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Biomarkers and outcomes in hospitalised patients with COVID-19: a prospective registry

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ABSTRACT

Objectives To determine association of biomarkers—high-sensitivity C reactive protein (hsCRP), D-dimer, interleukin-6 (IL-6), lactic dehydrogenase (LDH), ferritin and neutrophil–lymphocyte ratio (NLR)—at hospitalisation with outcomes in COVID-19.

Design and Setting Tertiary-care hospital based prospective registry.

Participants Successive virologically confirmed patients with COVID-19 hospitalised from April 2020 to July 2021 were prospectively recruited. Details of clinical presentation, investigations, management and outcomes were obtained.

Primary and secondary outcome measures All biomarkers were divided into tertiles to determine associations with clinical features and outcomes. Primary outcome was all-cause deaths and secondary outcome was oxygen requirement, non-invasive and invasive ventilation, dialysis, duration of stay in ICU and hospital. Numerical data are presented in median and interquartile range (IQR 25–75). Univariate and multivariate (age, sex, risk factors, comorbidities, treatments) ORs and 95% CIs were calculated.

Results 3036 virologically confirmed patients with COVID-19 were detected and 1251 hospitalised. Men were 70.0%, aged >60 years 44.8%, hypertension 44.1%, diabetes 39.6% and cardiovascular disease 18.9%. Median symptom duration was 5 days (IQR 4–7) and oxygen saturation 95% (90%–97%). Total white cell count was 6.9×10^9/L (5.0–9.8), neutrophils 79.2% (68.1%–88.2%), lymphocytes 15.8% (8.7%–25.5%) and creatinine 0.93 mg/dL (0.78–1.22). Median (IQR) for biomarkers were hsCRP 6.9 mg/dL (2.2–18.9), D-dimer 464 ng/dL (201–982), IL-6 20.1 ng/dL (6.5–60.4), LDH 284 mg/dL (220–396) and ferritin 351 mg/dL (159–676). Oxygen support at admission was in 38.6%, subsequent non-invasive or invasive ventilatory support in 11.0% and 11.6%, and haemodialysis in 38 (3.1%). 173 (13.9%) patients died and 15 (1.2%) transferred to hospice care. For each biomarker, compared with the first, those in the second and third tertiles had more clinical and laboratory abnormalities, and oxygen, ventilatory and dialysis support. Multivariate-adjusted ORs (95% CI) for deaths in second and third versus first tertiles, respectively, were hsCRP 2.24 (1.11 to 4.50) and 12.56 (6.76 to 23.35); D-dimer 3.44 (1.59 to 7.44) and 14.42 (7.09 to 29.30); IL-6 2.56 (1.13 to 5.10) and 10.85 (5.82 to 20.22); ferritin 2.88 (1.49 to 5.58) and 8.19 (4.41 to 15.20); LDH 1.75 (0.81 to 3.75) and 9.29 (4.75 to 18.14); and NLR 3.47 (1.68 to 7.14) and 17.71 (9.12 to 34.39) ($p<0.001$).

Conclusion High levels of biomarkers—hsCRP, D-dimer, IL-6, LDH, ferritin and NLR—in COVID-19 are associated with more severe illness and higher in-hospital mortality. NLR, a widely available investigation, provides information similar to more expensive biomarkers.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Prognostic evaluation of multiple biomarkers at admission in COVID-19.
⇒ Comprehensive clinical details available for all the patients.
⇒ We did not evaluate associations with biochemical markers of cardiac, renal, hepatic and gastrointestinal dysfunction.

INTRODUCTION

The pandemic of SARS-CoV-2 infection and COVID-19 continues unabated in many regions of the world.[1] The current wave of the epidemic has been triggered by the omicron variants of COVID-19 although previous variants (ancestral, alpha, beta, gamma and delta) are also present in certain parts of the world.[2 3] Studies have reported that adverse outcomes in the omicron wave are not as severe as the delta variant; a significant number of patients are being hospitalised and high transmission rate of these variants has resulted in more hospital admissions as compared with the previous waves in some countries.[3–6] A number of prognostic markers have been identified to indicate severity of COVID-19.[7 8] These include clinical markers such as tachypnoea, tachycardia and hypoxia, radiological abnormalities, and abnormalities of hepatic, renal and cardiac functions. Novel biomarkers that have been identified as important include interleukins (ILs) 6, 4 and 10, procalcitonin, C reactive protein (CRP), serum amyloid A, neutrophil, lymphocyte,

Table 1 High-sensitivity C reactive protein tertiles, clinical features and outcomes

	Tertile 1 (N=377, ≤3.53)	Tertile 2 (N=377, 3.54–13.58)	Tertile 3 (N=376, ≥13.59)	Statistics, p value
Men	241 (63.9)	263 (69.8)	286 (76.1)	<0.001
Women	136 (36.1)	114 (30.2)	90 (23.9)	<0.001
Age ≤45 years	105 (27.9)	72 (19.1)	58 (15.4)	<0.001
Age 46–60 years	131 (34.7)	145 (38.5)	122 (32.4)	0.041
Age >60 years	141 (37.4)	160 (42.4)	196 (52.1)	<0.001
Medical comorbidities				
Hypertension	161 (42.7)	170 (45.1)	175 (46.5)	0.565
Cardiovascular disease	63 (16.7)	58 (15.4)	78 (20.7)	0.147
Diabetes	131 (34.7)	163 (43.2)	164 (43.6)	0.020
Chronic obstructive pulmonary disease	08 (2.1)	08 (2.1)	11 (2.9)	0.474
Clinical features and investigations				
Fever	295 (78.2)	322 (85.4)	327 (87.0)	0.001
Shortness of breath	126 (33.4)	168 (44.6)	225 (59.8)	<0.001
Pulse (per minute)	86.0 (78.0–97.5)	88.0 (79.0–99.5)	93.0 (82.0–105.0)	<0.001
Oxygen (SpO_2, %)	97.0 (95.0–98.0)	95.0 (92.0–97.0)	91.0 (82.2–95.0)	<0.001
Systolic BP, mm Hg	130 (121.5–142.0)	130.0 (120.0–142.0)	132.0 (120.0–148.0)	0.314
Haemoglobin (g/L)	128 (117–141)	128 (115–140)	126 (111–138)	0.019
White cell count (10^9/L)	5.8 (4.5–7.6)	6.8 (5.1–9.5)	8.2 (5.9–11.9)	<0.001
Neutrophils (%)	71.3 (60.6–79.8)	78.9 (70.7–86.7)	86.9 (79.6–91.9)	<0.001
Lymphocytes (%)	23.4 (15.5–32.5)	15.7 (9.5–23.7)	9.6 (5.9–16.2)	<0.001
Platelet count (10^9/L)	2.2 (1.7–2.7)	2.1 (1.7–2.7)	2.2 (1.6–2.9)	0.807
D-dimer (ng/dL)	348.5 (167.0–835.2)	421.0 (190.5–744.0)	602.3 (268.5–1454.7)	<0.001
Interleukin-6 (ng/dL)	8.3 (2.9–22.7)	21.1 (6.8–53.2)	45.8 (16.5–117.3)	<0.001
Ferritin (mg/dL)	190.1 (101.0–365.3)	356.9 (171.4–655.1)	530.0 (296.1–1008.5)	<0.001
Lactic dehydrogenase (mg/dL)	213.0 (181.0–265.0)	283.0 (236.0–376.0)	368.0 (284.0–516.3)	<0.001
Creatinine (mg/dL)	0.87 (0.74–1.08)	0.92 (0.77–1.12)	1.03 (0.84–1.42)	<0.001
Sodium (mEq/L)	139.0 (137.0–142.0)	138.0 (135.0–141.0)	137.0 (134.0–141.0)	<0.001
Potassium (mEq/L)	4.3 (4.0–4.7)	4.4 (4.1–4.8)	4.6 (4.1–5.0)	<0.001
Serum aspartate transaminase (units)	25.0 (17.7–39.7)	29.1 (19.3–47.5)	31.0 (19.4–48.8)	0.001
Serum glutamate transaminase (units)	25.2 (19.0–36.3)	32.5 (22.7–50.5)	35.7 (25.3–53.9)	<0.001
HRCT scan thorax score (out of 25)	9 (4.0–12.0)	13.0 (9.5–17.0)	16.0 (11.0–19.0)	<0.001
Medicines				
Steroids	343 (91.0)	363 (96.3)	366 (97.3)	<0.001
Remdesivir	301 (79.8)	344 (91.2)	349 (92.8)	<0.001
Anticoagulants	346 (91.8)	359 (95.2)	354 (94.1)	0.180
Tocilizumab/bevacizumab	4 (1.1)	11 (2.9)	57 (13.2)	<0.001
Outcomes				
Oxygen requirement at admission	50 (13.3)	135 (25.8)	252 (67.0)	<0.001
Oxygen duration (days)	0 (0.0–0.0)	0.0 (0.0–5.0)	6 (2–11)	<0.001
Proning	137 (36.7)	226 (59.9)	301 (80.1)	<0.001
High-flow nasal cannula	9 (2.4)	29 (7.7)	97 (25.8)	<0.001
BiPaP support	8 (2.1)	26 (6.9)	95 (25.3)	<0.001
Invasive ventilation	8 (2.1)	21 (5.6)	101 (26.9)	<0.001
Secondary infection	16 (4.2)	19 (5.0)	95 (25.3)	<0.001
Dialysis	8 (2.1)	9 (2.4)	15 (4.0)	0.248
Total length of stay (day)	7.0 (6.0–9.0)	8.0 (6.0–8.0)	9.0 (7.0–12.7)	<0.001
ICU (days)	7.0 (3.0–9.2)	7.0 (5.0–10.0)	9.0 (5.0–14.0)	0.003
Transfer to hospice care	24 (6.4)	39 (10.3)	61 (16.2)	<0.001
Deaths	12 (3.2)	27 (7.2)	117 (31.1)	<0.001

