Relationship of Ferritin, Interleukin-8, and D-Dimer Levels with PaO_2/FiO_2 Ratio and Mortality in ARDS COVID-19

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ABSTRACT

This is an analytic observational study with prospective cohort design. Clinical and laboratories data were collected in Dr. Soetomo Hospital's COVID-19 isolation room between July and October 2020. PaO_2/FiO_2 Ratio was derived from calculation: PaO_2 divided by FiO_2. Mortality obtained from medical record. Spearman test, Mann-Whitney test, and ROC curves were used in statistical analysis. There were 77 patients included in this study. Mortality rate was 51.9%. Ferritin levels did not correlate with PaO_2/FiO_2 Ratio (p=0.769). Median of ferritin in non-survivor vs survivor group was not significantly different (p =0.079). There was a significant correlation between IL-8 and PaO_2/FiO_2 Ratio (p=0.009; r_s=0.298). IL-8 did not different significantly in survivor or non-survivor group (p=0.091). D-Dimer level did not correlate significantly with and PaO_2/FiO_2 Ratio (p=0.418). Median of D-Dimer in non-survivor group was significantly higher than survivor group (p=0,044). D-Dimer cutoff value as mortality predictor was >1,070 ng/ml. Plasma IL-8 level significantly correlates with PaO_2/FiO_2 Ratio in ARDS COVID-19 patients. D-Dimer level is useful as a mortality predictor in ARDS COVID-19 patients.
Keywords: COVID-19, D-Dimer, Ferritin, Interleukin-8, Mortality, PaO_2/FiO_2 Ratio

1. INTRODUCTION

In December 2019, the city of Wuhan, the capital of China's Hubei province, became the epicenter of an outbreak of unknown cause pneumonia. On January 7, 2020, Chinese scientists isolated a new type of Coronavirus, namely Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2, previously known as 2019-nCov)[1]. The clinical spectrum of SARS-CoV-2 infection is very wide, ranging from asymptomatic, mild upper respiratory tract symptoms, to severe pneumonia with respiratory failure that can lead to death. According to the meta-analysis, 32.8% of patients had Acute Respiratory Distress Syndrome (ARDS), 13% had acute cardiac injury, 7.9% had acute renal failure, 20.3% of COVID-19 patients required ICU care, 6.2 % went into shock, and 13.9% died[2]. Respiratory failure due to ARDS is the leading cause of death[3].

One of the characteristics of the lesions in ARDS is the extensive destruction of the alveolar epithelium and the entry of protein-rich exudate into the neutrophil-rich alveolar spaces. Leukocyte migration occurs due to the presence of large amounts of chemotactic cytokines. Interleukin-8 (IL-8) is the main chemokine of neutrophils. From the study before, it was found that IL-8 levels were significantly increased in ARDS patients who died compared to those who still survived on the 7th day[4]. Research associated IL-8 with PaO_2/FiO_2 Ratio in ARDS COVID-19 is still rare. A cross-sectional study in 100 patients, found that in mild-moderate COVID-19 patients, IL-8 values were within normal limits (<62 µg/mL), and there was a significant difference between IL-8 levels in critically ill patients compared to mild categories ($p<0.01$)[5]. Other parameter which can be used to assess immune and inflammatory responses is ferritin. There is a significant increase in ferritin levels in severe and critical COVID-19 cases compared to mild-moderate cases[6].

Several journals stated that ARDS in COVID-19 may differ from classic ARDS, because patients suffer from severe hypoxia early in the disease, and pulmonary compliance in some patients is only slightly reduced, although at an advanced stage the clinical picture is consistent with classic ARDS. It is suspected that there are other underlying

mechanisms besides hyperinflammation. Several recent studies mention the presence of coagulopathy and hypercoagulability in COVID-19 patients, thus facilitating the occurrence of thrombosis. The underlying pathophysiology remains unclear[7].

Several autopsy studies have found evidence of thrombotic microangiopathy with fibrin thrombi in the glomerular capillaries and Deep Vein Thrombosis (DVT) in 58% of patients (bilateral legs, not suspected before death). In 12 patients who underwent autopsy, there was evidence of thrombosis in 5 patients, 4 patients died of pulmonary embolism, 2 patients had D-Dimer >20,000 ng/ml and 1 patient >100,000 ng/ml. In a multicenter study in China, 260 of 560 patients (46.4%) with confirmed COVID-19 had an increase in D-Dimer (>0.5 mg/L), and this increase was more significant in severe cases (59.6%) compared to non-severe cases (43.2%)[8].

