Correlation of Neutrophil Elastase to D-Dimer, PaO^2/FiO^2 Ratio and SOFA Score In COVID-19 Patients

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Abstract

Hypercoagulability, thromboembolic events and multi-organ injury in COVID-19 cases are thought to be due to the immunothrombosis process. Immunothrombosis degrees are determined by Neutrophil Extracellular Traps (NETs) activity, and Neutrophil Elastase (NE) is the main enzyme that triggers NETs. SOFA score and PaO^2/FiO^2 ratio as parameters to determine organ injury, while D-Dimer is a parameter of hypercoagulability status and has been proven as a prognostic factor to determine the severity and mortality of COVID-19 cases. This study aims to assess the influence of Neutrophil Elastase on D-Dimer, PaO^2/FiO^2 ratio and SOFA score in COVID-19 patients. This is an observational analytic study with a cross-sectional design. The study population was patients treated in isolation wards and ICU RSUD dr Soetomo in the period July 2020 - October 2020. Inclusion criteria were patients with a primary diagnosis of Confirmed COVID-19 through PCR examination and aged ≥18 years. The study sample was taken from medical record data of COVID-19 patients for the period July 2020 - October 2020 which met the inclusion and exclusion criteria. Data collection includes NE serum levels, D-Dimer, PaO^2/FiO^2 ratio and SOFA score were examined upon admission to the hospital (day 0). This study includes a total of 67 subjects. The mean and standard deviation of NE serum levels 25.67 ± 19.01 ng/mL, D-Dimer 3433.54 ± 6033.15 g/L, PaO^2/FiO^2 ratio 178.21 ± 102.90 and SOFA score 3.75 ± 2.12. The data normality test was carried out using the Kolmogorov Smirnov test and the results of NE, D-Dimer, PaO^2/FiO^2 ratio and SOFA scores data were not normally distributed, so the analysis was carried out using the Spearman correlation test. The results of the Spearman correlation test showed a significant correlation between NE serum levels and D-Dimer with a strong positive correlation strength ($p < 0.001$; $rs = 0.755$); a significant correlation between NE and SOFA scores with a weak positive correlation strength ($p = 0.003$; $rs = 0.352$). There was no significant correlation between NE serum levels and PaO^2/FiO^2 ratio ($p = 0.068$). NE Serum levels have a strong positive correlation with D-Dimer, where the higher NE serum levels, the higher D-Dimer level in COVID-19 patients. There is a weak positive correlation between NE serum levels and SOFA Score, but there is no correlation between NE serum levels and PaO^2/FiO^2 ratio. This may be due to other factors besides NE which are more dominant in influencing PaO^2/FiO^2 ratio.

Keywords : COVID-19, NETs, Neutrophil Elastase, D-Dimer, SOFA score.

1. Introduction

International studies had studied the pathophysiology of COVID-19 and stated that formerly, acute respiratory distress syndrome (ARDS) was the main cause of mortality due to COVID-19, but it was developed that coagulation impairment and thromboembolic events contributed to the high mortality rates due to CVOID-19.(1) Coagulation impairments and thromboembolic events in COVID-19 were produced by the immunothrombosis process beginning from the SARS-COV-2 infection. SARS-CVO-2 infection causes the

injury of epithelial alveolar cells thus resulting in the inflammatory response and activation of mononuclear and neutrophil cells. These events subsequently damage the endothelial capillary cells. Injury to the epithelial and endothelial capillary cells increase the pro-coagulation cascade accompanied by the thrombocyte dysfunction. Both of these pathophysiologic alterations generate the microvascular thrombosis, and become one of the main factors contributing in multiorgan failure in COVID-19 cases.(2,3)