BiPaP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; ICU, intensive care unit; SpO_2, oxygen saturation.

Table 2 D-dimer tertiles, clinical features and outcomes

	Tertile 1 (N=287, ≤266)	Tertile 2 (N=287, 266–720)	Tertile 3 (N=287, ≥721)	Statistics
Male	196 (68.3)	182 (63.4)	209 (72.8)	0.053
Female	91 (31.7)	105 (36.6)	78 (27.2)	0.072
Age ≤45 years	67 (23.3)	49 (17.1)	41 (14.3)	<0.001
Age 46–60 years	125 (43.6)	111 (38.7)	76 (26.5)	<0.001
Age >60 years	95 (33.1)	127 (44.3)	170 (59.2)	<0.001
Medical comorbidities				
Hypertension	112 (39.0)	142 (49.5)	140 (48.8)	0.019
Cardiovascular disease	42 (14.6)	43 (15.0)	69 (24.0)	0.003
Diabetes	102 (35.5)	121 (42.2)	125 (43.6)	0.051
Chronic obstructive pulmonary disease	02 (0.7)	03 (1.0)	11 (3.8)	0.010
Clinical features and investigations				
Fever	252 (87.8)	252 (87.8)	235 (81.9)	0.042
Shortness of breath	100 (34.8)	126 (43.9)	177 (61.7)	<0.001
Pulse (per minute)	88.0 (80.0–100.0)	89.0 (80.0–100.0)	90.0 (80.0–104.0)	0.462
Oxygen (SpO_2, %)	96.0 (93.0–97.0)	95.0 (91.0–97.0)	92.0 (83.0–96.0)	<0.001
Systolic BP, mm Hg	131.0 (120.0–144.0)	134.0 (123.5–150.0)	130.0 (120.0–143.0)	0.099
Haemoglobin (g/L)	133 (122–144)	127 (116–139)	121 (107–135)	<0.001
White cell count (10^9/L)	6.5 (4.8–8.6)	6.5 (5.0–9.2)	7.8 (5.7–12.0)	<0.001
Neutrophils (%)	77.4 (64.3–85.6)	79.0 (69.2–87.6)	85.6 (76.5–91.5)	<0.001
Lymphocytes (%)	17.6 (11.3–30.0)	15.7 (9.2–24.8)	11.0 (5.9–19.6)	<0.001
Platelet count (10^9/L)	2.3 (1.7–2.8)	2.1 (1.7–2.7)	2.2 (1.8–2.9)	0.349
hsCRP (mg/dL)	5.9 (2.1–16.3)	9.0 (3.8–20.5)	13.3 (4.1–34.1)	<0.001
Interleukin-6 (ng/dL)	13.4 (3.6–36.3)	20.3 (7.8–53.2)	36.1 (14.3–113.2)	<0.001
Ferritin (mg/dL)	252.9 (118.5–560.8)	339.6 (178.3–649.4)	481.8 (212.3–993.0)	<0.001
Lactic dehydrogenase (mg/dL)	258.0 (208.0–314.0)	290.0 (228.7–390.0)	369.0 (252.2–545.2)	<0.001
Creatinine (mg/dL)	0.89 (0.77–1.06)	0.93 (0.80–1.20)	1.0 (0.79–1.40)	<0.001
Sodium (mEq/L)	139.0 (135.5–141.0)	138.0 (135.0–141.0)	138.0 (134.0–142.0)	0.805
Potassium (mEq/L)	4.5 (4.2–4.8)	4.4 (4.1–4.8)	4.5 (4.1–4.9)	0.861
Serum aspartate transaminase (units)	27.5 (18.8–42.8)	28.3 (18.4–46.4)	28.2 (19.4–46.6)	0.622
Serum glutamate transaminase (units)	28.1 (20.2–39.9)	32.0 (22.0–47.4)	34.2 (25.2–57.8)	<0.001
HRCT scan thorax score (out of 25)	11.0 (7.2–15.0)	12.0 (8.0–17.0)	15.5 (10.0–20.0)	<0.001
Medicines				
Steroids	276 (96.2)	280 (97.6)	276 (96.2)	1.00
Remdesivir	266 (92.7)	274 (95.5)	260 (90.6)	0.329
Anticoagulants	277 (96.5)	279 (97.2)	270 (94.1)	0.139
Tocilizumab/bevacizumab	06 (2.1)	13 (4.5)	41 (14.3)	<0.001
Outcomes				
O_2 required	69 (24.0)	110 (38.3)	182 (63.4)	<0.001
Duration (days)	0.0 (0.0–4.0)	2 (0.0–6.0)	5.0 (0.0–9.0)	<0.001
Proning	204 (71.1)	206 (71.8)	196 (68.3)	0.626
Nasal cannula	66 (23.0)	94 (32.8)	120 (41.8)	<0.001
High-flow nasal cannula	19 (6.6)	36 (12.5)	54 (18.8)	<0.001
BiPaP support	16 (5.6)	31 (10.8)	60 (20.9)	<0.001
Invasive ventilation	09 (3.1)	23 (8.0)	78 (27.2)	<0.001
Secondary infection	14 (4.9)	24 (8.4)	69 (24.0)	<0.001
Dialysis	2 (0.7)	10 (3.5)	16 (5.6)	0.004
Total length of stay (day)	7.0 (6.0–9.0)	8.0 (6.0–10.0)	9.0 (7.0–12.0)	<0.001
ICU (days)	9.0 (5.0–12.0)	8.0 (5.0–14.0)	8.0 (5.7–13.0)	0.002
Transfer to hospice care	11 (3.8)	25 (8.7)	43 (15.0)	<0.001
Deaths	09 (3.1)	28 (9.8)	91 (31.7)	<0.001

BiPAP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; hsCRP, high-sensitivity C reactive protein; ICU, intensive care unit; SpO_2, oxygen saturation.

monocyte and platelet counts, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), creatine kinase (CK), CK-MB isoenzyme, activated partial thromboplastin time (aPTT) and prothrombin time.[7] Thrombotic biomarkers are also important and include markers of platelet activation, platelet aggregation, endothelial cell activation or injury, coagulation and fibrinolysis, and fibrinogen, D-dimer and aPTT.[8] A systematic review and meta-analysis reported significant association of lymphopenia, thrombocytopenia and elevated levels of CRP, procalcitonin, LDH and D-dimer with adverse outcomes in COVID-19.[9]

Limited studies have evaluated the prognostic importance of novel biomarkers in developing countries where the burden of COVID-19 is the highest.[10] Only small studies are available from developing countries including India.[11–17] Measurement of laboratory biomarkers is expensive and not routinely available in most developing countries. We initiated a prospective COVID-19 registry at our hospital to evaluate disease pattern and outcomes.[18] The present study aims to evaluate association of select novel biomarkers—high-sensitivity CRP (hsCRP), D-dimer, IL-6, LDH, ferritin and neutrophil–lymphocyte ratio (NLR) at hospital admission with clinical presentation, investigations and outcomes in COVID-19. We also evaluated whether NLR, a low-cost and widely available biomarker, is as important as others for prognostication.

