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Please cite this article as: Eman Alsayed, Aljohara Mohmoud Hamza, Warda Demerdash Khalifa Ali, Nagwa Hassanein, Waddah Albassa, Mohammad Barry, Abdullah Mofareh Mousa AlFaifi, Khalid Abdullah Sulaiman Altayyar, Nuha Abdulrahman M Aboabat, Wafa Khaled Fahad Alshaiddi, Howayda Mohammad Abu Sabbah, Ahmed Hameed Alamri and Sara Abdullah Hamad Albabtain, Relation Between Macrophage Inflammatory Protein-1 and Intercellular adhesion molecule-1 and Computed Tomography Findings in Critically-ill Saudi Covid-19 P a t i e n t s , *Journal of Infection and Public Health,* (2022) doi:https://doi.org/10.1016/j.jiph.2022.10.023

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Relation Between Macrophage Inflammatory Protein-1 and Intercellular adhesion molecule-1 and Computed Tomography Findings in Critically-ill Saudi Covid-19 Patients

Eman Alsayed[a]*, Aljohara Mohmoud Hamza[b], Warda Demerdash Khalifa Ali[c], Nagwa Hassanein[d], Waddah Albassa[e], Mohammad Barry[e], Abdullah Mofareh Mousa AlFaifi [f], Khalid Abdullah Sulaiman Altayyar [f], Nuha Abdulrahman M Aboabat[f], Wafa Khaled Fahad Alshaiddi[f], Howayda Mohammad Abu Sabbah[f] , Ahmed Hameed Alamri[f], Sara Abdullah Hamad Albabtain[g]

[a] Department of Clinical Pathology, Minia University, Egypt.

[b] Department of Anesthesia, Princess Nourah Bint Abdulrahman University, Kingdom of Saudi Arabia.

[c] Department of Anesthesia and ICU, Al-Azhar University, Assiut branch, Egypt.

[d] Department of Clinical Pathology, Al-Azhar University, Egypt.

[e] Radiology Department, King Abdullah Bin Abdulaziz University Hospital, Kingdom of Saudi Arabia.

[f] Department of pathology and Laboratory Medicine, King Abdullah Bin Abdulaziz University Hospital, Kingdom of Saudi Arabia.

[g] Health Sciences Research Center, Princess Nourah Bint Abdulrahman University, Kingdom of Saudi Arabia.

* Corresponding author

Eman Alsayed Abouahmad

Email: Emanlab2003@yahoo.com

Phone Number: 00966565283019

Ethical Consideration

The study protocol was approved by the ethical committee of King Abdulla Bin Abdulaziz University Hospital with IRB registration Number (20-0273):H-01-R-059 (July,13,2020). A written informed consent was obtained from all patients.

Abstract

Background: Several, clinical and biochemical factors were suggested as risk factors for more severe forms of Covid-19. Macrophage inflammatory protein-1 alpha (MIP-1α, CCL3) is a chemokine mainly involved in cell adhesion and migration. Intracellular adhesion molecule 1 (ICAM-1) is an inducible cell adhesion molecule involved in multiple immune processes. The present study aimed to assess the relationship between baseline serum MIP-1α and ICAM-1 level in critically-ill Covid-19 patients and the severity of computed tomography (CT) findings.

Methods: The study included 100 consecutive critically-ill patients with Covid-19 infection. Diagnosis of infection was established on the basis of RT-PCR tests. Serum MIP-1α and ICAM-1 level were assessed using commercially available ELISA kits. All patients were subjected to a high-resolution computed tomography assessment.

Results: According to the computed tomography severity score, patients were classified into those with moderate/severe (n=49) and mild (n=51) pulmonary involvement. Severe involvement was associated with significantly higher MIP-1α and ICAM-1 level. Correlation analysis identified significant positive correlations between MIP-1α and age, D-dimer, IL6, in contrast, there was an inverse correlation with INF-alpha. Additionally, ICAM-1 showed significant positive correlations with age, D-Dimer, TNF—α, IL6,while an inverse correlation with INF-alpha was observed.

