Original Article

Role of biomarkers in prognostication of moderate and severe COVID-19 cases

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Abstract

Background: COVID-19 pandemic demanded upgrading of laboratory medicine to limit morbidity, disability and mortality from moderate and severe SARS-COV-2 infections. **Objective:** To assess among moderate and severe COVID-19 patients, C-reactive protein (CRP), procalcitonin (PCT), ferritin, D-dimer, interleukin 6 (IL-6), lactate dehydrogenase (LDH), total and differential leucocyte count (TLC and DLC), neutrophil-to-lymphocyte ratio (NLR), absolute platelet count (APC), prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) to find their interdependence and role in prognosis. **Methods:** This open label analytical cross-sectional noninterventional study evaluated array of independent biochemical, haematological and coagulopathy markers, viz. CRP, PCT, ferritin, D-dimer, IL-6, LDH, TLC, DLC, NLR, absolute platelet count, PT, APTT and INR on consecutive 100 patients with diagnosis of moderate and severe COVID-19 from July to August 2021. **Results:** In our study, on consecutive designated 100 cases (55 cases moderate and 45 cases severe), more severity were reported as the age progressed; gender difference was not noted. Among independent markers, CRP, PCT, ferritin, D-dimer, IL-6 and LDH had statistically significant relation in comparison with severity of the disease as Chi-square calculated value ($P < 0.05$). TLC, DLC and APC showed no significant relation in comparison with severity of the disease; NLR had highly significant relation. PT showed significant relation in comparison with severity, though APTT and INR did not show significant relation. **Conclusion:** Our research group felt that CRP, PCT, ferritin, D-dimer, IL-6, LDH and NLR should be in included in clinical practice guidelines to prognosticate COVID-19 cases. Furthermore, translational researches are needed at all levels of healthcare to improve validity for practices of primary care physicians.

Keywords: COVID-19, moderate, prognostic marker, SARS-COV-2, severe

Introduction

The colossal scale of morbidity, mortality and disability from SARS-CoV-2 have ushered novel experiences for healthcare forces across disciplines, levels and geographic borders. Amid imbalanced growth of public health around the world highest brunt of humanitarian crisis fell upon low- and middle-income countries (LMICs). Furthermore, COVID-19 has put laboratory medicine at the crossroads with uncountable incremental qualitative and quantitative workload for valid laboratory data, which are backbone to combat this unprecedented pandemic. Laboratory medicine personnel had to frame innovative approaches of research and development of inflammatory biomarkers for early as well as uneventful

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recovery from SARS-CoV-2 infection. All the nations have evolved ground-breaking Clinical Practice Guidelines from diagnosis through holistic interventions inclusive of combating concurrent complications, viz. opportunistic infections and handle ever-increasing inventory of long COVID.[1,2] Globally, 768,187,096 confirmed cases of COVID-19 including 6,945,714 deaths were reported by THE World Health Organization till date affecting 219 countries and territories.[2] Healthcare workers experienced trauma amid selfless services while learning to categorize stages for early diagnosis and prompt treatment supported by lab medicine.[3] Instant identification of clinical laboratory predictors of disease progression helps to stratify risks, differentiate severe cases from mild to moderate ones, guide treatment and therapeutic monitoring.[4] However, summary of previous research works indicated gaps of judicious use of biomarkers to afford confident interpretation of disease progression. In the current scenario, it is relevant for the general primary care providers and family physicians to get updated with reinforcement at regular intervals by continuing education. Our research group assessed CRP, LDH, PCT, ferritin, IL-6, PT, APTT, INR, D-dimer, TLC, DLC, NLR and absolute platelet count among moderate and severe COVID-19 patients and explored interrelation among these biomarkers in diagnosis and prognosis to halt the pandemic.

Methods

The present study compared biomarkers and explored their interdependence on each other in moderate and severe COVID-19 cases to predict favourable/unfavourable outcomes. The independent biochemical, haematological and coagulopathy markers studied were C-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin (PCT), ferritin, interleukin 6 (IL-6), prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), D-dimer, total and differential leucocyte count, neutrophil-to-lymphocyte ratio (NLR) and absolute platelet count among moderate and severe COVID-19 patients, and their role in prognosis of these patients at the notified State Government run COVID hospital at Kolkata since pandemic was declared.