METHODS

We initiated a registry of all patients with virologically confirmed COVID-19 admitted to our hospital since April 2020.[18]

Setting: this is a 220-bed tertiary care hospital with major focus on critical care and cardiovascular sciences. It was designated an advanced COVID-19 care hospital by the Government of Rajasthan and more than 20% beds in general wards and intensive care units (ICUs) were initially reserved for patients with COVID-19. This proportion was later increased to 50% and 75% as the number of critically ill patients in the region increased. The hospital provided subsidised treatment to all the admitted patients according to the state government regulations. We developed a protocol for admission so that only those patients fulfilling definite clinical criteria were hospitalised.[18] The case report form was updated and modified from that available at the regional COVID-19 care hospital at Health Sciences University.[19]

Patients: all patients presenting to medical and emergency departments with symptoms suggestive of upper respiratory infections were screened with reverse transcriptase-PCR (RT-PCR). Details of the sample collection and testing protocol have been reported.[20] We obtained details of case history; vital monitoring for all patients was recorded and a flow chart of all in-hospital investigations maintained. Haematological investigations included complete blood counts performed using XS-1000i, a six-part fully automated analyser from Sysmex, USA. Biochemical investigations focused on measurement of blood glucose, renal function tests (urea, creatinine, electrolytes, uric acid) and liver function tests (bilirubin, AST, ALT, alkaline phosphatase, proteins, albumin, gamma glutaryl transferase) that were measured using Roche Cobas 6000, a fully automated molecular biochemistry and immunoassay analyser. COVID-19-specific biomarkers measured at admission were hsCRP, IL-6, LDH and ferritin using Roche Cobas 6000. D-dimer was measured using a fully automated coagulation analyser, ECL-760 ERBA and ACL Pro, from Instrumentation Laboratories, USA.

All the hospitalised patients received clinical management according to national and international protocols.[18] Essentially, hydration and oxygenation were maintained using oral or intravenous fluids, and nasal cannula-based oxygen supplementation was provided as needed. Steroids were used only in patients needing non-invasive or invasive ventilatory support. Remdesivir was used according to international guidelines.[21 22] Anti-IL drugs (tocilizumab, bevacizumab) were used infrequently, and non-evidence-based therapies such as oral hydroxychloroquine, ivermectin, anti-viral drugs (eg, favipiravir or ritonavir) or plasma therapy were not recommended for hospitalised patients.

Statistical analyses

All the data were computerised and entered in MS Excel worksheets. We focused on clinical history at presentation, admission haematological and biochemical investigations, radiological imaging-CT scan, medical therapies, oxygenation, ventilation and in-hospital outcomes. Long-term follow-up data are not yet available. Descriptive analyses have been performed using SPSS package (V.22). The categorical variables have been reported as numbers and per cent, while continuous variables are reported as medians and 25th–75th percentile IQR. As most of the biochemical variables had a skewed distribution, we used medians and IQR for descriptive statistics. Intergroup comparisons have been performed using χ^2 test for categorical variables and Kruskal-Wallis test for non-parametric continuous variables. The biochemical variables (hsCRP, IL-6, LDH, D-dimer, ferritin) and haematological variables (NLR) were divided into tertiles, and clinical and other details tabulated accordingly. To identify correlation among various biomarkers, we performed both parametric (Pearson's r) and non-parametric (Spearman's r) analyses. Receiver operating characteristics (ROC) curves and area under the curve (AUC) were created for each of the biomarker for identification of sensitivity and specificity. To determine ORs and 95% CIs for deaths in second and third tertiles of various biomarkers versus first tertile, we initially performed a univariate logistic regression. Multivariate logistic regression was performed using variables likely to confound the outcomes such as age, sex, risk factors, comorbidities and medical treatments (steroids, remdesivir, tocilizumab, anticoagulants). We did not adjust for disease severity and mortality outcomes,

Table 3 Interleukin-6 tertiles, clinical features and outcomes

	Tertile 1 (N=346, ≤10.3)	Tertile 2 (N=346, 10.4–39.7)	Tertile 3 (N=346, ≥39.8	Statistics
Male	224 (64.7)	250 (72.3)	259 (74.9)	0.004
Female	122 (35.3)	96 (27.7)	87 (25.1)	<0.001
Age ≤45 years	92 (26.6)	77 (22.3)	41 (11.8)	<0.001
Age 46–60 years	132 (38.2)	123 (35.5)	123 (35.5)	<0.001
Age >60 years	122 (35.3)	146 (42.2)	182 (52.6)	<0.001
Medical comorbidities				
Hypertension	138 (39.9)	152 (43.9)	176 (50.9)	0.004
Cardiovascular disease	48 (13.9)	58 (16.8)	73 (21.1)	0.012
Diabetes	122 (35.3)	134 (38.7)	164 (47.4)	0.001
Chronic obstructive pulmonary disease	07 (2.0)	06 (1.7)	11 (3.2)	0.408
Clinical features and investigations				
Fever	283 (81.8)	306 (88.4)	297 (85.8)	0.045
Shortness of breath	125 (36.1)	149 (43.1)	194 (56.1)	<0.001
Pulse (per minute)	86.0 (78.0–97.0)	89.0 (80.0–100.0)	92.0 (82.0–106.0)	<0.001
Oxygen (SpO_2, %)	96.0 (94.0–98.0)	95.0 (92.0–97.0)	92.5 (85.0–96.0)	<0.001
Systolic BP, mm Hg	131.0 (122.7–145.0)	131.0 (122.0–142.2)	130.0 (120.0–144.0)	0.418
Haemoglobin (g/L)	130 (118–141)	129 (118–141)	124 (111–138)	<0.001
White cell count (10^9/L)	6.0 (4.6–8.6)	6.5 (4.7–8.9)	8.0 (6.0–11.6)	<0.001
Neutrophils (%)	75.5 (64.8–84.7)	78.4 (67.8–88.5)	83.7 (75.2–90.5)	<0.001
Lymphocytes (%)	19.3 (11.6–28.7)	17.1 (8.5–27.9)	12.1 (7.0–19.0)	<0.001
Platelet count (10^9/L)	2.4 (1.9–2.9)	2.1 (1.6–2.7)	2.1 (1.6–2.7)	<0.001
hsCRP (mg/dL)	3.0 (0.89–9.2)	7.0 (2.7–17.7)	15.3 (6.8–35.5)	<0.001
D-dimer (ng/dL)	306.6 (167.0–614.9)	450.0 (184.1–869.5)	622.5 (292.7–1368.7)	<0.001
Ferritin (mg/dL)	230.9 (102.1–504.4)	348.6 (173.8–614.1)	477.1 (244.2–884.2)	<0.001
Lactic dehydrogenase (mg/dL)	241.5 (192.0–320.0)	276.5 (224.0–375.7)	345.5 (264.2–499.7)	<0.001
Creatinine (mg/dL)	0.87 (0.75–1.06)	0.94 (0.78–1.19)	1.02 (0.83–1.42)	<0.001
Sodium (mEq/L)	140 (136.5–142.0)	138.0 (135.0–141.0)	137.0 (134.0–140.0)	<0.001
Potassium (mEq/L)	4.5 (4.1–4.8)	4.4 (4.1–4.8)	4.4 (4.0–4.9)	0.663
Serum aspartate transaminase (units)	28.1 (18.8–44.0)	26.6 (17.5–43.8)	29.7 (19.5–47.9)	0.440
Serum glutamate transaminase (units)	26.7 (19.6–39.6)	30.1 (21.5–47.5)	37.8 (25.0–57.4)	<0.001
HRCT scan thorax score (out of 25)	10.0 (4.2–14.0)	12.0 (7.0–16.0)	15.0 (12.0–19.0)	<0.001
Medicines				
Steroids	332 (96.0)	338 (97.7)	336 (97.7)	0.379
Remdesivir	307 (88.7)	322 (93.1)	321 (92.8)	0.056
Anticoagulants	329 (95.1)	333 (96.2)	325 (93.9)	0.372
Tocilizumab/bevacizumab	04 (1.2)	12 (3.5)	54 (15.6)	<0.001
Outcomes				
O_2 required	68 (19.7)	122 (35.3)	219 (63.3)	<0.001
Duration (days)	0.0 (0.0–0.0)	0.0 (0.0–6.0)	5.0 (0.0–9.0)	<0.001
Proning	179 (51.7)	220 (63.6)	242 (69.9)	<0.001
Nasal cannula	60 (17.3)	109 (31.5)	150 (43.4)	<0.001
High-flow nasal cannula	16 (4.6)	40 (11.6)	73 (21.1)	<0.001
BiPaP support	15 (4.3)	32 (9.2)	76 (22.0)	<0.001
Invasive ventilation	13 (3.8)	23 (6.6)	86 (24.9)	<0.001
Secondary infection	19 (5.5)	24 (6.9)	82 (23.7)	<0.001
Dialysis	8 (2.3)	5 (1.4)	16 (4.6)	0.032
Length of stay (day)	7.0 (6.0–9.0)	8.0 (6.0–10.0)	9.0 (7.0–12.0)	<0.001
ICU (days)	7.0 (4.0–10.2)	8.0 (6.0–12.5)	8.0 (5.0–13.7)	0.639
Transfer to hospice care	25 (7.2)	31 (9.0)	50 (14.3)	<0.001
Deaths	12 (3.5)	30 (8.7)	100 (28.9)	<0.001

