Resti Yudhawati <resti.yudhawati2021@gmail.com> Fri, Nov 26, 2021 at 5:28 AM
To: restiyudhawati@gmail.com, dhadhari1788@gmail.com

---------- Forwarded message ---------
Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Kam, 17 Jun 2021 pukul 07.52
Subject: Thank you for your submission to Annals of Medicine and Surgery
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Dear Mrs Yudhawati,

Thank you for sending your manuscript Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study with Prospective Design for consideration to Annals of Medicine and Surgery. Please accept this message as confirmation of your submission.
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For Annals of Medicine and Surgery, the average editorial time (in weeks) from submission to first decision is: 5.07 and from submission to final decision is: 10.13.

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Many thanks again for your interest in Annals of Medicine and Surgery.

Kind regards,

Dr. Riaz Agha
Editor-in-Chief
Annals of Medicine and Surgery

If you require further assistance, you are welcome to contact our Researcher Support team 24/7 by live chat and email or 24/5 by phone: http://support.elsevier.com

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Resti Yudhawati <resti.yudhawati2021@gmail.com> Fri, Nov 26, 2021 at 5:31 AM
To: restiyudhawati@gmail.com, dhadhari1788@gmail.com

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Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Kam, 24 Jun 2021 pukul 15.16
Subject: Editor handles AMSU-D-21-00618
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Ms. Ref. No.: AMSU-D-21-00618
Title: Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

Your submission "Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling" will be handled by Editor in Chief Riaz Ahmed Agha, BSc, MBBS, MRCSEng, MSc, D.Phil, FRSPH.

You may check on the progress of your paper by logging on to the Editorial Manager as an author.

Thank you for submitting your work to this journal.

Kind regards,

Editorial Manager
Annals of Medicine and Surgery

****

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Resti Yudhawati <resti.yudhawati2021@gmail.com> Fri, Nov 26, 2021 at 5:31 AM
To: restiyudhawati@gmail.com, dhadhari1788@gmail.com

---------- Forwarded message ---------
Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Min, 25 Jul 2021 pukul 14.05
Subject: Your Submission
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Ms. Ref. No.: AMSU-D-21-00618
Title: Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

The reviewers have commented on your above paper. They indicated that it is not acceptable for publication in its present form.

However, if you feel that you can suitably address the reviewers' comments (included below), I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

a) outline each change made (point by point) as raised in the reviewer comments

AND/OR

b) provide a suitable rebuttal to each reviewer comment not addressed

c) Supply a revised manuscript with track changes - Your revised manuscript with track changes added or your revisions highlighted in bold/red.

d) Supply a revised manuscript un-tracked - A clean unmarked copy of your revised manuscript.

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I look forward to receiving your revised manuscript.

Yours sincerely,

Jo Frankland
Editorial Office

Annals of Medicine and Surgery

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Reviewers' comments:

Managing Editor

Please can you make the following changes/checks:

1) Ensure your work is fully compliant with the STROCSS criteria www.strocssguideline.com, which should be cited within the methods section of your article and please submit a completed STROCSS checklist stating the page numbers where you completed each item (your work will be returned if this is not done).

Please also ensure your methods section states that the work has been reported in line with the STROCSS criteria and cite the paper as follows:

Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C and Mathew G, for the STROCSS Group. The STROCSS 2019 Guideline: Strengthening the Reporting of Cohort Studies in Surgery. International Journal of Surgery 2019;72:156-165.

2) Please ensure you submit your work with a Research Registry UIN: e.g. from www.researchregistry.com – it can't progress without being registered – even it its retrospective research. Please ensure you also state your registration unique identifying number (UIN) in your methods section and reference it including a hyperlink to it.

3) Please go through your paper and proofread it to correct spelling, grammar and syntax errors. If you need our author support services, you can access them here: https://www.ijspg.com/services/author-support

4. If you haven't already, please include your "highlights" which are 3-5 bullet points summarising the novel aspects and/or learning points (maximum 85 characters, including spaces, per bullet point).

5. Please add the following statement above references:

Provenance and peer review
Not commissioned, externally peer-reviewed

Reviewer #1: - Please discuss what the future implications of this work are, the research that can be conducted in response to these findings and work, and what the future will hold.

****

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Annals of Medicine and Surgery

Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling

--Manuscript Draft--

Manuscript Number:	AMSU-D-21-00618R1
Article Type:	Cross-sectional Study
Keywords:	Covid-19; KL-6; AT2
Corresponding Author:	Resti Yudhawati INDONESIA
First Author:	Titah Dhadhari Suryananda
Order of Authors:	Titah Dhadhari Suryananda
	Resti Yudhawati
Abstract:	Background : The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated. Objective: Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia. Methods : This study was an analytical study with a prospective design on 75 COVID-19 patients who were treated. Subjects were divided into two large groups according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired t-test and independent t-test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed. Result : In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. Conclusion : There was no significant association between serum KL-6 values on 0 days and 6 days in the severity of COVID-19.
Suggested Reviewers:	Miriana d'Alessandro delassandro.miriana@gmail.com
	AN Frix an.frix@chuliege.be
	Xiaoping Tang tangxiaopinggz@163.com
Response to Reviewers:	We have revised our manuscript and attached the answers from reviewers. please help us publish our manuscript. Thanks

Annals of Medicine and Surgery

The following information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned. If you have nothing to declare in any of these categories then this should be stated.

Please state any conflicts of interest
All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

The authors declare that they have no conflict of interest.

Please state any sources of funding for your research
All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

None.

Ethical Approval

Research studies involving patients require ethical approval. Please state whether approval has been given, name the relevant ethics committee and the state the reference number for their judgement.

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

Consent

Studies on patients or volunteers require ethics committee approval and fully informed written consent which should be documented in the paper.

Authors must obtain written and signed consent to publish a case report from the patient (or, where applicable, the patient's guardian or next of kin) prior to submission. We ask Authors to confirm as part of the submission process that such consent has been obtained, and the manuscript must include a statement to this effect in a consent section at the end of the manuscript, as follows: "Written informed consent was obtained from the 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".

Patients have a right to privacy. Patients' and volunteers' names, initials, or hospital numbers should not be used. Images of patients or volunteers should not be used unless the information is essential for scientific purposes and explicit permission has been given as part of the consent. If such consent is made subject to any conditions, the Editor in Chief must be made aware of all such conditions.

Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note.

Written informed consent was obtained from the patient.

Author contribution

Please specify the contribution of each author to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways should be listed as contributors.

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.

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In accordance with the Declaration of Helsinki 2013, all research involving human participants has to be registered in a publicly accessible database. Please enter the name of the registry and the unique identifying number (UIN) of your study.

You can register any type of research at http://www.researchregistry.com to obtain your UIN if you have not already registered. This is mandatory for human studies only. Trials and certain observational research can also be registered elsewhere such as: ClinicalTrials.gov or ISRCTN or numerous other registries.

1. Name of the registry: Health Reseach Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

2. Unique Identifying number or registration ID: 1953/KEPK/IV/2020

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

Guarantor

The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish

Resti Yudhawati is the person in charge for the publication of our manuscript.

To,

Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled **"Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Design"** in your esteemed journal as an Original Article.

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

In this paper, I/we report on association of Serum Kl-6 Levels on COVID-19 Severity. This is significant because give information about biomarker. The paper should be of interest to readers in the areas of infection.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

All authors have approved the manuscript and agree with its submission to Annals of Medicine and Surgery.

We hereby transfer, assign, or otherwise convey all copyright ownership, including any and all rights incidental thereto, exclusively to the journal, in the event that such work is published by the journal.

Thanking you,

Yours' sincerely,

Signature

Resti Yudhawati
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jalan Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: resti.yudhawati2021@gmail.com
Phone: +6231-5036047
Orcid ID: 0000-0002-0808-8524

Response to reviewers

Reviewers' comments:

Registration of Research

The registry must be accessible, please see public registry suggestions below.

The World Medical Association's Declaration of Helsinki 2013 states in article 35: 'Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject'. The Editors of AMS require that all types of research studies involving human participants should be registered prospectively, but failing that retrospectively. There are many places to register your research, and you can choose which is the most suitable for your needs:

*Clinicaltrials.gov - for all human studies – free

*Chinese Clinical Trial Registry chictr.org.cn - for all human studies – free

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Once registered, you will need to submit your assigned Unique Identifying Number (UIN) from your registration body as a mandatory part of your submission, please add the number and the URL of the registration site to the Author Form.

Author response: we have conducted a registration of research based on the Declaration of Helsinki at the Health Research Ethics Committee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia with certificate number “1953/KEPK/IV/2020”.

Highlight:

1. Most symptoms in COVID-19 were cough (84%), dyspnoea (78.6%) and fever (68%).
2. Serum KL-6 decreased on day 6 in COVID-19 patients.
3. KL-6 on day 0 does not have a significant correlation with the severity of COVID-19.

Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling

Running head: Serum Kl-6 in Covid-19 Patients

Titah Dhadhari Suryananda[1], Resti Yudhawati[2*]

[1] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

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Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling

Abstract

Background: The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated. **Objective:** Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia. **Methods**: This study was an analytical study with a prospectiveof design on 75 COVID-19 patients who were treated. Subjects were divided into a two large groups according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired t-test and 2 independent sample t-test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed. **Result**: In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. **Conclusion**: There was no significant association between serum KL-6 values on 0 days and 6 days in the severity of COVID-19.

Keywords: COVID-19, KL-6, AT2

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). COVID-19 was first discovered in Wuhan China at the end of December 2019, three months later the virus spread to 200 countries in the world so that the World Health Organization (WHO) declared it a global pandemic [1]. In September 2020, the worldwide incidence of COVID-19 reached 31 million. The American continent is the country with the most cases in the world, followed by Southeast Asia, Europe, the Central Mediterranean, and Africa [2]. Indonesia recorded 271,000 COVID-19 cases and 10,000 deaths, which is the highest number in Southeast Asia. East Java is the second province with the highest number of cases after DKI Jakarta, which reached 42,000 with a death rate of 3,000 people [3]. SARS-CoV2 shows mild-moderate manifestations and can develop into serious cases such as severe pneumonia to Acute Respiratory Distress Syndrome (ARDS) which is the main cause of death in COVID-19 [4].

SARS-CoV2 infects through a bond between the S protein and the host cell's angiotensin--converting enzyme 2 (ACE2) receptor. The upper respiratory tract is the first site of infection, but the main target of the virus is alveolar type II (AT2) cells. SARS-CoV2 ~~has the ability to~~can avoid or inhibit the innate immune system so that it easily reaches the lower respiratory tract and releases pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). The release of proinflammatory cytokines causes damage to AT2 cells. Severe damage to AT2 cells causes cytokine storms and results in ARDS characterized by diffuse alveolar damage (DAD), hyperplasia of pneumocytes, and microvascular thrombosis [5]. Biomarker studies help understand the underlying mechanisms and pathophysiology of lung damage and thus improve therapeutic strategies and evaluation. One of the specific biomarkers of pulmonary

alveolar cell damage that is currently being developed for research is von den lungen Kl~~k~~rebs (KL-6) [6].

KL-6 is a mucin glycoprotein which is also referred to as MUC1. MUC1 is a single glycoprotein consisting of cytoplasm, transmembrane, and extracellular domains. MUC1 has a high molecular weight with ~~a~~ large size of 200-500 nm. KL-6 is produced on the surface of AT2 epithelial cells. KL-6 is also produced in the epithelial cells of the bronchi, basal cells, and terminal bronchioles in lower numbers than AT2. KL-6 is increased in injured AT2 cells. The production of KL-6 can increase and decrease through the activity of the TNF-α converting enzyme (TACE). The expression of KL-6 protein correlated with changes in alveolar capillary permeability, indicating the association between increased KL-6 and barrier epithelial dysfunction in ARDS. KL-6 shows a correlation between bronchoalveolar lavage (BAL) and serum so that KL-6 produced in the lungs will be visible in the bloodstream [7].

Ruiz declared that the potential use of serum KL-6 in COVID-19 is a prognosis of disease activity and fibrosis [8]. Alessandro et al said that serum KL-6 was higher in the severe group of COVID-19 compared to the non-severe group. The cut-~~-~~off value of serum KL-6 in that study was 406 U / mL with a sensitivity of 89% and a specificity of 83% [9]. Awano, Xue, and Deng et al said that KL-6 serum in COVID-19 patients was higher than in healthy patients. Serum KL-6 value of the severe group of COVID-19 was higher than the non-severe group. The study also stated that serum KL-6 undergoes dynamic changes with the patient's clinical condition so that it can be used as a biomarker of severity [10-12]. Other several studies have shown less significant results. Arnold et al declared that serum KL-6 has a sensitivity of 86% and a specificity of 45% [13]. Kondo et al said that serum KL-6 in ARDS patients was only increased in BAL compared to serum [14]. Based on the description above, we are interested in analyzing the association between serum KL-6 and the degree of severity of COVID-19.

Methods

Participants in this study were COVID-19 patients who met the inclusion and exclusion criteria. Participant inclusion criteria included patients diagnosed with COVID-19 and aged >18 years. Participants' exclusion criteria included patients who had unsuccessful blood draws by day 6 and patients diagnosed with lung, breast, and pancreatic cancer. Participants who were willing to take part in the research first received an explanation of the rights and obligations of the participants, in which they voluntarily filled out the informed consent form.