Place of the study

State COVID Hospital: Medical College, Kolkata

Study population

Of consecutive 100 patients with documented history and diagnosis of moderate and severe COVID-19 cases, 55 cases were moderate and 45 cases were designated as severe by the Clinical Practice guidelines practiced in this State COVID hospital.

Study protocol

In this open label, analytical cross-sectional noninterventional study was conducted from July 2021 to August 2021 among COVID-19 cases with following selection criteria:

Inclusion criteria

All adult moderate and severe COVID-19 cases admitted as per COVID hospital protocol with documented history and confirmed diagnosis (using RT-PCR).

Exclusion criteria

Children and adolescents below 18 years of age, nonconsenting, known coagulopathy, bleeding disorder, haemoglobinopathy and serious comorbidities.

PRIMARY OUTCOME VARIABLE: CRP, LDH, PCT, ferritin, IL-6, PT, APTT, INR, D-dimer, TLC, DLC, NLR and absolute platelet count of moderate and severe COVID-19 cases.

Case definitions

Disease classification was performed according to clinical guidance by ICMR-COVID-19 National task force, Ministry of Health and Family Welfare, Govt. of India.[5]

Mild disease: Upper respiratory tract symptom (±fever) minus shortness of breath or hypoxia.

Moderate disease: Respiratory rate ≥24/min, breathlessness, SpO2 90-93% on room air.

Severe disease: Respiratory rate >30/min, breathlessness, SpO2 <90% on room air.

Instruments

Fully automated Hemostasis Analyzer (STA Compact Max 3) was utilized for the estimation of parameters of coagulation. INR was calculated as patient's PT/mean normal PT [ISI]

CRP (C-reactive protein), LDH, procalcitonin: done by commercial kits in fully automated Modular Biochemistry Analyzer Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

Ferritin was performed by Advia Centaur XP Immunoassay system (Siemens Healthineers).

TLC, DLC, absolute platelet count: measured by six-part automated cell counter Sysmex XT 4000i utilizing the principle of fluorescence flow cytometry.

The quality of results was validated with internal quality control (IQC) procedures and participation to an External Quality Assessment Scheme (EQAS).

Examination of peripheral blood smear was initiated based on abnormal findings from an automated count and detailed analysis of morphology of peripheral blood cells. Slide preparation was performed by trained medical laboratory technologist, ensuring quality slides for microscopy. Smear was stained by Leishman stain composed of polychrome methylene blue (basic component) and eosin (acidic component). Standardization of adequate contact

time with each new batch of stain made or procured to maintain uniform staining intensity. Slides were reviewed by Pathologist using Olympus Magnus Binocular Microscope Model Ch20i.

Data collection procedure

Data were collected by the Principal and Co-Principal investigators to ensure sanctity and uniformity to reduce bias to the minimum, kept strictly confidential with the investigators and used exclusively for this study. After approval from Institute Research Committee and Institute Ethics Committee, the study was initiated. Ethical principles were adhered to with strict confidentiality as per Helsinki declaration in letter and spirit. Informed consent process was followed with best possible sincerity and 100 consecutive patients were recruited after obtaining consent from all the participants before inclusion in the study. Each patient was individually counselled regarding the objectives of the study and was ensured that the data will only be used for research purpose. Furthermore, it was ensured that their consent or not will not hamper the course of treatment irrespective of their participation in the study and/or even if they leave the study for any reason after consent in the midway. Then, written informed consent was obtained from each participant or 'Legally Authorized Representative' without coercion. The standard operative procedure on COVID -19 protocols from national stakeholders was strictly applied regarding patient preparation and collection of sample and approximately 10 ml fasting blood sample(s) was collected from each patient in single needle prick. The sample was aseptically collected in the primary containers and transferred to secondary container at the Central laboratory for further storage/analysis. To reduce risk of biohazards in this unparalleled pandemic, all the additional precautions were strictly followed during the collection, transport, storage, biochemical testing, report writing and returning back report to the individual patient, and their caregivers was directly supervised by the investigators. All the suggested protocols from ICMR and State government were undertaken to prevent biohazards, viz. use of protection mask, double gloves, physical distancing and all the sanitization measures of donning and doffing at dedicated places. In the laboratory, there was dedicated infrastructure, instruments and logistics for suspected/confirmed infected body fluid/materials, viz. single use of sterile materials and disposal using universal precaution and standard operative procedures on biomedical waste management.

