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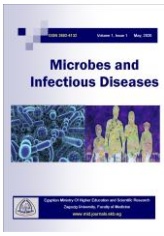

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Original article

Evaluation of the predictive value of c-reactive protein, interleukin-6 and their derived immune-inflammatory indices in COVID-19 Egyptian patients

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ABSTRACT

Background: In coronavirus disease 2019 (COVID-19), finding sensitive biomarkers is critical for detecting severe cases early and intervening effectively. **Aim of the work:** To compare and evaluate the value of pretreatment c-reactive protein (CRP), interleukin-6 (IL-6), and their derived immune-inflammatory indices (CRP/albumin (CRP/alb), lymphocyte/CRP (L/CRP), and lymphocyte/IL-6 (L/IL-6)) in the prediction of COVID-19 severity and in-hospital mortality. **Methods:** This cross-sectional study included 85 confirmed COVID-19 patients, their complete blood count with differential, as well as albumin and IL-6 levels on the day of their hospital admission, were assessed and compared. We followed all patients till their in-hospital death or discharge from the hospital. **Results:** On admission levels of CRP, IL-6, and CRP/alb were significantly higher (p=0.001) in severe patients and non-survivors, but L/CRP and L/IL-6 were significantly lower (p=0.001) compared to non-severe patients and non-survivors. CRP/alb and L/CRP at cut-offs of 1.65 and 260.86, respectively, were the best predictors for COVID-19 severity, while IL-6 and L/IL-6 at cut-offs of 120 pg/ml and 5.40, respectively, were the best predictors for COVID-19 in-hospital mortality. IL-6 was an independent risk factor associated with severe disease development (odds ratio (OR): 1.033; 95% confidence interval (CI): 1.002-1.066). **Conclusions:** Pretreatment levels of CRP, IL-6, and their derived indices should be included in the diagnostic work-up of COVID-19 to determine the severity and predict the outcome.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic is presently sweeping the globe, affecting millions of individuals [1]. Coronavirus disease 2019 is more contagious than seasonal influenza, has a longer incubation time, and is linked to a greater hospitalization rate and overall mortality. The virus spreads through the respiratory tract and quickly progresses to severe infection, multiorgan failure, and death [2].

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Although most COVID-19 patients recover quickly, patients with moderate severity, particularly those with risk factors like old age, obesity, and associated co-morbidities, can rapidly worsen and become severe, increasing ICU admission and mechanical ventilation, indicating a high mortality rate [3]. Thus, early identification of potentially severe patients is critical for halting disease progression at an early stage.

Coronavirus disease 2019 progression may be facilitated by an abnormal inflammatory response and cytokine storm that can lead to multiorgan failure and death in people with severe illness [4]. Inflammatory indicators like C-reactive protein (CRP) and interleukin (IL)-6 were proved to be elevated in this hyper-inflammatory state [5,6], and IL-6 have been linked to lymphopenia by causing widespread lymphocyte mortality [7]. Another study found a link between high IL-6 serum levels and impaired T and natural killer (NK) cell cytotoxicity in COVID-19 patients [8].

Therefore, our study was performed to evaluate the predictive efficacy of individual and combined baseline inflammatory indicators; C-reactive protein and IL-6 together with their derived immune-inflammatory indices (CRP/albumin (CRP/alb), lymphocyte/CRP (L/CRP), and lymphocyte/IL-6 (L/IL-6)) in a cohort of Egyptian COVID-19 patients.

Materials and Methods

Patients

In this cross-sectional study we recruited 85 COVID-19 adult patients (aged ≥ 18 years) from Isolation Hospitals of Ain-Shams University (ASU), Cairo, Egypt. A real-time polymerase chain reaction (RT-PCR) test confirmed COVID-19 infection in all the patients. Pregnant women and patients with hematological diseases, immunodeficiencies, or autoimmune disorders were excluded. All participants were classified according to their disease severity using the COVID-19 management guideline provided by Ain Shams University Hospitals [9]. Mild asymptomatic and moderate symptomatic cases were grouped in the non-severe group while severe and critical patients were grouped in the severe group.

Intensive care management for severe and critical cases

Severe and critical cases were transferred to intensive care management if they met one or more of the following criteria: Respiratory rate ≥30 breaths/min; Oxygen saturations< 93% at a rest state; Arterial partial pressure of oxygen (PaO2)/ Fraction of inspired oxygen (FiO2) <300 mmHg. Patients with more than 50% lesion progression within 24 to 48 hours in lung imaging. Occurrence of respiratory failure requiring mechanical ventilation. Development of shock or other organ failure requiring monitoring and treatment in ICU [9].

