ORIGINAL ARTICLE

Role of Biomarkers and Its Trend to Predict the Outcome of COVID-19 Patients: A Retrospective Study

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Abstract

Background: Biomarkers have been extensively studied and used in the diagnosis and management of various diseases. The trend of biomarkers helps in prognosticating and managing critically ill patients. In resource-limited settings, the availability and feasibility of using these biomarkers are challenging.

Our study aimed to see the trend of biomarkers and their effect on intensive care unit (ICU) mortality in coronavirus disease-2019 (COVID-19) patients.

Materials and methods: A retrospective observational study was done from 1 April 2020 to 30 September 2020. The primary objective was to evaluate the trend of biomarkers in patients with COVID-19 pneumonia and their effect on ICU mortality. The secondary objectives were the duration of mechanical ventilation and length of ICU stay.

Results: A total of 380 patients were included. The mean age was 54.9 (SD = 11.1) and 67% were males. The mean age, acute physiology and chronic health evaluation II (APACHE II) score was 29.54 (5.8). Among the biomarkers, total count (TC), ferritin, and procalcitonin (PCT) were higher in non-survivors than in survivors in bivariate analysis. The final multivariable logistic regression model showed age, APACHE II score, length of ICU stay, neutrophil:lymphocyte (NL) ratio, and ferritin as covariates. Among these variables, ferritin was the only biomarker [odds ratio (OR): 1.80, 95% confidence interval (CI) 1.17–2.77] with the APACHE II score (OR: 1.15, 95% CI 1.01–1.30) found to be significant.

Conclusion: Ferritin was the only significant biomarker with higher values in non-survivors than in survivors. The trend of biomarkers was not found to be useful in predicting outcome of the patients.

Keywords: Biomarkers, Coronavirus disease-2019, C-reactive protein, D-dimer, Ferritin, neutrophil:lymphocyte ratio.

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Highlights

- In severe COVID-19 patients, ferritin on day 1 with APACHE II score can help in prognostication. Higher values were seen in non-survivors than in survivors. The findings of this study need to be validated prospectively.
- Future studies evaluating the effect of therapeutic interventions on the trend of biomarkers are required.

Introduction

Biomarkers have been studied and used in the diagnosis and management of various diseases.[1] Novel coronavirus infection has an inconsistent clinical presentation varying from asymptomatic to milder symptoms to severe disease. In the ongoing pandemic of COVID-19, biomarkers have played a crucial role in the early suspicion, diagnosis, recognizing complications, management, monitoring, and prognostication of the disease state.

Multiple biomarkers have been identified to predict the severity of COVID-19 disease of which frequently studied parameters include hematological markers (neutrophil count, lymphocyte count, and NL ratio), inflammatory markers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and PCT], immunological parameter [interleukin 6 (IL-6)], and biochemical parameters [D-dimer, troponin I, lactate dehydrogenase (LDH) and ferritin].[2,3]

However, monitoring serial trends rather than a single measurement in conjunction with the clinical status and radiological features is the finest method in risk stratification.[2,3] Very few studies looked at the trend of biomarkers and their therapeutic implications.[4] Since the clinical course of novel coronavirus is unpredictable, the dynamic change in biomarkers helps in prognosticating and managing critically ill patients.[3] In resource-limited settings with a lack of availability of mechanical ventilators and beds in ICU, inflammatory biomarkers that predict mortality can act as an adjunct in clinical practice in allocating the available resources; however, the feasibility of using these biomarkers has been challenging.

Our study aimed to evaluate the role of biomarkers and their serial trend to predict ICU mortality.

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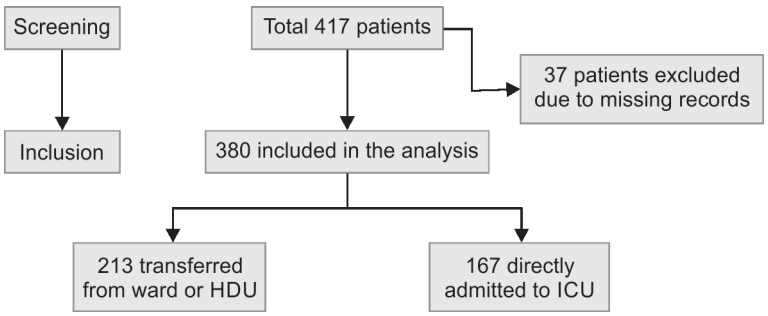
Flowchart 1: Strobe diagram