Statistical analysis

The collected data were cleaned, collated and entered in the MS Excel sheet 'Master table', and statistical analysis was performed using SPSS software version 21. The data were tabulated with biomarkers as independent variables and linked their levels above normal cut-off values with moderate and severe COVID-19 cases. Statistical analysis was performed using the Chi-square test to find significant correlation between study variables at alpha level of five per cent, which was considered significant in all analysis; values of the Chi-square test on the independent markers were compared to their corresponding tabulated values. Later on, Pearson correlation coefficient analysis was performed to find positive strengths of associations of different biochemical, haematological and coagulopathy factors – large: 0.5 to 1.0; medium: 0.3 to <0.5; small: 0.1 to <0.3.

Results

Consecutive 100 cases (55 moderate and 45 severe) were recruited in our study; no significant ($P > 0.05$) relation was noted in comparison with moderate and severe cases of the disease with age and gender.

Biochemical markers were statistically analysed between moderate and severe cases of the COVID-19. CRP (10 mg/L cut-off), PCT (0.1 ng/mL cut-off), Ferritin (250 ng/mL cut-off), IL-6 (80 pg/mL cut-off) and LDH (500 U/L cut-off): highly significant ($P = .000$) relation in comparison with moderate and severe cases of the COVID-19. D-dimer (1.5 μg/mL cut-off) is a significant ($P = 0.034$) relation in comparison with moderate and severe cases of the disease. Among haematological markers, total and differential leucocyte count and absolute platelet count, no significant ($P > 0.05$) relation was noted. NLR had highly significant ($P = 0.003$) relation in comparison with moderate and severe cases of the COVID-19 (2.5 cut-off) [Table 1].

Among coagulopathy markers, APTT and INR had no significant relation with severity; PT showed significant ($P = 0.003$) relation in comparison with severity of the disease [Table 1]. Pearson's correlation coefficient analysis was performed to find positive strengths of association of different biochemical, haematological and coagulopathy factors: large (L) and medium (M). Positive strengths of associations were noted in moderate COVID-19 cases among biomarkers: in CRP: IL-6(L), LDH, ferritin (M); in LDH: CRP, ferritin, IL-6, D-dimer (M), D-dimer (M); in PCT: D-dimer (M); in ferritin: CRP, LDH, IL-6, TC (M); in IL-6: CRP (L), LDH and NLR (M); in PT: INR (L); APTT: INR (M); in INR: PT (L) and APTT (M); in D-dimer: LDH, PCT and NLR (M); in TC: ferritin, NLR (M); in NLR: IL-6, D-dimer, TC (M) [Table 2].

Positive strengths of association were noted in severe COVID-19 patients among biomarkers as: in LDH: ferritin and IL-6 (L), D-dimer (M); in PCT: TC (L); in ferritin: LDH (L), D-dimer (M); in IL-6: LDH (L), ferritin (M); in PT: APTT, INR, APC (M); in APTT: PT (M); in INR: PT (M); in D-dimer: LDH and ferritin (M); in TC: PCT, NLR (L); in NLR: TC (L) [Table 3].

Discussion

Global researchers are working on valid biomarkers in search of predictors of outcomes of severity of COVID-19. In the light of the increasing complexity of diagnosis and interventions, the primary care providers and family physicians need upgrading to manage load of comorbidities with limited infrastructures. These are critical to provide spectrum of early interventions as well as preventive measures to reduce morbidity, mortality and disability; true especially for economically developing countries where

Table 1: Biochemical, haematological and coagulopathy markers in moderate and severe COVID-19 cases

Parameter Cut-off	Moderate (*n*=55)		Severe (*n*=45)		Chi-square test	
	Male *n*=31	Female *n*=24	Male *n*=24	Female *n*=21	Pearson Chi-square Asym. *P*. Sig 2-sided	Likelihood ratio Asym. *P*. Sig 2- sided
C-reactive protein (CRP)						
<10 mg/L	04	07	01	01	35.416	42.894
≥10 mg/L	27	17	23	20	0.000	0.000
Procalcitonin (PCT)						
<0.1 ng/mL	26	23	02	04	58.852	69.258
>0.1 ng/mL	05	01	22	17	0.000	0.000
Ferritin						
<250 ng/mL	24	21	03	04	47.803	55764
>250 ng/mL	07	03	21	17	0.000	0.000
D-dimer						
<1.5 µg/mL	23	20	12	10	15.184	18.632
>1.5 µg/mL	08	04	12	11	0.034	0.009
Interleukin 6 (IL-6)						
<80 pg/mL	31	23	09	05	65.307	88.241
>80 pg/mL	00	01	15	16	0.000	0.000
Lactate dehydrogenase (LDH)						
<500 U/L	04	11	00	01	39.070	48.092
>500 U/L	27	13	24	20	0.000	0.000
Neutrophil-to-leukocyte ratio (NLR)						
<2.5	05	06	01	01	24.880	28.041
>2.5	26	18	23	20	0.003	0.001
Prothrombin time (PT)						
<13 s	09	08	02	01	21.834	24,469
>13 s	22	16	22	20	0.003	0.001

intensive care set-up might not match the increasing demand of the quantity and quality of medical care.[6]

