. [3] Based on these observations, several studies have been conducted in patients with COVID-19 linking CRP with severe COVID-19 outcomes. However, further research is needed to understand which biomarkers, alone or in combination with other patient factors, best predict mortality.

The severity of illness and mortality predictive scores have been developed to aid in early treatment decisions, triage, and the allocation of resources. The COVID-GRAM score developed by Liang et al uses 10 variables and can predict the risk of critical illness.[8] However, this score has not found widespread use primarily because of the large number of variables required for the calculation. A simpler score was proposed by Zhou et al using the product of CRP, D-Dimer, and N/L ratio. This score had a sensitivity of 89% and a specificity of 67% but hasn't been fully validated.[9]

We conducted a study to evaluate the predictive model proposed by Zhou et al and explore the serum levels of individual inflammatory markers in patients with COVID-19 and assess their utility in predicting the need for ICU admission and mortality. We aimed to refine existing prognostic models for severe disease and mortality in COVID-19 patients. This study describes our assessment of the existing Zhou model and the development of prognostic models that better predict severe outcomes within our population.

2. Materials and Methods:

2.1 Study Design and Data Collection:

We conducted an electronic chart review of patients with a confirmed diagnosis of COVID 19, who were admitted to the Sentara healthcare system, the largest healthcare system in the Eastern region of Virginia. Data was collected on COVID-19 patients that were admitted to and discharged from the hospital between January 2020 and June 2020. Patients were adult (18 and above) and had confirmed SARS-Cov-2 infection diagnosed by nasal PCR. ICD-10 codes were utilized to extract clinical data from the electronic medical record (EMR) system

(EPIC, Verona, WI). The study was approved by the Eastern Virginia Medical School (EVMS) IRB (#20-07-XX-0148) and informed consent was waived since it was a retrospective study.

The raw EMR data received from Sentara Health System was organized, managed, and analyzed by the EVMS-Sentara Healthcare Analytics and Delivery Science Institute (HADSI) staff. Epidemiological, clinical, laboratory, treatment, and outcomes data were obtained from the EMR. Admission levels of CRP, D-Dimer, ferritin, absolute lymphocyte and neutrophil counts, and procalcitonin were obtained. The neutrophil/lymphocyte (N/L/) ratio was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The dataset included demographic variables including age, gender, race, and ethnicity, as well as outcomes of admission to ICU and in-hospital mortality. The sample size was determined by the number of patients meeting eligibility criteria during the study period. Missing lab values were imputed using multiple imputations with the Markov chain Monte Carlo method.

2.2 Statistical analysis:

Descriptive analysis was performed to examine the distributions of age, CRP, D-Dimer, ferritin, N/L ratio, and procalcitonin. Associations between patient characteristics and in-hospital mortality as well as ICU admission were assessed using t-tests (for continuous baseline variables) and Pearson's chi-square tests (for categorical variables).

Bivariate logistic regression was used to assess the relationship between each initial inflammatory marker and the outcomes of mortality and ICU admission. To assess the predictive ability of the score developed by Zhou et al we also ran several logistic regression models involving the key variables CRP, D-Dimer, and N/L ratio as a composite score. First, we applied the score as specified by Zhou, using the product of CRP, D-Dimer, and N/L ratio (CRP*D-Dimer*N/L ratio). We also applied the score as the sum of these three variables (CRP + D-Dimer + N/L ratio). We used receiver operator characteristic (ROC) curves to visualize the predictive accuracy of individual models and calculated area under the curve (AUC). Finally, we assessed the role of all five individual inflammatory markers as well as age and gender as predictors of mortality and ICU admission in logistic regression models. We further assessed models with CRP and age alone and constructed ROC curves for all models tested to contrast with the Zhou composite score. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

3 Results:

A total of 701 patients met eligibility criteria and were included in the analysis. Data on outcomes of mortality and ICU admission were available for all patients. Missing data was limited to lab values since not all labs were collected for all patients. Percent missing ranged from 5% (for Neutrophils and Lymphocytes) to 33% for Procalcitonin. Baseline analysis demonstrated that age, CRP, D-Dimer, and N/L ratio differed significantly between patients who died in

the hospital and those who survived to hospital discharge (Table 1). Additionally, ferritin and procalcitonin differed significantly between patients who were admitted to the ICU as compared to those admitted to the medical floor. In bivariate logistic regression, CRP, D-Dimer, and N/L ratio were individually associated with both increased odds of ICU admission and mortality (Table 2). When assessing the CRP, D-Dimer, and N/L ratio composite scores both the multiplicative and additive scores were significant predictors of mortality (Table 3), and both performed similarly in ROC analysis with AUC = 0.69 (0.62-0.76) and AUC = 0.70 (0.64-0.76) respectively (Figure 1). For the outcome of ICU admission, both scores were also significant predictors, though they demonstrated lower discrimination in ROC analysis with AUC = 0.61 (0.57-0.66) and AUC = 0.61 (0.56-0.66) respectively. In final models with age and CRP as predictors, both were significantly associated with mortality (OR 1.06, 95% CI 1.04-1.08, and OR 1.06, 95% CI 1.03-1.10 respectively) as well as ICU admissions (OR 1.02, 95% CI 1.01-1.03 and OR 1.03, 95% CI 1.01-1.06 respectively) (Table 4). Further, the model with age and CRP as predictors of mortality yielded an AUC of 0.77 (0.72-0.82).