This study aims were to examine the parameters of IL-8 on PaO_2/FiO_2 Ratio and mortality in COVID-19 patients because IL-8 is the main chemokines for neutrophils recruitment. From previous studies, neutrophils have an important role in the pathogenesis of ARDS and mortality in ARDS patients. Meanwhile, ferritin is an acute phase reactant which is one of the markers of inflammation. Cytokine storm is involved in pathogenesis of COVID-19, which leads to ARDS and death. D-Dimer was chosen because hypercoagulability was found in COVID-19 patients.

2. METHOD

This prospective cohort study was conducted in July 2020 - October 2020 at COVID-19 Isolation Room Dr. Soetomo Hospital Surabaya. The study aims were to analyze correlation between ferritin, Interleukin-8 (IL-8), and D-Dimer with severity (PaO_2/FiO_2 Ratio) and mortality in COVID-19 ARDS population. Ethical clearance was issued by Clinical Research Unit Dr. Soetomo Hospital Surabaya. The study population was COVID-19 confirmed patients with ARDS who were admitted to Isolation Room Dr. Soetomo Hospital Surabaya and met the inclusion and exclusion criteria.

Inclusion criteria were adult ($\geq$18 years old), COVID-19 confirmed with positive RT-PCR results from nasopharyngeal swab, received treatment according to clinical guidelines in Dr, Soetomo hospital, met the Berlin criteria for ARDS, gave informed consent. Exclusion criteria were HIV patient, history of autoimmune disease or receiving immunosuppressant therapy, history of malignancy, history of iron supplementation, pregnant women and in the puerperium period, history of asthma, trauma, or in the perioperative period. Drop-out criteria were refused treatment at own request and lysis or incomplete blood sample.

The study sample was taken by total sampling of inpatients in Dr. Soetomo Hospital Surabaya's COVID-19 Isolation Room. Sample that met inclusion and exclusion criteria were recorded for identity, demography data, and clinical condition. Furthermore, ferritin, IL-8, RT-PCR, D-Dimer, and PaO_2/FiO_2 ratio were also taken in the first 24 hours of admission in Isolation Room. Then, data of PaO_2/FiO_2 ratio were divided into three groups consist of PaO_2/FiO_2 ratio $\leq$100, 100,1 – 200, 200,1 – 300 (Berlin Criteria of ARDS). Mortality data were recorded among this groups. Later, the data will be processed with the SPSS Statistic Ver 25.

Characteristics of research subjects presented in descriptive statistical analysis. The significance of differences between groups was analyzed using a comparative test according to the type of data (ANOVA, Chi Square, Fisher's Exact, or Mann-Whitney test). Significant p value was < 0,05. Relationship between Ferritin, IL-8, and D-dimer levels with PF ratio was analyzed using Spearman test. Relationship between Ferritin, IL-8, and D-dimer levels with mortality status was analyzed using Mann-Whitney test. Parameter which had a significant difference was analyzed with ROC curve to get the cut off value of mortality.

3. RESULT

3.1 Characteristics of Research Subjects

A total of 82 patients met the criteria at the beginning of this study, then 5 patients dropped out because 1 patient refused treatment at their own request, and 4 patients had inappropriate samples and incomplete data, so that the remaining 77 study subjects were included in the analysis.

Subject characteristics based on PaO_2/FiO_2 ratio and mortality are presented in Tabel 1 and 2. Overall, median age was 53 years, 56% was male, 44.2% patients were overweight, and median of length of stay (LOS) was 15 days. There was a significant difference in SOFA score at hospital admission between three groups of PaO_2/FiO_2 Ratio (2 vs 4 vs 4.5; p = < 0.001). Decrease of PaO_2/FiO_2 Ratio associated with increase of SOFA score (table 1). There were significant differences in LOS, SOFA score at hospital admission, highest SOFA score during hospitalization, mechanical ventilation requirement, sepsis and septic shock between non-survivor and survivor groups (Table 2).