Conclusions: MIP-1α and ICAM-1 level are related to CT radiological severity in Covid-19 patients. Moreover, these markers are well-correlated with other inflammatory markers suggesting that they can be used as reliable prognostic markers in Covid-19 patients.

1. Background

Despite the significant successes achieved in the battle against Covid-19, the pandemic is thought to continue as a predominant global health threat for years to come. The unique virological, epidemiological, and clinical characteristics of Covid-19 infection had shaped the unprecedented worldwide combat against the pandemic with many questions remaining unanswered [1]. One of the most challenging issues in the management of Covid-19 is the early identification of patients liable for a worse prognosis; so that, resources can be focused on their follow-up and management. Several genetic, clinical, and biochemical factors were suggested as risk factors for more severe forms of Covid-19 [2].

Genetic risk factors entail variations within the angiotensin-converting enzyme 2 (ACE2) gene, genes regulating multiple Toll-like receptors, and many complement pathways and others [3-5]. Clinical risk factors include obesity diabetes poor diabetic control, and vitamin D deficiency [6-9]. In addition, there is a wide spectrum of biochemical markers that were studied as correlates of Covid-19 severity including immune parameters [10], coagulation factors [11], metabolic mediators [12], and inflammatory markers [13].

In spite of the fact that many of these risk factors proved to successfully predict bad prognostic scenarios in some studies, other studies failed to document such relations. So, the pursuit of other prognostic markers remains a clinical priority. Macrophage inflammatory protein-1 alpha (MIP-1α, CCL3) is a CC chemokine mainly involved in cell adhesion and migration [14]. Clinical and experimental reports recognized probable contributions of MIP-1α

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in the pathogenesis of different diseases including traumatic brain injury [15] atrial fibrillation [16] chronic rhinosinusitis [17] diabetic nephropathy [18] and cancers [19-21]. In Covid-19 patients, MIP-1α has been linked to the cytokine storm [22]. Intracellular adhesion molecule 1 (ICAM-1) is an inducible cell adhesion molecule involved in multiple immune processes [23]. One report noted a pronounced rise of ICAM-1 level in a convalescent Covid-19 patient [24]. Despite these findings, data on the relation between MIP-1 and ICAM-1 and the severity of COVID-19 infection are lacking in the Kingdom of Saudi Arabia, Therefore, the current study aimed to assess the relationship between baseline serum MIP-1α and ICAM-1 levels in Covid-19 patients and the severity of computed tomography (CT) findings.

2. Methods and materials

2.1. Study setting and population

The present study was conducted at a University Hospital during the period from November 2020 to July 2021. The study protocol was approved by the hospital ethics committee with the Institutional review board (IRB) Registration Number (20-0273): H-01-R-059 on 13/7/2020.

This was a cross-sectional study that included 100 critically-ill patients with Covid-19 infection that were recruited consecutively. Diagnosis of infection was established on the basis of a real-time polymerase chain reaction (RT-PCR) test. Patients with known associated infections, immunocompromised conditions, or conditions with manifested coagulopathy were excluded.

2.2. Subjects' assessment

Data for all patients who were proved to have COVID-19 infection based on RT-PCR results were collected through the medical files' review and included the following: demographic

data, co-morbidities, and signs and symptoms related to COVID-19 infection. Additionally, the intensive care unit (ICU) admission, related length of stay, and the in-hospital mortality rate for patients with acute respiratory distress syndrome (ARDS) or critical cases were collected.

Routine laboratory assessments were performed and included complete blood count, and differential leukocytic count using ADVIA 2120i Hematology System (Siemens Healthcare Diagnostics Inc., NY, USA). Erythrocyte sedimentation rate (ESR) using the manual method, liver, and renal functions were assessed using Beckman Coulter Unicel DxC Synchron 800 (Beckman Coulter, CA 92821, USA), while coagulation profile was assessed using an automated coagulation analyzer, Sysmex CA-7000, Kobe, Japan. Serum MIP-1α and ICAM-1 levels, in addition to other pro-inflammatory proteins (INF-alpha, IL-6, TNF-α), were assessed using Evolis Fully Automated ELISA Processor (Bio-Rad Laboratories, CA, USA) using commercially available ELISA kits (Abcam, Minneapolis, USA).