Table 1: Baseline characteristics of study population by outcome

	Hospital survival (n=623)	**In-Hospital Mortality (n=78)**		**Not Admitted to ICU (n=513)**	**Admitted to ICU (n=188)**	
	Mean(SD)	**Mean(SD)**	***P*-value**	**Mean(SD)**	**Mean(SD)**	***P*-value**
Age	60 (17)	73 (13)	**<.0001**	60 (18)	65 (14)	**0.0008**
CRP µg/ml	10 (9)	17 (13)	**0.0002**	10 (9)	14 (11)	**<.0001**
D-Dimer ng/ml	2.83 (6.11)	7.32 (11.06)	**0.0020**	2.56 (5.91)	5.21 (8.86)	**0.0006**
Ferritin ng/ml	1161 (1879)	1406 (1518)	0.2579	1045 (1220)	1477 (2636)	**0.0484**
N/L Ratio	6 (5)	9 (6)	**0.0003**	6 (5)	8 (6)	**0.0006**
Procalcitonin ng/ml	0.8 (4.1)	7.3 (26.9)	0.0596	0.7 (3.5)	3.7 (17.6)	**0.0350**
	Frequency (%)	**Frequency (%)**	***P*-value**	**Frequency (%)**	**Frequency (%)**	***P*-value**
Gender-Male	321 (52)	49 (63)	0.0596	253 (49)	117 (62)	**0.0024**
Gender-Female	302 (49)	29 (37)		260 (51)	71 (38)	
Race-Black	246 (39)	34 (44)	0.0904	191 (37)	89 (47)	**0.0188**
Race-White	193 (31)	30 (38)		164 (32)	59 (31)	
Race-Other	184 (30)	14 (18)		158 (31)	40 (21)	
Hypertension	209 (34)	27 (35)	0.8507	169 (33)	67 (36)	0.5036
Diabetes	254 (41)	38 (49)	0.1795	181 (35)	111 (59)	**<.0001**
Hyperlipidemia	258 (41)	52 (67)	**<.0001**	195 (38)	115 (61)	**<.0001**
Congestive Heart Failure	115 (18)	34 (44)	**<.0001**	82 (16)	67 (36)	**<.0001**

a. Bolded *P*-values indicate significance at the 0.05 level

Table 2: Summary of Bivariate Logistic Regression Analysis for Individual Score Components Predicting Mortality and ICU Admission

Outcome	Parameter	Estimate	OR	95% CI	p-value
Mortality	CRP	0.05	1.05	1.03-1.08	**0.0001**
	D-Dimer	0.05	1.06	1.03-1.08	**<.0001**
	N/L Ratio	0.06	1.06	1.03-1.10	**0.0007**
ICU Admission	CRP	0.03	1.03	1.01-1.05	**0.0014**
	D-Dimer	0.03	1.03	1.01-1.06	**0.0145**
	N/L Ratio	0.05	1.05	1.02-1.08	**0.001**

a. Bolded *P*-values indicate predictor significance at the 0.05 level

Table 3: Summary of Bivariate Logistic Regression Analysis for Composite Scores Predicting Mortality and ICU Admission

Outcome	Predictor	Estimate	Odds Ratio	95% CI	p-value
Mortality	CRP*D-Dimer*NLR	0.0	1.00	1.00-1.00	**0.0007**
	CRP + D-Dimer + NLR	0.04	1.04	1.03-1.06	**<.0001**
ICU	CRP*D-Dimer*NLR	0.00	1.00	1.00-1.00	**0.0125**
	CRP + D-Dimer + NLR	0.03	1.03	1.01-1.04	**<.0001**

a. Bolded *P*-values indicate predictor significance at the 0.05 level

Table 4: Summary of Multiple Logistic Regression Models with Age and CRP Predicting Mortality and ICU Admission