Materials and Methods

After obtaining ethical committee approval (IEC No.267/2020, CTRI/2020/10/028436), a retrospective study was conducted from 1 April 2020 to 30 September 2020 over 6 months. Patients of COVID-19 pneumonia with positive reverse transcriptase-polymerase chain reaction (RT-PCR) or rapid antigen test (RAT) and requiring ICU admission were included in the study. Patients with non-COVID-19 illness and negative RT-PCR were excluded. Clinical features and demographic details were recorded. Biomarkers, CRP, D-dimer, ferritin, LDH, PCT, troponin I, NL ratio, and IL-6 were collected if available within 7 days of ICU admission. However, the analysis was restricted to biomarkers measured in the first 5 days of ICU admission. The ordering of the biomarkers was left to the clinician's discretion. The primary outcome was ICU mortality. Secondary outcomes were the length of ICU stay and duration of mechanical ventilation.

Statistical Analysis

The data collected were entered in the Microsoft Excel and analyzed using STATA, v.16.0 (StataCorp. 2019, Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC). Continuous data were represented as mean (SD) or median [interquartile range (IQR)] as applicable. The categorical data were presented in percentage. The trend of biomarker measured over the first 5 days of admission was examined using a mixed linear model. Among the variables, ferritin had lognormal distribution and for the mixed model, this variable was used. The association of day 1 biomarkers with duration of ventilation and length of ICU stay were examined in separate multivariable logistic regression models. The inclusion of variables in the multiple regression model was based on literature review and the level of significance of the covariate in univariate models. Statistical significance in the final models was at the 5% level.

Results

A total of 417 patients were screened; 37 patients were excluded due to missing records. A total of 380 patients were included in the study. Out of 380 patients, 213 (56.05%) were transferred from the ward or high-dependency units (HDUs) and 167 (43.94%) were directly admitted to the ICU (Flowchart 1). The mean age of the patients admitted was 54.9 (SD = 11.1) and 67% were males. The age of non-survivors was 5 years higher compared to survivors ($p = 0.001$). There was no effect of gender on ICU mortality. The common comorbidities were diabetes mellitus (DM) followed by hypertension. Higher proportion of patients had ischemic heart disease (IHD), hypertension, and chronic kidney disease (CKD) in non-survivors than in survivors. Moreover, APACHE II score and sequential organ failure assessment (SOFA) score, on day 1 of ICU admission were also higher in the non-survivors than in survivors (Table 1).

Among the various biomarkers, TC, NL ratio, ferritin, D-dimer, and PCT were commonly ordered by the treating team during the ICU stay. Day-1 parameters such as TC, ferritin, and PCT were significant with higher values in non-survivors than survivors (Table 2). As age and APACHE II score at baseline were different between survivors and non-survivors, they were included in a multiple variable logistic regression model of significant laboratory parameters (Table 3) for mortality. The final multivariable model showed ferritin and APACHE II score were associated with higher odds of mortality of (OR = 1.80, 95% CI: 1.17–2.77 and OR 1.15, 95% CI: 1.01–1.30, respectively). The discrimination of this model was excellent as suggested by area under the receiver operator characteristics (ROC) curve of 0.85 (Fig. 1).

Day-wise trend (first 5 days of ICU stay) for commonly used laboratory biomarkers such as CRP, ferritin, D-dimer, and NL ratio was not statistically significant among survivors and non-survivors in mixed linear model analysis. Although slightly higher values were seen in non-survivors, the trend in time was not different between survivors and non-survivors, as suggested by p value (Fig. 2). There was no interaction effect with the duration of mechanical ventilation or with the duration of ICU stay.

Discussion

This study showed among the various biomarkers day 1 ferritin and APACHE II scores were found to be significant with higher values among non-survivors than survivors. The OR for ferritin was 1.8 (1.17–2.77) as shown in Table 4, indicating 1.8 times higher risk of death. The ICU mortality observed was 63.16%. Similarly, the length of ICU stay was also higher in non-survivors than in survivors [OR of 1.09 (1.00–1.18)] (Table 4).

The study by Wang et al., showed among the laboratory parameters, D-dimer was higher in non-survivors than survivors.[5]

Two meta-analyses, one including 31 and another with 32 studies showed inflammatory, hematological, and biochemical biomarkers can be used for prognostication and risk stratification of COVID-19 patients. Among inflammatory biomarkers, PCT and CRP were found to be significant in both the studies.[6,7] In our study, PCT and ferritin on day 1 were significant with higher values in non survivors as shown in bivariate analysis (Table 2).