Neutrophils has an important role in the generation of immunothrombosis, by the development of NETSs (Neutrophils Extracellular Traps). NETs is a DNA structure and protein forming like a net that are produced by the neutrophils to trap pathogens. But, the dysregulated NETs will initiate the spread of inflammation and thrombosis. NETs' overproduction facilitate the micro-thrombosis and leads to blood vessel's obstruction and the subsequent permanent organ damage in the lungs, cardiovascular system, and renal. (1,4) NETs' activity could be detected qualitatively using microscope, and quantitatively could be approached by measuring influencing the NETs development. Neutrophil elastase (NE) is one of the main enzymes for NETs development. NE has an antimicrobials effect because of its potential in degrading and phagocyting the pathogens. It contributes to inflammation by increasing the vascular permeability and induction of pro-inflammatory cytokines release (IL-6, IL-8).(5)

D-Dimer is a product from the degradation of fibrin cross-linking, and reflecting the activation from the homeostasis system. A high D-dimer concentration represents the hypercoagulability state and predicts the severity and mortality in COVID-19.(6) hypercoagulability state increase the risk of multiple organ damage in COVID-19, which can be measured using SOFA (Sequential Organ Failure Assessment) score. SOFA Score is one of parameter to assess the functional severity of organs dysfunction in severe and critical illness of COVID-19. Severe COVID-19 could be manifested as ARDS, ACS (Acute Coronary Syndrome), AKI (Acute Kidney Injury) and MODs (multiple organ dysfunctions)(7). Not only in SOFA score, PaO^2/FiO^2 ratio independently represents severity of pulmonary injury and determines ARDS severity according to Berlin criteria.(8,9) Moreover, D-Dimer concentration, SOFA score, and PaO^2/FiO^2 reflect the prognosis and severity of COVID-19 patient especially for the severe and critical manifestation, and NE as the enzyme to produce the NETs could be correlated with this inflammatory and hypercoagulability that leads to the worse prognosis and severity of COVID-19. Therefore, we aimed to investigate the effects of NE serum levels towards D-Dimer, PaO^2/FiO^2 ratio and SOFA score in COVID-19 patients.

2. Methods

2.1 Study design and population

This analytic observational study with cross sectional design was conducted in isolation wards and ICU RSUD dr Soetomo in the period July 2020 - October 2020, The medical record data of COVID-19 patients which met the inclusion and exclusion criteria were collected as study sample.

Patients with a primary diagnosis of Confirmed COVID-19 through PCR examination and aged ≥18 years were included in this study. Patients with surgery, trauma, gestational and in postpartum period, history of abnormal blood coagulation including history of anticoagulation drug, autoimmune disease and immunocompromised condition, history of cancer and chemotherapy were excluded, thus 67 subjects were included.

2.2 Measurements

Medical records of the study participants contained the data of patients' baseline characteristics such as: age, gender, body mass index, medications, present and past medical histories, comorbidities, NE serum levels, D-Dimer, PaO2/FiO2 ratio and SOFA score upon admission to the hospital. Diagnosis of COVID-19 was confirmed by RT-PCR test from the patients' nasopharyngeal swab samples and ELISA method (Human NE/ELA2 ELISA Kit by Elabscience®) was used to measure the NE serum levels.

2.3 Statistical analysis

Statistical analysis was calculated and presented by using IBM SPSS Statistics 23.0®. The data of NE serum levels, D-Dimer, PaO^2/FiO^2 ratio and SOFA score were tested with Kolmogorov-Smirnov for normality. NE serum levels, as the independent data, was analyzed for the correlation with the D-dimer, PaO2/FiO2 ratio, and SOFA score upon the hospital admission, as the dependent data. Correlation between variables was analyzed with linear regression test. If the normality and the other requirements for linear regression test were not fulfilled, the data were analyzed using Spearman correlation test, with P value of ≤ 0.05.

3. Results

67 patients with confirmed COVID 19 were included in this study during July 2020 - October 2020. Most of the samples were dominated with 41 (61,2%) males and 51 – 60 years old, with 52,94 ± 12,33 as the average age and 27,14 kg/m^2 as the average BMI. Most patients (88,1%) were found having comorbidities with obesity (58%) as the most prevalent comorbidities.