The design of this study was observational analytic with a prospective design ~~which~~that used consecutive sampling. This study reported the data based on the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15]. The number of participants in this study was 75 participants were divided into two large groups namely severe (76%) and non-severe (24%). Severe groups consist of severe to critical categories~~y~~. Non-severe groups consist of mild to moderate categor~~y~~ies. Data collection was carried out at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during the period May – October 2020. Data collection included participant characteristics, levels of KL-6, and the degree of severity of COVID-19.

The severity of COVID-19 in this study was assessed using WHO criteria at the time of the initial examination of the patient, which distinguished the severity of COVID-19 from being non-severe (mild-moderate category) and severe (severe-critical category). Mild: Symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate: clinical symptoms of pneumonia (fever, cough, dyspnoea, rapid breathing) but no signs of severe pneumonia, including SpO2 ≥90% in room air or PaO2 ≥60 mmHg. (PaO2 measurements were obtained from patient medical records). Weight: obtained clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid

breathing) plus one of~~:~~ respiratory rate >30 times/minute; severe respiratory distress or SpO2 <90% or PaO2 ≤59 mmHg. (PaO2 measurements were obtained from patient medical records). Critically, in patients with ARDS, sepsis, and septic shock. Mild ARDS: 200 mmHg <PaO2/FiO2a ≤300 mmHg (with PEEP or CPAP ≥5 cmH2O). Moderate ARDS: 100 mmHg <PaO2/FiO2 ≤200 mmHg (with PEEP ≥5 cmH2O). ARDS weight: PaO2/FiO2 ≤100 mmHg (with PEEP ≥5 cmH2O).

KL-6 is a mucin glycoprotein that is expressed on alveolar type II (AT2) epithelial cells that are injured/regenerated. The increased height of KL-6 reflects the degree of severity of COVID-19. Serum KL-6 levels were measured using the Enzyme-~~-~~linked immunosorbent assay (ELISA) method of the Bioassay technology labor~~l~~atory E1980Hu brand in U/ml units. KL-6 was measured by blood serum taken on day 0, namely when the diagnosis was made and on day 6 after diagnosis.

The data are recorded in data collection sheets that have been arranged, processed, and analyzed descriptively using computer software or manually. The statistical analysis used to test the hypothesis is paired and independent t-~~-~~test ~~and free sample t 2 test~~ for normally distributed data, Wilcoxon test, and Mann Whitney test for data that are not normally distributed. The association between data from the results of statistical analysis is shown in tables, diagrams, and text.

Result

Participant characteristics

The mean age of the participants was 48.04 ± 11.66 years with an age range of 24-73 years. Most of the male participants were 46 participants (61.3%) and the rest were female as many as 29 participants (38.7%). Some of the participants experienced clinical symptoms as follows: fever as many as 51 participants (68.0%), coughing as many as 63 participants

(84.0%), dyspnoea as many as 59 participants (78.6%), diarrhea as many as 10 participants (13.0%), anosmia as many as 5 participants (6.0%)), 1 participant of muscle pain (2.0%), 9 participants of nausea & vomiting (12.0%), 9 participants of sore throat (12.0%), 7 participants of colds (9.3%), and 14 participants of weakness (18.6 %). Most of them recovered after receiving treatment as many as 62 participants (82.7%) and the rest died as many as 13 participants (17.3%). Only 7 participants (9.3%) had a history of smoking. Some of the participants also had comorbid diseases such as hypertension as many as 34 participants (45.3%), diabetes mellitus as many as 24 participants (32.0%), COPD as many as 1 participant (1.3%), asthma as many as 3 participants (4.0%), tuberculosis as many as 5 participants (6.7 %), obesity as many as 8 participants (10.6%), and heart disease as many as 8 participants (10.6%).

The mean KL-6 level at curry 0 was 56.43 ± 31.20 U/mL with a median value of 45.1 (8.5 – 151.4) U/mL. The mean KL-6 level in the 6th curry was 52.61 ± 37.99 U/mL with a median value of 41.5 (4.6 – 163.7) U/mL. The results of the 75 participant examination showed several categories of the severity of COVID-19, as follows: 18 participants (24.0%) moderate category, 35 participants (47.0%) severe category, and 22 participants (29.0%) critical categories. Details of participant characteristics can be seen in Table 1.

Changes in the Value of KL-6 Serum on Day 0 and 6

The results showed that the KL-6 value on day 0 for the non-severe group was 44.85 (11.4 - 151.4) U/mL and the severe group wasere 45.70 (8.5 - 131.8) U/mL. The KL-6 value on day 6 for the non-severe group was 41.95 (4.6 - 157.4) U/mL and the severe group wereas 41.30 (4.6 - 163.7) U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. Based on the data obtained, there was no significant association between serum KL-6 values and the severity of COVID-19 day 0 (p = 0.895; table 2).

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Discussion

Awano et al declared that the mean serum KL-6 value of all participants on day 0 was 229 U/mL with a minimum value of 184 U/mL and a maximum of 336 U/mL. On day 6, the mean score was 283 U/mL with a minimum value of 222 U/mL and a maximum value of 540 U/mL. When compared with previous studies, the mean serum KL-6 levels in this study showed lower values. The difference can be due to differences in methods and kits where previous studies used the Nanopia KL-6 reagent kit, Sexui medical. Co., Tokyo Japan antigen agglutination technique [10]. This study used Bioassay technology laboratory E1980Hu with the *Enzyme linked immunosorbent assay* (ELISA) method. Racial and genetic differences also affect serum KL-6 values. Horimasu et al said that the average serum KL-6 score in was higher than in Japan due to Germany. The dominant participant in Germany containing the genotype A/A and Japan G/G [16].

Mason said that as many as 80% of patients infected with COVID-19 experience non-severe symptoms, 14% of which require hospital treatment. As many as 20% of patients show severe symptoms, most of whom require treatment at the hospital and 5% of this number are experiencing a critical phase so they require treatment in an intensive care unit (ICU) [17]. This study is different from the study that conducted by Mason where there were was a more severe group hospitalized. The explanation for this is because Dr. Soetomo General Academic Hospital, Surabaya is a referral center hospital for eastern Indonesia. Old age is more susceptible to infection and occurs with severe manifestations due to several reasons, namely a decrease in the system called immunosenescence. Cells also experience a decrease in a function called cellular senescence. Old age also occurs with systemic inflammation called inflammaging [18].

Gender did not show a significant difference in the degree of severity even though men were infected more. Cevix said that gender is related to the immune response. Men have higher congenital cytokines and chemokines than women. Women have stronger T cell activation than men. Increasing age in men leads to decreased T cell activation. This also suggests that older men are associated with a greater degree of disease severity and ~~a~~ risk of death [19].

Shortness of breath and diarrhea are clinical symptoms that show a significant difference in severity. Shortness of breath is caused by damage to AT2 cells by viruses. The dysregulation of the immune system causes the virus to reach target cells directly. Diarrhea was more common in the severe-critical category. Diarrhea is associated with ARDS, use of mechanical ventilators, and care in the ICU but the link between diarrhea and severity is not fully understood and requires further clarification [20]. This study found no significant difference between smoking and severity and accordance to research conducted by Rosatto et al [21].

In this study, the laboratory value showed a significant difference in the degree of severity. The laboratory for the non-severe degree group showed normal values. In the severe group, WBC showed normal/increased values and decreased lymphocytes, while neutrophils, CRP, procalcitonin, D dimer, and ferritin increased. Biomarkers such as WBC, neutrophil procalcitonin lymphocytes, CRP, D-dimer, ferritin showed significant differences with the WHO severity [22].

Dysregulation of the immune system causes the release of proinflammatory cytokines. The release of proinflammatory cytokines causes cell damage. Damaged cells release toxins that increase the release of pro-inflammatory cytokines, causing a cytokine storm. Cytokine storms cause neutrophil lymphocytes and macrophages to be released to the site of infection. Macrophages and IL-6 release ferritin, IL-6 also releases CRP. Stress conditions cause an

increase in the hormone cortisol, as a result, lymphocytes migrate to the peripheral circulatory system, then lymphocyte destruction and apoptosis occurs. Depression of T and B cells also causes low lymphocytes. Apart from being caused by damage to lung cells, the degree of severity is also caused by damage to other organs. D-dimers is~~are~~ released upon activation of coagulation and fibrinolysis [23].

This study assessed serum KL-6 is based on the degree of severity. On day 0, the median KL-6 value for the non-severe group was 44.85 U/mL, while the severe group was 45.70 U/mL. On day 6, the median serum KL-6 value for the non-severe grade group was 41.95 U/mL, while the severe group value was 41.30 U/mL. The KL-6 value in the severe group was higher on that two days examination. The higher the serum KL-6 value caused by the more severe/extensive AT2 cell damage. That damage or regeneration of AT2 are the main source of serum KL-6 [6].

This study assessed changes in the KL-6 serum on days 0 and 6 based on the severity. Serum KL-6 values in both groups decreased on day 6. The main decreased serum KL-6 in the non-severe group was 2.9 U/mL and in the severe group was 4.4 U/~~.~~mL. The decrease was greater in the severe group. Xue et al said that serum KL-6 value decreased on day 4 then increased sharply on day 8 and decreased again on day 16. On days 24 and 46 the serum KL-6 value increased again but not as much as on day 16. Serum KL-6 values were consistent with the clinical condition of the participant. Changes were found in the severe group, while the non-severe group tended to be stable [11]. Deng et al said that serum KL-6 value has increased and reaches a peak for up to 1 month, then has a gradual decrease until the 6th month [12]. Awano et al said that serum KL-6 experienced an increase on day 6 in the severe group, while the non-severe group tended to be stable [10]. Langer said that serum KL-6 can dynamically assess disease activity and pathophysiology [24]. This study and previously conducted serially showed dynamic changes in serum KL-6.

Awano et al, Deng et al, Xue et al and Alessandro et al said that serum KL-6 value in the severe group was higher than that in the non-severe group. Deng et al~~.~~, Xue et al and Alessandro et al said that serum KL-6 normal pa~~t~~sients have the same ~~with~~as the non-severe group [9-12]. This study and the previous study show different results. In this study, there was no significant association between serum KL-6 value on day 0 to the degree of severity. The serum KL-6 value in the severe group was not much different from the KL-6 value for the serum in the non-severe group this differenc~~e~~t can be caused by several reasons. First is the number of samples, this study involved more subjects (75 samples) and more severity samples. In the previous study, the sample used was less in number and more in the non-severe.

Second, the cause~~d~~ of the degree severity is not only due to damage to AT2 cells. Apart from AT2 cells, endothelial cells are also the main targets of SARS CoV-2. SARS-CoV2 can infect endothelial cells directly [5]. Viral infection causes endothelial dysfunction. Endothelial dysfunction results in platelet adhesion, leukocyte aggregation, complement activation, and cytokine release leading to microvascular complications such as pulmonary embolism and deep vein thrombosis (DVT) [25]. There are also features of apoptosis, pyroptosis, and lymphocytic inflammation. These histopathologic features are associated with organ ischemia, tissue edema, and procoagulant status [5].

Limitation of the study, further research is needed on the role of serum KL-6 in COVID-19 with ~~a~~ more average sample size. Further research is needed on the role of serum KL-6 in COVID-19 involving healthy controls. It is necessary to classify the severity of the subject on day 6.

SARS-CoV2 that enters the body destroys AT2 cells resulting in increased production of Kl-6. Kl-6 was mostly found in the pulmonary alveoli where the more severe the inflammation in the lungs, the higher the KL-6 level. This study is used to identify the

severity of COVID-19 patients in the developing world with low resource settings to improve patient care management. In future research, it is expected that the data collection time for Kl-6 can be >6 days, the participant group will be compared to several groups (normal, mild, and moderate severity), and the number of participants will be more and represent each age group.

Conclusion

Participants were mostly male (61.73%). The mean age of the participants was 48 years. Most symptoms were cough (84%), dyspnoea (78.6%), and fever (68%). As many as 9.3% of the participants were smokers. The most common comorbidities were hypertension (45.3%) and diabetes mellitus (32%). The mortality rate was 17.3% which occurred in the severe group. The mean value of KL-6 serum participants on day 0 was 56.43 ± 31.204 U/mL and day 6 of 52.61 ± 37.99 U/mL. Participants in the severe group were 76%, while the non-severe group was 24%. On day 6, there was a decrease in serum KL-6 values in both groups. There was no significant association between serum KL-6 on day 0 and severity.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author's cContributor

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.

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Ethical Approval

We have conducted an ethical approval base on the Declaration of Helsinki with registration of research at the Health Research Ethicals Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1953/KEPK/IV/2020).

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Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study Design with Purposive Sampling

Abstract

Background: The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated. **Objective:** Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia. **Methods**: This study was an analytical study with a prospective design on 75 COVID-19 patients who were treated. Subjects were divided into two large groups according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired t-test and independent t-test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed. **Result**: In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. **Conclusion**: There was no significant association between serum KL-6 values on 0 days and 6 days in the severity of COVID-19.