BiPaP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; hsCRP, high-sensitivity C reactive protein; ICU, intensive care unit; SpO_2, oxygen saturation.

as these were the primary focus in the present study. P values of <0.05 are considered significant.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

RESULTS

In the successive waves of COVID-19 epidemic from April 2020 to July 2021, we evaluated 16 146 suspected patients with nasopharyngeal samples for SARS-CoV-2 antigen RT-PCR test. Of these, 3036 (18.8%) tested positive for the virus and 1251 (41.2%) were hospitalised. Data of these 1251 successively admitted patients have been obtained and analysed. Men were 876 (70.0%), aged >60 years were 560 (44.8%) and prevalence of important comorbidities were in: hypertension 551 (44.1%), diabetes 495 (39.6%), cardiovascular disease 241 (18.9%), and asthma or chronic obstructive pulmonary disease 72 (2.7%). Median symptom duration was 5 days (IQR 4–7) and oxygen saturation (SpO_2) at admission was 95% (90%–97%). Total white cell count was 6.9×10^9/L (5.0–9.8), neutrophils 79.2% (68.1–88.2), lymphocytes 15.8% (8.7%–25.5%) and platelets 2.2×10^9/L (1.7–2.8). At admission, median creatinine level was 0.93 mg/dL (0.78–1.22), serum creatinine >1.2 mg/dL (laboratory upper limits of normal) was in 312 (25.4%) and >2.0 mg/dL in 104 (8.5%). Levels of various biomarkers were hsCRP 6.9 mg/dL (2.2–18.9), D-dimer 464 ng/dL (201–982), IL-6 20.1 ng/dL (6.5–60.4), LDH 284 mg/dL (220–396) and ferritin 351 mg/dL (159–676). Other details including liver functions tests and radiography have been reported earlier.[18] Oxygen support at admission was in 480 (38.6%), and during hospitalisation, nasal cannula-based oxygen support was provided in 357 (28.7%), non-rebreather masks in 139 (11.2%), non-invasive ventilatory support in 137 (11.0%) and invasive ventilation in 144 (11.6%). Prone positioning (proning) was performed in 676 (54.3%) patients. All the patients received antibiotics, and clinically and microbiologically confirmed secondary infection developed in 133 (10.7%) patients. Although acute kidney injury was observed in 104 (8.5%), incidence of severely deranged renal function requiring haemodialysis was in 38 (3.1%). Of the total hospitalised patients, 1055 (84.9%) were discharged to home-based care, 15 (1.2%) transferred to hospice care and 173 (13.9%) died.

The patients have been divided into tertiles of various biomarkers—hsCRP (table 1, n=1130), D-dimer (table 2, n=961), IL-6 (table 3, n=1038), LDH (table 4, n=721), ferritin (table 5, n=998) and NLR (table 6, n=1214). Details of demography, risk factors, comorbid conditions, clinical features and investigations, medical management and outcomes for each of the biomarkers are provided in tables 1–6.

For each biomarker, those in the highest tertile have the most adverse clinical characteristics and significantly greater levels of clinical abnormalities—fever, shortness of breath, hypoxia, leucocytosis, lymphopenia—and higher levels of other biomarkers. Use of steroids, anticoagulants, remdesivir and anti-inflammatory drugs as well as proning, oxygenation and non-invasive and invasive ventilatory support is also greater in those in the second and third tertiles (tables 1–6). Evidence of bacteriologically confirmed secondary infection requiring escalation of antibiotics was observed in the highest tertile groups for all the biomarkers. Acute kidney injury advance disease requiring haemodialysis was low (3.1%) and was significantly more in the highest tertile groups only for D-dimer, IL-6 and ferritin biomarkers.

There is a strong correlation among various biomarkers (table 7). Results of parametric (Pearson's) and non-parametric (Spearman's) correlation among various biomarkers show significant intercorrelation among all the biomarkers (p<0.001). Non-parametric analysis shows greater correlation (r) of hsCRP with IL-6 (0.42), LDH (0.54), ferritin (0.42) and NLR (0.48), and NLR with hsCRP (0.48), LDH (0.48) and ferritin (0.38) (table 7). ROC curves are shown in figure 1. The AUC statistics were significant for all the biomarkers with AUC of >0.70, which is considered as of fair instrumental sensitivity for true positive outcomes. The highest AUC ±1 SD was observed for hsCRP (0.793±0.032) followed by NLR (0.778±0.031), LDH (0.773±0.032), D-dimer (0.752±0.034), ferritin (0.735±0.032) and IL-6 ((0.072±0.034).

Incidence of deaths in various biomarker groups is shown in figure 2. For all the biomarkers, incidence of death is the highest in the third tertile. Univariate, age and sex, multivariate (age, sex, risk factors, comorbidities) and multivariate and treatment-adjusted ORs (95% CI) for deaths in second and third versus first tertiles are in table 8. As compared with the lowest tertile (reference group, T1), deaths were significantly greater in second and third tertiles for all the biomarkers (figure 3). Multivariate-adjusted ORs (95% CI) in second and third tertiles, compared with the first, respectively, were for hsCRP 2.24 (1.11 to 4.50) and 12.56 (6.76 to 23.35); D-dimer 3.44 (1.59 to 7.44) and 14.42 (7.09 to 29.30); IL-6 2.56 (1.13 to 5.10) and 10.85 (5.82 to 20.22); ferritin 2.88 (1.49 to 5.58) and 8.19 (4.41 to 15.20); LDH 1.75 (0.81 to 3.75) and 9.29 (4.75 to 18.14); and NLR 3.47 (1.68 to 7.14) and 17.71 (9.12 to 34.39) (table 8). We also calculated multivariate ORs (95% CI) for comparison of second and third tertiles (table 8). Compared with the second tertile, the ORs were significantly greater in the third tertile for all the biomarkers—hsCRP 5.60 (3.57 to 8.78), D-dimer 4.20 (2.64 to 6.68), IL-6 4.23 (2.72 to 6.58), ferritin 2.84 (1.86 to 4.33), LDH 5.32 (3.12 to 9.05) as well as NLR 5.14 (3.40 to 7.78).