Table 1. Baseline characteristics

Characteristics	N = 67	%
Gender		
Males	41	61,2
Females	26	38,8
Age (Years)		
21-30	3	4,5
31-40	6	8,9
41-50	16	23,9
51-60	24 35,9	
61-70	12	17,9
> 71	6	8,9
Comorbidities		
Obesity	39	58,20
Diabetes	32	47,80
Hypertension	20	29,90
Heart failure	2	2,90
Cholelithiasis	1	1,50
Allergy	1	1,50
Epilepsy	1	1,50
Tuberculosis	1	1,50

NE serum levels, D-Dimer, PaO2/FiO2 ratio and SOFA score upon admission to the hospital were measured and Kolmogorov-Smirnov test was used as the normality test for the numerical variables in this study (Table 2).

Table 2. Biomarkers and Kolmogorov-Smirnov test

Biomarkers	Average ± SD	Kolmogorov-Smirnov test
Neutrophil Elastase (ng/mL)	25,67 ± 19,01	p<0,001
D-Dimer (µg/L)	3433,54 ± 6033,15	p=0,03
PaO^2/FiO^2 ratio	178,21 ± 102,90	p<0,001
SOFA Score	3,75 ± 2,12	p=0,003

Kolmogorov-Smirnov test of NE serum levels, D-Dimer, PaO^2/FiO^2 ratio and SOFA Score showed that none of them were normally distributed (p<0,05), therefore Spearman correlation test was used to determine the correlation of the variables. Correlation between NE serum levels and each dependent variables then measured and we found strong positive correlation between serum NE levels dan D-Dimer with p<0,05 and rs = 0,755. NE serum levels and SOFA score also found correlated positively with p<0,05 and rs = 0,352 while we found no correlation between NE serum levels and PaO^2/FiO^2 ratio (p>0,068).

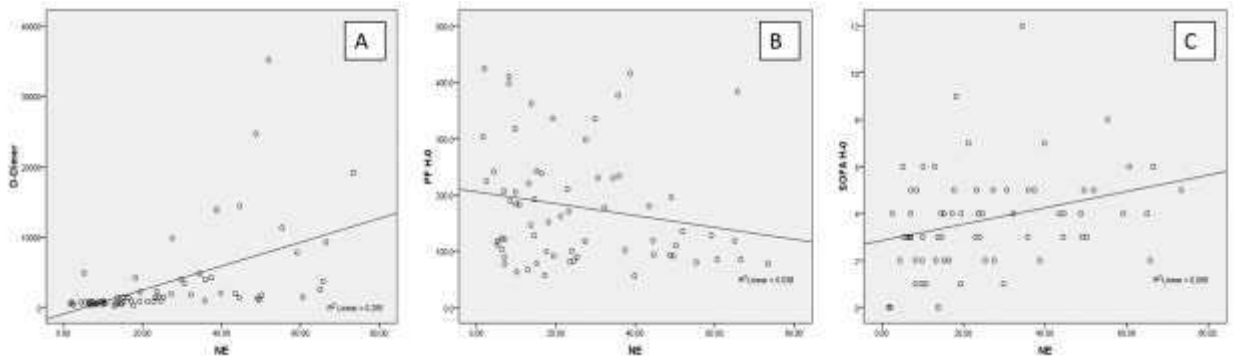

Fig.1. Scatterplot diagram of the correlation of NE serum levels towards (A) D-Dimer, (B) PaO2/FiO2 ratio, and (C) SOFA score

4. Discussion

Consecutively from July to October 2020, a total of 41 male patients (61,2%) and 26 female patients (38,8%) admitted to this study. A prior study also stated that 64% of 1314 confirmed COVID-19 patients were male.(10) Differences in sexual hormone and ACE2 expression between male and female were hypothesized to be the cause of the different COVID-19 prevalence. Female tends to have more effective adaptive immunity compared to the male, that may be caused by the genes related to immunity were found more in X chromosome.(11)