All patients underwent assessment by high-resolution computed tomography (CT) of the chest and the findings were evaluated by two experienced radiologists. These frequently included bilateral, multilobar, or posterior peripheral ground-glass opacities [25, 26]. Furthermore, the severity of COVID-19 pneumonia was assessed by CT of the chest using a scoring system adopted by Saeed et al. [27] based on the work of Chang et al [28]. This scoring system has been developed by using the percentage of lobar involvement, as follows: lobar involvement of 5% or less given score 1, 5-25 % is score 2, 26-49% is score 3, 50-75% is score 4, and > 75% is score 5. Then, for each patient, a total score is obtained by summing up the lobar scores of the five lobes. The resulting number should represent the total lung involvement of a given patient, who gets categorized into mild (score 7 or less), moderate (8-17), and severe 18 or more.

In our work, we adopted the same steps for CT assessment of lung involvement. Thus, the lobar scores were summed up to yield the total CT score as a measure of the total lung involvement in a given patient. The total lung involvement was categorized according to the total score into groups, which for the purposes of this study we reduced to two categories only, mild (≤7) and moderate/ severe (≥8).

1.3. Data management and analysis

Data were presented as number and percent or median and interquartile range (IQR). Categorical variables were compared using the chi-square test while numerical variables were compared using the Mann-Whitney U test. Pearson correlation analysis was used to identify correlates of numerical variables. Receiver operator characteristic (ROC) analysis was used to identify the diagnostic performance of investigated markers. All statistical operations were processed using SPSS 25 (IBM, USA) with a p-value less than 0.05 considered statistically significant.

3. Results

The present study included 100 Covid-19 patients. They comprised 44 males and 56 females with an age of [median (IQR): 54.5 (42.0 - 62.0)] year. According to the CT severity score, patients were classified into patients with moderate/severe (n=49) and mild (n=51) pulmonary involvement (Table-1). Comparison between the studied groups regarding the clinical and laboratory data revealed that patients with moderate/severe involvement had significantly higher D-dimer [median (IQR): 1.51 (0.99-2.51) versus 0.73 (0.4-0.93) mg/L, p<0.001], lower INF-alpha [median (IQR): 54.1 (48.2-65.1) versus 68.8 (59.4-82.9) pg/mL, p<0.001], higher IL-6 [median (IQR): 51.7 (32.9-124.3) versus 25.1 (14.9-45.4) pg/mL, p<0.001] and higher TNF-α [median (IQR): 35.2 (32.1-44)   versus 31.3(23.2-35.3) pg/mL, p<0.001 when compared with

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patients with mild involvement (Table-1). Moreover, moderate/severe involvement was associated with significantly higher MIP-1α [median (IQR): 8.38 (7.27-10.69) versus 6.45 (5.14-7.3) pg/mL, p<0.001] and ICAM-1 [median (IQR): 216381(100513-319289) versus 73033(52595-111681) pg/mL, p<0.001] (Table-1). Patients with moderate/severe involvement had significantly longer ICU stay [17.0 (9.0-35.5) versus 7.0 (4.0-10.0) days, p<0.001] and higher mortality rate (18.4 % versus 0 %, p<0.001) (Table-1).

Correlation analysis identified significant positive correlations between MIP-1α and age (r=0.3), D-dimer (r=0.592), TNF-α (r=0.42), IL6 (r=0.368) and inverse correlation with INF-alpha (r=-0.225) (Table-2). Also, ICAM-1 showed significant positive correlations with patients' age (r=0.241), D-Dimer (r=0.746), TNF—α (r=0.471), IL6 (r=0.475) and inverse correlation with INF-alpha (r=-0.336) (Table-3).