Table 1. Subject characteristics based on PaO_2/FiO_2 Ratio

Characteristic	PaO_2/FiO_2 Ratio 200,1-300 (N=13)	PaO_2/FiO_2 Ratio 100,1-200 (N=40)	PaO_2/FiO_2 Ratio ≤ 100 (N=24)	P
Age (years)*	49.0 (42.0-58.0)	54.5 (46.3-64.5)	54.0 (45.8-57.5)	0.207[a]
Sex				
Male	8 (61.5%)	30 (75%)	18 (75%)	0.610[b]
Female	5 (38.5%)	10 (25%)	6 (25%)	
BMI (kg/m^2)				
< 25	5 (38.5%)	16 (40%)	8 (33.3%)	0.866[b]
≥ 25	8 (61.5%)	24 (60%)	16 (66.7%)	
Comorbidities				
Diabetes mellitus	5 (38.5%)	19 (47.4%)	7 (29.2%)	0.347[b]
Hypertension	5 (38.5%)	18 (45%)	12 (50%)	0.795[b]
Coronary heart disease	2 (15.4%)	3 (7.5%)	2 (8.3%)	0.683[b]
Others (CVA, Epilepsy)	0 (0%)	2 (5.0%)	0 (0%)	0.626[b]
Duration of symptoms at hospital admission (days)*	7 (3.5-8.5)	7 (5-9)	8 (5-13.5)	0.310[c]
Length of stay at hospital (days)*	20 (13-22)	12 (6-21.75)	17 (6.25-24.75)	0.290[c]
SOFA score at hospital admission*	2 (2-3.5)	4 (3-5)	4.5 (4-5.75)	<0.001[c]
Highest SOFA score during hospitalization*	4 (3.5-6)	5.5 (4-8.75)	6.5 (5-9)	0.067[c]
Mortality	3 (23.1%)	23 (57.5%)	14 (58.3%)	0.073[b]
Mechanical Ventilation	2 (15.4%)	17 (42.5%)	13 (54.2%)	0.072[b]
Complication				
Acute Kidney Injruy	6 (46.2%)	10 (25%)	9 (37.5%)	0.300[b]
Sepsis	10 (76.9%)	32 (80%)	21 (87.5%)	0.664[b]
Septic shock	1 (7.7%)	15 (37.5%)	8 (33.3%)	0.126[b]
Secondary infection	2 (15.4%)	12 (30%)	7 (29.2%)	0.571[b]

*Median (Q1-Q3); [a]Analysis using ANOVA test; [b]Analysis using Chi-Square test; [c]Analysis using Kruskal-Wallis test

Table 2. Subject characteristics based on mortality

Characteristic	Non-Survivor N=40	Survivor N=37	P
Age (years)*	54 (45-65)	54 (46-59)	0,218[a]
Sex			
Male	29 (72,5%)	27 (73%)	0,963[b]
Female	11 (27,5%)	10 (27%)	
BMI (kg/m^2)			
< 25	15 (37,5%)	14 (37,8%)	0,976[b]
≥ 25	25 (62,5%)	23 (62,2%)	
Comorbidities			
Diabetes mellitus	17 (42,5%)	14 (37,8%)	0,677[b]
Hypertension	21 (52,5%)	14 (37,8%)	0,197[b]
Coronary heart disease	3 (7,5%)	4 (10,8%)	0,705[d]
Others (Stroke, Epilepsy)	2 (5,0%)	0 (0%)	1,000[d]
Duration of symptoms at hospital admission (days)*	7 (4-11)	7 (5,5-9)	0,621[c]
Length of stay (LOS) (days)*	8 (4-13)	22 (17-25,5)	<0,001[c]
SOFA score at hospital admission*	4 (4-5)	3 (3-5)	0,007[c]
Highest SOFA score during hospitalization*	8 (5-10)	4 (3-5,5)	<0,001[c]
PaO_2/FiO_2 Ratio			
200,1-300	3 (7,5%)	10 (27%)	0,073[b]
100,1-200	23 (57,5%)	17 (45,9%)	
≤ 100	14 (35%)	10 (27%)	
Mechanical ventilation	27 (67,5%)	5 (13,5%)	<0,001[b]
Complication			
Acute Kidney Injury	17 (42,5%)	8 (21.6%)	0,051[b]
Sepsis	37 (92,5%)	26 (70,3%)	0,012[b]
Septic shock	21 (52,5%)	3 (8,1%)	<0,001[b]
Secondary infection	14 (35%)	7 (18,9%)	0,113[b]

*Median (Q1-Q3); [a]Analysis using independent-t test; [b]Analysis using Chi-Square test; [c]Analysis using Mann-Whitney test; [d]Analysis using Fisher's exact test.