The average of NE concentration based on the gender were 26,72 ng/mL in male patients and 23,99 ng/mL in the female patients. There were no significant differences between the NE serum levels in male and female patients in this population. On the contrary, the average of NE concentration was significantly higher in elder patients, they were 24,79 ng/mL in patients whose age were <65 years old and 30,63 ng/mL in patients whose age were >65 years old. A study from Greenwood et al also showed a consistent result that NE concentration was significantly higher in elder patients (10,18 ± 0,87 vs 25,92 ± 3,45; p=0,002)(12). COVID-19 patients with the advancing age had higher risk of developing severe or critical illness, respiratory failure, longer hospital stay, which may because of the higher comorbidities' incidence and the lower immunity status among this population.(13)

Comorbidities were also a significant risk factors of the worse outcome in COVID-19 patients. As many as 59 patients (88,1%) in our study had comorbidities, in which the most prevalent comorbidity was obesity (58,2%), diabetes mellitus (47,7%) and hypertension (29,8%). There was an obvious association between obesity and basal inflammation which were marked by the increase of IL-6 and CRP. Adipose tissue was pro-inflammatory and stimulate the increase of the adipokine, Obesity also caused the dysregulation of tissue leukocyte expression and inflammatory subset from macrophage. It was also known for impairing the adaptive immune response in COVID-19 and other viral infections.(14)

Hypertension, diabetes mellitus, and cardiovascular disease often need medications that increase the protein ACE2 expression, whereas in COVID-19 patients, SARS-COV-2 was connected to ACE2 receptor to spread and cause tissue damage, more specifically in organ system that expressed the high ACE2 expression.(15) Tissue damage will furtherly cause the increase of the viral load and subsequently worsen the prognosis by initiating the ARDS, cytokine storm, and mortality.(10)

Hyperactivity of coagulation system was commonly found in severe COVID-19, marked by the high D-dimer and fibrinogen concentration, and low thrombin levels. NETs was one of the leading cause of systemic thrombus in the lungs of COVID-19 patients.(4) A prior study showed a tendency of NETs formation in the isolated Neutrophils from COVID-19 patients, thus confirming that COVID-19 generate a convenient environment for NETs formation.(3) NETs was also increase significantly in COVID-19 with thrombotic events. (16)

Our study showed a significantly strong correlation between NE serum levels and D-Dimer (p<0,001; rs = 0.755). The connector between inflammation and thrombosis is the NETs, where the activated neutrophils release the DNA, histone, and antimicrobial protein to the extracellular environments in procoagulant and prothrombotic tissue.(6) The antithrombotic medication for COVID-19 patients were generally the Heparin administration. By giving Heparin, hypercoagulation process could be degraded and monitored with the decrease of D-Dimer concentration. Our study proved that NETs had a role in hypercoagulation pathology in COVID-19 patients and was related to the high D-dimer concentration. Therefore, medications toward the NETs will be required beside Heparin administration.

The other therapeutic strategies were to prevent the NETs formation, increase NETs degradation, and neutralize the harmful degradation byproducts.(1) NE inhibitor (Sivelestat) could reduce the incidence of acute lung injury through the improvements of alveolar epithelial cells and vascular endothelial, and improving vascular permeability mediated by neutrophils. In ARDS and SIRS patients (non-COVID-19), Sivelestat significantly improved respiratory function, marked by the increase of PaO2/FiO2 ratio, and shorter mechanical ventilation duration, but showed no significance difference in the mortality rate.(17) There is no study that investigate the effects of NE inhibitor administration for COVID-19 patients yet.