Receiver operator characteristic analysis showed both markers (MIP-1α and ICAM-1) had good performance in distinguishing moderate/severe from mild lung involvement with an AUC of 0.852 and 0.829 respectively (Figures 1 and 2). The performance of other parameters compared to MIP-1α and ICAM-1 is shown in table-4

1. **Discussion**

The present study identified significant relations between MIP-1α and also ICAM-1 levels and the severity of pulmonary involvement in Covid-19 patients. Moreover, both markers were well-correlated with inflammatory and coagulation markers related to Covid-19 infection. To the best of our knowledge, no previous study documented a relation between these markers and the extent of lung involvement in similar patients. The relation between MIP-1α and pro-inflammatory markers (IL-6 and TNF-α) reflects a probable contribution of this mediator in the Covid-19-related cytokine storm.

Yang et al., [29] published their findings in China, where they examined the CT scan results of 102 people infected with COVID-19 and discovered that patients with severe COVID-19 infections had a significantly higher total CT severity score than those with moderate infections.

In support of our conclusions, Fonseca et al [30], noted an association between elevated MIP-1α levels and ICU admission and mortality among African American Covid-19 patients. In another work, cytokine profiling including MIP-1α was performed during the early and late phases of Covid-19 onset. Results showed that MIP-1α in the early and late phases of illness could reliably distinguish mild from severe cases [31]. Moreover, the study of Pons et al [32], reported an association between elevated MIP-1α levels and Covid-19 severity in Peruvian patients. Similar conclusions were reported by Young et al., [33]. Chi et al., [34] and Patterson et al [35], using a bioinformatics approach.

The relation between ICAM-1 level and Covid-19 severity was previously reported by many studies. The retrospective study of Tong et al., [36]. found a link between ICAM-1 level and Covid-19 severity. This finding was confirmed by other studies [37]. Moreover, Kaur et al., [38] found that elevated ICAM-1 level is related to 28-day mortality. In another work, an association was detected between Covid-19 viral RNA load and ICAM-1 level [39].

The findings of our work may have therapeutic implications. The study of Bermejo-Martin et al., [40] studied the antiviral and anti-inflammatory activities of a traditional Chinese agent against Covid-19. The investigators demonstrated that the efficacy of this agent was associated with a significant decline in MIP-1α levels. Likewise, it was shown that the use of bromelain and acetylcysteine resulted in a significant reduction of MIP-1α levels in the tracheal aspirate of Covid-19 patients [41].

2. Conclusions

In conclusion, MIP-1α and ICAM-1 levels are related to CT-scored radiological severity in Covid-19 patients regardless of the severity of clinical illness. Moreover, these markers are well-correlated with other inflammatory markers suggesting that they can be used as reliable prognostic markers in Covid-19 patients. ROC curve results showed the performance of MIP-1α and ICAM-1 level in identifying cases with higher CT chest severity scores.

acknowledge the research and scientific Center in Sultan Bin Abdulaziz Humanitarian city for

the assisting us in paper submission.

Research involving Human Participants and/or Animals: Not applicable.

Informed consent: Informed consent was obtained from all patients.

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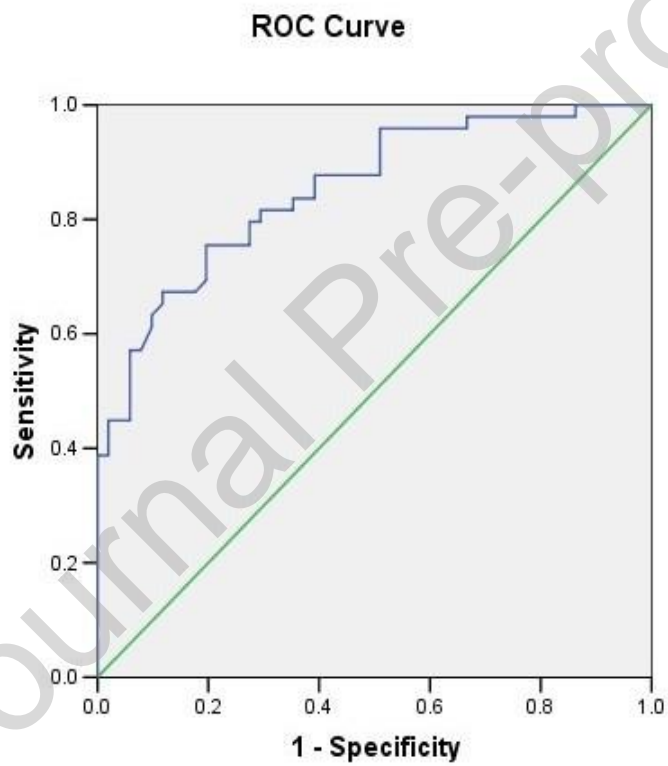
Figure Legends:

