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Cross-sectional Study

Interleukin-6 (IL-6) expression of lung tissue in COVID-19 patient severity through core biopsy post mortem

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ABSTRACT

Introduction: In COVID-19 patients, Interleukin-6 (IL-6) will increase, and the production of antigens will be excessive, which will cause excessive inflammation of the tissues, especially the respiratory tract, which causes fibrosis in the lungs and can lead to death.
Objective: To analyze IL-6 expression of lung tissue in COVID-19 patient severity.
Methods: The study is an observational analytic design from July to December 2020. COVID-19 patient severity who died was examined for IL-6 expression on lung tissue. The lung tissue sampling uses the core biopsy method.
Results: The total number of samples obtained was 38 samples. Characteristics of patients with a mean age of patients were 48 years, male, the most common chief complaint was shortness of breath, mean symptom onset was 5 days, patient length of stay was 10 days, the most common cause of death was a combination of septic shock and ARDS and the most common comorbid diabetes mellitus. There is an increased WBC, neutrophils, platelets, procalcitonin, CRP, BUN, creatinine serum, AST, ALT, and D-dimer. In this study, the average tissue IL-6 expression was 72.63, with the highest frequency of strong positive 47.4%.
Conclusion: An increase in IL-6 expression on lung tissue showed the severity of COVID-19 infection.

1. Introduction

Indonesia reported its first case of Coronavirus Disease 2019 (COVID-19) on March 2, 2020, and the number continues to grow until now. As of June 30, 2020, the Ministry of Health reported 56,385 confirmed cases of COVID-19, with 2875 cases of death (CFR 5.1%) spread across 34 provinces. As many as 51.5% of cases occurred in men. Most cases occurred in the age range of 45–54 years and the least occurred at 0–5 years. The highest mortality rate was found in patients aged 55–64 years [1].

In COVID-19, an inflammatory process involves the production of several proinflammatory cytokines, including Interleukin-6 (IL-6). Increased IL-6 correlates with the occurrence of activity and progression of this disease [2]. The method used to measure levels of IL-6 uses tissue biopsy examination by knowing the bond between antigen and antibody IL-6 [3]. IL-6 is a pleiotropic proinflammatory cytokine and is a response regulator in COVID-19. The role of IL-6 in biological activities, including regulation of immune response, inflammation and hematopoiesis. IL-6 is essential role in the pathogenesis of pulmonary fibrosis in COVID-19 patients [3,4]. In patients who died from Covid 19, pulmonary fibrosis

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was found in lung biopsy tissue [5].

In a previous study conducted for histopathological examination of lung tissue in COVID-19 patients who died in less than 12 h, positive IL-6 expression was found but no quantitative assessment was carried out for tissue IL-6 positivity and it has not been quantitatively associated with the severity of pulmonary fibrosis [6]. Previous studies have classified tissue IL-6 expression in colon cancer and rheumatoid arthritis patients but not in COVID-19 patients [4,7]. This study aims to analyze IL-6 expression of lung tissue in COVID-19 patient severity.

2. Method

The study's design was an observational analytic study and the report is based on STROCSS 2021 [8]. Participants in this study were COVID-19 patients who had died in June–December 2020. This study has received ethical approval from the health research committee in the hospital. The number of participants in the study was 38 participants. Diagnosis of severe acute respiratory syndrome coronavirus 2 (SAR--Cov-2) based on positive and chest X-rays' real-time polymerase chain reaction [5–7]. Participant inclusion criteria include >18 years old, diagnosed with COVID-19 [1,9,10], and died. Participant exclusion criteria included core biopsy subjects with a malignancy history that could not be analyzed. Participant family/guardians must fill out an informed consent form without coercion before the research is carried out.

Postmortem needle core biopsies were performed on the lungs 2 h after death in a negative air isolation ward. The procedures were performed without ultrasound guidance, but the patients' last radiographic images and surface anatomic landmarks were used as references. The tissues were received fixed in neutral buffered formalin for over 24 h and then routinely processed under standard biosafety measures. Other data were taken from the patient's medical record data.

Tissue specimens were collected from all subjects to determine IL-6 expression levels and degree of fibrosis. The IL-6 area was measured in each sample stained by the IL-6 antibody (GTX17623) in per cent data. The estimation was carried out microscopically for inflammatory cells expressing IL-6 and divided into 3 groups, such as weakly positive (1–50%), moderate (51–75%), and strong (>76%).

The analysis used in this study was descriptive and assisted by IBM SPSS Statistics software Version 23.0 (IBM Corp., Armonk, NY, USA).