DISCUSSION

This study shows that among consecutive patients hospitalised with COVID-19, increasing levels of biomarkers: hsCRP, D-dimer, IL-6, LDH, ferritin and high NLR are

Table 4 Lactic dehydrogenase tertiles, clinical features and outcomes

	Tertile 1 (N=241, ≤242)	Tertile 2 (N=240, 243–347)	Tertile 3 (N=240, ≥348)	Statistics
Male	149 (61.8)	181 (75.4)	170 (70.8)	0.032
Female	92 (38.2)	59 (24.6)	70 (29.2)	0.043
Age ≤45 years	64 (26.6)	42 (17.5)	35 (14.6)	<0.001
Age 46–60 years	87 (36.1)	98 (40.8)	95 (39.6)	0.236
Age >60 years	90 (37.3)	100 (41.7)	110 (45.8)	<0.001
Medical comorbidities				
Hypertension	98 (40.7)	119 (49.6)	110 (45.8)	0.254
Cardiovascular disease	41 (17.0)	41 (17.1)	36 (15.0)	0.551
Diabetes	94 (39.0)	102 (42.5)	92 (38.3)	0.882
Chronic obstructive pulmonary disease	08 (3.3)	02 (0.8)	06 (2.5)	0.167
Clinical features and investigations				
Fever	195 (80.9)	210 (87.5)	205 (85.4)	0.177
Shortness of breath	69 (28.6)	108 (45.0)	167 (69.6)	<0.001
Pulse (per minute)	86.0 (78.0–98.5)	90.0 (81.0–101.7)	90.0 (80.0–104.7)	0.135
Oxygen (SpO_2, %)	97.0 (95.0–98.0)	96.0 (92.0–97.0)	90.0 (80.0–94.0)	<0.001
Systolic BP, mm Hg	130.0 (120.0–141.5)	133.0 (122.2–146.0)	133.0 (122.0–146.0)	0.082
Haemoglobin (g/L)	128 (116–140)	128 (116–142)	128 (115–139)	0.762
White cell count (10^9/L)	5.8 (4.5–7.3)	6.7 (4.7–9.7)	8.4 (5.9–12.1)	<0.001
Neutrophils (%)	71.9 (59.8–81.3)	79.2 (70.6–88.2)	87.0 (80.0–91.5)	<0.001
Lymphocytes (%)	22.1 (14.2–32.2)	14.9 (8.5–23.4)	9.4 (6.0–16.5)	<0.001
Platelet count (10^9/L)	2.2 (1.7–2.8)	2.2 (1.6–2.8)	2.1 (1.6–2.9)	0.729
hsCRP (mg/dL)	2.3 (0.75–6.7)	8.8 (4.2–20.0)	18.2 (8.4–39.6)	<0.001
D-dimer (ng/dL)	292.0 (167.0–633.1)	346.5 (182.7–668.1)	758.4 (382.2–1715.1)	<0.001
Interleukin-6 (ng/dL)	10.8 (3.8–28.1)	25.3 (9.6–66.2)	42.8 (13.1–112.3)	<0.001
Ferritin (mg/dL)	180.9 (84.3–320.7)	357.2 (169.3–618.9)	592.0 (374.0–1173.0)	<0.001
Creatinine (mg/dL)	0.88 (0.75–1.08)	0.94 (0.78–1.14)	1.01 (0.84–1.37)	0.001
Sodium (mEq/L)	139.0 (137.0–141.0)	137.0 (134.0–140.0)	138.0 (134.0–141.0)	<0.001
Potassium (mEq/L)	4.3 (4.0–4.7)	4.5 (4.1–4.8)	4.6 (4.1–5.1)	0.002
Serum aspartate transaminase (units)	21.6 (15.4–30.7)	32.9 (21.0–48.6)	37.0 (23.1–63.1)	<0.001
Serum glutamate transaminase (units)	22.5 (17.4–29.6)	32.6 (23.6–46.0)	45.6 (31.5–68.7)	<0.001
HRCT scan thorax score (out of 25)	7.0 (2.0–11.0)	13.0 (10.0–16.0)	17.0 (13.2–21.0)	<0.001
Medicines				
Steroids	214 (88.8)	236 (98.3)	236 (98.3)	<0.001
Remdesivir	199 (82.6)	226 (94.2)	221 (92.1)	0.001
Anticoagulants	216 (89.6)	229 (95.4)	233 (97.1)	0.001
Tocilizumab/bevacizumab	03 (1.2)	13 (5.4)	42 (17.5)	<0.001
Outcomes				
O_2 required	42 (17.4)	85 (35.4)	178 (74.2)	<0.001
Duration (days)	0.0 (0.0–0.0)	0.0 (0.0–6.0)	6.0 (2.0–10.5)	<0.001
Proning	116 (48.1)	151 (62.9)	190 (79.2)	<0.001
Nasal cannula	39 (16.2)	78 (32.5)	119 (49.6)	<0.001
High-flow nasal cannula	10 (4.1)	19 (7.9)	66 (27.5)	<0.001
BiPaP support	07 (2.9)	19 (7.9)	68 (28.3)	<0.001
Invasive ventilation	08 (3.3)	15 (6.3)	70 (29.2)	<0.001
Secondary infection	11 (4.6)	20 (8.3)	55 (22.9)	<0.001
Dialysis	9 (3.7)	3 (1.3)	7 (2.9)	0.233
Length of stay (day)	7.0 (6.0–8.0)	8.0 (6.0–10.7)	9.0 (7.0–13.0)	<0.001
ICU (days)	8.0 (4.0–10.0)	9.0 (6.0–15.0)	8.0 (5.0–14.0)	0.265
Transfer to hospice care	27 (11.2)	17 (7.1)	46 (19.2)	0.237
Deaths	11 (4.6)	20 (8.3)	80 (33.3)	<0.001

BiPaP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; hsCRP, high-sensitivity C reactive protein; ICU, intensive care unit; SpO_2, oxygen saturation.