Keywords: COVID-19, KL-6, AT2

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). COVID-19 was first discovered in Wuhan China at the end of December 2019, three months later the virus spread to 200 countries in the world so that the World Health Organization (WHO) declared it a global pandemic [1]. In September 2020, the worldwide incidence of COVID-19 reached 31 million. The American continent is the country with the most cases in the world, followed by Southeast Asia, Europe, the Central Mediterranean, and Africa [2]. Indonesia recorded 271,000 COVID-19 cases and 10,000 deaths, which is the highest number in Southeast Asia. East Java is the second province with the highest number of cases after DKI Jakarta, which reached 42,000 with a death rate of 3,000 people [3]. SARS-CoV2 shows mild-moderate manifestations and can develop into serious cases such as severe pneumonia to Acute Respiratory Distress Syndrome (ARDS) which is the main cause of death in COVID-19 [4].

SARS-CoV2 infects through a bond between the S protein and the host cell's angiotensin-converting enzyme 2 (ACE2) receptor. The upper respiratory tract is the first site of infection, but the main target of the virus is alveolar type II (AT2) cells. SARS-CoV2 can avoid or inhibit the innate immune system so that it easily reaches the lower respiratory tract and releases pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). The release of proinflammatory cytokines causes damage to AT2 cells. Severe damage to AT2 cells causes cytokine storms and results in ARDS characterized by diffuse alveolar damage (DAD), hyperplasia of pneumocytes, and microvascular thrombosis [5]. Biomarker studies help understand the underlying mechanisms and pathophysiology of lung damage and thus improve therapeutic strategies and evaluation. One of the specific biomarkers of pulmonary alveolar cell damage that is currently being developed for research is von den lungen Krebs (KL-6) [6].

KL-6 is a mucin glycoprotein which is also referred to as MUC1. MUC1 is a single glycoprotein consisting of cytoplasm, transmembrane, and extracellular domains. MUC1 has a high molecular weight with large size of 200-500 nm. KL-6 is produced on the surface of AT2 epithelial cells. KL-6 is also produced in the epithelial cells of the bronchi, basal cells, and terminal bronchioles in lower numbers than AT2. KL-6 is increased in injured AT2 cells. The production of KL-6 can increase and decrease through the activity of the TNF-α converting enzyme (TACE). The expression of KL-6 protein correlated with changes in alveolar capillary permeability, indicating the association between increased KL-6 and barrier epithelial dysfunction in ARDS. KL-6 shows a correlation between bronchoalveolar lavage (BAL) and serum so that KL-6 produced in the lungs will be visible in the bloodstream [7].

Ruiz declared that the potential use of serum KL-6 in COVID-19 is a prognosis of disease activity and fibrosis [8]. Alessandro et al said that serum KL-6 was higher in the severe group of COVID-19 compared to the non-severe group. The cut-off value of serum KL-6 in that study was 406 U / mL with a sensitivity of 89% and a specificity of 83% [9]. Awano, Xue, and Deng et al said that KL-6 serum in COVID-19 patients was higher than in healthy patients. Serum KL-6 value of the severe group of COVID-19 was higher than the non-severe group. The study also stated that serum KL-6 undergoes dynamic changes with the patient's clinical condition so that it can be used as a biomarker of severity [10-12]. Other several studies have shown less significant results. Arnold et al declared that serum KL-6 has a sensitivity of 86% and a specificity of 45% [13]. Kondo et al said that serum KL-6 in ARDS patients was only increased in BAL compared to serum [14]. Based on the description above, we are interested in analyzing the association between serum KL-6 and the degree of severity of COVID-19.

Methods

Participants in this study were COVID-19 patients who met the inclusion and exclusion criteria. Participant inclusion criteria included patients diagnosed with COVID-19 and aged >18 years. Participants' exclusion criteria included patients who had unsuccessful blood draws by day 6 and patients diagnosed with lung, breast, and pancreatic cancer. Participants who were willing to take part in the research first received an explanation of the rights and obligations of the participants, in which they voluntarily filled out the informed consent form.

The design of this study was observational analytic with a prospective design that used consecutive sampling. This study reported the data based on the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15]. The number of participants in this study was 75 participants were divided into two large groups namely severe (76%) and non-severe (24%). Severe groups consist of severe to critical categories. Non-severe groups consist of mild to moderate categories. Data collection was carried out at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during the period May – October 2020. Data collection included participant characteristics, levels of KL-6, and the degree of severity of COVID-19.

The severity of COVID-19 in this study was assessed using WHO criteria at the time of the initial examination of the patient, which distinguished the severity of COVID-19 from being non-severe (mild-moderate category) and severe (severe-critical category). Mild: Symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate: clinical symptoms of pneumonia (fever, cough, dyspnoea, rapid breathing) but no signs of severe pneumonia, including SpO2 ≥90% in room air or PaO2 ≥60 mmHg. (PaO2 measurements were obtained from patient medical records). Weight: obtained clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of respiratory rate >30 times/minute; severe respiratory distress or SpO2 <90% or PaO2 ≤59 mmHg. (PaO2 measurements were obtained from patient medical

records). Critically, in patients with ARDS, sepsis, and septic shock. Mild ARDS: 200 mmHg <PaO2/FiO2a ≤300 mmHg (with PEEP or CPAP ≥5 cmH2O). Moderate ARDS: 100 mmHg <PaO2/FiO2 ≤200 mmHg (with PEEP ≥5 cmH2O). ARDS weight: PaO2/FiO2 ≤100 mmHg (with PEEP ≥5 cmH2O).

KL-6 is a mucin glycoprotein that is expressed on alveolar type II (AT2) epithelial cells that are injured/regenerated. The increased height of KL-6 reflects the degree of severity of COVID-19. Serum KL-6 levels were measured using the Enzyme-linked immunosorbent assay (ELISA) method of the Bioassay technology laboratory E1980Hu brand in U/ml units. KL-6 was measured by blood serum taken on day 0, namely when the diagnosis was made and on day 6 after diagnosis.

The data are recorded in data collection sheets that have been arranged, processed, and analyzed descriptively using computer software or manually. The statistical analysis used to test the hypothesis is paired and independent t-test for normally distributed data, Wilcoxon test, and Mann Whitney test for data that are not normally distributed. The association between data from the results of statistical analysis is shown in tables, diagrams, and text.

Result

Participant characteristics

The mean age of the participants was 48.04 ± 11.66 years with an age range of 24-73 years. Most of the male participants were 46 participants (61.3%) and the rest were female as many as 29 participants (38.7%). Some of the participants experienced clinical symptoms as follows: fever as many as 51 participants (68.0%), coughing as many as 63 participants (84.0%), dyspnoea as many as 59 participants (78.6%), diarrhea as many as 10 participants (13.0%), anosmia as many as 5 participants (6.0%)), 1 participant of muscle pain (2.0%), 9 participants of nausea & vomiting (12.0%), 9 participants of sore throat (12.0%), 7

participants of colds (9.3%), and 14 participants of weakness (18.6 %). Most of them recovered after receiving treatment as many as 62 participants (82.7%) and the rest died as many as 13 participants (17.3%). Only 7 participants (9.3%) had a history of smoking. Some of the participants also had comorbid diseases such as hypertension as many as 34 participants (45.3%), diabetes mellitus as many as 24 participants (32.0%), COPD as many as 1 participant (1.3%), asthma as many as 3 participants (4.0%), tuberculosis as many as 5 participants (6.7 %), obesity as many as 8 participants (10.6%), and heart disease as many as 8 participants (10.6%).

The mean KL-6 level at curry 0 was 56.43 ± 31.20 U/mL with a median value of 45.1 (8.5 – 151.4) U/mL. The mean KL-6 level in the 6th curry was 52.61 ± 37.99 U/mL with a median value of 41.5 (4.6 – 163.7) U/mL. The results of the 75 participant examination showed several categories of the severity of COVID-19, as follows: 18 participants (24.0%) moderate category, 35 participants (47.0%) severe category, and 22 participants (29.0%) critical categories. Details of participant characteristics can be seen in Table 1.

Changes in the Value of KL-6 Serum on Day 0 and 6

The results showed that the KL-6 value on day 0 for the non-severe group was 44.85 (11.4 - 151.4) U/mL and the severe group was 45.70 (8.5 - 131.8) U/mL. The KL-6 value on day 6 for the non-severe group was 41.95 (4.6 - 157.4) U/mL and the severe group was 41.30 (4.6 - 163.7) U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. Based on the data obtained, there was no significant association between serum KL-6 values and the severity of COVID-19 day 0 ($p = 0.895$; table 2).

Discussion

Awano et al declared that the mean serum KL-6 value of all participants on day 0 was 229 U/mL with a minimum value of 184 U/mL and a maximum of 336 U/mL. On day 6, the mean score was 283 U/mL with a minimum value of 222 U/mL and a maximum value of 540 U/mL. When compared with previous studies, the mean serum KL-6 levels in this study showed lower values. The difference can be due to differences in methods and kits where previous studies used the Nanopia KL-6 reagent kit, Sexui medical. Co., Tokyo Japan antigen agglutination technique [10]. This study used Bioassay technology laboratory E1980Hu with *the* ELISA method. Racial and genetic differences also affect serum KL-6 values. Horimasu et al said that the average serum KL-6 score was higher than in Japan due to Germany. The dominant participant in Germany containing the genotype A/A and Japan G/G [16].

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Gender did not show a significant difference in the degree of severity even though men were infected more. Cevix said that gender is related to the immune response. Men have higher congenital cytokines and chemokines than women. Women have stronger T cell activation than men. Increasing age in men leads to decreased T cell activation. This also

suggests that older men are associated with a greater degree of disease severity and risk of death [19].

Shortness of breath and diarrhea are clinical symptoms that show a significant difference in severity. Shortness of breath is caused by damage to AT2 cells by viruses. The dysregulation of the immune system causes the virus to reach target cells directly. Diarrhea was more common in the severe-critical category. Diarrhea is associated with ARDS, use of mechanical ventilators, and care in the ICU but the link between diarrhea and severity is not fully understood and requires further clarification [20]. This study found no significant difference between smoking and severity and accordance to research conducted by Rosatto et al [21].

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Dysregulation of the immune system causes the release of proinflammatory cytokines. The release of proinflammatory cytokines causes cell damage. Damaged cells release toxins that increase the release of pro-inflammatory cytokines, causing a cytokine storm. Cytokine storms cause neutrophil lymphocytes and macrophages to be released to the site of infection. Macrophages and IL-6 release ferritin, IL-6 also releases CRP. Stress conditions cause an increase in the hormone cortisol, as a result, lymphocytes migrate to the peripheral circulatory system, then lymphocyte destruction and apoptosis occurs. Depression of T and B cells also causes low lymphocytes. Apart from being caused by damage to lung cells, the

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This study assessed serum KL-6 is based on the degree of severity. On day 0, the median KL-6 value for the non-severe group was 44.85 U/mL, while the severe group was 45.70 U/mL. On day 6, the median serum KL-6 value for the non-severe grade group was 41.95 U/mL, while the severe group value was 41.30 U/mL. The KL-6 value in the severe group was higher on that two days examination. The higher the serum KL-6 value caused by the more severe/extensive AT2 cell damage. That damage or regeneration of AT2 is the main source of serum KL-6 [6].

This study assessed changes in the KL-6 serum on days 0 and 6 based on the severity. Serum KL-6 values in both groups decreased on day 6. The main decreased serum KL-6 in the non-severe group was 2.9 U/mL and in the severe group was 4.4 U/mL. The decrease was greater in the severe group. Xue et al said that serum KL-6 value decreased on day 4 then increased sharply on day 8 and decreased again on day 16. On days 24 and 46 the serum KL-6 value increased again but not as much as on day 16. Serum KL-6 values were consistent with the clinical condition of the participant. Changes were found in the severe group, while the non-severe group tended to be stable [11]. Deng et al said that serum KL-6 value has increased and reaches a peak for up to 1 month, then has a gradual decrease until the 6th month [12]. Awano et al said that serum KL-6 experienced an increase on day 6 in the severe group, while the non-severe group tended to be stable [10]. Langer said that serum KL-6 can dynamically assess disease activity and pathophysiology [24]. This study and previously conducted serially showed dynamic changes in serum KL-6.

Awano et al, Deng et al, Xue et al and Alessandro et al said that serum KL-6 value in the severe group was higher than that in the non-severe group. Deng et al, Xue et al and Alessandro et al said that serum KL-6 normal patients have the same as the non-severe group

[9-12]. This study and the previous study show different results. In this study, there was no significant association between serum KL-6 value on day 0 to the degree of severity. The serum KL-6 value in the severe group was not much different from the KL-6 value for the serum in the non-severe group This difference can be caused by several reasons. First is the number of samples, this study involved more subjects (75 samples) and more severity samples. In the previous study, the sample used was less in number and more in the non-severe.

Second, the cause of the degree severity is not only due to damage to AT2 cells. Apart from AT2 cells, endothelial cells are also the main targets of SARS CoV-2. SARS-CoV2 can infect endothelial cells directly [5]. Viral infection causes endothelial dysfunction. Endothelial dysfunction results in platelet adhesion, leukocyte aggregation, complement activation, and cytokine release leading to microvascular complications such as pulmonary embolism and deep vein thrombosis (DVT) [25]. There are also features of apoptosis, pyroptosis, and lymphocytic inflammation. These histopathologic features are associated with organ ischemia, tissue edema, and procoagulant status [5].

Limitation of the study, further research is needed on the role of serum KL-6 in COVID-19 with more average sample size. Further research is needed on the role of serum KL-6 in COVID-19 involving healthy controls. It is necessary to classify the severity of the subject on day 6.