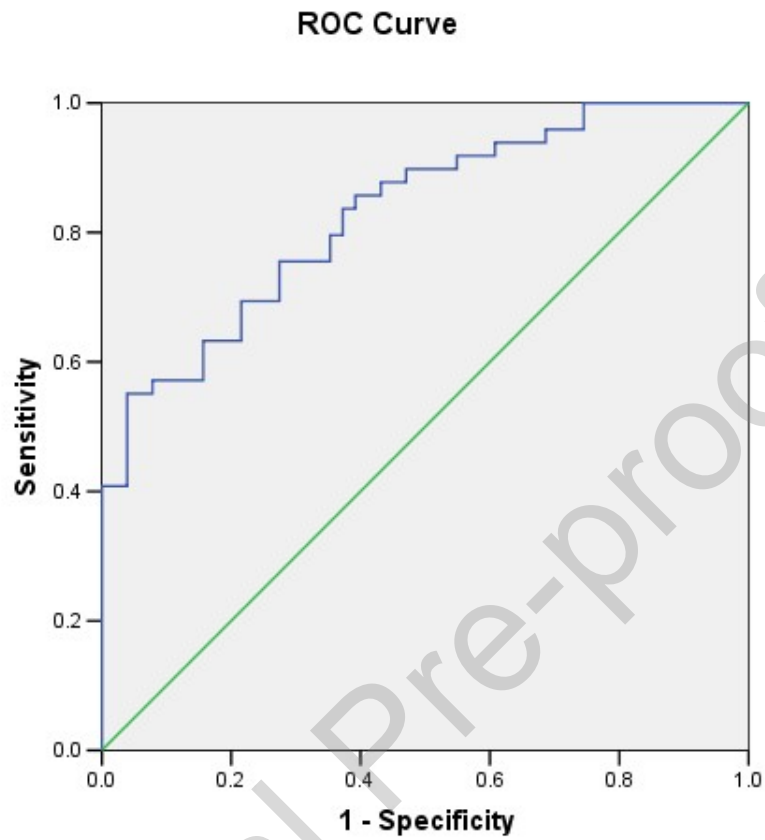**Fig.1:** Receiver operator curve (ROC) for MIP1a and radiological severity

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Fig.2: Receiver operator curve (ROC) for ICAM1 and radiological severity

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Table-1 Clinical and laboratory findings in the studied patients (n=100)

	All patients N=100	Moderate/Severe n=49	Mild n=51	p value
Age (years)	54.5 (42.0 - 62.0)	54.0 (45.0-63.0)	55.0(40.0-62.0)	0.44
Male/female n	44/56	22/27	22/29	0.031
Co-morbidities n (%)				
Obesity	9 (9.0)	6 (12.2)	3 (5.9)	0.27
Diabetes mellitus	31 (31.0)	16 (32.7)	15 (29.4)	0.73
Hypertension	26 (26.0)	14 (28.6)	12 (23.5)	0.57
Smoking	5 (5.0)	4 (8.2)	1 (2.0)	0.16
CAD	4 (4.0)	2 (4.1)	2 (3.9)	0.97
Clinical findings n (%)				
Fever	18 (18.0)	15 (30.6)	3 (5.9)	0.001*
ARDS	21 (21.0)	21 (42.9)	-	<0.001*
Sepsis	13 (13.0)	10 (20.4)	3 (5.9)	0.031
RR > 30 breath/min.	35 (35.0)	34 (69.4)	1 (2.0)	<0.001*
$SaO_2 < 90\%$	39 (39.0)	37 (75.5)	2 (3.9)	<0.001*
Laboratory data median (IQR)				
Hb (g/dL)	13.0 (11.4-14.1)	12.8 (11.1-13.7)	13.4 (11.6-14.3)	0.35
Platelets ($\times10^3$/μL)	236.0 (174.0-299.0)	219.0 (153.0-274.0)	240.0(184.0-338.0)	0.080
TLC ($\times10^3$/μL)	5.3 (4.2 - 7.0)	5.6 (4.4-9.3)	5.0 (3.6-6.5)	0.12
Lymphocytes ($\times10^3$/μL)	0.99 (0.76-1.4)	0.8 (0.62-0.99)	1.32 (0.97-1.57)	<0.001*
ESR (mm/hr)	51.0 (30.0-69.0)	55.0 (35.0-75.0)	45.0(22.0-66.0)	0.034
ALT (IU/L)	25.0 (17.0-49.0)	31.0 (20.0-54.0)	19.0 (15.0-33.0)	0.003*
AST (IU/L)	26.0 (22.0-31.0)	27.0 (23.5-33.0)	25 (21-28)	0.2