Table 5 Ferritin tertiles, clinical features and outcomes

	Tertile 1 (N=333, ≤2078)	Tertile 2 (N=333, 207–526)	Tertile 3 (N=332, ≥527)	Statistics
Male	179 (53.8)	245 (73.6)	280 (84.3)	<0.001
Female	154 (46.2)	88 (26.4)	52 (15.7)	<0.001
Age ≤45 years	78 (23.4)	59 (17.7)	65 (19.6)	0.253
Age 46–60 years	119 (35.7)	120 (36.0)	117 (35.2)	0.653
Age >60 years	136 (40.8)	154 (46.2)	150 (45.2)	0.168
Medical comorbidities				
Hypertension	149 (44.7)	152 (45.6)	152 (45.8)	0.788
Cardiovascular disease	65 (19.5)	58 (17.4)	53 (16.0)	0.229
Diabetes	136 (40.8)	136 (40.8)	134 (40.4)	0.900
Chronic obstructive pulmonary disease	09 (2.7)	08 (2.4)	08 (2.4)	0.804
Clinical features and investigations				
Fever	261 (78.4)	283 (85.8)	299 (90.1)	<0.001
Shortness of breath	103 (30.9)	156 (46.8)	207 (62.3)	<0.001
Pulse (per minute)	86.0 (78.0–97.0)	90.0 (80.0–102.0)	90.0 (80.0–103.0)	0.001
Oxygen (SpO_2, %)	97.0 (95.0–98.0)	95.0 (91.0–97.0)	92.0 (85.0–96.0)	<0.001
Systolic BP, mm Hg	132.0 (121.0–144.0)	130.0 (121.2–141.7)	131.0 (120.0–147.0)	0.780
Haemoglobin (g/L)	126 (112–136)	129 (118–142)	130 (116–143)	0.007
White cell count (10^9/L)	5.9 (4.5–7.8)	7.0 (5.2–9.8)	7.8 (5.5–11.5)	<0.001
Neutrophils (%)	72.1 (60.1–81.4)	79.8 (70.7–88.6)	85.7 (77.1–91.1)	<0.001
Lymphocytes (%)	22.2 (14.1–32.6)	15.0 (8.5–24.0)	11.1 (6.3–18.5)	<0.001
Platelet count (10^9/L)	2.2 (1.7–2.7)	2.2 (1.7–2.9)	2.1 (1.6–2.8)	0.598
hsCRP (mg/dL)	3.4 (0.98–8.9)	7.7 (2.3–18.7)	14.8 (6.3–37.0)	<0.001
D-dimer (ng/dL)	336.1 (167.0–724.7)	489.0 (234.0–855.2)	587.1 (265.0–1283.7)	<0.001
Interleukin-6 (ng/dL)	11.5 (3.9–30.0)	24.5 (9.0–67.2)	33.8 (12.1–86.8)	<0.001
Lactic dehydrogenase (mg/dL)	233.5 (187.2–276.7)	292.0 (232.0–383.5)	385.0 (281.0–531.0)	<0.001
Creatinine (mg/dL)	0.88 (0.73–1.06)	0.93 (0.78–1.2)	1.0 (0.85–1.40)	<0.001
Sodium (mEq/L)	139.0 (136.0–141.0)	138.0 (135.0–141.0)	137.5 (134.0–141.0)	0.034
Potassium (mEq/L)	4.4 (4.1–4.7)	4.4 (4.1–4.8)	4.6 (4.1–5.0)	0.005
Serum aspartate transaminase (units)	22.6 (15.5–32.6)	27.9 (20.1–44.0)	39.1 (24.0–64.7)	<0.001
Serum glutamate transaminase (units)	24.5 (18.2–34.6)	29.9 (22.3–42.2)	42.2 (29.9–67.4)	<0.001
HRCT scan thorax score (out of 25)	9.0 (3.0–13.0)	13.0 (9.0–17.0)	15.5 (12.0–19.0)	<0.001
Medicines				
Steroids	306 (91.9)	327 (98.2)	323 (97.3)	0.001
Remdesivir	278 (83.5)	305 (91.6)	306 (92.2)	<0.001
Anticoagulants	307 (92.2)	322 (96.7)	315 (94.9)	0.125
Tocilizumab/bevacizumab	04 (1.2)	28 (8.4)	35 (10.5)	<0.001
Outcomes				
O_2 required	65 (19.5)	128.0 (38.4)	205.0 (61.7)	<0.001
Duration (days)	0.0 (0.0–0.0)	0.0 (0.0–6.0)	05 (0.0–9.0)	<0.001
Proning	174 (52.3)	193 (58.0)	236 (71.1)	<0.001
Nasal cannula	58 (17.4)	107 (32.1)	141 (42.5)	<0.001
High-flow nasal cannula	12 (3.6)	42 (12.6)	69 (20.8)	<0.001
BiPaP support	11 (3.3)	36 (10.8)	73 (22.0)	<0.001
Invasive ventilation	12 (3.6)	28 (8.4)	79 (23.8)	<0.001
Secondary infection	13 (3.9)	39 (11.7)	64 (19.3)	<0.001
Dialysis	7 (2.1)	5 (1.5)	17 (5.1)	0.012
Total length of stay (days)	7.0 (6.0–9.0)	8.0 (6.0–10.0)	8.0 (7.0–11.7)	<0.001
ICU stay (days)	7.0 (4.0–9.0)	9.0 (5.0–13.5)	8.0 (5.0–13.0)	0.055
Transfer to hospice care	31 (9.3)	38 (11.4)	41 (12.3)	<0.001
Deaths	13 (3.9)	38 (11.4)	88 (26.5)	<0.001

BiPaP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; hsCRP, high-sensitivity C reactive protein; ICU, intensive care unit; SpO_2, oxygen saturation.

Table 6 Neutrophil:lymphocyte ratio tertiles, clinical features and outcomes

	Tertile 1 (N=405, ≤3.34)	Tertile 2 (N=405, 3.35–7.75)	Tertile 3 (N=404, ≥7.76)	Statistics
Male	255 (63.0)	276 (68.1)	319 (79.0)	<0.001
Female	150 (37.0)	129 (31.9)	85 (21.0)	<0.001
Age ≤45 years	112 (27.7)	78 (19.3)	62 (15.3)	<0.001
Age 46–60 years	134 (33.1)	159 (39.3)	130 (32.2)	0.346
Age >60 years	159 (39.3)	168 (41.5)	212 (52.5)	<0.001
Medical comorbidities				
Hypertension	158 (39.0)	181 (44.7)	197 (48.8)	0.005
Cardiovascular disease	71 (17.5)	71 (17.5)	86 (21.3)	0.172
Diabetes	142 (35.1)	169 (41.7)	171 (42.3)	0.035
Chronic obstructive pulmonary disease	09 (2.2)	14 (3.5)	09 (2.2)	1.00
Clinical features and investigations				
Fever	320 (79.0)	323 (79.8)	324 (80.2)	0.675
Shortness of breath	118 (29.1)	176 (43.5)	252 (62.5)	<0.001
Pulse (per minute)	85.0 (78.0–95.0)	90.0 (80.0–100.0)	91.0 (80.0–104.7)	<0.001
Oxygen (SpO_2, %)	97.0 (95.0–98.0)	95.0 (92.0–97.0)	91.0 (82.0–95.0)	<0.001
Systolic BP, mm Hg	130.0 (120.0–140.0)	130.0 (120.0–142.0)	133.0 (120.0–148.0)	0.145
Haemoglobin (g/L)	128 (118–140)	127 (113–140)	126 (111–139)	0.086
White cell count (10^9/µL)	5.3 (4.2–6.8)	6.7 (5.3–8.8)	10.0 (7.4–13.6)	<0.001
Neutrophils (%)	63.4 (56.1–68.5)	79.5 (76.4–82.6)	90.8 (88.1–93.4)	<0.001
Lymphocytes (%)	30.4 (25.5–35.9)	15.9 (13.4–18.9)	6.8 (4.4–8.8)	<0.001
Platelet count (10^9/µL)	2.1 (1.7–2.7)	2.2 (1.8–2.8)	2.2 (1.6–2.9)	0.487
hsCRP (mg/dL)	2.6 (0.82–7.3)	6.8 (2.6–16.0)	16.6 (6.7–36.6)	<0.001
D-dimer (ng/dL)	329.8 (167.0–618.7)	428.5 (187.3–806.8)	662.0 (294.0–1517.0)	<0.001
Interleukin-6 (ng/dL)	13.8 (4.0–36.2)	19.8 (5.9–54.8)	30.3 (11.9–102.8)	<0.001
Ferritin (mg/dL)	196.1 (113.1–416.2)	333.9 (169.8–709.9)	523.3 (314.4–996.0)	<0.001
Lactic dehydrogenase (mg/dL)	236 (188.5–285.0)	277.0 (219.0–376.0)	368.0 (281.0–518.0)	<0.001
Creatinine (mg/dL)	0.87 (0.74–1.04)	0.95 (0.76–1.20)	1.0 (0.86–1.42)	<0.001
Sodium (mEq/L)	140.0 (137.0–142.0)	138.0 (134.0–141.0)	137.5 (134.0–141.0)	<0.001
Potassium (mEq/L)	4.4 (4.1–4.7)	4.4 (4.1–4.8)	4.5 (4.1–5.0)	0.001
Serum aspartate transaminase (units)	25.7 (17.4–40.1)	26.8 (17.4–43.5)	32.7 (21.0–53.8)	0.007
Serum glutamate transaminase (units)	27.1 (19.7–41.0)	30.5 (21.3–45.1)	35.4 (22.7–55.4)	<0.001
HRCT scan thorax score (out of 25)	10.0 (4.0–12.0)	12.0 (8.0–17.0)	16.0 (12.0–20.0)	<0.001
Medicines				
Steroids	352 (86.9)	362 (89.4)	375 (92.8)	0.006
Remdesivir	315 (77.8)	334 (82.5)	351 (86.9)	0.001
Anticoagulants	354 (87.4)	367 (90.6)	363 (89.9)	0.261
Tocilizumab/bevacizumab	02 (0.5)	18 (4.4)	53 (13.1)	<0.001
Outcomes				
O_2 required	57 (14.1)	136 (33.6)	275 (68.1)	<0.001
Duration (days)	0 (0.0)	0.0 (0.0–5.0)	5.0 (1.0–9.0)	<0.001
Proning	169 (41.7)	218 (53.8)	283 (70.0)	<0.001
Nasal cannula	50 (12.3)	116 (28.6)	184 (45.5)	<0.001
High-flow nasal cannula	05 (1.2)	37 (9.1)	96 (23.8)	<0.001
BiPaP support	09 (2.2)	29 (7.2)	95 (23.5)	<0.001
Invasive ventilation	08 (2.0)	27 (6.7)	107 (26.5)	<0.001
Secondary infection	9 (2.2)	21 (5.2)	102 (25.2)	<0.001
Dialysis	13 (3.2)	9 (2.2)	16 (4.0)	0.363
Length of stay (day)	7.0 (5.0–8.0)	8.0 (6.0–9.0)	8.0 (6.0–12.0)	<0.001
ICU (days)	5.0 (2.0–8.0)	7.0 (3.7–10.0)	8.0 (4.0–13.0)	0.002
Transfer to hospice care	50 (12.3)	57 (14.1)	78 (19.3)	<0.001
Deaths	10 (2.5)	33 (8.1)	128 (31.7)	<0.001