Outcome	Parameter	Estimate	OR	95% CI	p-value
Mortality	Age	0.06	1.06	1.04-1.08	**<.0001**
	CRP	0.06	1.06	1.03-1.10	**0.0002**
ICU Admission	Age	0.02	1.02	1.01-1.03	**0.0028**
	CRP	0.03	1.03	1.01-1.06	**0.0016**

a. Bolded *P*-values indicate predictor significance at the 0.05 level

Figure 1

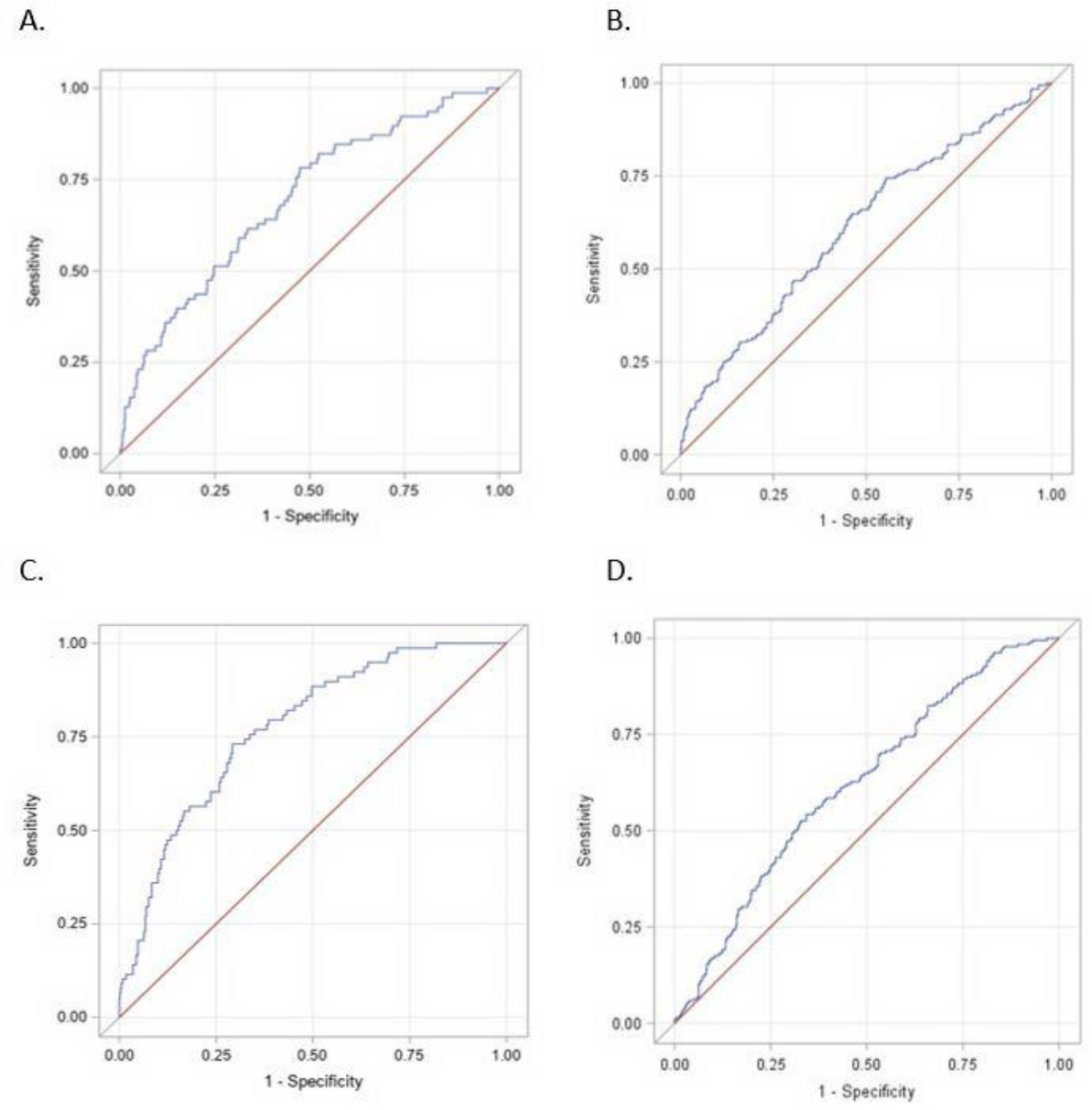

Figure 1:
A. ROC Curve for CRP*D-Dimer*NLR predicting Mortality; AUC=0.6941 (95% CI: 0.6320-0.7563)
B. ROC Curve for CRP*D-Dimer*NLR predicting ICU Admission; AUC=0.6130 (95% CI: 0.5655-0.6606)
C. ROC Curve for Age and CRP predicting Mortality; AUC = 0.7732 (95% CI: 0.7228-0.8236)
D. ROC Curve for Age and CRP predicting ICU Admission; AUC = 0.6211 (95% CI; 0.5758-0.6663)