According to ASU isolation hospitals protocols, active management included the standard ICU management; with empirical antibiotics (intravenous Meropenem and Vancomycin or intravenous Linozeloid) until cultures' results were obtained, symptomatic treatment with intravenous paracetamol for fever and myalgia, proton pump inhibitors and motility regulators. Prophylactic anticoagulation in the form of subcutaneous Enoxaparin 0.5 mg/kg/day was given. Antiviral drug: Lopinavir-Ritonavir (200/ 50mg) 2 tablets bid were given for 5-10 days. Tocilizumab was reserved for cases with all of the following findings: 1. Chest imaging consistent with COVID-19. 2. Rapidly worsening respiratory status requiring >4- 6 L/min oxygen. 3. Absence of systemic bacterial or fungal co-infection. 4. Elevated inflammatory markers (e.g., ferritin >600 ug/mL; D-dimer >1 mg/L). 5. Patients who didn't have a poor prognosis who were unlikely to survive >48 hours (in critical cases). 6. Mechanical ventilation for ≤48 hours (in Critical cases). Tocilizumab was given as a slow intravenous infusion. The first dose was calculated as 8 mg/kg, and the response was assessed; if the patient needed the second dose, it was calculated as 4 mg/kg after 12 hrs. If Tocilizumab was not available, methylprednisolone was given as 1 mg/kg/day IV for 5 days; then 0.5 mg/kg/day IV for 2 days. Prone positioning was encouraged (unless contraindicated) (30 min / 2 hours) or as tolerated. Fluid restriction policy was followed to keep balance either net or slightly negative, considering hemodynamics and renal perfusion. As for oxygen therapy, Supplemental oxygen therapy was started immediately with target SpO2 ≥ 94%. Oxygen therapy was escalated from the nasal cannula to the face mask with a reservoir. High Flow Nasal Oxygen (HFNO) and Non Invasive ventilation (NIV) were also used. Invasive ventilation was initiated when Spo2 was not stabilized (P/F less than 150), increased work of breathing and the use of accessory muscles. Mechanical ventilation was initiated using lower tidal volumes (8 ml/kg

predicted body weight, PBW) and decreased to 4ml/kg to maintain plateau pressure. Permissive hypercapnia was permitted. Deep sedation was used to control respiratory drive and achieve tidal volume targets and avoid ventilator dyssynchrony. If patients continued to deteriorate with a P/F ratio below 150, prone ventilation was initiated within 24-48 hours of ARDS onset and maintained for 12–16 hours per day.

Data collection

Data were collected from the medical records of all patients, including epidemiological and clinical data (such as age, sex, underlying diseases or comorbidities, length of stay in the hospital, the requirement for ICU admission, and patients' outcomes).

Blood sampling and analysis

A venous blood sample (5 mL) was taken via venipuncture from each enrolled patient at the time of their hospital admission. Collected blood was divided into two vacutainer tubes; an ethylenediamine tetra-acetic acid (EDTA) tube for CBC with differential count analysis by Sysmex XT-1800i autoanalyzer (Sysmex, Japan), and a plain biochemistry tube with clot activator for serum separation by centrifugation for 10 mins at 2500 ×g after complete blood clotting. Sera were immediately used to determine albumin levels using a Beckman Coulter AU480 autoanalyzer (Beckman Coulter, Inc., USA). The dynamic range of albumin was 1.5-6.0 g/dL. The remaining sera were kept at -80°C until they were tested for IL-6 using a commercially available Human IL-6 ELISA Kit from RayBiotech Life, Inc., GA, USA [CODE: ELH-IL6-1]. According to the supplier's instructions, the assay was carried out with a detection range of 3 pg/ml – 1000.

Calculations

Derived immune-inflammatory indices were calculated as follows: L/CRP = lymphocyte (number/µl)/CRP (mg/dl); L/IL-6 = lymphocyte (number/µl)/IL-6 (pg/ml); CRP/alb = CRP (mg/dl)/albumin (g/dl).

Statistical analysis

Statistical analysis was performed using the SPSS 20.0. Descriptive statistics were done using median and percentiles for quantitative nonparametric measures, while categorized data were described in numbers and percentages. The Wilcoxon Rank Sum test and Chi-square test were used to compare two groups of quantitative nonparametric and qualitative data, respectively. The receiver operating characteristic (ROC) curve assessed the predictive performance of each pretreatment biomarker alone and the clinical benefit of the biomarker combination. The odds ratio (OR) and 95% confidence interval (CI) were calculated using logistic regression analysis. The significant probability of error was set at 0.05.

Results

Demographic and clinical characterization of the study cohort

The study included 85 confirmed COVID-19 patients of them 48 (56.5%) were males, and 37 (43.5%) were females with a median (IQR) age of 55 years (42 – 65), 54.1% (n=46) of all included patients were non-severe while 45.9% (n=39) were severe. The most prevalent presenting symptoms were dyspnea (51.8%) and fever (40%). Hypertension (40.0%) and diabetes mellitus (36.5%) were the most common associated co-morbidities. The median (IQR) duration of hospitalization was 10 days (6 – 17), during which 38.8% (n=33) of patients required ICU admission, and 24.7% (n=21) died **(Table 1)**.