BiPaP, bilevel positive airway pressure; BP, blood pressure; HRCT, high-resolution CT; hsCRP, high-sensitivity C reactive protein; ICU, intensive care unit; SpO_2, oxygen saturation.

Table 7 Correlation matrix (parametric Pearson's r and non-parametric Spearman's r) among the biomarkers evaluated

Variable	hsCRP	D-dimer	Interleukin-6	Lactic dehydrogenase	Ferritin	Neutrophil–lymphocyte ratio
Pearson correlation (r)						
hsCRP	—	0.180**	0.142**	0.287**	0.269**	0.305**
D-dimer	0.180**	—	0.262**	0.346**	0.224**	0.225**
Interleukin-6	0.142**	0.262**	—	0.317**	0.166**	0.202**
Lactic dehydrogenase	0.287**	0.346**	0.377**	—	0.416**	0.308**
Ferritin	0.269**	0.224**	0.166**	0.416**	—	0.256**
Neutrophil–lymphocyte	0.305**	0.225**	0.202**	0.308**	0.256**	—
Spearman correlation (r)						
hsCRP	—	0.231**	0.424**	0.543**	0.420**	0.477**
D-dimer	0.231**	—	0.288**	0.357**	0.227**	0.282**
Interleukin-6	0.424**	0.288**	—	0.360**	0.280**	0.266**
Lactic dehydrogenase	0.543**	0.357**	0.360**	—	0.521**	0.477**
Ferritin	0.420**	0.227**	0.280**	0.521**	—	0.383**
Neutrophil–lymphocyte	0.477**	0.282**	0.266**	0.477**	0.383**	—

** p<0.01
hsCRP, high-sensitivity C reactive protein.

associated with greater illness severity, oxygenation, non-invasive and invasive ventilation and exponentially higher in-hospital mortality. We also show that NLR, a simple, widely available and inexpensive investigation, provides prognostic information that is similar to the more expensive biomarkers.

A meta-analysis that evaluated influence of biomarkers on poor outcomes (measured as SpO2 <90%, invasive mechanical ventilation, severe disease, ICU admission and mortality) among hospitalised patients with COVID-19 from December 2019 to August 2020 included 32 studies with 10 491 patients.[9] Biomarkers included were lymphocyte, platelets, D-dimer, LDH, CRP, AST, ALT, creatinine, procalcitonin and CK. This study reported a significant association between lymphopenia, thrombocytopenia and elevated levels of CRP, procalcitonin, LDH, D-dimer with COVID-19 severity with ORs varying from 3.33 (2.51 to 4.41) for lymphopenia, 4.37 (3.37 to 5.68) for elevated CRP and 5.48 (3.89 to 7.71) for LDH. We used mortality as the endpoint and the results of our study are not directly comparable; however, the ORs are similar when we compared tertile 2 with tertile 3 in our study (table 8). Another meta-analysis reviewed impact of biomarkers of endothelial dysfunction (von Willebrand factor, tissue type plasminogen activator, soluble thrombomodulin, plasminogen activator inhibitor-1) and reported significant association with poor outcomes among 1187 patients from 17 studies.[23] We did not evaluate these biomarkers and cannot directly compare our results; however, significance of D-dimer, another marker of coagulation and vascular dysfunction, in the present study suggests importance of coagulation markers in COVID-19. A more recent review reported that biomarkers useful for risk prediction in COVID-19 include several proinflammatory

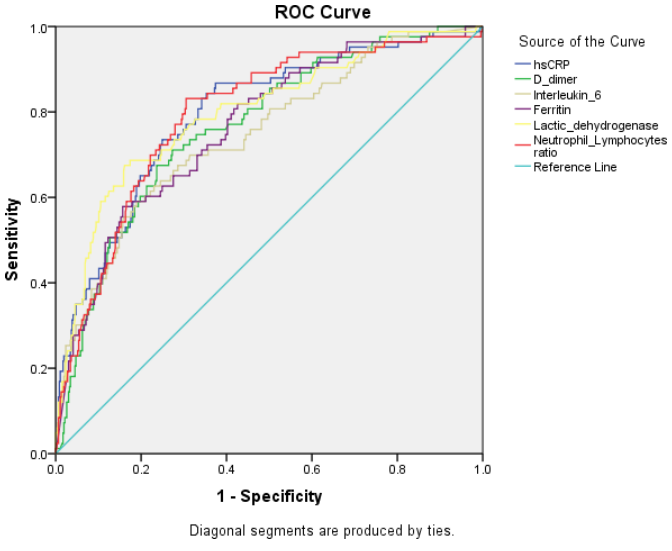

Figure 1 Receiver operating characteristics curve shows that area under the curve for each parameter was more than 0.7 (range 0.720–0.793, p<0.001). hsCRP, high-sensitivity C reactive protein; IL-6, interleukin-6; LDH, lactic dehydrogenase.

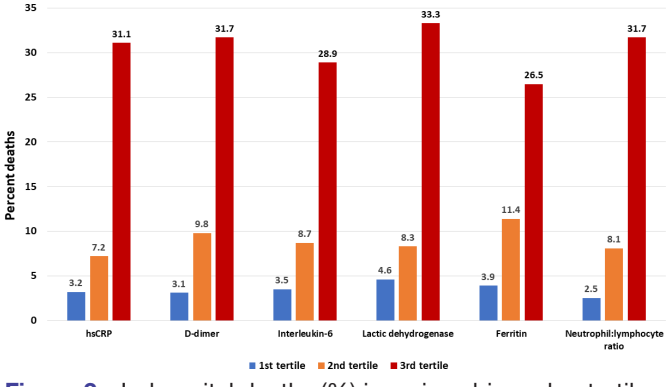

Figure 2 In-hospital deaths (%) in various biomarker tertiles. hsCRP, high-sensitivity C reactive protein.