Our study attempted to explore independent biochemical, haematological and coagulopathy markers viz. CRP, PCT, ferritin, D-dimer, IL-6, LDH, TLC, DLC, NLR, absolute platelet count, PT, APTT and INR to predict rationality of the role in moderate and severe COVID-19 cases.

Age

In our study, a more number of cases were reported as the age progressed. Yet, no significant ($P > 0.05$) relation in comparison with severity of the disease was noted. Research groups across globe have noted that chances of worst prognosis with COVID-19 go up as age advances may be related with chronic health problems, compromised immune system, lesser probability of healing of lung tissue; highest risk is in people 85 and older.[7] World Health Organization commented that COVID-19 is often more severe in people who are older than 60 years with or without comorbidities.[8]

Gender

In this study, the researchers did not find significant ($P > 0.05$) relation of gender in comparison with severity of the disease. COVID-19 exhibits differences in morbidity and mortality between sexes, more in males as a worldwide phenomenon.[9] A comprehensive approach was hypothesized to recognize uneven death rate among males as biological, psychological, behavioural and social factors put them at disproportionate risk of death which may explore stepwise approach to clinical, public health and policy interventions.[10]

C-reactive protein (CRP)

Higher CRP levels were noted with associated overproduction of inflammatory cytokines in severe COVID-19. Normal level of CRP is less than 10mg/L; rapidly rises within 6-8 hours and gives the highest peak in 48 hours of pathogenesis with half-life of 19 hours and its concentration decreases when inflammation or tissue damage gets resolved; falling concentration is useful marker for monitoring disease severity; unfavourable outcomes have been associated with higher levels of CRP along with other laboratory parameters.[11-15]

Procalcitonin (PCT)

Procalcitonin is a peptide precursor of the hormone calcitonin, synthesized by the parafollicular cells of thyroid gland, and involved in calcium homeostasis.[16] The reference value for adult procalcitonin is <0.1 ng/mL, while opinion differs to indicate the presence of suspected infections in levels above 0.25 ng/mL. Research group reported cytokine storms are common with severe-to-critical symptoms with elevated PCT levels; serial measurement promoted as indicator to predict prognosis and may contribute to reduce fatal outcomes of COVID-19.[17]

Table 2: Pearson's correlation coefficient analysis of biomarkers in moderate COVID-19 cases