3.2 Ferritin, Interleukin-8 and D-Dimer

Ferritin, IL-8, and D-Dimer levels from this study are presented in Table 3. All variables are not normally distributed ($p < 0.001$).

Table 3. Research Variables Taken at Hospital Admission

Parameter	Minimum-Maximum	Median (Q-Q3)	p*
Interleukin-8 (pg/ml)	3.7 – 602.33	109.72 (41.76-234.8)	<0.001
D-Dimer (ng/ml)	190 – 35200	1380 (590-3625)	<0.001
Ferritin (ng/ml)	38.8 – 7340.5	1328 (1003.9-1722)	<0.001

* Normality test with Kolmogorov-Smirnov, normal distribution if p>0.05

3.3 Relationship of Ferritin, Interleukin – 8 and D-dimer with PaO_2/FiO_2 Ratio of ARDS in Covid-19 patients

Ferritin, IL-8, and D-Dimer levels in each PaO_2/FiO_2 Ratio group are presented in Table 4. IL-8 levels were significantly correlate with PaO_2/FiO_2 Ratio ($p = 0.009$, r_s 0.298 – weak correlation).

Table 4. Correlation of Ferritin, IL-8, and D-Dimer Levels at Hospital Admission with the PaO_2/FiO_2 Ratio

	PaO_2/FiO_2 Ratio 200,1-300 (N=13)	**PaO_2/FiO_2 Ratio 100,1-200 (N=40)**	**PaO_2/FiO_2 Ratio ≤ 100 (N=24)**	**P†**	**R_s**
Ferritin (ng/ml)	1,244.0 (543.6-1,997.0)	1,387.0 (1,035.0-1,698.0)	1,270.0 (1,020.0-1,717.0)	0.769	
Interleukin-8 (pg/ml)	34.6 (18.8-86.7)	119.3 (56.0-259.0)	142.2 (58.1-329.6)	0.009	0.298
D-Dimer (ng/ml)	740.0 (545.0-3,020.0)	1,360.0 (802.5-3,737.5)	1,610.0 (582.5-7,615)	0.418	

†Analysis using Spearman Test

3.4 Relationship of Ferritin, Interleukin-8 and D-dimer with Mortality in Covid-19 patients

Ferritin, IL-8, and D-Dimer levels in each mortality group (non-survivor vs survivor) are presented in Table 5. There was significant difference of D-Dimer levels in non-survivor and survivor group (p = 0.044). D-Dimer value was analyzed further to get the cut off value to predict the mortality.

Table 5. Ferritin, IL-8, and D-Dimer Levels at ICU Admission in Non-Survivor and Survivor Group

	Non-Survivor (N=40)	**Survivor (N=37)**	**P‡**
Ferritin (ng/ml)	1,491.5 (1,039.3-1,976.0)	1,233.0 (725.2-1,571.4)	0.079
Interleukin-8 (pg/ml)	136.1 (56.8-276.6)	89.8 (31.0-205.8)	0.091
D-Dimer (ng/ml)	1,480.0 (927.5-4,205.0)	910.0 (570.0 – 2,410.0)	0.044

‡Analysis using Mann Whitney test

Statistical analysis (ROC curve) was used to analyze D-Dimer levels to obtain a cut off for predicting mortality. The cut-off value of D-Dimer level > 1,070 ng/ml can predict mortality (AUC = 0.633), with sensitivity and specificity of 72.5% and 59.5%, respectively. The relative risk of death with D-dimer levels > 1.070 ng/ml was 1.977 (95% CI = 1.167 – 3.350).

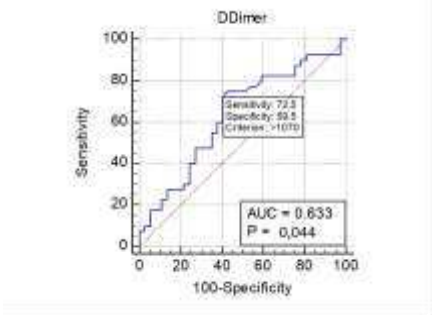

Figure 1. ROC curve of D-Dimer levels as mortality predictor.