4 Discussion:

A dysregulated host immune response is postulated to be the main driver behind severe COVID-19 disease.[10] Elevated levels of a diverse spectrum of proinflammatory cytokines and chemokines have been reported in severe disease and referred to as the "Cytokine Storm."[11] The cytokine storm is likely responsible for the elevation of the acute phase reactants (CRP and ferritin) observed in this study. Previous studies have shown these acute phase reactants to be associated with increased morbidity and mortality. [3,5,12]

In our study, age was a significant risk factor for both ICU admission and mortality; this association has been shown in multiple prior studies.[5,13,14] One of the potential mechanisms for this phenomenon is immunosenescence, whereby a failure to mount an effective adaptive immune response leads to the dysregulated release of cytokines. In addition, inflammaging, a chronic low-grade inflammation that develops with advanced age may contribute to the immune dysregulation and poorer outcome in elderly patients.[15]

Among the inflammatory markers in this study, CRP appeared to be the main driver for predicting ICU admission as well as mortality. D-dimer and N/L ratio were associated with poor outcomes but they were not significant in the multivariate logistic regression. CRP is a small protein and is part of the innate immune response. Originally described in bacterial infections, it has been demonstrated to be elevated in a variety of inflammatory conditions.[16] Its production is induced in hepatocytes by cytokines, notably by interleukin-1 (IL-1) and IL-6.[16,17] CRP can activate the classical complement pathway and may play a role in the pathogenesis of COVID-19. Moreover, since CRP rises early in the course of illness, it can be utilized for triaging patients.

Procalcitonin is a peptide precursor of the hormone calcitonin and is often found elevated in patients with sepsis and septic shock.[3] In patients with COVID-19 as in other viral infections, the PCT is usually normal, due to increased interferon-γ (which decreases PCT transcription).[18] Interestingly, in our study procalcitonin was associated with ICU admission (3.7 vs 0.7, p=0.035) and a trend toward increased mortality (7.3 vs 0.8, p=0.059). The elevated PCT is likely due to bacterial superinfection which contributes to a worse prognosis. Hence, serial monitoring of PCT might be useful in COVID-19 patients to exclude secondary bacterial infections. Of note, 67% of the patients in this study had an elevated procalcitonin implying that secondary bacterial infections are more common than previously assumed in COVID-19.

Elevated levels of ferritin are seen in hemophagocytic lymphohistiocytosis and have also been reported in conjunction with the cytokine storm that has been observed in severe COVID-19 infections.[14] In our study, elevated ferritin correlated with ICU admissions but not with overall mortality. This might be because the cytokine storm tends to develop later in the course of the disease and initial ferritin levels may not be elevated. [3] Nevertheless, a high ferritin

level on admission suggests a severe disease that may require ICU admission.

The product of CRP, D-Dimer, and N/L ratio performed reasonably well in predicting mortality as well as ICU admission (AUC 0.69 and 0.61 respectively) though the accuracy was less compared to that reported by Zhou et al (AUC 0.88). This might be because Zhou et al had a different definition of severity (fever, respiratory rate, ARDS, sepsis, septic shock) whereas we compared outcome variables of mortality and ICU admission. Interestingly, we found that a model of CRP and age performed reasonably well and may be a better predictor for mortality when compared to the product of CRP, D-Dimer, and N/L ratio (AUC 0.77 and 0.69 respectively), although there was some overlap between the confidence intervals of the two models. Risk scores and prediction models are important to screen critically ill patients for early ICU admission. They may also help patient triage and in the allocation of resources.

Our study has several limitations. First, we did not look at clinical data such as respiratory signs and symptoms. Furthermore, selection bias could overestimate the utility of the biomarkers studied. Our sample only included COVID-19 patients who were admitted to a hospital and did not include patients who were evaluated in the emergency room and discharged. If biomarkers in these discharged patients were also elevated, their predictive value could be diminished. However, checking inflammatory biomarkers is not the standard of care in the emergency department. Finally, some laboratory data were missing and had to be computed using multiple imputations.

5. Interpretation:

The inflammatory biomarkers CRP, D-Dimer, ferritin, N/L ratio, and procalcitonin have prognostic implications in COVID-19. In our study, CRP and age were the most important predictors of ICU admission and mortality. Procalcitonin may also have a role in predicting poor outcomes, possibly due to secondary bacterial infections. These parameters can be utilized in prognostication as well as the development of risk scores.

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Author Contributions: QA, SED, and PEM had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. Study concept and design: QA, SD, and PEM. Acquisition, analysis, or interpretation of data: SED, PEM, QA, and SD. Drafting of the manuscript: QA, SED, SD, PEM. Critical revision of the manuscript: all authors. Administrative and technical support: JFE, SD.

The authors have no competing interests to report.

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