SARS-CoV2 that enters the body destroys AT2 cells resulting in increased production of Kl-6. Kl-6 was mostly found in the pulmonary alveoli where the more severe the inflammation in the lungs, the higher the KL-6 level. This study is used to identify the severity of COVID-19 patients in the developing world with low resource settings to improve patient care management. In future research, it is expected that the data collection time for Kl-6 can be >6 days, the participant group will be compared to several groups (normal, mild,

and moderate severity), and the number of participants will be more and represent each age group.

Conclusion

Participants were mostly male (61.73%). The mean age of the participants was 48 years. Most symptoms were cough (84%), dyspnoea (78.6%), and fever (68%). As many as 9.3% of the participants were smokers. The most common comorbidities were hypertension (45.3%) and diabetes mellitus (32%). The mortality rate was 17.3% which occurred in the severe group. The mean value of KL-6 serum participants on day 0 was 56.43 ± 31.204 U/mL and day 6 of 52.61 ± 37.99 U/mL. Participants in the severe group were 76%, while the non-severe group was 24%. On day 6, there was a decrease in serum KL-6 values in both groups. There was no significant association between serum KL-6 on day 0 and severity.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author's Contributor

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.

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Ethical Approval

We have conducted an ethical approval base on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1953/KEPK/IV/2020).

Provenance and Peer Review

Not commissioned, externally peer-reviewed.

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1

Table and Legend

Table 1. Characteristics of the participant based on the severity of COVID-19

Characteristics	Non severe n = 18	Severe n = 57	p
Age	48.5 (24.0 – 78.0)	52.0 (25.0 – 73.0)	0.084
Gender			
Male	8 (44.4)	38 (66.7)	0.158
Female	10 (55.5)	19 (33.3)	
Clinical symptoms			
Fever	12 (66.7)	39 (68.4)	1.000
Cough	14 (77.7)	49 (85.9)	0.466
Crowded	7 (38.8)	52 (91.2)	<0.001*
Diarrhea	6 (33.3)	4 (7.0)	0.001
Anosmia	2 (1.1)	3 (5.2)	0.588
Muscle ache	0 (0.0)	1 (1.7)	1.000
Nauseous vomit	2 (11.1)	7 (12.2)	1.000
Sore throat	2 (11.1)	7 (12.2)	1.000
Cold	1 (5.0)	6 (10.5)	1.000
Weak body	5 (27.7)	9 (15.7)	0.303
Outcome			
Heal	18 (100.0)	44 (77.2)	-
Died	0 (0.0)	13 (22.8)	
Smoking history			
Smoker	1 (5.5)	6 (10.5)	1.000
Comorbid disease			
Hypertension	6 (33.3)	28 (49.1)	0.367
Diabetes mellitus	4 (22.2)	20 (35.0)	0.465
COPD	0 (0.0)	1 (1.7)	1.000
Asthma	0 (0.0)	3 (5.2)	1.000
Tuberculosis	0 (0.0)	5 (8.7)	0.329
Obesity	0 (0.0)	8 (14.0)	0.186
Heart disease	1 (5.5)	7 (12.2)	0.671
Laboratory Day 0			
Leukocytes	7,050 (2,870 – 14,110)	9,720 (4,530 – 25,150)	0.006*
Lymphocytes	18.8 (6.9 – 48.4)	10.3 (0.7 – 45.6)	0.001*
Neutrophils	68.7 (35.0 – 91.3)	80.2 (40.8 – 96.8)	0.007*
Procalcitonin	0.09 (0.01 – 0.47)	0.23 (0.01 – 7.04)	0.004*
CRP	1.4 (0.01 – 18.9)	6.2 (0.01 – 39.5)	0.011*
Ferritin	323.05 (24.1 –	1,180 (38.5 – 6,498.8)	0.001*
D-Dimer	1,724.7)	1,380 (190 – 35,200)	0.368
	980 (310 – 35,200)		
Laboratory Day 6			
Leukocytes	7,110 (5,080 – 13,160)	11,860 (3,610 – 29,320)	<0.001*
Lymphocytes	23.45 (5.6 – 39.8)	9.5 (1.1 – 33.8)	<0.001*
Neutrophils	64.4 (35.0 – 88.4)	82.6 (46.4 – 99.2)	<0.001*
Procalcitonin	0.07 (0.01 – 0.47)	0.1 (0.01 – 14.71)	0.049*
CRP	0.55 (0.1 – 8.4)	3.1 (0.1 – 31.9)	0.006*
Ferritin	417.8 (34.3 – 2,116)	983 (89.4 – 16,321)	0.009*
D-Dimer	920 (180 – 21,320)	2,060 (190 – 35,200)	0.004*

Treatment			
Lopivia	5 (27.0)	24 (42.1)	-
Oseltamivir	2 (11.0)	6 (10.5)	
Avugan	1 (5.0)	1 (1.7)	
Remdisivir	1 (5.0)	1 (1.7)	
Hyloquin	9 (50.0)	19 (33.3)	
Actemra	0 (0.0)	2 (3.5)	
Crime scene	0 (0.0)	4 (7.0)	
Vitamin C	4 (22.2)	24 (32.0)	
Vitamin D	0 (0.0)	5 (8.7)	
Steroids	1 (5.0)	34 (59.6)	
Lovenox	10 (55.5)	26 (34.6)	
Heparin	3 (16.7)	32 (56.1)	
Arixtra	0 (0.0)	1 (1.7)	
NAC	15 (8.3)	35 (61.4)	
Isoprenosine	13 (72.2)	32 (56.1)	
Zinc	1 (5.0)	4 (7.0)	
Mechanical ventilation	0 (0.0)	13 (22.8)	
Non-mechanical ventilation	0 (0.0)	13 (22.8)	

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories; *significant if p <0.05

Table 2. The serum KL-6 value is based on the degree of severity

KL-6	Covid-19 severity		p
	non-severe	severe	
Day 0	44.85 (11.4 – 151.4)	45.70 (8.5 – 131.8)	0.895
Day 6	41.95 (4.6 – 157.4)	41.30 (4.6 – 163.7)	
Delta	-3.2 (-66.4 – 39.5)	-3.1 (-109.9 – 111.8)	

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories.

The STROCSS 2019 Guideline		
Item no.	**Item description**	**Page**
TITLE		
1	Title: - The word cohort or cross-sectional or case-controlled is included - The area of focus is described (e.g. disease, exposure/intervention, outcome) - Key elements of study design are stated (e.g. retrospective or prospective)	1
ABSTRACT		
2a	Introduction: the following points are briefly described - Background - Scientific Rationale for this study	1
2b	Methods: the following areas are briefly described - Study design (cohort, retro-/prospective, single/multi-centred) - Patient populations and/or groups, including control group, if applicable - Interventions (type, operators, recipients, timeframes) - Outcome measures	1
2c	Results: the following areas are briefly described - Summary data (with statistical relevance) with qualitative descriptions, where appropriate	1
2d	Conclusion: the following areas are briefly described - Key conclusions - Implications to practice - Direction of and need for future research	1
3	Introduction: the following areas are described in full - Relevant background and scientific rationale - Aims and objectives - Research question and hypotheses, where appropriate	2-3
4a	Registration and ethics - Research Registry number is stated, in accordance with the declaration of Helsinki* - All studies (including retrospective) should be registered before submission *"*Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject*" (this can be obtained from: ResearchRegistry.com or ClinicalTrials.gov or ISRCTN)	12
4b	Ethical Approval: the following areas are described in full - Necessity for ethical approval - Ethical approval, with relevant judgement reference from ethics committees - Where ethics was unnecessary, reasons are provided	12
4c	Protocol: the following areas are described comprehensively - Protocol (*a priori* or otherwise) details, with access directions - If published, journal mentioned with the reference provided	4

4d	Patient Involvement in Research - Describe how, if at all, patients were involved in study design e.g. were they involved on the study steering committee, did they provide input on outcome selection, etc.	4
5a	Study Design: the following areas are described comprehensively - 'Cohort' study is mentioned - Design (e.g. retro-/prospective, single/multi-centred)	4
5b	Setting: the following areas are described comprehensively - Geographical location - Nature of institution (e.g. academic/community, public/private) - Dates (recruitment, exposure, follow-up, data collection)	4
5c	Cohort Groups: the following areas are described in full - Number of groups - Division of intervention between groups	4
5d	Subgroup Analysis: the following areas are described comprehensively - Planned subgroup analyses - Methods used to examine subgroups and their interactions	4
6a	Participants: the following areas are described comprehensively - Eligibility criteria - Recruitment sources - Length and methods of follow-up	4
6b	Recruitment: the following areas are described comprehensively - Methods of recruitment to each patient group - Period of recruitment	4
6c	Sample Size: the following areas are described comprehensively - Margin of error calculation - Analysis to determine study population - Power calculations, where appropriate	4
7a	Pre-intervention Considerations: the following areas are described comprehensively - Patient optimisation (pre-surgical measures) - Pre-intervention treatment (hypothermia/-volaemia/-tension; ICU care; bleeding problems; medications)	4
7b	Intervention: the following areas are described comprehensively - Type of intervention and reasoning (e.g. pharmacological, surgical, physiotherapy, psychological) - Aim of intervention (preventative/therapeutic) - Concurrent treatments (antibiotics, analgaesia, anti-emetics, NBM, VTE prophylaxis) - Manufacturer and model details where applicable	4
7c	Intra-Intervention Considerations: the following areas are described comprehensively - Administration of intervention (location, surgical details, anaesthetic, positioning, equipment needed, preparation, devices, sutures, operative time) - Pharmacological therapies include formulation, dosages, routes and durations - Figures and other media are used to illustrate	4

7d	Operator Details: the following areas are described comprehensively - Training needed - Learning curve for technique - Specialisation and relevant training	4
7e	Quality Control: the following areas are described comprehensively - Measures taken to reduce variation - Measures taken to ensure quality and consistency in intervention delivery	4-5
7f	Post-Intervention Considerations: the following areas are described comprehensively - Post-operative instructions and care - Follow-up measures - Future surveillance requirements (e.g. imaging, blood tests)	5
8	Outcomes: the following areas are described comprehensively - Primary outcomes, including validation, where applicable - Definitions of outcomes - Secondary outcomes, where appropriate - Follow-up period for outcome assessment, divided by group	5
9	Statistics: the following areas are described comprehensively - Statistical tests, packages/software used, and interpretation of significance - Confounders and their control, if known - Analysis approach (e.g. intention to treat/per protocol) - Sub-group analysis, if any	5
10a	Participants: the following areas are described comprehensively - Flow of participants (recruitment, non-participation, cross-over and withdrawal, with reasons) - Population demographics (prognostic features, relevant socioeconomic features, and significant numerical differences)	5-6
10b	Participant Comparison: the following areas are described comprehensively - Table comparing demographics included - Differences, with statistical relevance - Any group matching, with methods	5-6
10c	Intervention: the following areas are described comprehensively - Changes to interventions, with rationale and diagram, if appropriate - Learning required for interventions - Degree of novelty for intervention	6
11a	Outcomes: the following areas are described comprehensively - Clinician-assessed and patient-reported outcomes for each group - Relevant photographs and imaging are desirable - Confounders to outcomes and which are adjusted	6
11b	Tolerance: the following areas are described comprehensively - Assessment of tolerance - Loss to follow up, with reasons (percentage and fraction) - Cross-over with explanation	6
11c	Complications: the following areas are described comprehensively - Adverse events described - Classified according to Clavien-Dindo classification* - Mitigation for adverse events (blood loss, wound care, revision surgery	6

	should be specified) *Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. A New Proposal with Evaluation in a Cohort of 6336 Patients and Results of a Survey. Ann Surg. 2004; 240(2): 205-213	
12	Key Results: the following areas are described comprehensively - Key results, including relevant raw data - Statistical analyses with significance	6
13	Discussion: the following areas are described comprehensively - Conclusions and rationale - Reference to relevant literature - Implications to clinical practice - Comparison to current gold standard of care - Relevant hypothesis generation	7-10
14	Strengths and Limitations: the following areas are described comprehensively - Strengths of the study - Limitations and potential impact on results - Assessment of bias and management	10
15	Implications and Relevance: the following areas are described comprehensively - Relevance of findings and potential implications to clinical practice are detailed - Future research that is needed is described, with study designs detailed	10-11
16	Conclusions: - Key conclusions are summarised - Key directions for future research are summarised	11
17a	Conflicts of interest - Conflicts of interest, if any, are described	11
17b	Funding - Sources of funding (e.g. grant details), if any, are clearly stated	11

Annals of Medicine and Surgery

Association of Serum KI-6 Levels on COVID-19 Severity: A Cross-Sectional Study with Prospective Design

--Manuscript Draft--

Manuscript Number:	
Article Type:	Cross-sectional Study
Keywords:	COVID-19; KL-6; AT2
Corresponding Author:	Resti Yudhawati INDONESIA
First Author:	Titah Dhadhari Suryananda
Order of Authors:	Titah Dhadhari Suryananda
	Resti Yudhawati
Abstract:	Background : The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated. Objective: Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia. Methods : This study was an analytical study with a prospectif design on 75 COVID-19 patients who were treated. Subjects were divided into a two large group according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired t test and 2 sample t test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed. Result : In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than non-severe group. Conclusion : There was no significant association between serum KL-6 values on 0 day and 6 day in the severity of COVID-19.
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Annals of Medicine and Surgery

The following information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned. If you have nothing to declare in any of these categories then this should be stated.