Characteristics of COVID-19 patients according to disease severity and in-hospital mortality

Compared to the non-severe group, severe patients had significantly higher values of CRP ($p \leq 0.001$), IL-6 ($p \leq 0.001$) and CRP/alb ($p \leq 0.001$). And, significantly lower values of L/CRP ($p \leq 0.001$), L/IL-6 ($p \leq 0.001$), lymphocyte count (P=0.028) and albumin ($p \leq 0.001$). Regarding in-hospital mortality of COVID-19, CRP ($p \leq 0.001$), IL-6 ($p \leq 0.001$), and CRP/alb ($p \leq 0.001$) showed significantly higher values in non-survivors compared to survivors. In contrast, L/CRP ($p \leq 0.001$), L/IL-6 ($p \leq 0.001$) and albumin ($p = 0.001$) were significantly lower. But the change in lymphocyte count according to in-hospital mortality was not significant (p=0.178).

Predictive performance evaluation

Regarding COVID-19 severity, while CRP alone at a cut-off of 48 mg/L offered 0.861 AUC and 82.4% diagnostic accuracy, its derived indices, CRP/alb, and L/CRP, exhibited the best predictive performance compared to other biomarkers. At the cut-off point of 1.65 for CRP/alb and 260.86 for L/CRP, the AUC was 0.878 and 0.873, respectively, and the diagnostic accuracy was 84.7% for both. And when CRP/alb at a cut-off point of 1.65 was combined with L/CRP at a cut-off point of 2300 in a multi-ROC analysis, COVID-19 severity

prediction accuracy increased to 95.3% with an AUC of 0.922 **(Table 3** & **Figure 1)**.

Concerning COVID-19 in-hospital mortality, the baseline IL-6 level was the most highly predictive biomarker with 0.88 AUC at a cut-off point of 120 pg/ml and diagnostic accuracy of 87.1%. Comparable to IL-6 was L/IL-6 with 0.86 AUC at a cut-off point of 5.40 and a diagnostic accuracy of 85.9%. Adding IL-6 at a cut-off point of 120 pg/ml to L/IL-6 at a cut-off point of 8 has improved the overall in-hospital mortality prediction ability and increased the AUC to 0.946 and the diagnostic accuracy to 96.5% **(Table 3** & **Figure 2)**.

Risk factors assessment

Logistic regression analysis further evaluated the association between IL-6, CRP, and their derived indices with COVID-19 severity and in-hospital mortality. As to COVID-19 in-hospital mortality, the multivariate analysis showed that the odds ratios (ORs) of all studied biomarkers were not significantly different ($p > 0.05$). And only IL-6 was an independent risk that was significantly positively associated with severe disease development (OR: 1.033; 95% CI: 1.002 - 1.066; p=0.037). **Table 4** demonstrates logistic regression analysis for possible predictors of COVID-19 severity and in-hospital mortality.

Table 1. Baseline characteristics and laboratory findings of the studied population (n=85)**.**

Variable		All Cases (n=85)
Age (years)	Median (IQR)	55 (42 – 65)
	Range	21 – 85
Sex n, (%)	Male	48 (56.5%)
	Female	37 (43.5%)
Comorbidities n, (%)	DM	31 (36.5%)
	HTN	34 (40.0%)
	COPD	7 (8.2%)
	IHD	5 (5.9%)
	CLD	3 (3.5%)
	CKD	15 (17.6%)
Symptoms n, (%)	Cough	1 (1.2%)
	Cough and dyspnea	2 (2.4%)
	diarrhea	1 (1.2%)
	Dyspnea	44 (51.8%)
	fever	34 (40.0%)
	Fever and Cough	3 (3.5%)
ICU admission n, (%)	Positive	33 (38.8%)
	Negative	52 (61.2%)
Severity n, (%)	Non-severe	46 (54.1%)
	severe	39 (45.9%)
Laboratory findings Median (IQR)	Hemoglobin gm/dl	12.6 (10.2 – 14.2)
	Platelets $\times 10^3$/cmm	227 (171.5 – 273.5)
	Total leukocytic count $\times 10^3$/	6.9 (4.35 – 10.55)
	Lymphocytes	1.34 (0.7 – 2.2)
	Neutrophils	4.28 (2.39 – 7.86)
	Albumin (g/dl)	3.5 (3 – 4.2)
	CRP (mg/L)	32 (9.5 – 132.5)
	IL– 6 (pg/ml)	55 (25 – 120)
	L/CRP	348.83 (99.78 – 1545.32)
	CRP/alb	0.94 (0.28 – 3.87)
	L/IL-6	18.97 (8.25 – 51.71)
Hospital stay (days)	Median (IQR)	10 (6 – 17)
	Range	(2 – 30)
Fate	Discharged	64 (75.3%)
	Died	21 (24.7%)

CKD: Chronic kidney disease; CLD: Chronic liver disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; HTN: Hypertension; ICU: Intensive care unit; IHD: Ischemic heart disease; IQR: interquartile range.

Table 2. Comparisons of baseline characteristics and laboratory findings according to COVID-19 severity and in-hospital mortality.