4. DISCUSSION

4.1 Characteristics of Research Subject

Most of the research subjects were male (72.7%). Males have higher plasma ACE-2 levels than female, this may lead to higher expression of ACE-2 receptors which has a role in SARS-CoV-2 infection. In addition, the X chromosome has a high density of immune-related gene, therefore, women have stronger innate and adaptive immune

responses. Differences in immune response can also be caused by sex hormones, such as estrogen, progesterone, and androgens [9].

In this study, only 37.7% of patients had normal BMI, while majority were in the overweight/obese category. Obesity is a risk factor for severe symptoms [10], increased need for mechanical ventilation, and death in COVID-19 19 [11]. Obesity causes lung restriction, ventilation-perfusion mismatch, and fatigue of the respiratory muscles, resulting in reduced ventilation capacity. Obesity affects the compliance of the chest wall and lungs due to fat depositions in the thorax and abdominal cavity. The movement of the diaphragm and chest wall will be restricted, so the functional residual capacity (FRC) will be reduced. Obesity is associated with endothelial dysfunction due to chronic inflammation. In obese patients, there is an increase in pro-inflammatory cytokines, such as TNF- and interleukins, where if SARS-CoV-2 infection occurs, the inflammatory response will increase, and will affect the severity and mortality of the disease [12].

AKI and septic shock increase mortality in ARDS COVID-19 patients in previous study [13]. In this study, 32.5% of patients had AKI and 31.2% of patients experienced septic shock during treatment in ICU. In a study by Chen et al. (2020), as many as 65% of ARDS patients deteriorate rapidly and die from multiple organ dysfunction[14]. SOFA score is an assessment for organ dysfunction [15]. SOFA score is able to predict mortality in severe and critical COVID-19 categories. SOFA score 3 is associated with mortality in severe and critical COVID-19 patients. From the SOFA score parameter, 5 variables (mean arterial pressure, platelet count, bilirubin, creatinine, PaO_2/FiO_2 Ratio) were significantly different between patients who died and patients who lived. Higher serum creatinine levels at the start of treatment are one of the predictors of the severity of COVID-19 [16].

4.2 Relationship of Ferritin, Interleukin – 8 and D-dimer with PaO_2/FiO_2 Ratio and Mortality of ARDS in Covid-19 patients

Median of ferritin levels in this study was above the normal value (15-300 µg/L [17]) in all PaO_2/FiO_2 Ratio groups. All subjects were diagnosed with ARDS, so they were included in the critical category of COVID-19. This finding is in line with a previous study, which found increased ferritin levels at first hospital admission especially in severe cases [18]. Elevated serum ferritin reflects an exaggerated inflammatory response of the host, which underlies the development of ARDS and multiple organ failure, while also representing increased secretion as a protective response to oxidative stress[19]. SARS-CoV-2 infection causes macrophages to produce pro-inflammatory cytokines such as IL-6 which can stimulate the production of ferritin, then ferritin can stimulate the production of pro-inflammatory cytokines, such as IL-1β [20]. In this study, ferritin was not associated with PaO_2/FiO_2 Ratio. Confounding factors of ferritin, namely age, gender, BMI, history of diabetes, and secondary infection[17,21–23].

In this study, in all study subject's ferritin value in non-survivor was higher than survivor group, but the difference was not statistically significant. This finding is similar with another study in China, which found a significant increase in serum ferritin in non-survivor compared to survivor group ($p = 0.0008$)[24]. The high value of ferritin is influenced by the massive production of pro-inflammatory cytokines. Research on interventions or drugs for severe symptoms of COVID-19, including dealing with cytokine storms has been widely studied, such as IL-1 receptor antagonist (Anakinra), anti-IL-6 receptor monoclonal antibody (Tocilizumab). These drugs have been shown to reduce the likelihood of using mechanical ventilation in COVID-19 patients. Other non-pharmacological interventions, such as continuous renal replacement therapy (CRRT) as hemofiltration for clearance of proinflammatory cytokines and therapeutic plasma exchange (TPE). This method in several studies is said to be able to reduce ferritin followed by clinical improvement and reduce mortality [25].