Please state any conflicts of interest
All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

The authors declare that they have no conflict of interest.

Please state any sources of funding for your research
All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

None.

Ethical Approval

Research studies involving patients require ethical approval. Please state whether approval has been given, name the relevant ethics committee and the state the reference number for their judgement.

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

Consent

Studies on patients or volunteers require ethics committee approval and fully informed written consent which should be documented in the paper.

Authors must obtain written and signed consent to publish a case report from the patient (or, where applicable, the patient's guardian or next of kin) prior to submission. We ask Authors to confirm as part of the submission process that such consent has been obtained, and the manuscript must include a statement to this effect in a consent section at the end of the manuscript, as follows: "Written informed consent was obtained from the 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".

Patients have a right to privacy. Patients' and volunteers' names, initials, or hospital numbers should not be used. Images of patients or volunteers should not be used unless the information is essential for scientific purposes and explicit permission has been given as part of the consent. If such consent is made subject to any conditions, the Editor in Chief must be made aware of all such conditions.

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Written informed consent was obtained from the patient.

Author contribution

Please specify the contribution of each author to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways should be listed as contributors.

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.

Registration of Research Studies

In accordance with the Declaration of Helsinki 2013, all research involving human participants has to be registered in a publicly accessible database. Please enter the name of the registry and the unique identifying number (UIN) of your study.

You can register any type of research at http://www.researchregistry.com to obtain your UIN if you have not already registered. This is mandatory for human studies only. Trials and certain observational research can also be registered elsewhere such as: ClinicalTrials.gov or ISRCTN or numerous other registries.

1. Name of the registry: -

2. Unique Identifying number or registration ID: -

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

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The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish

Resti Yudhawati is the person in charge for the publication of our manuscript.

To,

Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled **"Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study with Prospective Design"** in your esteemed journal as an Original Article.

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

In this paper, I/we report on pulmonology division. This is significant because give information about biomarker. The paper should be of interest to readers in the areas of infection.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

All authors have approved the manuscript and agree with its submission to Annals of Medicine and Surgery.

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Thanking you,

Yours' sincerely,

Signature

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Highlight:

1. Most symptoms in COVID-19 were cough (84%), dyspnoea (78.6%) and fever (68%).
2. Serum KL-6 decreased on day 6 in COVID-19 patients.
3. KL-6 on day 0 does not have a significant correlation with the severity of COVID-19.

Association of Serum Kl-6 Levels on COVID-19 Severity: A Cross-Sectional Study with Prospective Design

Running head: Serum Kl-6 in Covid-19 Patients

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Association of Serum KL-6 Levels on Covid-19 Severity: A Cross-Sectional Study with Prospective Design

Abstract

Background: The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated. **Objective:** Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia. **Methods**: This study was an analytical study with a prospectif design on 75 COVID-19 patients who were treated. Subjects were divided into a two large group according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired t test and 2 sample t test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed. **Result**: In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than non-severe group. **Conclusion**: There was no significant association between serum KL-6 values on 0 day and 6 day in the severity of COVID-19.

Keywords: COVID-19, KL-6, AT2

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). COVID-19 was first discovered in Wuhan China at the end of December 2019, three months later the virus spread to 200 countries in the world so that the World Health Organization (WHO) declared it a global pandemic [1]. In September 2020, the worldwide incidence of COVID-19 reached 31 million. The American continent is the country with the most cases in the world, followed by Southeast Asia, Europe, the Central Mediterranean and Africa [2]. Indonesia recorded 271,000 COVID-19 cases and 10,000 deaths, which is the highest number in Southeast Asia. East Java is the second province with the highest number of cases after DKI Jakarta, which reached 42,000 with a death rate of 3,000 people [3]. SARS-CoV2 shows mild-moderate manifestations and can develop into serious cases such as severe pneumonia to Acute Respiratory Distress Syndrome (ARDS) which is the main cause of death in COVID-19 [4].

SARS-CoV2 infects through a bond between the S protein and the host cell's angiotensin coverting enzyme 2 (ACE2) receptor. The upper respiratory tract is the first site of infection, but the main target of the virus is alveolar type II (AT2) cells. SARS-CoV2 has the ability to avoid or inhibit the innate immune system so that it easily reaches the lower respiratory tract and releases proinflammatory cytokines (IL-1β, IL-6, TNF-α). The release of proinflammatory cytokines causes damage to AT2 cells. Severe damage to AT2 cells causes cytokine storms and results in ARDS characterized by diffuse alveolar damage (DAD), hyperplasia of pneumocytes and microvascular thrombosis [5]. Biomarker studies help understand the underlying mechanisms and pathophysiology of lung damage and thus improve therapeutic strategies and evaluation. One of the specific biomarkers of pulmonary alveolar cell damage that is currently being developed for research is von den lungen krebs (KL-6) [6].

KL-6 is a mucin glycoprotein which is also referred to as MUC1. MUC1 is a single glycoprotein consisting of cytoplasm, transmembrane and extracellular domains. MUC1 has a high molecular weight with a large size of 200-500 nm. KL-6 is produced on the surface of AT2 epithelial cells. KL-6 is also produced in the epithelial cells of the bronchi, basal cells and terminal bronchioles in lower numbers than AT2. KL-6 is increased in injured AT2 cells. The production of KL-6 can increase and decrease through the activity of TNF-α converting enzyme (TACE). The expression of KL-6 protein correlated with changes in alveolar capillary permeability, indicating association between increased KL-6 and barrier epithelial dysfunction in ARDS. KL-6 shows a correlation between bronchoalveolar lavage (BAL) and serum so that KL-6 produced in the lungs will be visible in the bloodstream [7].

Ruiz declared that the potential use of serum KL-6 in COVID-19 is a prognosis of disease activity and fibrosis [8]. Alessandro et al said that serum KL-6 was higher in the severe group of COVID-19 compared to the non-severe group. The cut off value of serum KL-6 in that study was 406 U / mL with a sensitivity of 89% and a specificity of 83% [9]. Awano, Xue and Deng et al said that KL-6 serum in COVID-19 patients was higher than healthy patients. Serum KL-6 value of the severe group of COVID-19 was higher than the non-severe group. The study also stated that serum KL-6 undergoes dynamic changes with the patient's clinical condition so that it can be used as a biomarker of severity [10-12]. Other several studies have shown less significant result. Arnold et al declared that serum KL-6 has a sensitivity of 86% and a specificity of 45% [13]. Kondo et al said that serum KL-6 in ARDS patients was only increased in BAL compared to serum [14]. Based on the description above, we are interested in analyzing the association between serum KL-6 and the degree of severity of COVID-19.

Methods

Participants in this study were COVID-19 patients who met the inclusion and exclusion criteria. Participant inclusion criteria included patients diagnosed with COVID-19 and aged >18 years. Participants' exclusion criteria included patients who had unsuccessful blood draws by day 6 and patients diagnosed with lung, breast and pancreatic cancer. Participants who were willing to take part in the research first received an explanation of the rights and obligations of the participants, in which they voluntarily filled out the informed consent form.

The design of this study was observational analytic with a prospective design which used consecutive sampling. This study reported the data based on the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15]. The number of participants in this study was 75 participants were divided into two large groups namely severe (76%) and non-severe (24%). Severe groups consist of severe to critical category. Non-severe groups consist of mild to moderate category. Data collection was carried out at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during the period May – October 2020. Data collection included participant characteristics, levels of KL-6, and the degree of severity of COVID-19.

The severity of COVID-19 in this study was assessed using WHO criteria at the time of the initial examination of the patient, which distinguished the severity of COVID-19 from being non-severe (mild-moderate category) and severe (severe-critical category). Mild: Symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate: clinical symptoms of pneumonia (fever, cough, dyspnoea, rapid breathing) but no signs of severe pneumonia, including SpO2 ≥90% in room air or PaO2 ≥60 mmHg. (PaO2 measurements were obtained from patient medical records). Weight: obtained clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of: respiratory rate >30 times/minute; severe respiratory distress or SpO2 <90% or PaO2 ≤59 mmHg. (PaO2 measurements were obtained from patient medical

records). Critically, in patients with ARDS, sepsis and septic shock. Mild ARDS: 200 mmHg <PaO2/FiO2a ≤300 mmHg (with PEEP or CPAP ≥5 cmH2O). Moderate ARDS: 100 mmHg <PaO2/FiO2 ≤200 mmHg (with PEEP ≥5 cmH2O). ARDS weight: PaO2/FiO2 ≤100 mmHg (with PEEP ≥5 cmH2O).

KL-6 is a mucin glycoprotein that is expressed on alveolar type II (AT2) epithelial cells that are injured/regenerated. The increase height of KL-6 reflects the degree of severity of COVID-19. Serum KL-6 levels were measured using the Enzyme linked immunosorbent assay (ELISA) method of the Bioassay technology labolatory E1980Hu brand in U/ml units. KL-6 was measured by blood serum taken on day 0, namely when the diagnosis was made and on day 6 after diagnosis.

The data recorded in data collection sheets that have been arranged, processed and analyzed descriptively using computer software or manually. The statistical analysis used to test the hypothesis is paired t test and free sample t 2 test for normally distributed data, Wilcoxon test and Mann Whitney test for data that are not normally distributed. The association between data from the results of statistical analysis is shown in tables, diagrams and text.

Result

Participant characteristics

The mean age of the participants was 48.04 ± 11.66 years with an age range of 24-73 years. Most of the male participants were 46 participants (61.3%) and the rest were female as many as 29 participants (38.7%). Some of the participants experienced clinical symptoms as follows: fever as many as 51 participants (68.0%), coughing as many as 63 participants (84.0%), dyspnoea as many as 59 participants (78.6%), diarrhea as many as 10 participants (13.0%), anosmia as many as 5 participants (6.0%)), 1 participant of muscle pain (2.0%), 9

participants of nausea & vomiting (12.0%), 9 participants of sore throat (12.0%), 7 participants of colds (9.3%), and 14 participant of weakness (18.6 %). Most of them recovered after receiving treatment as many as 62 participants (82.7%) and the rest died as many as 13 participants (17.3%). Only 7 participants (9.3%) had a history of smoking. Some of the participants also had comorbid diseases such as hypertension as many as 34 participants (45.3%), diabetes mellitus as many as 24 participants (32.0%), COPD as many as 1 participant (1.3%), asthma as many as 3 participants (4.0%), tuberculosis as many as 5 participants (6.7 %), obesity as many as 8 participants (10.6%), and heart disease as many as 8 participants (10.6%).

The mean KL-6 level at curry 0 was 56.43 ± 31.20 U/mL with a median value of 45.1 (8.5 – 151.4) U/mL. The mean KL-6 level in the 6th curry was 52.61 ± 37.99 U/mL with a median value of 41.5 (4.6 – 163.7) U/mL. The results of the 75 participant examination showed several categories of the severity of COVID-19, as follows: 18 participants (24.0%) moderate category, 35 participants (47.0%) severe category, and 22 participant (29.0%) critical categories. Details of participant characteristics can be seen in Table 1.

Changes in the Value of KL-6 Serum on Day 0 and 6

The results showed that the KL-6 value on day 0 for non-severe group was 44.85 (11.4 - 151.4) U/mL and severe group were 45.70 (8.5 - 131.8) U/mL. The KL-6 value on day 6 for non-severe group was 41.95 (4.6 - 157.4) U/mL and severe group were 41.30 (4.6 - 163.7) U/mL. Serum KL-6 in the severe group experienced an even greater decrease than non-severe group. Based on the data obtained, there was no significant association between serum KL-6 values and the severity of COVID-19 day 0 ($p = 0.895$; table 2).

Discussion

Awano et al delacred that the mean serum KL-6 value of all participants on day 0 was 229 U/mL with a minimum value of 184 U/mL and a maximum of 336 U/mL. On day 6, the mean score was 283 U/mL with a minimum value of 222 U/mL and a maximum value of 540 U/mL. When compared with previous studies, the mean serum KL-6 levels in this study showed lower values. The difference can be due to differences in methods and kits where previous studies used the Nanopia KL-6 reagent kit, Sexui medical. Co., Tokyo Japan antigen agglutination technique [10]. This study used Bioassay technology labolatory E1980Hu with *Enzyme linked immunosorbent assay* (ELISA) method. Racial and genetic differences also affect serum KL-6 values. Horimasu et al said that the average serum KL-6 score in was higher than in Japan due to Germany. The dominant participant in Germany containing the genotype A/A and Japan G/G [16].

Mason said that as many as 80% of patients infected with COVID-19 experience non-severe symptoms, 14% of which require hospital treatment. As many as 20% of patients show severe symptoms, most of whom require treatment at the hospital and 5% of this number are experiencing a critical phase so they require treatment in an intensive care unit (ICU) [17]. This study is different from study that conducted by Mason where there were more severe group hospitalized. Explanation for this is because Dr. Soetomo General Academic Hospital, Surabaya is a referral center hospital for eastern Indonesia. Old age is more susceptible to infection and occurs with severe manifestations due to several reasons, namely a decrease in the system called immunosenenscene. Cells also experience a decrease in a function called cellular senescence. Old age also occurs with systemic inflammation called inflammaging [18].