Variable		Non– severe	Severe	*p*– value	Survivors	Non–survivors	*p*– value
		(n=46)	(n=39)		(n=64)	(n=21)	
Age (years)	Median (IQR)	55.5 (41.5 – 63)	55 (43 – 67)	0.250	55.5 (42 – 64.75)	55 (47 – 69.5)	0.454
Sex n, (%)	Male	23 (50.0%)	25 (64.1%)	0.191	34 (53.1%)	14 (66.7%)	0.277
	Female	23 (50.0%)	14 (35.9%)		30 (46.9%)	7 (33.3%)	
Comorbidities n, (%)	DM	13 (28.3%)	18 (46.2%)	0.088	19 (29.7%)	12 (57.1%)	0.023
	HTN	15 (32.6%)	19 (48.7%)	0.131	22 (34.4%)	12 (57.1%)	0.065
	COPD	2 (4.3%)	5 (12.8%)	0.157	3 (4.7%)	4 (19.0%)	0.038
	IHD	0 (0.0%)	5 (12.8%)	0.012	3 (4.7%)	2 (9.5%)	0.414
	CLD	2 (4.3%)	1 (2.6%)	0.657	3 (4.7%)	0 (0.0%)	0.312
	CKD	4 (8.7%)	11 (28.2%)	0.019	10 (15.6%)	5 (23.8%)	0.393
Laboratory parameters Median (IQR)	Lymp $\times 10^3$/cmm	1.49	0.9	0.028	1.4	0.9	0.178
		(1 – 2.22)	(0.5 – 2.2)		(0.77 – 2.2)	(0.55 – 1.96)	
	CRP (mg/L)	12	129)	≤ 0.001	18	152	≤ 0.001
		(6.5 – 32)	(50 – 152		(7.7 – 65.75)	(57.8 – 152)	
	Albumin (g/dl)	4	3.2	≤ 0.001	3.75	3	0.001
		(3.1 – 4.8)	(2.9 – 3.6)		(3.02 – 4.35)	(2.75 – 3.6)	
	IL–6 (pg/ml)	32	120	≤ 0.001	45	168	≤ 0.001
		(18 – 50)	(80 – 220)		(20 – 97.5)	(110 – 275)	
	L/IL-6	40.83	8.33	≤ 0.001	29.55	5.33	≤ 0.001
		(25.20– 98.71)	(4.20– 14.54)		(11.90 – 79)	(3.34 – 14.55)	
	CRP/alb	0.35	3.87	≤ 0.001	0.52	4.3	≤ 0.001
		(0.15 – 0.64)	(1.66 – 4.90)		(0.20 – 1.70)	(1.82 – 5.52)	
	L/CRP	1211.96	105.26	≤ 0.001	703.27	105.26	≤ 0.001
		(449.82–2500)	(56.96-230.26)		(186.18 - 2005.35)	(43.56 – 212.5)	
ICU admission n, (%)	Positive	9 (19.6%)	24 (61.5%)	≤ 0.001	17 (26.6%)	16 (76.2%)	≤ 0.001
	Negative	37 (80.4%)	15 (38.5%)		47 (73.4%)	5 (23.8%)	
Hospital stay (days)	Median (IQR)	10.5 (6.75 – 17.5)	9 (5 – 14)	0.112	9.5 (6 – 17)	10 (7 – 16)	0.759
Fate	Discharged	43 (67.2%)	21 (32.8%)	≤ 0.001			
	Died	3 (14.3%)	18 (85.7%)				

CKD: Chronic kidney disease; CLD: Chronic liver disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; HTN: Hypertension; ICU: Intensive care unit; IHD: Ischemic heart disease; IQR: Interquartile range; Lymp: Lymphocytes. Statistical significance set at 0.05.

Table 3. Diagnostic validity and receiver operating curve (ROC) analysis for the ability of CRP, IL-6 and their derived indices to distinguish COVID-19 infected patients according to severity and mortality of the disease.

Indicators	Cut off	AUC	Specificity %	Sensitivity %	NPV %	PPV %	Accuracy %
Severity							
CRP	48	0.861	84.8	79.5	83.0	81.6	82.4
IL– 6	50	0.873	78.3	87.2	87.8	77.3	82.4
L/IL-6	11.66	0.883	95.7	71.8	80.0	93.3	84.7
CRP/alb	1.65	0.878	91.3	76.9	82.4	88.2	84.7
L/CRP	260.86	0.873	89.1	79.5	83.7	86.1	84.7
CRP/alb at 1.65 + L/CRP at:2300	--	0.922	91.3	100.0	100.0	90.7	95.3
In- hospital mortality							
CRP	151	0.801	92.2	57.1	86.8	70.6	83.5
IL–6	120	0.888	92.2	71.4	90.8	75.0	87.1
L/IL-6	5.40	0.866	95.3	57.1	87.1	80.0	85.9
CRP/alb	4.21	0.812	90.6	57.1	86.6	66.7	82.4
L/CRP	129.03	0.787	79.7	61.9	86.4	50.0	75.3
IL-6 at 120 pg/ml + L/IL-6 at:8	--	0.946	100.0	85.7	95.5	100.0	96.5

AUC: area under curve; CRP/alb = CRP (mg/dl)/albumin (g/dl); CRP: C-reactive protein in mg/L; IL-6: interleukin-6 in pg/mL; L/CRP = lymphocyte (number/µl)/CRP (mg/dl); L/IL-6 = lymphocyte (number/µl)/IL-6 (pg/ml); NPV: negative predictive value; PPV: positive predictive value.