Table 8 Univariate, age and sex, multivariate (age, sex, risk factors, comorbidities) and multivariate (plus treatments)-adjusted OR for association of various biomarkers with in-hospital deaths among second and third tertiles compared with the first

	Unadjusted		Age/sex adjusted		Multivariate adjusted*		Multivariate* plus treatment adjusted		
	Tertile 1 vs 2	Tertile 1 vs 3	Tertile 1 vs 2	Tertile 1 vs 3	Tertile 1 vs 2	Tertile 1 vs 3	Tertile 2 vs 3	Tertile 1 vs 2	Tertile 1 vs 3	Tertile 2 vs 3
High-sensitivity C reactive protein	2.34 (1.17 to 4.70)*	13.74 (7.43 to 25.42)***	2.37 (1.18 to 4.77)*	14.30 (7.70 to 26.55)***	2.29 (1.14 to 4.60)*	13.39 (7.23 to 24.80)***	5.83 (3.72 to 9.14)***	2.24 (1.11 to 4.50)*	12.56 (6.76 to 23.35)***	5.60 (3.57 to 8.78)***
D-dimer	3.34 (1.54 to 7.21)**	14.34 (7.06 to 29.13)***	3.20 (1.48 to 6.92)**	13.98 (6.87 to 28.42)***	3.26 (1.31 to 7.05)**	13.89 (6.87 to 28.27)***	4.26 (2.68 to 6.77)***	3.44 (1.59 to 7.44)**	14.42 (7.09 to 29.30)***	4.20 (2.64 to 6.68)***
Interleukin-6	2.64 (1.33 to 5.25)**	11.43 (6.14 to 21.26)***	2.66 (1.34 to 5.29)**	11.41 (6.13 to 21.24)***	2.61 (1.31 to 5.18)**	10.96 (5.88 to 20.43)***	4.22 (2.71 to 6.56)***	2.56 (1.13 to 5.10)**	10.85 (5.82 to 20.22)***	4.23 (2.72 to 6.58)***
Ferritin	3.17 (1.65 to 6.07)***	8.88 (4.84 to 16.23)***	3.68 (1.90 to 7.12)***	11.68 (6.23 to 21.90)***	3.19 (1.66 to 6.11)***	9.13 (4.97 to 16.78)***	2.86 (1.88 to 4.35)***	2.88 (1.49 to 5.58)**	8.19 (4.41 to 15.20)***	2.84 (1.86 to 4.33)***
Lactic dehydrogenase	1.90 (0.89 to 4.06)	10.45 (5.39 to 20.26)***	1.93 (0.90 to 4.13)	10.75 (5.52 to 20.93)***	1.85 (0.87 to 3.97)	10.51 (5.41 to 20.41)***	5.62 (3.30 to 9.58)***	1.75 (0.81 to 3.75)	9.29 (4.75 to 18.14)***	5.32 (3.12 to 9.05)***
Neutrophil–lymphocyte ratio	3.50 (1.70 to 7.21)**	18.32 (9.45 to 35.50)***	3.47 (1.68 to 7.15)**	19.63 (10.08 to 38.23)***	3.34 (1.62 to 6.89)**	17.52 (9.03 to 34.0)***	5.23 (3.45 to 7.91)***	3.47 (1.68 to 7.14)**	17.71 (9.12 to 34.39)***	5.14 (3.40 to 7.78)***

ORs for comparison of third tertile with the second are also provided.
* p<0.05; ** p<0.01; *** p<0.001
*Multivariate ORs are adjusted for age, sex, risk factors, comorbidities and without and with treatments (steroids, remdesivir, tocilizumab, bevacizumab and anticoagulants).

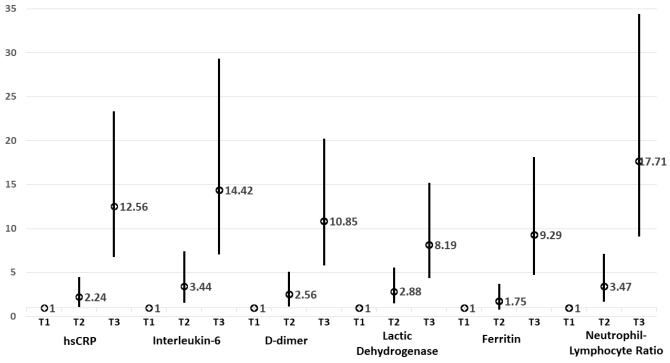

Figure 3 Multivariate (age, sex, risk factors, comorbidity, treatments)-adjusted ORs and 95% CIs for association of various biomarkers with in-hospital mortality. T1 (reference), T2 and T3 are the respective tertiles. hsCRP, high-sensitivity C reactive protein.

cytokines, neuron-specific enolase, LDH, AST, neutrophil count, NLR, troponins, CK-MB, myoglobin, D-dimer, brain natriuretic peptide and N-terminal pro-hormone. Some of these biomarkers can be readily used to predict disease severity, hospitalisation, ICU admission and mortality, while markers of metabolomic and proteomic analysis have not yet been translated to clinical practice.[24] The International COVID-19 Thrombosis Biomarkers Colloquium and international agencies recommend routine use of some of these biomarkers for prognostication.[8 22] Our study shows a strong correlation among various biomarkers (table 7) and suggests that clinical use of any one or two of these biomarkers may be used for prognostication.

Our study also shows that a widely available biomarker—NLR—is as predictive as other biomarkers. This is similar to previous studies,[9 24] although the magnitude of risk is much higher as compared with them (figure 2). The relative risk is similar to previous studies on comparison of second and third tertiles (table 8). Regolo *et al*[25] compared NLR with CRP in 411 elderly patients with COVID-19; 33% of patients died during hospitalisation. When divided into tertiles according to NLR values, it was observed that NLR was a better predictor of mortality than CRP with largest AUC (0.772) and high specificity (71.9%) and sensitivity (72.9%). Other studies, considerably smaller than ours, have also identified the role of NLR in identification of severe disease with similar AUC on ROC analyses (figure 1).[26 27] Reviews have concluded that this is an important marker,[7 8 23 28] and the present study, which is larger than most of the previous studies, confirms this observation. This finding is important in the context of India and low and lower middle-income countries as the cost of this test is minuscule compared with the biomarkers evaluated in the present study.

Our study has limitations and strengths, apart from those mentioned above. This is one of the larger studies that has evaluated multiple biomarkers in mild, moderate and severe COVID-19 cases and shows an exponential increase in deaths with rising levels of hsCRP, D-dimer,

IL-6, LDH, ferritin and NLR, and the strength of association is maintained after multivariate adjustment (figure 3). This is also one of the larger studies from India—a country with one of the highest deaths from COVID-19.[10 29 30] We did not assess well-known markers of COVID-19 severity such as serum creatinine and other markers of renal dysfunction, liver enzymes (transaminases, etc), markers of cardiac damage (troponins, myocardial-bound CK) or pulmonary involvement in the present study, and this is a study limitation. Other limitations include lack of assessment of novel biomarkers of coagulation and endothelial dysfunction.[8 9 23 24] We also do not have long-term data in these patients and cannot comment on importance of biomarkers in incidence of post-acute syndrome of COVID-19. Only few studies have addressed this question.[31 32]

In conclusion, the present study in hospitalised patients with COVID-19 shows that higher levels of multiple biomarkers—hsCRP, D-dimer, IL-6, LDH, ferritin and NLR—at admission are associated with greater illness severity and significantly higher in-hospital mortality. The study also shows that NLR, a universally available investigation, provides prognostic information similar to the less available biomarkers. Our study is important in the context of occurrence of multiple waves of COVID-19 in many developed and developing countries.[1–3 33 34] We demonstrate that a simple investigation—NLR—can provide important prognostic information and can inexpensively triage patients presenting to primary care into low risk—who can be advised home-based care—and the intermediate and high-risk groups to more intensive care settings.

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Contributors RSK and RG initiated the project. KM, HCA, JBG, YS and AM collected the data. RG and KS performed all statistical analyses. RG had main responsibility of writing the article. SSingh and SSharma performed the biochemical and pathological investigations. RSK, RG and KS contributed to the structure and content of the manuscript, and all authors have read and approved the final draft. RSK acts as the guarantor.

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Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the ethics committee of Eternal Heart Care Centre and Research Institute, Jaipur, India (Government of India registration, CDSCO No. ECR/615/Inst/RJ/2014/RR-20) before initiation of the study. Informed consent from all the patients or next of kin was obtained for anonymised data publication.

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