Correlations												
	CRP	**LDH**	**PCT**	**FERRITIN**	**IL6**	**PT**	**APTT**	**INR**	**DDIMER**	**TC**	**NLR**	
CRP												
Pearson Correlation	1	0.378	-0.089	0.460**	0.523**	-0.195	0.246	- 055	0.127	0.245		
Sig. (2-tailed)		0.004	0.516	0.000	0.000	0.154	0.071	0.691	0.356	0.071	0.063	0.084
n	55	55	55	55	55	55	55	55	55	55	55	55
LDH												
Pearson Correlation	0.378	1	0.132	0.388**	0.495**	0.175	0.228	0.206	0.324*	-0.013	0.116	-0.256
Sig. (2-tailed)	0.004		0.338	0.003	0.000	0.200	0.095	0.130	0.016	0.922	0.399	0.059
n	55	55	55	55	55	55	55	55	55	55	55	55
PCT												
Pearson Correlation	-0.089	0.132	1	0.002	0.032	-0.043	-0.092	-0.173	0.327*	-0.033	-0.096	-0.060
Sig. (2-tailed)	0.516	0.338		0.990	0.816	0.757	0.504	0.208	0.015	0.812	0.484	0.665
n	55	55	55	55	55	55	55	55	55	55	55	55
FERRITIN												
Pearson Correlation	0.460**	0.388**	0.002	1	0.481**	-0.076	0.156	-0.091	-0.093	0.463**	0.048	-0.086
Sig. (2-tailed)	0.000	0.003	0.990		0.000	0.580	0.255	0.507	0.499	0.000	0.729	0.532
n	55	55	55	55	55	55	55	55	55	55	55	55
IL6												
Pearson Correlation	0.523**	0.495**	0.032	0.481**	1	-0.283*	0.106	-0.190	0.205	0.258	0.432**	-0.174
Sig. (2-tailed)	0.000	0.000	0.816	0.000		0.036	0.441	0.164	0.134	0.058	0.001	0.203
n	55	55	55	55	55	55	55	55	55	55	55	55
PT												
Pearson Correlation	-0.195	0.175	-0.043	-0.076	-0.283*	1	0.240	0.796**	0.013	-0.194	-0.215	-0.145
Sig. (2-tailed)	0.154	0.200	0.757	0.580	0.036		0.077	0.000	0.923	0.156	0.115	0.293
n	55	55	55	55	55	55	55	55	55	55	55	55
APTI												
Pearson Correlation	0.246	0.228	-0.092	0.156	0.106	0.240	1	0.392**	0.054	-0.156	0.000	-0.201
Sig. (2-tailed)	0.071	0.095	0.504	0.255	0.441	0.077		0.003	0.693	0.256	0.998	0.140
n	55	55	55	55	55	55	55	55	55	55	55	55
INR												
Pearson Correlation	-0.055	0.206	-0.173	-0.091	-0.190	0.796	0.392	1	0.033	-0.121	-0.119	-0.203
Sig. (2-tailed)	0.691	0.130	0.208	0.507	0.164	0.000	0.003		0.811	0.377	0.387	0.138
n	55	55	55	55	55	55	55	55	55	55	55	55
DDIMER												
Pearson Correlation	0.127	0.324*	0.327*	-0.093	0.205	0.013	0.054	0.033	1	-0.068	0.382**	-0.070
Sig. (2-tailed)	0.356	0.016	0.015	0.499	0.134	0.923	0.693	0.811		0.622	0.004	0.610
n	55	55	55	55	55	55	55	55	55	55	55	55
TC												
Pearson Correlation	0.245	-0.013	-0.033	0.463**	0.258	-0.194	-0.156	-0.121	-0.068	1	0.366**	0.228
Sig. (2-tailed)	0.071	0.922	0.812	0.000	0.058	0.156	0.256	0.377	0.622		0.006	0.094
n	55	55	55	55	55	55	55	55	55	55	55	55
NLR												
Pearson Correlation	0.252	0.116	-0.096	0.048	0.432**	-0.215	0.000	-0.119	0.382**	0.366**	1	0.011
Sig. (2-tailed)	0.063	0.399	0.484	0.729	0.001	0.115	0.998	0.387	0.004	0.006		0.934
n	55	55	55	55	55	55	55	55	55	55	55	55
plt in lakh												
Pearson Correlation	-0.235	-0.256	-0.060	-0.086	-0.174	-0.145	-0.201	-0.203	-0.070	0.228	0.011	1
Sig. (2-tailed)	0.084	0.059	0.665	0.532	0.203	0.293	0.140	0.138	0.610	0.094	0.934	
n	55	55	55	55	55	55	55	55	55	55	55	55

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

Ferritin

Normal ferritin levels are upto 250 ng/mL of blood across ages and genders. A higher-than-normal level of ferritin is indicative of an iron accumulation in the organs; prognostically leads to destruction and loss of normal function. Higher values of ferritin were reported by different research groups in COVID-19 patients with severe-to-critical symptoms.[11-15]

D-dimer

D-dimer produced in human body by the degradation of

Table 3: Pearsons correlation coefficient analysis of biomarkers in severe COVID-19 cases