Albumin (g/L)	30.0 (24.3-35.0)	34.0 (32.0-38.0)	42.0 (40.0-47.0)	<0.001*
Creatinine (mg/dL)	0.71 (0.56-1.03)	0.73 (0.55-1.04)	0.7 (0.56-0.98)	0.81
Urea (mg/dL)	25.5 (15.3-42.4)	36.0 (24.0-47.0)	17.0 (11.0-33.0)	<0.001*
BUN (mg/dL)	11.9 (7.1-19.8)	17.0 (11.0-22.0)	8.0 (6.0-15.0)	<0.001*
LDH (iu/L)	240.0 (181.0-364.0)	432.0 (331.0-536.0)	231.0(187.0-282.0)	<0.001*
PT (sec)	11.4 (10.9-12.1)	11.4 (10.7-12.0)	11.4 (10.9-12.3)	0.77
PTT (sec)	28.0 (23.6-30.5)	28.0 (23.8-30.4)	28.0 (23.6-31.0)	0.86
INR	1.07 (0.99-1.14)	1.07 (0.98-1.135)	1.07 (1.01-1.16)	0.91
D-Dimer (mg/L)	0.95 (0.58-1.5)	1.51 (0.99-2.51)	0.73 (0.4-0.93)	<0.001*
INF-alpha (pg/mL)	60.3 (52.4-72.8)	54.1 (48.2-65.1)	68.8 (59.4-82.9)	<0.001*
IL-6 (pg/mL)	41.2 (17.8-78.7)	51.7 (32.9-124.3)	25.1 (14.9-45.4)	<0.001*
TNF-α (pg/mL)	33.5 (25.8-37.75)	35.2 (32.1-44)	31.3(23.2-35.3)	<0.001*
MIP1a (pg/mL)	7.28 (6.02-8.76)	8.38 (7.27-10.69)	6.45 (5.14-7.3)	<0.001*
ICAM-1 (pg/mL)	104890 (65620-224257)	216381(100513-319289)	73033(52595-111681)	<0.001*
ICU stay (days)	13.0 (5.0-37.0)	17.0 (9.0-35.5)	7.0 (4.0-10.0)	<0.001*
Mortality n (%)	9 (9.0)	9 (18.4)	-	<0.001*

ALT: Alanine aminotransferase, ARDS: Acute respiratory distress syndrome, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CAD: Coronary artery disease, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, ICAM: Intercellular adhesion molecule, ICU: Intensive care unit, IL: Interleukin, INF: Interferon, INR: international normalized ratio, LDH: Lactic dehydrogenase, MIP1a: Macrophage inflammatory protein, PT: Prothrombin time, PTT: Partial thromboplastin time, RR: Respiratory rate, SaO2: Oxygen saturation of the arterial blood, TLC: Total leucocytic count, TNF: Tumor necrosis factor.

Continuous data are reported as median (Interquartile range). Categorical data as frequency(percentage).