Various studies showed different kinds of biomarkers can be used for prognostication.[4,6–11] Hashem et al., showed simple biomarkers such as anemia, NL ratio, Platelet: Lymphocyte ratio,

Table1: Baseline characteristics

Parameter	*All (n = 380)*	*Survivors (n = 140)*	*Non-survivors (n = 240)*	*p-value*
Age[¥]	54.90 (15.10)	51.71 (15.12)	56.75 (14.80)	0.001
Gender	M/F 255/125 (67.11/32.89)	M/F (93/47) (66.43/33.57)	M/F (162/78) (67.50/32.50)	0.830
DM	210 (55.26)	70 (50)	140 (58.33)	0.115
Hypertension	203 (53.42)	63 (45)	140 (58.33)	0.012
CLD	8 (2.11)	3 (2.14)	5 (2.08)	0.969
CKD	53 (13.95)	13 (9.29)	40 (16.67)	0.045
CVA	23 (6.05)	5 (3.57)	18 (7.5)	0.121
IHD	51 (13.42)	8 (5.71)	43 (17.92)	0.001
COPD	13 (3.42)	4 (2.86)	9 (3.75)	0.644
Bronchial asthma	16 (4.21)	3 (2.14)	13 (5.42)	0.125
ILD	4 (1.05)	1 (0.71)	3 (1.25)	0.622
TB	7 (1.84)	2 (1.43)	5 (2.08)	0.647
Immunosuppressants	13 (3.42)	4 (2.86)	9 (3.75)	0.644
Malignancy	2 (0.53)	1 (0.71)	1 (0.42)	0.699
APACHE II score	29.54 (5.8)	27.58 (5.96)	30.70 (5.38)	<0.001
SOFA score	8 (4–11)	5 (3–8)	9 (6–12)	<0.001

Values are *n* (%), *p* value from Chi-squared test of association; [¥]Mean (SD), independent sample *t*-test was used for comparison; CKD, chronic kidney disease; CLD, chronic liver disease; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; DM, diabetes mellitus; IHD, ischemic heart disease; ILD, interstitial lung disease; TB, tuberculosis

Table 2: Laboratory parameters

Day 1 Parameters	*N*	*All n = 380*	*Survivors n = 140*	*Non-survivors n = 240*	*p-value*
TC	213	14.09 (9.38–19.89)	13.17 (8.87–13.03)	15.25 (10.06– 21.06)	0.015
NL ratio[§]	213	16.61 (8.05–25.94)	14.17 (7.73–20.46)	18.07 (8.21–29.61)	0.108
CRP[§]	99	12.80 (5.49–22.18)	13.79 (5.47–20.82)	11.26 (5.55–27.76)	0.904
Ferritin[§]	135	793.80 (322.80–2264)	378.50 (206.30–1228.60)	1,237.60 (704.05–2878.50)	<0.001
PCT[§]	112	0.84 (0.28–4.50)	0.34 (0.18–1.36)	1.64 (0.58–7.78)	<0.001
D-dimer[§]	131	1,093 (678–3673)	1,010 (489–1469)	1,218 (800–4122)	0.105
LDH[§]	89	700 (500–843)	649 (395–766.50)	716 (538–966)	0.055
Troponin I[§]	99	0.10 (0.02–0.51)	0.06 (0.01–0.21)	0.13 (0.03–0.55)	0.057
Outcomes					
Primary outcome					
ICU mortality	380		140 (36.84%)	240 (63.16%)	
Secondary outcomes					
ICU length of stay	380	8 (4–13)	6 (4–10)	8 (4–14)	0.090
Days on mechanical ventilation	380	7 (4–13)	8 (6–10.5)	7 (4–13)	0.494

Values are *n* (%), *p* value from Chi-squared test of association; [§]Median (IQR), *p* value from Mann–Whitney U test; Units of measurement: D-dimer, ferritin, PCT, and troponin I (ng/mL); CRP (mg/dL); LDH (U/L)

Table 3: Multiple variable logistic regression (Adjusted for age and APACHE score)