Table 4. Predictors of COVID-19 progression.

Indicator	Uni-variate				Multi-variate			
	p-value	Odds ratio (OR)	95% C.I.		*p*-value	Odds ratio (OR)	95% C.I.	
Mortality								
L/IL-6	**0.001**	0.872	0.803	0.947	0.374	0.92	0.766	1.105
CRP/alb	**≤ 0.001**	1.921	1.431	2.579	0.439	2.602	0.231	29.268
L/CRP	**0.065**	0.999	0.999	1	0.164	1	1	1.001
CRP (mg/L)	**≤ 0.001**	1.021	1.011	1.031	0.758	0.988	0.917	1.065
IL– 6 (pg/ml)	**≤ 0.001**	1.019	1.01	1.028	0.152	1.013	0.995	1.032
Severity								
L/IL-6	**0.001**	0.945	0.914	0.976	0.652	1.006	0.981	1.032
CRP/alb	**≤ 0.001**	2.787	1.821	4.264	0.371	7.401	0.092	593.008
L/CRP	**≤ 0.001**	0.997	0.996	0.999	0.285	0.999	0.996	1.001
CRP (mg/L)	**≤ 0.001**	1.03	1.018	1.043	0.495	0.956	0.84	1.088
IL– 6 (pg/ml)	**≤ 0.001**	1.032	1.018	1.047	**0.037**	1.033	1.002	1.066

CI: Confidence interval

Figure 1. Diagnostic performance of CRP/alb (AUC:0.878; 95%CI: 0.805-0.952); L/CRP (AUC: 0.873; 95%CI: 0.798-0.948) and their combination (AUC: 0.922) for discriminating severe COVID-19 patients from those non-severe.

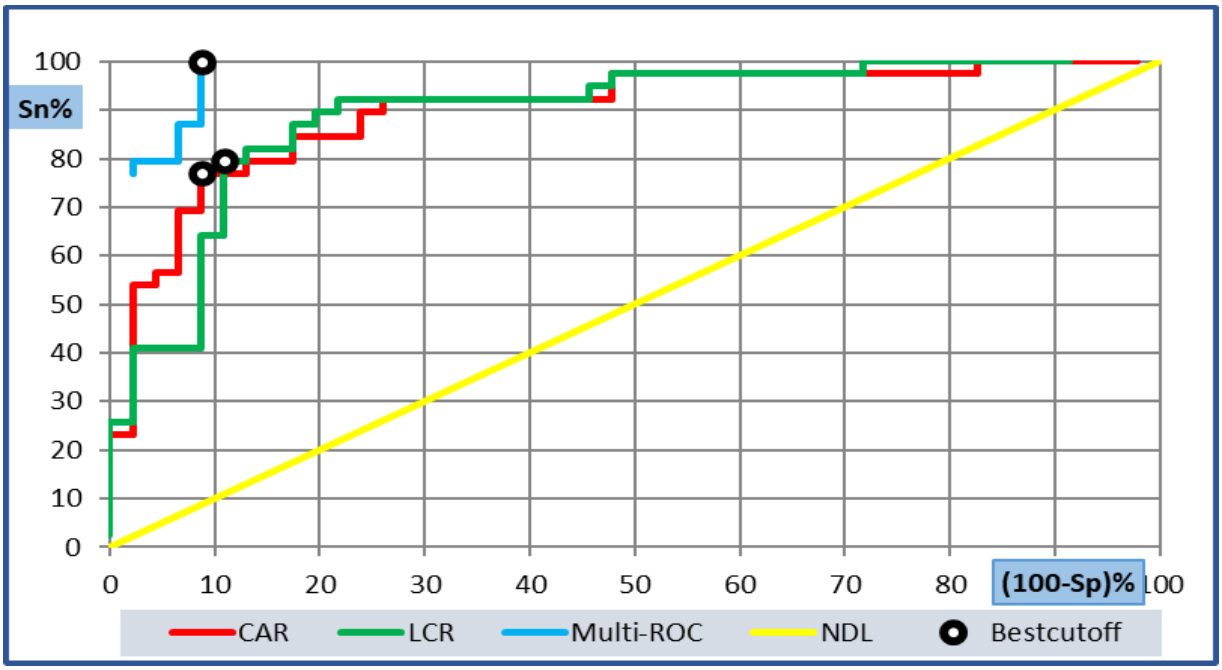
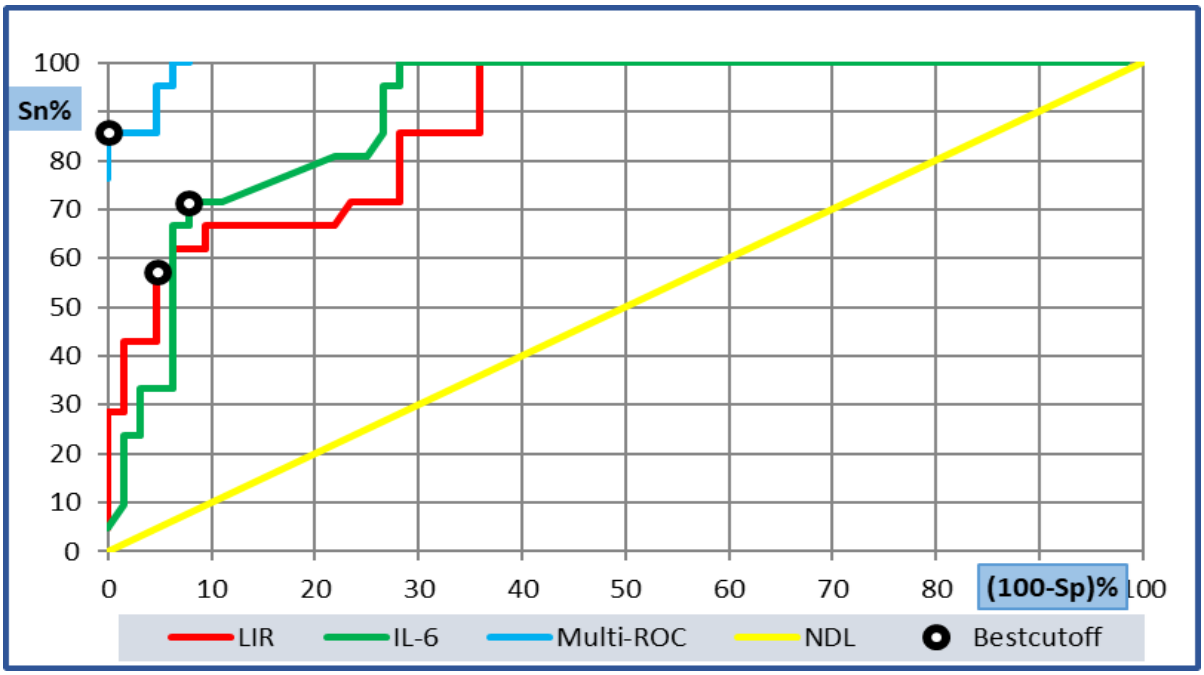