	Correlation											
	CRP	**LDH**	**PCT**	**FERRITIN**	**IL6**	**PT**	**APTT**	**INR**	**DDIMER**	**TC**	**NLR**	**plt in lakh**
CRP												
Pearson Correlation	1	0.186	-0.096	0.230	0.200	-0.153	-0.078	-0.277	0.261	0.014	0.042	0.221
Sig. (2-tailed)		0.222	0.530	0.128	0.189	0.317	0.610	0.065	0.084	0.929	0.782	0.145
n	45	45	45	45	45	45	45	45	45	45	45	45
LDH												
Pearson Correlation	0.186	1	0.282	0.529**	0.572**	0.046	-0.026	0.124	0.361*	-0.073	-0.216	-0.166
Sig. (2-tailed)	0.222		0.061	0.000	0.000	0.763	0.865	0.418	0.015	0.631	0.154	0.277
n	45	45	45	45	45	45	45	45	45	45	45	45
PCT												
Pearson Correlation	-0.096	0.282	1	0.231	0.180	0.103	-0.258	0.104	0.257	0.578**	0.142	-0.137
Sig. (2-tailed)	0.530	0.061		0.128	0.238	0.500	0.087	0.495	0.089	0.000	0.353	0.368
n	45	45	45	45	45	45	45	45	45	45	45	45
FERRITIN												
Pearson Correlation	0.230	0.529**	0.231	1	0.314*	0.024	-0.009	0.061	0.349*	0.194	-0.050	0.033
Sig. (2-tailed)	0.128	0.000	0.128		0.035	0.875	0.955	0.689	0.019	0.203	0.743	0.828
n	45	45	45	45	45	45	45	45	45	45	45	45
IL6												
Pearson Correlation	0.200	0.572**	0.180	0.314*	1	0.081	-0.183	0.161	0.110	-0.013	-0.095	0.014
Sig. (2-tailed)	0.189	0.000	0.238	0.035		0.599	0.230	0.290	0.473	0.93 1	0.536	0.930
n	45	45	45	45	45	45	45.	45.	45	45	45	45
PT												
Pearson Correlation	-0.153	0.046	0.103	0.024	0.081	1	0.436**	0.483**	-0.069	0.141	0.280	-0.438**
Sig. (2-tailed)	0.317	0.763	0.500	0.875	0.599		0.003	0.001	0.654	0.357	0.062	0.003
n	45	45	45	45	45	45	45	45	45	45	45	45
APTT												
Pearson Correlation	-0.078	-0.026	-0.258	-0.009	-0.183	0.436**	1	0.270	0.159	-0.150	-0.131	-0.221
Sig. (2-tailed)	0.610	0.865	0.087	0.955	0.230	0.003		0.073	0.296	0.325	0.390	0.144
n	45	45	45	45	45	45	45	45	45	45	45	45
INR												
Pearson Correlation	-0.277	0.124	0.104	0.061	0.161	0.483**	0.270	1	0.113	-0.035	0.081	-0.305*
Sig. (2-tailed)	0.065	0.418	0.495	0.689	0.290	0.001	0.073		0.458	0.818	0.599	0.041
n	45	45	45	45	45	45	45	45	45	45	45	45
DDIMER												
Pearson Correlation	0.261	0.361*	0.257	0.349*	0.110	-0.069	0.159	0.113	1	0.214	-0.058	0.035
Sig. (2-tailed)	0.084	0.015	0.089	0.019	0.473	0.654	0.296	0.458		0.157	0.704	0.819
n	45	45	45	45	45	45	45	45	45	45	45	45
TC												
Pearson Correlation	0.014	-0.073	0.578**	0.194	-0.013	0.141	-0.150	-0.035	0.214	1	0.503**	0.123
Sig. (2-tailed)	0.929	0.631	0.000	0.203	0.931	0.357	0.325	0.818	0.157		0.000	0.422
n	45	45	45	45	45	45	45	45	45	45	45	45
NLR												
Pearson Correlation	0.042	-0.216	0.142	-0.050	-0.095	0.280	-0.131	0.081	-0.058	0.503**	1	-0.184
Sig. (2-tailed)	0.782	0.154	0.353	0.743	0.536	0.062	0.390	0.599	0.704	0.000		0.225
n	45	45	45	45	45	45	45	45	45	45	45	45
plt in lakh												
Pearson Correlation	0.221	-0.166	-0.137	0.033	0.014	-0.438**	-0.22 1	-0.305*	0.035	0.123	-0.184	1
Sig. (2-tailed)	0.145	0.277	0.368	0.828	0.930	0.003	0.144	0.041	0.819	0.422	0.225	
n	45	45	45	45	45	45	45	45	45	45	45	45

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

cross-linked (by factor XIII) fibrin showing ongoing activation of haemostatic system.[18-20] Elevated D-dimer levels reflect ongoing activation of the haemostatic and thrombolytic system, providing clinical utility in: a) evaluation of thrombus formation, b) ruling out deep vein thrombosis and c) disseminated intravascular coagulation (DIC). D-dimer value on admission is an accurate biomarker for predicting mortality in patients with COVID-19 and 1.5 μg/ml is the optimal cut-off value for predicting mortality. Research groups predicted cytokine storms with severe-to-critical symptoms with elevated D-dimer levels. Above

normal range of 0–16.4 pg/mL for IL-6 (95% CIs), different short- and long-term complications of COVID-19 were reported including acute kidney injury. Researchers reported elevated level of IL-6 was associated with high case fatality of COVID-19.