Parameters	*OR (95% CI)*	*p-value*
CRP	0.99 (0.95–1.03)	0.717
D-dimer	1.00 (0.99–1.00)	0.886
Ferritin	1.71 (1.25–2.33)	0.001
LDH	1.00 (0.99–1.00)	0.225
PCT	1.02 (0.98–1.06)	0.265
TC	1.04 (1.00–1.09)	0.040
Neutrophil	0.99 (0.95–1.02)	0.691
Lymphocytes	1.00 (0.96,1.05)	0.736
NL ratio	1.01 (0.99,1.03)	0.052
Troponin I	1.03 (0.93,1.14)	0.484

CI: confidence interval

and D-dimer at the time of admission can be used for prediction of severe COVID-19 infection requiring ICU admission.[12]

Meta-analysis including 163 studies showed ferritin was associated with severe disease and poorer outcomes, but the studies included had significant heterogeneity.[13] Deng et al. showed that ferritin can be used as an independent predictor of mortality. This study included non-ICU and ICU patients.[14] Similarly, in our study, ferritin was the only biomarker found to be associated with higher mortality. We included ICU patients, hence comparison between the non-ICU and ICU population was not possible. Also, for patients who were transferred from the ward or HDUs, values of the biomarkers from hospital admission till ICU were not included in the analysis.

The study on monitoring trend of inflammatory biomarkers by Muller et al. showed that trend of CRP was useful in predicting

disease progression. In our study, serial trend of parameters done during ICU stay was not found to be significant.[4]

There are several strengths found in our study. We used easily available and routinely advised biomarkers by the treating physician. In resource-limited settings, using biomarkers, especially ferritin along with APACHE II done on day 1 of ICU admission may help in prognostication as shown by our study.

There were several limitations of this study. This was a retrospective study; hence, the study findings will have limitations. It will require further validation by a larger prospective study. Another limitation was, the initial objective was to see the trend of these biomarkers over 7 days. The ordering of the biomarkers was left to the discretion of the treating physician and due to retrospective study design, many patients did not have consecutive values of biomarkers to use as a trend for further analysis. Although with the available serial parameters, change of biomarkers was not significant among survivors and non-survivors (Flowchart 1).

We did not evaluate the effect of therapeutic interventions, such as steroids, immunomodulators or antivirals on the biomarkers and thereby on ICU mortality. We did not monitor the trend of biomarkers from hospital admission till ICU discharge or ICU mortality as it would have helped in knowing the disease progression.

At present, there is no single biomarker for prognostication consistently seen in all the studies. There are hematological biomarkers such as NL ratio and lymphopenia, but biochemical and inflammatory biomarkers are variable across the studies. The possible reasons are the duration of symptoms, effect of different comorbidities like presence of renal dysfunction altering the serum levels of biomarkers. Therapeutic interventions can alter the biomarkers. Also with severe disease, the clinical utility of the biomarkers will be limited as therapeutic options will be limited.

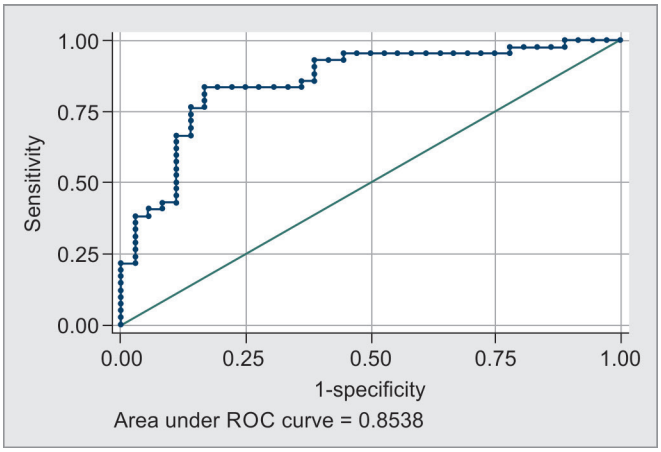


Fig. 1: Receiver operator characteristics curve

Conclusion

This study showed ferritin with APACHE II score can be used for predicting the outcome of COVID-19 patients admitted to the ICU, but the trend of biomarkers was not found to be useful. As there are several limitations in using biomarkers for prognostications,