Figure 2. Diagnostic performance of L/IL-6 (AUC: 0.866; 95CI: 0.762-0.970); IL-6(AUC: 0.888; 95CI: 0.819-0.957), and their combination (AUC: 0.946) for discriminating survivors from non-survivors in COVID-19.



Discussion

During the current pandemic of COVID-19, since immune dysfunction, hyper-inflammation, and hyper-cytokinaemia have been closely linked to the rapid progression of the disease, continuous finding of sensitive inflammatory biomarkers for timely and effective identification of disease progression is necessary [10]. C-reactive protein, an inflammatory molecule that plays a vital role in host resistance to infections, was highly linked to acute lung injury and unfavorable outcomes in COVID-19 patients. As a result, detecting CRP levels is quite

useful in determining the severity of COVID-19 patients [11].

In our study, CRP increased significantly with COVID-19 severity and mortality. Similarly, several studies have reported significantly higher CRP values in the more severe patients and those who died from the disease [12-14]. On the contrary, **Zhang et al.**, revealed no significant change in CRP levels in patients after being admitted to ICU [15]. Also, **Ferrari and colleagues**, based on statistical analysis, stated that CRP might not be very useful in discriminating between patients with or without COVID-19 [16].

Interleukin-6 is a multifunctional cytokine that regulates immunological and inflammatory responses. It plays a key role in the development and progression of coronavirus pneumonia. In COVID-19 patients with the hyper-inflammatory syndrome, circulating IL-6 concentrations have been linked to disease severity, the incidence of acute lung damage, and mechanical ventilation need, suggesting that measuring IL-6 levels can help guide therapy decisions [17].

In our study, IL-6 levels were significantly higher in severe patients and non-survivors. Similarly, **Sun and co-workers**, reported significantly higher IL-6 values in the more severe patients [17]. Also, **Coomes and Haghbayan**, have reported similar results in their systemic review [18].

Furthermore, several studies have reported the distinct clinical significance of IL-6 at varying cut-off values. **Gao et al.** 2020, studied a cohort of 43 cases and reported that combining IL-6 at a cut-off value of 24.3 pg/ml with the D-Dimer helped in the early detection of severity progression [19]. In another cohort by Grifoni et al., 2020, serum IL-6 at a cut-off value of 25 pg/ml represented an independent risk of COVID-19 progression [20]. In a cohort study of 40 patients in Munich, COVID-19 patients' need for mechanical ventilation increased 22 times with the elevated IL-6 levels (> 80 pg/ml) [21].

In a meta-analysis, **Coomes and Haghbayan**, discovered statistical heterogeneity in IL-6 levels in subjects with varying degrees of disease severity. They attributed this to inconsistencies of patients' characteristics in each trial and discrepancies in the timing of IL-6 testing and immunomodulatory medicines received [18].

Significant hypo-albuminemia and lymphopenia were discovered in severe patients in the current study; however, only significant hypo-albuminemia and not lymphopenia was detected in non-survivors. Returning to the fact that albumin levels drop in inflammatory conditions, hypoalbuminemia has been noted in COVID-19 patients with severe hyperinflammation [22].