Gender did not show a significant difference in the degree of severity even though men were infected more. Cevix said that gender is related to the immune response. Men have higher congenital cytokines and chemokines than women. Women have stronger T cell

activation than men. Increasing age in men leads to decreased T cell activation. This also suggests that older men are associated with a greater degree of disease severity and a risk of death [19].

Shortness of breath and diarrhea are clinical symptoms that show a significant difference in severity. Shortness of breath is caused by damage to AT2 cells by viruses. The dysregulation of the immune system causes the virus to reach target cells directly. Diarrhea was more common in the severe-critical category. Diarrhea is associated with ARDS, use of mechanical ventilators and care in the ICU but the link between diarrhea and severity is not fully understood and requires further clarification [20]. This study found no significant difference between smoking and severity and accordance to research conducted by Rosatto et al [21].

In this study, the laboratory value showed a significant difference in the degree of severity. The laboratory for the non-severe degree group showed normal values. In the severe group, WBC showed normal/increased values and decreased lymphocytes, while neutrophils, CRP, procalcitonin, D dimer, and ferritin increased. Biomarkers such as WBC, neutrophil procalcitonin lymphocytes, CRP, D-dimer, ferritin showed significant differences with the WHO severity [22].

Dysregulation of the immune system causes the release of proinflammatory cytokines. The release of proinflammatory cytokines causes cell damage. Damaged cells release toxins that increase the release of pro-inflammatory cytokines, causing a cytokine storm. Cytokine storms cause neutrophil lymphocytes and macrophages to be released to the site of infection. Macrophages and IL-6 release ferritin, IL-6 also releases CRP. Stress conditions cause an increase in the hormone cortisol, as a result, lymphocytes migrate to the peripheral circulatory system, then lymphocyte destruction and apoptosis occurs. Depression of T and B cells also causes low lymphocytes. Apart from being caused by damage to lung cells, the

degree of severity is also caused by damage to other organs. D-dimers are released upon activation of coagulation and fibrinolysis [23].

This study assessed serum KL-6 is based on the degree of severity. On day 0, the median KL-6 value for the non-severe group was 44.85 U/mL, while the severe group was 45.70 U/mL. On day 6, the median serum KL-6 value for the non-severe grade group was 41.95 U/mL, while the severe group value was 41.30 U/mL. The KL-6 value in the severe group was higher on that two days examination. The higher the serum KL-6 value caused by the more severe/extensive AT2 cell damage. That damage or regeneration of AT2 are the main source of serum KL-6 [6].

This study assessed changes in the KL-6 serum on days 0 and 6 based on the severity. Serum KL-6 values in both groups decreased on day 6. The main decreased serum KL-6 in the non-severe group was 2.9 U/mL and in severe group was 4.4 U.mL. The decrease was greater in severe group. Xue et al said that serum KL-6 value decreased on day 4 then increased sharply on day 8 and decreased again on day 16. On day 24 and 46 the serum KL-6 value increased again but not as much as on day 16. Serum KL-6 values were consistent with the clinical condition of the participant. Changes were found in the severe group, while the non-severe group tended to be stable [11]. Deng et al said that serum KL-6 value has increased and reaches a peak for up to 1 month, then has a gradual decrease until the 6th month [12]. Awano et al said that serum KL-6 experienced an increase on day 6 in the severe group, while the non-severe group tended to be stable [10]. Langer said that serum KL-6 can dynamically assess disease activity and pathophysiology [24]. This study and previous conducted serially showed dynamic changes in serum KL-6.

Awano et al, Deng et al, Xue et al and Alessandro et al said that serum KL-6 value in the severe group was higher than that in the non-severe group. Deng et al , Xue et al and Alessandro et al said that serum KL-6 normal pasien have the same with non-severe group

[9-12]. This study and previous study show different result. In this study there was no significant association between serum KL-6 value on day 0 to the degree of severity. The serum KL-6 value in the severe group was not much different from the KL-6 value for the serum in the non-severe group This different can be caused by several reasons. First is the number of samples, this study involved more subjects (75 samples) and more severity samples. In previous study, the sample used was less in number and more in the non-severe.

Second, caused of the degree severity not only due to damage to AT2 cells. Apart from AT2 cells, endothelial cells are also the main targets of SARS CoV-2. SARS-CoV2 can infect endothelial cells directly [5]. Viral infection causes endothelial dysfunction. Endothelial dysfunction results in platelet adhesion, leukocyte aggregation, complement activation and cytokine release leading to microvascular complications such as pulmonary embolism and deep vein thrombosis (DVT) [25]. There are also features of apoptosis, pyroptosis and lymphocytic inflammation. These histopathologic features are associated with organ ischemia, tissue edema and procoagulant status [5].

Limitation of the study, Futher research is needed on the role of serum KL-6 in COVID-19 with a more average sample size. Futher research is needed on the role of serum KL-6 in COVID-19 involving healthy controls. It is necessary to classify the severity of the subject on day 6.

Conclusion

Participants were mostly male (61.73%). The mean age of the participants was 48 years. Most symptoms were cough (84%), dyspnoea (78.6%) and fever (68%). As many as 9.3% of the participants were smokers. The most common comorbidities were hypertension (45.3%) and diabetes mellitus (32%). The mortality rate was 17.3% which occurred in the severe group. The mean value of KL-6 serum participant on day 0 was 56.43 ± 31.204 U/mL and day 6 of

52.61 ± 37.99 U/mL. Participants in the severe group were 76%, while the non-severe group was 24%. On day 6, there was a decrease in serum KL-6 values in both groups. There was no significant association between serum KL-6 on day 0 and severity.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author's Contributor

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.

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Ethical Approval

We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1953/KEPK/IV/2020).

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Table and Legend

Table 1. Characteristics of the participant based on the severity of COVID-19

Characteristics	Non severe n = 18	Severe n = 57	p
Age	48.5 (24.0 – 78.0)	52.0 (25.0 – 73.0)	0.084
Gender			
Male	8 (44.4)	38 (66.7)	0.158
Female	10 (55.5)	19 (33.3)	
Clinical symptoms			
Fever	12 (66.7)	39 (68.4)	1.000
Cough	14 (77.7)	49 (85.9)	0.466
Crowded	7 (38.8)	52 (91.2)	<0.001*
Diarrhea	6 (33.3)	4 (7.0)	0.001
Anosmia	2 (1.1)	3 (5.2)	0.588
Muscle ache	0 (0.0)	1 (1.7)	1.000
Nauseous vomit	2 (11.1)	7 (12.2)	1.000
Sore throat	2 (11.1)	7 (12.2)	1.000
Cold	1 (5.0)	6 (10.5)	1.000
Weak body	5 (27.7)	9 (15.7)	0.303
Outcome			
Heal	18 (100.0)	44 (77.2)	-
Died	0 (0.0)	13 (22.8)	
Smoking history			
Smoker	1 (5.5)	6 (10.5)	1.000
Comorbid disease			
Hypertension	6 (33.3)	28 (49.1)	0.367
Diabetes mellitus	4 (22.2)	20 (35.0)	0.465
COPD	0 (0.0)	1 (1.7)	1.000
Asthma	0 (0.0)	3 (5.2)	1.000
Tuberculosis	0 (0.0)	5 (8.7)	0.329
Obesity	0 (0.0)	8 (14.0)	0.186
Heart disease	1 (5.5)	7 (12.2)	0.671
Laboratory Day 0			
Leukocytes	7,050 (2,870 – 14,110)	9,720 (4,530 – 25,150)	0.006*
Lymphocytes	18.8 (6.9 – 48.4)	10.3 (0.7 – 45.6)	0.001*
Neutrophils	68.7 (35.0 – 91.3)	80.2 (40.8 – 96.8)	0.007*
Procalcitonin	0.09 (0.01 – 0.47)	0.23 (0.01 – 7.04)	0.004*
CRP	1.4 (0.01 – 18.9)	6.2 (0.01 – 39.5)	0.011*
Ferritin	323.05 (24.1 – 1,724.7)	1,180 (38.5 – 6,498.8)	0.001*
D-Dimer	980 (310 – 35,200)	1,380 (190 – 35,200)	0.368
Laboratory Day 6			
Leukocytes	7,110 (5,080 – 13,160)	11,860 (3,610 – 29,320)	<0.001*
Lymphocytes	23.45 (5.6 – 39.8)	9.5 (1.1 – 33.8)	<0.001*
Neutrophils	64.4 (35.0 – 88.4)	82.6 (46.4 – 99.2)	<0.001*
Procalcitonin	0.07 (0.01 – 0.47)	0.1 (0.01 – 14.71)	0.049*
CRP	0.55 (0.1 – 8.4)	3.1 (0.1 – 31.9)	0.006*
Ferritin	417.8 (34.3 – 2,116)	983 (89.4 – 16,321)	0.009*
D-Dimer	920 (180 – 21,320)	2,060 (190 – 35,200)	0.004*

Treatment			
Lopivia	5 (27.0)	24 (42.1)	-
Oseltamivir	2 (11.0)	6 (10.5)	
Avugan	1 (5.0)	1 (1.7)	
Remdisivir	1 (5.0)	1 (1.7)	
Hyloquin	9 (50.0)	19 (33.3)	
Actemra	0 (0.0)	2 (3.5)	
Crime scene	0 (0.0)	4 (7.0)	
Vitamin C	4 (22.2)	24 (32.0)	
Vitamin D	0 (0.0)	5 (8.7)	
Steroids	1 (5.0)	34 (59.6)	
Lovenox	10 (55.5)	26 (34.6)	
Heparin	3 (16.7)	32 (56.1)	
Arixtra	0 (0.0)	1 (1.7)	
NAC	15 (8.3)	35 (61.4)	
Isoprenosine	13 (72.2)	32 (56.1)	
Zinc	1 (5.0)	4 (7.0)	
Mechanical ventilation	0 (0.0)	13 (22.8)	
Non-mechanical ventilation	0 (0.0)	13 (22.8)	

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories; *significant if p <0.05

Table 2. The serum KL-6 value is based on the degree of severity

KL-6	Covid-19 severity		p
	non-severe	severe	
Day 0	44.85 (11.4 – 151.4)	45.70 (8.5 – 131.8)	0.895
Day 6	41.95 (4.6 – 157.4)	41.30 (4.6 – 163.7)	
Delta	-3.2 (-66.4 – 39.5)	-3.1 (-109.9 – 111.8)	

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories.

The STROCSS 2019 Guideline		
Item no.	**Item description**	**Page**
TITLE		
1	Title: - The word cohort or cross-sectional or case-controlled is included - The area of focus is described (e.g. disease, exposure/intervention, outcome) - Key elements of study design are stated (e.g. retrospective or prospective)	1
ABSTRACT		
2a	Introduction: the following points are briefly described - Background - Scientific Rationale for this study	1
2b	Methods: the following areas are briefly described - Study design (cohort, retro-/prospective, single/multi-centred) - Patient populations and/or groups, including control group, if applicable - Interventions (type, operators, recipients, timeframes) - Outcome measures	1
2c	Results: the following areas are briefly described - Summary data (with statistical relevance) with qualitative descriptions, where appropriate	1
2d	Conclusion: the following areas are briefly described - Key conclusions - Implications to practice - Direction of and need for future research	1
3	Introduction: the following areas are described in full - Relevant background and scientific rationale - Aims and objectives - Research question and hypotheses, where appropriate	2-3
4a	Registration and ethics - Research Registry number is stated, in accordance with the declaration of Helsinki* - All studies (including retrospective) should be registered before submission *"*Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject*" (this can be obtained from: ResearchRegistry.com or ClinicalTrials.gov or ISRCTN)	11
4b	Ethical Approval: the following areas are described in full - Necessity for ethical approval - Ethical approval, with relevant judgement reference from ethics committees - Where ethics was unnecessary, reasons are provided	11
4c	Protocol: the following areas are described comprehensively - Protocol (*a priori* or otherwise) details, with access directions - If published, journal mentioned with the reference provided	4

4d	Patient Involvement in Research - Describe how, if at all, patients were involved in study design e.g. were they involved on the study steering committee, did they provide input on outcome selection, etc.	4
5a	Study Design: the following areas are described comprehensively - 'Cohort' study is mentioned - Design (e.g. retro-/prospective, single/multi-centred)	4
5b	Setting: the following areas are described comprehensively - Geographical location - Nature of institution (e.g. academic/community, public/private) - Dates (recruitment, exposure, follow-up, data collection)	4
5c	Cohort Groups: the following areas are described in full - Number of groups - Division of intervention between groups	4
5d	Subgroup Analysis: the following areas are described comprehensively - Planned subgroup analyses - Methods used to examine subgroups and their interactions	4
6a	Participants: the following areas are described comprehensively - Eligibility criteria - Recruitment sources - Length and methods of follow-up	4
6b	Recruitment: the following areas are described comprehensively - Methods of recruitment to each patient group - Period of recruitment	4
6c	Sample Size: the following areas are described comprehensively - Margin of error calculation - Analysis to determine study population - Power calculations, where appropriate	4
7a	Pre-intervention Considerations: the following areas are described comprehensively - Patient optimisation (pre-surgical measures) - Pre-intervention treatment (hypothermia/-volaemia/-tension; ICU care; bleeding problems; medications)	4
7b	Intervention: the following areas are described comprehensively - Type of intervention and reasoning (e.g. pharmacological, surgical, physiotherapy, psychological) - Aim of intervention (preventative/therapeutic) - Concurrent treatments (antibiotics, analgaesia, anti-emetics, NBM, VTE prophylaxis) - Manufacturer and model details where applicable	4
7c	Intra-Intervention Considerations: the following areas are described comprehensively - Administration of intervention (location, surgical details, anaesthetic, positioning, equipment needed, preparation, devices, sutures, operative time) - Pharmacological therapies include formulation, dosages, routes and durations - Figures and other media are used to illustrate	4