There was an increase of median value of IL-8 in the three groups of PaO_2/FiO_2 Ratio. This result was similar with previous study in China [5]. In the lung, SARS-CoV-2 infects alveolar cells (type 1 and 2 pneumocytes and alveolar macrophages), and then replicates in tissues. Infection of the epithelium of the respiratory system by SARS-CoV-2 triggers secretion of various cytokines, chemokines, and DAMPs. Bronchoalveolar lavage fluid (BALF) of COVID-19 patients exhibits an increase in CXCL-2 and IL-8, chemokines that lead to recruitment of PMNs to the site of

infection [26]. The role of IL-8 in the occurrence of ARDS in COVID-19 may underlie the statistically significant relationship with the PaO_2/FiO_2 Ratio in this study. This relationship can also form the basis of a therapy with IL-8 as a target. There is a currently ongoing clinical trial of potential anti-IL-8 monoclonal antibodies - BMS-986253 in COVID-19 [27]. Another study using Sulforaphane in vitro can inhibit the upregulation of IL-6 and IL-8 due to SARS-CoV-2 infection [28]. The use of IL-8 as a marker to see the therapeutic response in COVID-19 may be further investigated. IL-8 is known as a marker for tumor burden and is used to monitor the effectiveness of immunotherapy in cancer [29].

The median of D-Dimer value in all PaO_2/FiO_2 Ratio groups were higher than normal, and the highest median was in the lowest PaO_2/FiO_2 Ratio group. D-Dimer level was also higher in the non-survivor than survivor group. This findings was similar with the previous study [1,8,30,31].

D-Dimer is an indirect marker of active coagulation and thrombin formation. D-Dimer is released when plasmin, a fibrinolytic enzyme, cleaves fibrin for clot degradation and reflects the process of endovascular thrombosis. In the inflammatory process, the release of cytokines such as IL-1, TNF-α, and complement factors will cause upregulation of plasminogen activator inhibitor (PAI)-1. The inflammatory process also results in increased expression of chemoattractant and adhesion molecules on endothelial cells, which activate mononuclear cells and transform macrophages. Mononuclear cells are stimulated by cytokines to produce tissue factor, which is a trigger and initiator of the extrinsic coagulation cascade. In addition, proinflammatory cytokines, such as IL-6 and IL-8, induce platelet activation, which also plays a role in the coagulation process. The products of coagulation will cause leukocyte stimulation, which will then increase the production of cytokines such as IL-1 and IL-6, which eventually play a role in the coagulation mechanism [32].

Autopsies on COVID-19 patients found evidence of thrombotic microangiopathy with fibrin thrombi in the glomerular capillaries, DVT in 58% of patients. In 12 patients who underwent autopsy, there was evidence of thrombosis in 5 patients, 4 patients died of pulmonary embolism, 2 patients had D-Dimer >20,000 ng/ml and 1 patient >100,000 ng/ml. Another study comparing lung pathology in 7 patients who died from COVID-19 found severe endothelial injury, extensive thrombosis with microangiopathy and increased angiogenesis, and was significantly prominent in the lungs of patients who died from COVID-19 [7].

A significant relationship between D-Dimer and mortality shows the danger of hypercoagulation in COVID-19 patients, so it requires appropriate intervention. The study by Tang et al. (2020) stated that 28-day mortality in patients with high D-Dimer who were given anticoagulants (heparin or LMWH) was lower than those who were not given heparin [33]. Low molecular weight heparin (LMWH) should be used over direct oral anticoagulants (DOACs) because of possible drug interactions with antiviral drugs (especially anti-HIV protease inhibitors such as ritonavir) and antibacterial agents (such as azithromycin). Such therapy may increase the risk of bleeding or decrease the antithrombotic effect of DOACs [34].

There were some limitations of this study. Ferritin, IL-8, and D-Dimer measurements in this study were not carried out in healthy people, so there were not any controls. Measurement of all parameters were only done once (at hospital admission). The number of male and female subjects was not the same, so it could be biased in the ferritin value. Research subjects with uncontrolled DM could be a confounder on ferritin values.

5. CONCLUSION

Plasma IL-8 level significantly correlates with PaO_2/FiO_2 Ratio in ARDS COVID-19. D-Dimer level is significantly different in non-survivor vs survive group and useful as a mortality predictor in ARDS COVID-19 patients. Suggestions from this study are (1) Ferritin, IL-8, and D-Dimer measurements were serially measured to get the better description in ARDS COVID-19 patients; (2) Confounding factors, such as gender and uncontrolled diabetes

mellitus, must be taken into account in analysis; (3) Measurement all parameters in healthy subjects as a control in the next study.

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