3. Result

Of 41 patients, 38 fulfilled the inclusion criteria, and 3 exclusion samples showed no lung tissue. 38 patients were distinguished by age, sex, chief complaint, symptom onset, length of stay, dead cause and comorbidities. The characteristics of the sample are presented in Table 1.

Characteristics obtained by the patient's average age is 48 years, with more men than women. At the time of admission to the hospital, the main complaint was shortness of breath, with a mean symptom onset of 5 days and a patient's length of stay of 10 days. The most common cause of death was a combination of septic shock and ARDS, which was 86.8%. The highest comorbidities are diabetes mellitus 76%.

Characteristics obtained with WBC average of 22.85 × 103/μL, neutrophil with an average of 88.5%, lymphocyte with an average of 6.08%, an average NLR of 22.4, platelets on average 263 × 10^3/μL, BUN on average 64.7 mg/dL, creatinine serum on average 3.18 mg/dL, average AST of 102.7 U/L, average ALT of 80.2 U/L, procalcitonin on average 19.79 ng/ml, CRP average 15.76 mg/dL, D-dimer average 5835 g/L, INR average 1.18 (Table 2). The IL-6 expression has obtained an average of 72.63 with the highest frequency on a strong positive result of 47.4% (Table 3 & Fig. 1).

Table 1
characteristics of participant.

Characteristics	Result				
	n (%)	Mean ± SD	Median	Min	Max
Age	13 (31.7)	48.4 ± 13	49	23	69
18–44 years	21 (51.2)				
45–64 years	4 (9.8)				
65–74 years					
Sex	27 (71.1)				
Male	11 (28.9)				
Female					
Chief complaint	32 (84.2)				
Dyspnea	5 (13.2)				
Fever	1 (2.6)				
Body weakness					
Symptom onset (days)		5.8 ± 2.9	7	1	18
Length of stay (days)		10.5 ± 5.4	11	3	21
Dead cause	33 (86.8)				
Septic Shock & ARDS	4 (10.5)				
Septic Shock	1 (2.6)				
ARDS					
Comorbidities	29 (76)				
Diabetes Mellitus	19 (50)				
Hypertension	16 (42)				
Obesity	5 (13)				
Chronic Kidney Disease	2 (5)				
Gravid	1 (2)				
Hepatitis	1 (2)				
CHD	1 (2)				
Asthma					

4. Discussion

Characteristics of the sample with the mean age of the patients who were the study subjects were 48 years, the most age group was 45–64 years, the youngest age was 23 years, and the oldest was 69 years. The prevalence of COVID-19 in Indonesia as of June 2020 was 27,676 cases, and the highest age was 31–45 years (29.3%). The percentage of deaths from COVID-19 increases with age and the number of comorbidities [11]. In this study, male sex was obtained as much as 71.1%. This is follows other studies, which found that the prevalence of COVID-19 in Indonesia in males was higher than in females by 54.5% [12].

Upon admission to the hospital, the most common chief complaint was shortness of breath (84.2%) of the total sample, with a mean symptom onset of 5 days and a patient's length of stay of 10 days. To Surendra et al.'s research, COVID-19 patients will generally complain of respiratory symptoms, namely coughing 76.2%, fever 47.1% and shortness of breath 41.6% of the sample of subjects with COVID-19 [13]. In another study, the mean symptom onset to hospital admission was 5 days for patients who died, and the length of stay was 24 days for patients who died [14]. The most common cause of death was a combination of septic shock & ARDS, which was 86.8%. In Elezkurtaj et al.'s study of 26 COVID-19 patients who died, 19 cases (73.1%) died from infections including sepsis, septic shock and sepsis-related multi-organ failure, and 4 cases (15.4%) due to ARDS or respiratory failure [15]. Comorbidities were found in diabetes mellitus 76%, hypertension 50% and obesity 42%. In Lagana et al.'s study on COVID-19 patients, hypertension was found 341 (52.1%), diabetes mellitus 220 (33.6%), cardiovascular disease 137 (20.9%) [16].

In the study of Zhu Z et al. as many as 16 severe COVID-19 patients, the average WBC was 5.35 × 10^9/μL, Neutrophil of 75.7%, Lymphocyte of 23.6%, NLR of 4.24, platelets of 155 × 10^9/μL, and CRP of 36.64, mg/dL, these results show that more many patients in the severe condition group had increased inflammation [17]. This is consistent with this study, where the exaggerated inflammatory response in fatal COVID-19 patients was characterized by increased WBC, Neutrophils, NLR, and CRP. This increase in procalcitonin corresponds to the high secondary infection rate in COVID-19 patients. It is associated with the frequency of causes of death in the study subjects, namely septic shock and ARDS

Table 2
Laboratory characteristics before death in study participant.