NE is a Serine protease that gradually increase in COVID-19 patients. In COVID-19 patients admitted to ICU, the increase of Elastase 2 concentration could contribute to the increase of the lungs vascular permeability

and the lungs injury. Elastase 2 has been shown to be increased in various severe inflammatory conditions and contributes to the development of ARDS.(18)

Dysregulation of neutrophils activation by releasing cytotoxic chemicals including Reactive oxygen species (ROS) and proteinase such as leukocyte elastase contributed to the pathogenesis of inflammation injury in renal, cardiovascular, gastrointestinal tract, and the lungs, with the subsequent necrosis and apoptosis of these tissues.(19) Our study showed that there was no significant strong correlation between NE and PaO2/FiO2 ratio (p = 0,068; rs = -0,224). The other dominant factors besides the NE might influence the PaO2/FiO2 ratio result. Some other potential factors that cause the epithelial and endothelial damage in lungs are: SARS CoV-2 virulence that damage the alveolar type II cells, cytokine storms (TNF-α, IL-1, IL-6, L-8), another neutrophils' product (ROS and MMP). Cytokine storm increase the recruitment and activation of Neutrophils in all organ, including lungs. The increasing number of Neutrophils was followed by the activation and release of not only NE, but also ROS and MMP, and subsequently cause diffuse alveolar injury and damage in the lungs' alveolo-capillary membrane. Both of these processes are specific characteristic changes in the beginning of ALI (Acute lung injury) and ARDS development.(20)

SOFA score, one of multiorgan failure assessment scoring system, could predict severity and outcome in several diseases. Most of the severely and critically ill COVID-19 patients develop the multiple organ failure, thus, SOFA score could assess the multiple organ injury caused by the SARS-COV-2 infection, comprehensively.(21) Severe and critical COVID-19 patients could be manifested as ARDS, ACS, AKI, MODs, and even mortality, with the characteristic of the comprehensive dysfunction of these organs.(7) NETs was responsible for the incidence of MODs in COVID-19. NETs development in the lung tissue could impair microcirculation and expanded to the alveolus, whereas histone could be accumulated rapidly in the lung capillary. Histone was proved to activate and recruit leukocyte, activate the erythrocytes, damage the alveolar macrophage, endothelial and epithelial cells of the lungs. Histone in the NETs stimulate inflammatory response that causes the microvascular leakage and endothelial dysfunction, induce a pro-coagulation and immunothrombosis and resulted in the multiorgan failure.(22)

Our study showed a significantly strong correlation between NE serum concentration and the SOFA score (p = 0,003; rs : 0,352). These results were consistent with the study from Dupont et al that showed the significant increase of endothelial and nucleosomal damage in severe COVID-19 patients who developed the MODS.(23) This study could prove that the dysregulation of immune system, specifically the NETs formation, initiate the immunothrombosis through the endothelial damage in the pathogenesis of severe COVID-19. Some other relevant and supporting evidences that largely correlated the NETs dysregulation to ARDS, explained that the neutrophils from the ARDS pneumonia patients had the tendency to form NETs. The circulating NETs were also significantly associated with the disease severity and mortality. NETs, as the extracellular histone source contribute to the ARDS and sepsis severity. The mechanical obstruction of blood vessel by NETs is one of the main pathogenesis on how COVID-19 could cause the endothelial damage, thrombosis, and multiple organ dysfunctions.(23) Dysregulation of immune response (including neutrophils and NETs) could be a novel therapeutic approach to prevent inflammation and thrombosis, and improve the COVID-19 outcome.

5. Conclusion

There was a significantly strong correlation between NE serum levels and D-dimer (p<0,001; rs = 0,755), the higher NE serum levels, the higher the D-dimer concentration. No significant correlation was documented between NE serum levels and PaO^2/FiO^2 ratio that might be caused by the more dominant other contributing factors such as: SARS-COV-2 virulence, cytokine storm, and the other Neutrophil products like ROS and MMP. NE concentration was significantly correlated with the SOFA score but with a low strength of correlation (p = 0,003; rs = 0,352) that might be caused by the PaO^2/FiO^2 ratio as one of the SOFA score components.

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