7d	Operator Details: the following areas are described comprehensively - Training needed - Learning curve for technique - Specialisation and relevant training	4
7e	Quality Control: the following areas are described comprehensively - Measures taken to reduce variation - Measures taken to ensure quality and consistency in intervention delivery	4-5
7f	Post-Intervention Considerations: the following areas are described comprehensively - Post-operative instructions and care - Follow-up measures - Future surveillance requirements (e.g. imaging, blood tests)	5
8	Outcomes: the following areas are described comprehensively - Primary outcomes, including validation, where applicable - Definitions of outcomes - Secondary outcomes, where appropriate - Follow-up period for outcome assessment, divided by group	5
9	Statistics: the following areas are described comprehensively - Statistical tests, packages/software used, and interpretation of significance - Confounders and their control, if known - Analysis approach (e.g. intention to treat/per protocol) - Sub-group analysis, if any	5
10a	Participants: the following areas are described comprehensively - Flow of participants (recruitment, non-participation, cross-over and withdrawal, with reasons) - Population demographics (prognostic features, relevant socioeconomic features, and significant numerical differences)	5-6
10b	Participant Comparison: the following areas are described comprehensively - Table comparing demographics included - Differences, with statistical relevance - Any group matching, with methods	5-6
10c	Intervention: the following areas are described comprehensively - Changes to interventions, with rationale and diagram, if appropriate - Learning required for interventions - Degree of novelty for intervention	6
11a	Outcomes: the following areas are described comprehensively - Clinician-assessed and patient-reported outcomes for each group - Relevant photographs and imaging are desirable - Confounders to outcomes and which are adjusted	6
11b	Tolerance: the following areas are described comprehensively - Assessment of tolerance - Loss to follow up, with reasons (percentage and fraction) - Cross-over with explanation	6
11c	Complications: the following areas are described comprehensively - Adverse events described - Classified according to Clavien-Dindo classification* - Mitigation for adverse events (blood loss, wound care, revision surgery	6

	should be specified) *Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. A New Proposal with Evaluation in a Cohort of 6336 Patients and Results of a Survey. Ann Surg. 2004; 240(2): 205-213	
12	Key Results: the following areas are described comprehensively - Key results, including relevant raw data - Statistical analyses with significance	6
13	Discussion: the following areas are described comprehensively - Conclusions and rationale - Reference to relevant literature - Implications to clinical practice - Comparison to current gold standard of care - Relevant hypothesis generation	7-10
14	Strengths and Limitations: the following areas are described comprehensively - Strengths of the study - Limitations and potential impact on results - Assessment of bias and management	10
15	Implications and Relevance: the following areas are described comprehensively - Relevance of findings and potential implications to clinical practice are detailed - Future research that is needed is described, with study designs detailed	10
16	Conclusions: - Key conclusions are summarised - Key directions for future research are summarised	10-11
17a	Conflicts of interest - Conflicts of interest, if any, are described	11
17b	Funding - Sources of funding (e.g. grant details), if any, are clearly stated	11

Cross-sectional Study

Association of serum KL-6 levels on COVID-19 severity: A cross-sectional study design with purposive sampling

Q1

Q4

The corrections made in this section will be reviewed and approved by a journal production editor.

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Abstract

***Background*:** The main target of SARS-CoV2 is the alveolar type II (AT2) cells of the lung. SARS-CoV2 evades the innate immune system resulting in the release of proinflammatory cytokines (IL-1β, IL-6, TNF-α) which causes AT2 cell damage. Krebs von den Lungen (KL-6) is a specific biomarker of AT2 cell damage. KL-6 is produced in AT2 cells that are injured/regenerated.

***Objective*:** Research that discusses the role of KL-6 in COVID-19 is still being debated and not much has been done in Indonesia.

***Methods*:** This study was an analytical study with a prospective design on 75 COVID-19 patients who were treated. Subjects were divided into two large groups according to their degree of severity, 57 subjects with severe degrees and 18 subjects with non-severe degrees. The serum KL-6 levels were measured on days 0 and 6. Data were analyzed using paired *t*-test and independent *t*-test for data were normally distributed and Wilcoxon test and Mann Whitney test for data that were not normally distributed.

***Result*:** In this study, the mean serum KL-6 for day 0 in the severe group was higher than the non-severe group with values of 45.70 U/mL and 44.85 U/mL. On day 6, the mean serum KL-6 in the severe group was lower than that in the non-severe group with values of 41.3 U/mL and 41.95 U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group.

***Conclusion*:** There was no significant association between serum KL-6 values on 0 days and 6 days in the severity of COVID-19.

Keywords:

COVID-19, KL-6, AT2

1 Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). COVID-19 was first discovered in Wuhan China at the end of December 2019, three months later the virus spread to 200 countries in the world so that the World Health Organization (WHO) declared it a global pandemic [1]. In September 2020, the worldwide incidence of COVID-19 reached 31 million. The American continent is the country with the most cases in the world, followed by Southeast Asia, Europe, the Central Mediterranean, and Africa [2]. Indonesia recorded 271,000 COVID-19 cases and 10,000 deaths, which is the highest

number in Southeast Asia. East Java is the second province with the highest number of cases after DKI Jakarta, which reached 42,000 with a death rate of 3000 people [3]. SARS-CoV2 shows mild-moderate manifestations and can develop into serious cases such as severe pneumonia to Acute Respiratory Distress Syndrome (ARDS) which is the main cause of death in COVID-19 [4].

SARS-CoV2 infects through a bond between the S protein and the host cell's angiotensin-converting enzyme 2 (ACE2) receptor. The upper respiratory tract is the first site of infection, but the main target of the virus is alveolar type II (AT2) cells. SARS-CoV2 can avoid or inhibit the innate immune system so that it easily reaches the lower respiratory tract and releases pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). The release of proinflammatory cytokines causes damage to AT2 cells. Severe damage to AT2 cells causes cytokine storms and results in ARDS characterized by diffuse alveolar damage (DAD), hyperplasia of pneumocytes, and microvascular thrombosis [5]. Biomarker studies help understand the underlying mechanisms and pathophysiology of lung damage and thus improve therapeutic strategies and evaluation. One of the specific biomarkers of pulmonary alveolar cell damage that is currently being developed for research is von den lungen Krebs (KL-6) [6].

KL-6 is a mucin glycoprotein which is also referred to as MUC1. MUC1 is a single glycoprotein consisting of cytoplasm, transmembrane, and extracellular domains. MUC1 has a high molecular weight with large size of 200–500 nm. KL-6 is produced on the surface of AT2 epithelial cells. KL-6 is also produced in the epithelial cells of the bronchi, basal cells, and terminal bronchioles in lower numbers than AT2. KL-6 is increased in injured AT2 cells. The production of KL-6 can increase and decrease through the activity of the TNF-α converting enzyme (TACE). The expression of KL-6 protein correlated with changes in alveolar capillary permeability, indicating the association between increased KL-6 and barrier epithelial dysfunction in ARDS. KL-6 shows a correlation between bronchoalveolar lavage (BAL) and serum so that KL-6 produced in the lungs will be visible in the bloodstream [7].

Ruiz declared that the potential use of serum KL-6 in COVID-19 is a prognosis of disease activity and fibrosis [8]. Alessandro et al. said that serum KL-6 was higher in the severe group of COVID-19 compared to the non-severe group. The cut-off value of serum KL-6 in that study was 406 U/mL with a sensitivity of 89 % and a specificity of 83 % [9]. Awano, Xue, and Deng et al. said that KL-6 serum in COVID-19 patients was higher than in healthy patients. Serum KL-6 value of the severe group of COVID-19 was higher than the non-severe group. The study also stated that serum KL-6 undergoes dynamic changes with the patient's clinical condition so that it can be used as a biomarker of severity [10–12]. Other several studies have shown less significant results. Arnold et al. declared that serum KL-6 has a sensitivity of 86 % and a specificity of 45 % [13]. Kondo et al. said that serum KL-6 in ARDS patients was only increased in BAL compared to serum [14]. Based on the description above, we are interested in analyzing the association between serum KL-6 and the degree of severity of COVID-19.

2 Methods

Participants in this study were COVID-19 patients who met the inclusion and exclusion criteria. Participant inclusion criteria included patients diagnosed with COVID-19 and aged >18 years. Participants' exclusion criteria included patients who had unsuccessful blood draws by day 6 and patients diagnosed with lung, breast, and pancreatic cancer. Participants who were willing to take part in the research first received an explanation of the rights and obligations of the participants, in which they voluntarily filled out the informed consent form.

The design of this study was observational analytic with a prospective design that used consecutive sampling. This study reported the data based on the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [15]. The number of participants in this study was 75 participants were divided into two large groups namely severe (76 %) and non-severe (24 %). Severe groups consist of severe to critical categories. Non-severe groups consist of mild to moderate categories. Data collection was carried out at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during the period May–October 2020. Data collection included participant characteristics, levels of KL-6, and the degree of severity of COVID-19.

The severity of COVID-19 in this study was assessed using WHO criteria at the time of the initial examination of the patient, which distinguished the severity of COVID-19 from being non-severe (mild-moderate category) and severe (severe-critical category). Mild: Symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate: clinical symptoms of pneumonia (fever, cough, dyspnoea, rapid breathing) but no signs of severe pneumonia, including SpO2 ≥90 % in room air or PaO2 ≥60 mmHg. (PaO2 measurements were obtained from patient medical records). Weight: obtained clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of respiratory rate >30 times/minute; severe respiratory distress or SpO2 <90 % or PaO2 ≤59 mmHg. (PaO2 measurements were obtained from patient medical records). Critically, in patients with ARDS, sepsis, and septic shock. Mild ARDS: 200 mmHg < PaO2/FiO2a ≤300 mmHg (with PEEP or CPAP ≥5 cmH2O). Moderate ARDS: 100 mmHg < PaO2/FiO2 ≤200 mmHg (with PEEP ≥5 cmH2O). ARDS weight: PaO2/FiO2 ≤100 mmHg (with PEEP ≥5 cmH2O).

KL-6 is a mucin glycoprotein that is expressed on alveolar type II (AT2) epithelial cells that are injured/regenerated. The increased height of KL-6 reflects the degree of severity of COVID-19. Serum KL-6 levels were measured using the Enzyme-linked immunosorbent assay (ELISA) method of the Bioassay technology laboratory E1980Hu brand in U/ml units. KL-6 was measured by blood serum taken on day 0, namely when the diagnosis was made and on day 6 after diagnosis.

The data are recorded in data collection sheets that have been arranged, processed, and analyzed descriptively using computer software or manually. The statistical analysis used to test the hypothesis is paired and independent *t*-test for normally distributed data, Wilcoxon test, and Mann Whitney test for data that are not normally distributed. The association between data from the results of statistical analysis is shown in tables, diagrams, and text.

3 Result

3.1 Participant characteristics

The mean age of the participants was 48.04 ± 11.66 years with an age range of 24–73 years. Most of the male participants were 46 participants (61.3 %) and the rest were female as many as 29 participants (38.7 %). Some of the participants experienced clinical symptoms as follows: fever as many as 51 participants (68.0 %), coughing as many as 63 participants (84.0 %), dyspnoea as many as 59 participants (78.6 %), diarrhea as many as 10 participants (13.0 %), anosmia as many as 5 participants (6.0 %)), 1 participant of muscle pain (2.0 %), 9 participants of nausea & vomiting (12.0 %), 9 participants of sore throat (12.0 %), 7 participants of colds (9.3 %), and 14 participants of weakness (18.6%). Most of them recovered after receiving treatment as many as 62 participants (82.7 %) and the rest died as many as 13 participants (17.3 %). Only 7 participants (9.3 %) had a history of smoking. Some of the participants also had comorbid diseases such as hypertension as many as 34 participants (45.3 %), diabetes mellitus as many as 24 participants (32.0 %), COPD as many as 1 participant (1.3 %), asthma as many as 3 participants (4.0 %), tuberculosis as many as 5 participants (6.7%), obesity as many as 8 participants (10.6 %), and heart disease as many as 8 participants (10.6 %).

The mean KL-6 level at curry 0 was 56.43 ± 31.20 U/mL with a median value of 45.1 (8.5–151.4) U/mL. The mean KL-6 level in the 6th curry was 52.61 ± 37.99 U/mL with a median value of 41.5 (4.6–163.7) U/mL. The results of the 75 participant examination showed several categories of the severity of COVID-19, as follows: 18 participants (24.0 %) moderate category, 35 participants (47.0 %) severe category, and 22 participants (29.0 %) critical categories. Details of participant characteristics can be seen in Table 1.

alt-text: Table 1

Table 1

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Characteristics of the participant based on the severity of COVID-19.