* Highly significant p value (<0.005)

Table-2 Pearson correlation between MIP1a levels and clinical and laboratory data

	MIP1a	
	r	P-value
Age	0.301	0.002*
TLC	0.304	0.002*
Lymphocytes	-0.368	<0.001*
Hb	-0.203	0.046
Platelets	-0.049	0.633
ESR	0.197	0.050
ALT	0.178	0.083
AST	0.052	0.608
Albumin	-0.552	<0.001*
Creatinine	0.237	0.018
Urea	0.448	<0.001*
BUN	0.447	<0.001*
LDH	0.604	<0.001*
PT	0.168	0.094
PTT	0.139	0.168
INR	0.161	0.110
ICU stay	0.286	0.004*
SaO2	-0.663	<0.001*
D-Dimer	0.592	<0.001*
TNF-α	0.415	<0.001*
IL6	0.368	<0.001*
INF-alpha	-0.225	0.025
ICAM-1	0.663	<0.001*
CT severity score	0.621	<0.001*

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CAD: Coronary artery disease, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, ICAM: Intercellular adhesion molecule, IL: Interleukin, INF: Interferon, INR: international normalized ratio, LDH: Lactic dehydrogenase, MIP1a: Macrophage inflammatory protein, PT: Prothrombin time, PTT: Partial thromboplastin time, RR: Respiratory rate, SaO2: Oxygen saturation of the arterial blood, TLC: Total leucocytic count, TNF: Tumor necrosis factor

* Highly significant p value (<0.005)

Table-3 Pearson Correlation between ICAM levels and clinical and laboratory data

	ICAM	
	r	P-value
Age	0.241	0.016
TLC	0.318	0.001
Lymphocytes	-0.391	<0.001*
Hb	-0.119	0.245
Platelets	0.010	0.926
ESR	0.144	0.154
ALT	0.195	0.057
AST	-0.043	0.668
Albumin	-0.561	<0.001*
Creatinine	0.067	0.510
Urea	0.381	<0.001*
BUN	0.378	<0.001*
LDH	0.608	<0.001*
PT	0.165	0.101
PTT	0.101	0.316
INR	0.146	0.146
ICU stay	0.372	<0.001*
SaO2	-0.675	<0.001*
D-Dimer	0.746	<0.001*
TNF-α	0.471	<0.001*
IL6 (pg/mL)	0.475	<0.001*
INF-α	-0.336	0.001*
CT severity score	0.600	<0.001*

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, CAD: Coronary artery disease, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, ICAM: Intercellular adhesion molecule, IL: Interleukin, INF: Interferon, INR: international normalized ratio, LDH: Lactic dehydrogenase, MIP1a: Macrophage inflammatory protein, PT: Prothrombin time, PTT: Partial thromboplastin time, RR: Respiratory rate, SaO2: Oxygen saturation of the arterial blood, TLC: Total leucocytic count, TNF: Tumor necrosis factor

* Highly significant p value (<0.005)

Table 4. Performance of acute inflammatory proteins in identifying cases with CT determined severity of lung involvement

	AUC	CI (LL-UL)		SE	Cutoff	Sensitivity	Specificity	PPV	NPV	P-value
D-Dimer	0.878	0.813	0.942	0.033	>1.01	0.735	0.882	0.857	0.776	<0.001
MIP1a	0.852	0.779	0.925	0.037	7.280	0.755	0.745	0.740	0.760	<0.001
ICAM-1	0.829	0.751	0.907	0.040	>126279	0.633	0.843	0.795	0.705	<0.001
IFN-α	0.782	0.693	0.872	0.046	59.550	0.745	0.694	0.723	0.717	<0.001
IL6	0.754	0.660	0.848	0.048	>44.3	0.633	0.725	0.689	0.673	<0.001
TNF- α	0.740	0.645	0.835	0.049	>33.8	0.612	0.725	0.674	0.649	<0.001

AUC=Area under the curve; CI=95% confidence interval; LL=Lower limit; UL=Upper limit;

SE=Standard error; PPV= Positive predictive value; NPV=Negative predictive value