In several studies [3,23], COVID-19 severity and progression have been linked to lymphopenia, a reliable and accurate prediction. According to a meta-analysis study, the more serious the condition, the fewer lymphocytes there were [24], which could be attributed to the disease's effect on T cell subsets, resulting in immunological dysfunction [25,26]. Several studies have emphasized that when the lymphocyte count is less than $1.5 \times 10^3/\mu L$ lymphopenia should be addressed [13].

Using an inflammatory biomarker alone to evaluate patients infected with COVID-19 could be influenced by several factors. As a result, the generated indices from the most important inflammatory biomarkers may more accurately and thoroughly indicate immunological dysregulation [11,27].

Coronavirus disease 2019 triggers an inflammatory reaction throughout the body; therefore, the L/CRP could be a useful prognostic biomarker for disease severity and outcome. On exploring the role of L/CRP in the prognosis of our studied COVID-19 patients, we found that it declined significantly with the increase in disease severity and mortality. Similarly**, Ullah and co-workers**, have found that a low L/CRP was a good predictor of complications and mortality in COVID-19 patients [28].

High CRP/alb was previously linked to mortality in critically ill patients of various conditions [29]. In our study, we found a significant elevation of the CRP/alb as the disease progressed. Similarly, **El-Shabrawy and colleagues**, have found that the CRP/alb was a predictive factor of COVID-19 mortality [30].

In our study, the L/IL-6 was much lower in severe COVID-19 patients and non-survivors. In the same context, because high IL-6 levels and low lymphocyte counts have been connected to the severity and in-hospital mortality of COVID-19, in a study **by Yang and colleagues**, the IL-6/lymphocyte ratio (IL-6/L, reversed) was described as a novel biomarker to conduct risk stratification in

COVID-19 patients [11] Other studies have found that having a high IL-6/L is an independent risk factor for disease progression and a poor prognosis [31,32]. The decrease in lymphocyte numbers caused by IL-6-mediated inhibition of T cell activation explained the high in COVID-19 [33]. Furthermore, in a study by **Liu et al.**, T cell numbers were inversely associated with the studied cytokine levels, including IL-6 [34]. As a result, the balance of IL-6 and lymphocytes appeared to be crucial in the immune system's homeostasis.

In the multi-ROC curve analysis, we integrated biomarkers that exhibited the best predictive performance for COVID-19 progression to achieve the maximum overall test efficacy and improve the predictive ability. The combination of CRP/alb and L/CRP improved disease severity prediction accuracy. Similarly, combining IL-6 with the L/IL-6 enhanced in-hospital mortality prediction accuracy. Finally, only IL-6 was positively correlated with disease severity, but none of the investigated biomarkers were shown to be an independent predictor of in-hospital mortality. This could be attributed to the small sample size and the disproportion between the study groups, and these were the study's major limitations.

More research on a larger scale and for a longer period is needed to determine the predictive usefulness of the proposed inflammatory indices compared to the other existing biomarkers to slow the disease's progression and put the epidemic to an end.

Conclusions

In conclusion, pretreatment levels of IL-6 was the only risk factor for COVID-19 severity. L/IL-6 and IL-6 had the best diagnostic performance for COVID-19 in-hospital mortality, while CRP/alb and L/CRP had the best diagnostic performance for COVID-19 severity. Using these indicators in combinations rather than a single biomarker alone would improve the overall performance in disease progression prediction.

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Authors' contribution

All authors contributed significantly to this work.

Data availability statement

On reasonable request, the corresponding author will provide the datasets used and/or analyzed during the current work.

Conflicts of interest disclosure: None.

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Ethics

The current study protocol received ethical approval from the Ain Shams University Faculty of Medicine Research Ethics Committee FWA 00017585. All procedures were explained to all participants or their first-degree relatives, with informed consent obtained from them.

References

1-**Chan AS, Rout A.** Use of Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in COVID-19. J Clin Med Res 2020 ;12(7):448-453.

2-**Feng X, Li S, Sun Q, Zhu J, Chen B, Xiong M, et al.** Immune-Inflammatory Parameters in COVID-19 Cases: A Systematic Review and Meta-Analysis. Front Med (Lausanne) 2020;7:301.

3-**Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al.** Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther. 2020;5(1):33.

4-**Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, et al.** Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. Int J Infect Dis 2020 ;95:332-339.

5-**Fouad SH, Allam MF, Taha SI, Okba AA, Hosny A, Moneer M, et al.** Comparison of hemoglobin level and neutrophil to lymphocyte ratio as prognostic markers in patients with COVID-19. J Int Med Res 2021;49(7):3000605211030124.

6-**Taha SI, Samaan SF, Baioumy SA, Shata AK, Moussa AH, Abdalgeleel SA, et al.** Role of hemogram derived ratios and systemic-immune inflammation index in prediction of COVID-19 progression in Egyptian patients. Microbes Infect Dis 2021; 2(4): 613-622.

7-**Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M.** Lymphopenia during the

COVID-19 infection: What it shows and what can be learned. Immunol Lett 2020;225:31-32.

8-**Mazzoni A, Salvati L, Maggi L, Capone M, Vanni A, Spinicci M, et al.** Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest 2020;130(9):4694-4703.