Characteristics	Non severe n = 18	Severe n = 57	p
Age	48.5 (24.0–78.0)	52.0 (25.0–73.0)	0.084
Gender			
Male	8 (44.4)	38 (66.7)	0.158
Female	10 (55.5)	19 (33.3)	
Clinical symptoms			
Fever	12 (66.7)	39 (68.4)	1.000
Cough	14 (77.7)	49 (85.9)	0.466
Crowded	7 (38.8)	52 (91.2)	<0.001*
Diarrhea	6 (33.3)	4 (7.0)	0.001
Anosmia	2 (1.1)	3 (5.2)	0.588
Muscle ache	0 (0.0)	1 (1.7)	1.000
Nauseous vomit	2 (11.1)	7 (12.2)	1.000
Sore throat	2 (11.1)	7 (12.2)	1.000
Cold	1 (5.0)	6 (10.5)	1.000
Weak body	5 (27.7)	9 (15.7)	0.303

Outcome			
Heal	18 (100.0)	44 (77.2)	–
Died	0 (0.0)	13 (22.8)	
Smoking history			
Smoker	1 (5.5)	6 (10.5)	1.000
Comorbid disease			
Hypertension	6 (33.3)	28 (49.1)	0.367
Diabetes mellitus	4 (22.2)	20 (35.0)	0.465
COPD	0 (0.0)	1 (1.7)	1.000
Asthma	0 (0.0)	3 (5.2)	1.000
Tuberculosis	0 (0.0)	5 (8.7)	0.329
Obesity	0 (0.0)	8 (14.0)	0.186
Heart disease	1 (5.5)	7 (12.2)	0.671
Laboratory Day 0			
Leukocytes	7050 (2870–14,110)	9720 (4530–25,150)	0.006*
Lymphocytes	18.8 (6.9–48.4)	10.3 (0.7–45.6)	0.001*
Neutrophils	68.7 (35.0–91.3)	80.2 (40.8–96.8)	0.007*
Procalcitonin	0.09 (0.01–0.47)	0.23 (0.01–7.04)	0.004*
CRP	1.4 (0.01–18.9)	6.2 (0.01–39.5)	0.011*
Ferritin	323.05 (24.1–1724.7)	1180 (38.5–6498.8)	0.001*
D-Dimer	980 (310–35,200)	1380 (190–35,200)	0.368
Laboratory Day 6			
Leukocytes	7110 (5080–13,160)	11,860 (3610–29,320)	<0.001*
Lymphocytes	23.45 (5.6–39.8)	9.5 (1.1–33.8)	<0.001*
Neutrophils	64.4 (35.0–88.4)	82.6 (46.4–99.2)	<0.001*
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Ferritin	417.8 (34.3–2116)	983 (89.4–16,321)	0.009*
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Lopivia	5 (27.0)	24 (42.1)	–
Oseltamivir	2 (11.0)	6 (10.5)	
Avugan	1 (5.0)	1 (1.7)	
Remdisivir	1 (5.0)	1 (1.7)	
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Vitamin C	4 (22.2)	24 (32.0)	
Vitamin D	0 (0.0)	5 (8.7)	
Steroids	1 (5.0)	34 (59.6)	
Lovenox	10 (55.5)	26 (34.6)	
Heparin	3 (16.7)	32 (56.1)	
Arixtra	0 (0.0)	1 (1.7)	
NAC	15 (8.3)	35 (61.4)	
Isoprenosine	13 (72.2)	32 (56.1)	

Zinc	1 (5.0)	4 (7.0)	
Mechanical ventilation	0 (0.0)	13 (22.8)	
Non-mechanical ventilation	0 (0.0)	13 (22.8)	

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories; *significant if $p < 0.05$.

3.2 Changes in the value of KL-6 serum on day 0 and 6

The results showed that the KL-6 value on day 0 for the non-severe group was 44.85 (11.4–151.4) U/mL and the severe group was 45.70 (8.5–131.8) U/mL. The KL-6 value on day 6 for the non-severe group was 41.95 (4.6–157.4) U/mL and the severe group was 41.30 (4.6–163.7) U/mL. Serum KL-6 in the severe group experienced an even greater decrease than the non-severe group. Based on the data obtained, there was no significant association between serum KL-6 values and the severity of COVID-19 day 0 (p = 0.895; Table 2).

alt-text: Table 2

Table 2

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Expand

Previous version

The serum KL-6 value is based on the degree of severity.

KL-6	Covid-19 severity		*p*

Updated version

The serum KL-6 value is based on the degree of severity.

KL-6	Covid-19 severity		*p*
	non-severe	severe	
Day 0	44.85 (11.4–151.4)	45.70 (8.5–131.8)	0.895
Day 6	41.95 (4.6–157.4)	41.30 (4.6–163.7)	-
Delta	−3.2 (−66.4–39.5)	−3.1 (−109.9–111.8)	-

Note: non severe consisting of mild and moderate covid-19 severity; severe consisting of the severity of Covid-19, severe and critical categories.

4 Discussion

Awano et al. declared that the mean serum KL-6 value of all participants on day 0 was 229 U/mL with a minimum value of 184 U/mL and a maximum of 336 U/mL. On day 6, the mean score was 283 U/mL with a minimum value of 222 U/mL and a maximum value of 540 U/mL. When compared with previous studies, the mean serum KL-6 levels in this study showed lower values. The difference can be due to differences in methods and kits where previous studies used the Nanopia KL-6 reagent kit, Sexui medical. Co., Tokyo Japan antigen agglutination technique [10]. This study used Bioassay technology laboratory E1980Hu with *the* ELISA method. Racial and genetic differences also affect serum KL-6 values. Horimasu et al. said that the average serum KL-6 score was higher than in Japan due to Germany. The dominant participant in Germany containing the genotype A/A and Japan G/G [16].

Mason said that as many as 80 % of patients infected with COVID-19 experience non-severe symptoms, 14 % of which require hospital treatment. As many as 20 % of patients show severe symptoms, most of whom require treatment at the hospital and 5 % of this number are experiencing a critical phase so they require treatment in an intensive care unit (ICU) [17]. This study is different from the study conducted by Mason where there was a more severe group hospitalized. The explanation for this is because Dr. Soetomo General Academic Hospital, Surabaya is a referral center hospital for eastern Indonesia. Old age is more susceptible to infection and occurs with severe manifestations due to several reasons, namely a decrease in the system called immunosenescence. Cells also experience a decrease in a function called cellular senescence. Old age also occurs with systemic inflammation called inflammaging [18].

Gender did not show a significant difference in the degree of severity even though men were infected more. Cevix said that gender is related to the immune response. Men have higher congenital cytokines and chemokines than women.

Women have stronger T cell activation than men. Increasing age in men leads to decreased T cell activation. This also suggests that older men are associated with a greater degree of disease severity and risk of death [19].

Shortness of breath and diarrhea are clinical symptoms that show a significant difference in severity. Shortness of breath is caused by damage to AT2 cells by viruses. The dysregulation of the immune system causes the virus to reach target cells directly. Diarrhea was more common in the severe-critical category. Diarrhea is associated with ARDS, use of mechanical ventilators, and care in the ICU but the link between diarrhea and severity is not fully understood and requires further clarification [20]. This study found no significant difference between smoking and severity and accordance to research conducted by Rosatto et al. [21].

In this study, the laboratory value showed a significant difference in the degree of severity. The laboratory for the non-severe degree group showed normal values. In the severe group, WBC showed normal/increased values and decreased lymphocytes, while neutrophils, CRP, procalcitonin, D dimer, and ferritin increased. Biomarkers such as WBC, neutrophil procalcitonin lymphocytes, CRP, D-dimer, ferritin showed significant differences with the WHO severity [22].

Dysregulation of the immune system causes the release of proinflammatory cytokines. The release of proinflammatory cytokines causes cell damage. Damaged cells release toxins that increase the release of pro-inflammatory cytokines, causing a cytokine storm. Cytokine storms cause neutrophil lymphocytes and macrophages to be released to the site of infection. Macrophages and IL-6 release ferritin, IL-6 also releases CRP. Stress conditions cause an increase in the hormone cortisol, as a result, lymphocytes migrate to the peripheral circulatory system, then lymphocyte destruction and apoptosis occurs. Depression of T and B cells also causes low lymphocytes. Apart from being caused by damage to lung cells, the degree of severity is also caused by damage to other organs. D-dimers are released upon activation of coagulation and fibrinolysis [23].

This study assessed serum KL-6 is based on the degree of severity. On day 0, the median KL-6 value for the non-severe group was 44.85 U/mL, while the severe group was 45.70 U/mL. On day 6, the median serum KL-6 value for the non-severe grade group was 41.95 U/mL, while the severe group value was 41.30 U/mL. The KL-6 value in the severe group was higher on that two days examination. The higher the serum KL-6 value caused by the more severe/extensive AT2 cell damage. That damage or regeneration of AT2 is the main source of serum KL-6 [6].

This study assessed changes in the KL-6 serum on days 0 and 6 based on the severity. Serum KL-6 values in both groups decreased on day 6. The main decreased serum KL-6 in the non-severe group was 2.9 U/mL and in the severe group was 4.4 U/mL. The decrease was greater in the severe group. Xue et al. said that serum KL-6 value decreased on day 4 then increased sharply on day 8 and decreased again on day 16. On days 24 and 46 the serum KL-6 value increased again but not as much as on day 16. Serum KL-6 values were consistent with the clinical condition of the participant. Changes were found in the severe group, while the non-severe group tended to be stable [11]. Deng et al. said that serum KL-6 value has increased and reaches a peak for up to 1 month, then has a gradual decrease until the 6th month [12]. Awano et al. said that serum KL-6 experienced an increase on day 6 in the severe group, while the non-severe group tended to be stable [10]. Langer said that serum KL-6 can dynamically assess disease activity and pathophysiology [24]. This study and previously conducted serially showed dynamic changes in serum KL-6.

Awano et al. Deng et al., Xue et al. and Alessandro et al. said that serum KL-6 value in the severe group was higher than that in the non-severe group. Deng et al. Xue et al. and Alessandro et al. said that serum KL-6 normal patients have the same as the non-severe group [9–12]. This study and the previous study show different results. In this study, there was no significant association between serum KL-6 value on day 0 to the degree of severity. The serum KL-6 value in the severe group was not much different from the KL-6 value for the serum in the non-severe group This difference can be caused by several reasons. First is the number of samples, this study involved more subjects (75 samples) and more severity samples. In the previous study, the sample used was less in number and more in the non-severe.

Second, the cause of the degree severity is not only due to damage to AT2 cells. Apart from AT2 cells, endothelial cells are also the main targets of SARS CoV-2. SARS-CoV2 can infect endothelial cells directly [5]. Viral infection causes endothelial dysfunction. Endothelial dysfunction results in platelet adhesion, leukocyte aggregation, complement activation, and cytokine release leading to microvascular complications such as pulmonary embolism and deep vein thrombosis (DVT) [25]. There are also features of apoptosis, pyroptosis, and lymphocytic inflammation. These histopathologic features are associated with organ ischemia, tissue edema, and procoagulant status [5].

Limitation of the study, further research is needed on the role of serum KL-6 in COVID-19 with more average sample size. Further research is needed on the role of serum KL-6 in COVID-19 involving healthy controls. It is necessary to classify the severity of the subject on day 6.

SARS-CoV2 that enters the body destroys AT2 cells resulting in increased production of KL-6. KL-6 was mostly found in the pulmonary alveoli where the more severe the inflammation in the lungs, the higher the KL-6 level. This study is used to identify the severity of COVID-19 patients in the developing world with low resource settings to

improve patient care management. In future research, it is expected that the data collection time for KL-6 can be > 6 days, the participant group will be compared to several groups (normal, mild, and moderate severity), and the number of participants will be more and represent each age group.

5 Conclusion

Participants were mostly male (61.73 %). The mean age of the participants was 48 years. Most symptoms were cough (84 %), dyspnoea (78.6 %), and fever (68 %). As many as 9.3 % of the participants were smokers. The most common comorbidities were hypertension (45.3 %) and diabetes mellitus (32 %). The mortality rate was 17.3 % which occurred in the severe group. The mean value of KL-6 serum participants on day 0 was 56.43 ± 31.204 U/mL and day 6 of 52.61 ± 37.99 U/mL. Participants in the severe group were 76 %, while the non-severe group was 24 %. On day 6, there was a decrease in serum KL-6 values in both groups. There was no significant association between serum KL-6 on day 0 and severity.

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Author's contributor

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.

Consent

Written informed consent was obtained from the patient.

Registration of Research Studies

- Name of the registry: Health Reseach Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
- Unique Identifying number or registration ID: 1953/KEPK/IV/2020.
- Hyperlink to your specific registration (must be publicly accessible and will be checked):-.

Guarantor

Resti Yudhawati is the person in charge for the publication of our manuscript.

Ethical approval

We have conducted an ethical approval base on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (1953/KEPK/IV/2020).

Provenance and peer review

Not commissioned, externally peer-reviewed.

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.2021.102673.

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Highlights

- Most symptoms in COVID-19 were cough (84 %), dyspnoea (78.6 %) and fever (68 %).
- Serum KL-6 decreased on day 6 in COVID-19 patients.
- KL-6 on day 0 does not have a significant correlation with the severity of COVID-19.

Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

alt-text: Multimedia component 1

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