Characteristics	Result				
	n (%)	Mean ± SD	Median	Min	Max
WBC	0 (0)	22.85 ± 13	17.99	5.91	63.4
<3.37 × 10^3/uL	3 (7.9)				
3.37–10 × 10^3/uL	35 (92.1)				
>10 × 10^3/uL					
Neutrophil	0 (0)	88.5 ± 8.4	88.5	58.9	95.3
<39.8%	3 (7.9)				
39.8–70.5%	35 (92.1)				
>70.5%					
Lymphocyte	36 (94.7)	6.08 ± 5.13	3.95	1.6	24.8
<23.1%	2 (5.3)				
23.1–49.9%	0 (0)				
>49.9%					
NLR	1 (2.6)	22.4 ± 13.4	23.2	2.67	57.8
1–5	6 (15.8)				
6–9	8 (21.1)				
10–18	23 (60.5)				
>18					
Thrombocyte (PLT)	6 (15.8)	263 ± 164	219.5	25	732
<150 × 10^3/uL	24 (63.2)				
150–450 × 10^3/uL	8 (21.1)				
>450 × 10^3/uL					
BUN	0 (0)	64.7 ± 52.7	40	11	225
<7 mg/dL	7 (18.4)				
7–18 mg/dL	31 (81.6)				
>18 mg/dL					
Creatinine serum	1 (2.6)	3.18 ± 2.7	2.1	0.4	12.8
<0.6 mg/dL	15 (39.5)				
0.6–1.3 mg/dL	38 (57.9)				
>1.3 mg/dL					
SGOT	13 (34.2)	102.7 ± 180	54	22	1103
0–50 U/L	19 (50)				
51–100 U/L	6 (15.8)				
>100 U/L					
SGPT	17 (44.7)	80.2 ± 75.3	56.5	21	346
0–50 U/L	13 (34.2)				
51–100 U/L	8 (21.1)				
>100 U/L					
Procalcitonin	1 (2.6)	19.79 ± 33.3	1.55	0.01	100
<0.05 ng/mL (normal)	10 (26.3)				
0.05 - <0.5 ng/mL (local infection)	8 (21.1)				
0.5 - <2 ng/mL (systemic infection)	8 (21.1)				
2 - <10 ng/ml (severe sepsis)	11 (28.9)				
≥10 ng/ml (septic shock)					
CRP	18 (47.4)	15.76 ± 18.6	13.2	0.6	115.2
<10 mg/dL	12 (31.6)				
10–20 mg/dL	7 (18.4)				
20–75 mg/dL	1 (2.6)				
>75 mg/dL					
D-dimer	2 (5.3)	5835 ± 7995	3055	159	35200
0–500 μg/L	12 (31.6)				
500–2000 μg/L	24 (63.2)				
>2000 μg/L					
INR	17 (44.7)	1.18 ± 0.37	1.1	0.9	2.6
0–1.0	19 (50)				
1.1–2.0	2 (5.3)				
>2.0					

(86.8%). In the study, Liu et al. stated that serum interleukin-6 levels were associated with increased inflammatory markers such as neutrophils, platelets, NLR, and CRP [18].

In Huang et al.'s study conducted in Jiangsu province, China, BUN and serum creatinine were higher in severe COVID-19 patients. This is in line with this study, where the average was 3.18 mg/dL with the highest frequency >1.3 mg/dL [19]. In Sarin et al. it was found that the mean

Table 3
Positivity of interleukin-6 expression in study participant.

Characteristics	Result				
	n (%)	Mean ± SD	Median	Min	Max
Interleukin-6	8 (21.1%)	72.63 ± 16.9	75	35	100
Weak (1–50%)	12 (31.6%)				
Moderate (51–75%)	18 (47.4%)				
Strong (>76%)					

SGPT or SGOT of patients with severe COVID-19 symptoms was higher than those with mild symptoms and the PT-INR value increased in severe compared to mild symptoms [20].