9-**Abdelfattah EB, Ahmed S, El-Zahapy H, El Said A, Salem S, Tharwat A, et al.** Hospital response to COVID-19 a consensus: Report on Ain Shams University Hospital Strategy. ScienceOpen. [online] 2020. Available at https://www.scienceopen.com/hosted-document?doi=10.14293/S2199-1006.1.SOR-.PPD4QZX.v1 [Accessed 9 Feb. 2021].

10-**Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al.** COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395(10229):1033-1034.

11-**Yang B, Chang X, Huang J, Pan W, Si Z, Zhang C, et al.** The role of IL-6/lymphocyte ratio in the peripheral blood of severe patients with COVID-19. Int Immunopharmacol 2021;97:107569.

12-**Shang W, Dong J, Ren Y, Tian M, Li W, Hu J, et al.** The value of clinical parameters in predicting the severity of COVID-19. J Med Virol 2020;92(10):2188-2192.

13-**Kazemi E, Soldoozi Nejat R, Ashkan F, Sheibani H.** The laboratory findings and different COVID-19 severities: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob 2021;20(1):17.

14-**Danwang C, Endomba FT, Nkeck JR, Wouna DLA, Robert A, Noubiap JJ.** A meta-analysis of potential biomarkers associated with severity of coronavirus disease 2019 (COVID-19). Biomark Res 2020 ;8:37.

15-**Zhang Y, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q.** Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single centre in Wuhan city, China. Liver Int 2020;40(9):2095-2103.

16-**Ferrari D, Motta A, Strollo M, Banfi G, Locatelli M**. Routine blood tests as a potential diagnostic tool for COVID-19. Clin Chem Lab Med 2020;58(7):1095-1099.

17-**Sun H, Guo P, Zhang L, Wang F.** Serum Interleukin-6 Concentrations and the Severity of COVID-19 Pneumonia: A Retrospective Study at a Single Center in Bengbu City, Anhui Province, China, in January and February 2020. Med Sci Monit 2020;26:e926941.

18-**Coomes EA, Haghbayan H.** Interleukin-6 in Covid-19: A systematic review and meta-analysis. Rev Med Virol 2020;30(6):1-9.

19-**Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al.** Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol 2020;92(7):791-796.

20-**Grifoni E, Valoriani A, Cei F, Lamanna R, Gelli AMG, Ciambotti B, et al.** Interleukin-6 as prognosticator in patients with COVID-19. J Infect 2020;81(3):452-482.

21-**Herold T, Jurinovic V, Arnreich C, Hellmuth J, von Bergwelt-Baildon MV, Klein M, et al.** Level of IL-6 predicts respiratory failure in hospitalized symptomatic COVID-19 patients. *medRxiv* (preprint). 2020.

22-**Aziz M, Fatima R, Lee-Smith W, Assaly R.** The association of low serum albumin level with severe COVID-19: a systematic review and meta-analysis. Crit Care 2020;24(1):255.

23-**Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al.** Hematological findings and complications of COVID-19. Am J Hematol 2020;95(7):834-847.

24-**Pourbagheri-Sigaroodi A, Bashash D, Fateh F, Abolghasemi H.** Laboratory findings in COVID-19 diagnosis and prognosis. Clin Chim Acta 2020;510:475-482.

25-**Tufan A, Avanoğlu Güler A, Matucci-Cerinic M.** COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. Turk J Med Sci 2020;50(SI-1):620-632.

26-**Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al.** Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia. J Infect Dis 2020;221(11):1762-1769.

27-**Sun Y, Dong Y, Wang L, Xie H, Li B, Chang C, et al.** Characteristics and prognostic factors of disease severity in patients with COVID-19: The Beijing experience. J Autoimmun 2020;112:102473.

28-**Ullah W, Basyal B, Tariq S, Almas T, Saeed R, Roomi S, et al.** Lymphocyte-to-C-Reactive Protein Ratio: A Novel Predictor of Adverse Outcomes in COVID-19. J Clin Med Res 2020;12(7):415-422.

29-**Akirov A, Masri-Iraqi H, Atamna A, Shimon I.** Corrigendum to 'Low Albumin Levels Are Associated with Mortality Risk in Hospitalized Patients' American Journal of Medicine 2017:130(12):1465.e11-1465.e19.

30-**El-Shabrawy M, Alsadik ME, El-Shafei M, Abdelmoaty AA, Alazzouni AS, Esawy M, et al.** Interleukin-6 and C-reactive protein/albumin ratio as predictors of COVID-19 severity and mortality. Egypt J Bronchol 2021;15(1):5.

31-**Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al.** Detectable Serum Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load (RNAemia) Is Closely Correlated With Drastically Elevated Interleukin 6 Level in Critically Ill Patients With Coronavirus Disease 2019. Clin Infect Dis 2020;71(8):1937-1942.

32-**Chiappelli F, Khakshooy A, Greenberg G.** CoViD-19 Immunopathology and Immunotherapy. Bioinformation 2020 ;16(3):219-222.

33-**Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al .** Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020;382(18):1708-1720.

34-**Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al.** Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 2020;55:102763.