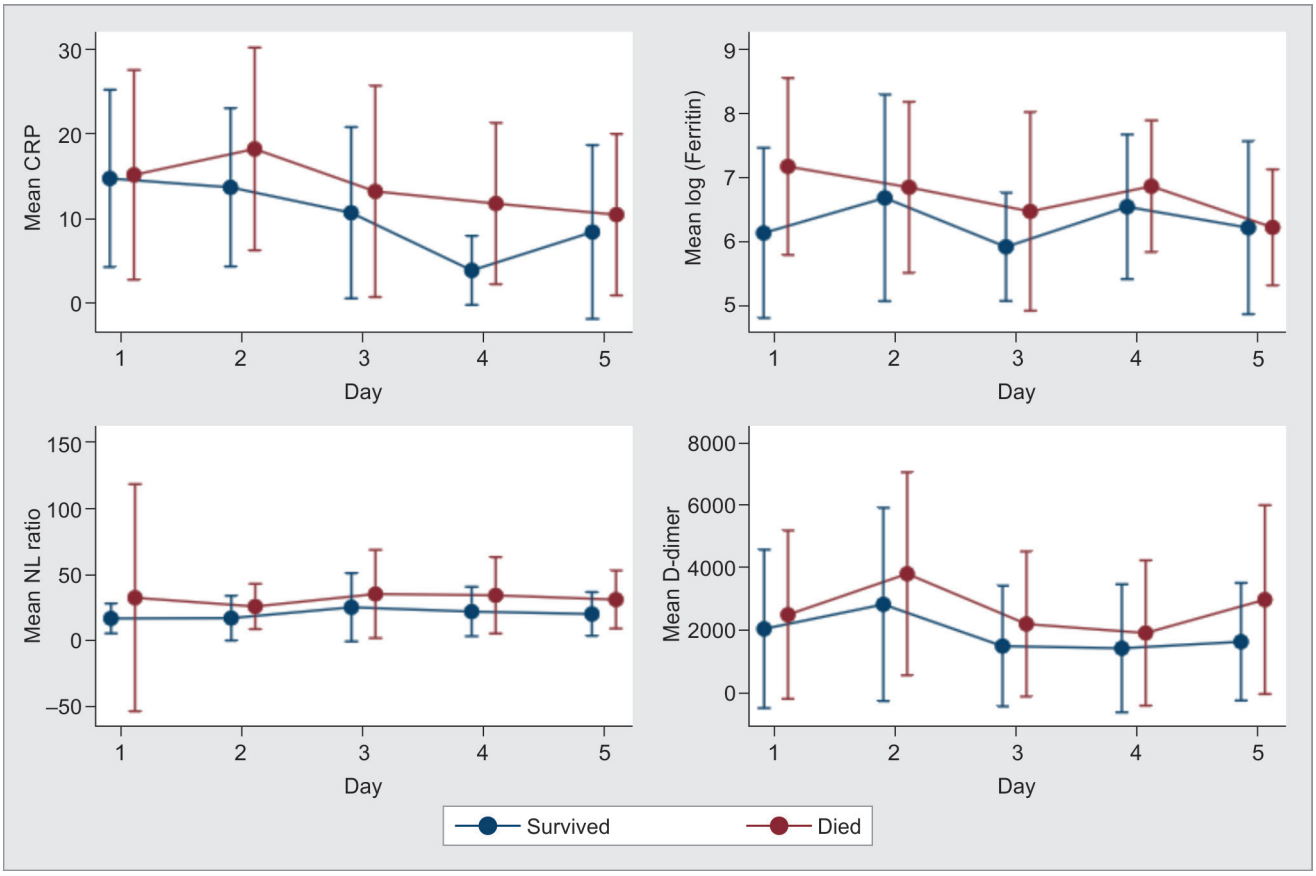


Fig. 2: Trend of biomarkers over 5 days

Table 4: Final logistic regression model

Parameters	*OR (95% CI)*	*p-value*
Age	0.98 (0.93–1.02)	0.470
APACHE II	1.15 (1.01–1.30)	0.028
Length of ICU stay	1.09 (1.00–1.18)	0.046
Ferritin	1.80 (1.17–2.77)	0.007
TC	1.05 (0.97–1.13)	0.178
NL ratio	1.03 (0.99–1.07)	0.085

CI: confidence interval

we suggest future studies to be designed, taking into account limitations stated in our study.

Authors' Contributions

Author AH helped in concept, design, conduct, writing, and finalizing manuscript; VL, VK, and CS helped in data collection; AH and VL helped in writing and finalizing the manuscript; JR and TT helped in statistical analysis. All the authors have approved the final manuscript.

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