Interleukin 6 (IL-6)

Research group noted that maximal IL-6 levels to predict respiratory failure, with cut-off level of 80 pg/ml, were 22 times higher compared to patients with lower levels. With overwhelmed intensive care units (ICUs) and overcrowded emergency rooms, correct triage is crucial, wherein IL-6 may be an effective marker to predict upcoming respiratory failure with higher accuracy to help physicians correctly prognosticate at an early stage.[21]

Lactate dehydrogenase (LDH)

Biomarker of tissue damage was found to be higher in our population with moderate and severe cases. In SARS-COV-2 infection, an acceptable study level of elevated LDH with an upper limit cut-off in the range of 240–255 U/L is required. Research group noted association between elevated LDH levels measured at earliest time point in hospitalization and disease outcomes in patients with COVID-19 with ~6-fold increase in odds to predict severe disease and ~16-fold increase in odds of mortality.[12, 22] Other research groups also correlated higher levels of LDH with worse outcomes from severe acute lung injury and mortality.[12-15] Elevated levels of LDH were correlated with unfavourable prognosis in COVID-19 cases, especially in diabetics, wherein reduced glycogen synthesis, altered glucose oxidative metabolism and higher nonoxidative glycolysis may play a role.[23]

Haematology

Routine haematology with total and differential leucocyte count, and absolute platelet count is backbone of the management of COVID-19. Release of neutrophil chemo-attractive elements and downstream effects on leukocyte population are global host response to SARS--2.[24] The neutrophil to lymphocyte ratio (NLR) is an independent risk factor for severe COVID-19, and neutrophilia forecasts poor outcomes in COVID-19 patients. In COVID-19 patients, several research groups reported increased levels of neutrophils along with lymphopenia as hallmark of severity. The combination of lymphopenia and increased neutrophil to lymphocyte ratio in SARS-CoV-2-infected cases on hospital admission were identified to predict severe acute lung injury and mortality.

Activated partial thromboplastin time (APTT) and prothrombin time (PT)

In our study, among haematological markers, significant relation was not noted with severity probably in APTT and INR as most of them were on anticoagulant regime on admission as per Standard Treatment Protocol for COVID-19 cases in this COVID hospital. However, PT showed significant relation in comparison with severity of the disease. APTT explores coagulation factors XII, XI, IX, VIII, X, V, II and I except platelets. Critical values that prompt a clinical alert: aPTT more than 70 seconds signifies spontaneous bleeding crisis.[18,25,26]

International normalized ratio (INR)

INR reference range (not on anticoagulation): 0.8-1.1; 3 is critical value above which there is increased risk of bleeding if on anticoagulant therapy.[26,27]

STRENGTHS OF THE STUDY: There is limited number of published study that assessed the role of laboratory markers in predicting disease severity and outcome in the Eastern Indian region. Furthermore, in this study we have studied biochemical, haematological and coagulopathy markers in COVID-19 cases.

Limitations of the study

There were several limitations. Firstly, as the study was conducted among moderate-to-severe COVID-19 cases, we had to strictly follow inclusion and exclusion criteria as per protocol that led to limited number of cases. Secondly, for moderate and severe cases, consent was major hindrance.

Future direction of the study

Since the number of infected case and mortality rate is reported to be increased in an overwhelmed manner, understanding the disease predictors in our setup is crucial.

Conclusions

Advancing age, male gender and biomarkers, viz. CRP, PCT, ferritin, D-dimer, IL-6, LDH and NLR were associated with higher morbidity, disability and mortality in COVID-19 cases. To halt this unprecedented pandemic, we needed translational researches to find user-friendly and cost-effective inflammatory biomarkers for predictions of disease outcomes vis-à-vis natural protective cytokine responses culmination into lethal pathogenic process.[28,29] Our research work will fill gaps in the current scenario regarding prognostication of COVID-19.

Key take-home message

The unique set of information emerging from this study is relevant to the interest of the journal readers who are in general primary care providers and family physicians.

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Conflicts of interest

There are no conflicts of interest.

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