Cui et al.'s study showed that D-dimer >1500 g/L was a predictor of venous thromboembolism in COVID-19 patients with a sensitivity of 85% and specificity of 88.5% [21]. In Frisoni et al.'s study of 7 samples from lung tissue, the expression of IL-6 in tissues with fatal COVID-19 infection was higher than in control tissues in patients. This is because activated resident macrophages and pneumocytes initiate an inflammatory response triggered by the presence of SARS-CoV-2 in the lung, leading to excessive production of proinflammatory cytokines and chemokines, which are involved in endothelial cell apoptosis, increased vascular permeability, pulmonary exudation, hypoxia, and multiple organ failure [6].

According to Jiali et al.'s study, 66 COVID-19 patients were examined for blood serum IL-6, significantly increasing serum IL-6 levels compared to regular patients. In critically ill patients with COVID-19, the increase in Interleukin-6 is also associated with age, complications, increased procalcitonin, CRP, and increased liver and kidney markers that lead to death [22,23]. IL-6 in fatal COVID-19 infection increases the inflammatory response to a cytokine storm and the STAT3 activation pathway that can cause pulmonary fibrosis [5]. IL-6, IL-8, IL-1β, GM-CSF, and other chemokines cause ARDS, cause pulmonary fibrosis and lead death [24].

5. Conclusion

There was a significant high Interleukin-6 expression in patients with fatal COVID-19 infection. In this study, the average tissue expression of Interleukin-6 was 72.63, with the highest frequency of strong positive 47.4%.

Ethical Approval

We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Sources of funding

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Author contribution

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Research registration

Name of the registry: Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Unique identifying number or registration ID: 1581/KEPK/X/2019.

Hyperlink to your specific registration (must be publicly accessible and will be checked): -.

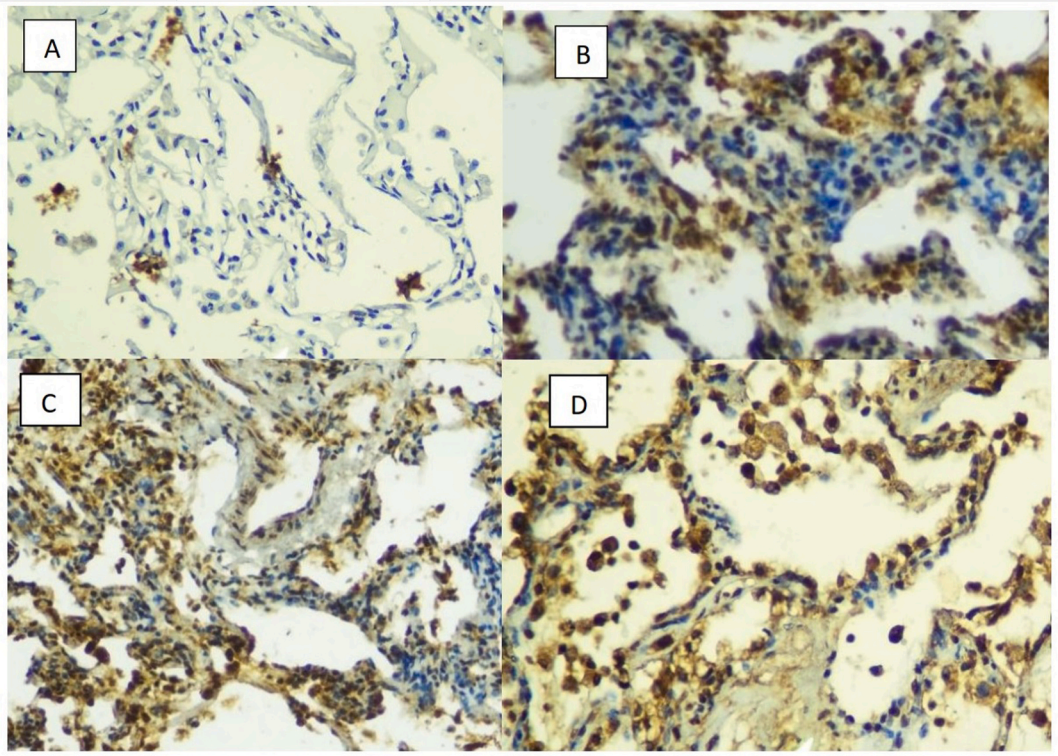

Fig. 1. (A) Control of lung tissue with IL-6 antibody staining, (B) Lung tissue stained with IL-6 antibody weak positive expression, (C) Lung tissue stained with IL-6 antibody with moderate positive expression, (D) Lung tissue stained with IL-6 antibody with strong positive expression.

Consent

Written informed consent was obtained from the family/guardian patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Guarantor

Isnin Anang Marhana.

Provenance and peer review

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.104648.

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