Gmail **Resti Yudhawati <restiyudhawati@gmail.com>**

Fwd: Your Submission

Resti Yudhawati <resti.yudhawati2021@gmail.com> Wed, Feb 2, 2022 at 9:24 PM
To: restiyudhawati@gmail.com

---------- Forwarded message ---------
Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Sen, 24 Jan 2022 pukul 19.04
Subject: Your Submission
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Ms. Ref. No.: AMSU-D-21-01406R1
Title: Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

I am pleased to inform you that your paper "Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study" has been accepted for publication in Annals of Medicine and Surgery.

This journal is fully open access; all articles will be immediately and permanently free for everyone to read and download. To provide Open Access, this journal has a publication fee which needs to be met by the authors or their research funders.
In the next few days, you will be receiving information via email to allow you to choose one of the CC license options, providing funding information and a link to our payment system.

Learn about publishing Open Access in this journal at http://www.elsevier.com/journals/Annals-of-Medicine-and-Surgery/2049-0801/open-access-journal.

Your manuscript will be published online in raw form 4 days from this acceptance.

We appreciate and value your contribution to Annals of Medicine and Surgery. We regularly invite authors of recently published manuscript to participate in the peer review process. If you were not already part of the journal's reviewer pool, you have now been added to it. We look forward to your continued participation in our journal, and we hope you will consider us again for future submissions.

Yours sincerely,

Dr Riaz Agha
Editorial Office
Annals of Medicine and Surgery

****

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EM via

interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Gmail **Resti Yudhawati <restiyudhawati@gmail.com>**

Fwd: Editor handles AMSU-D-21-01406R1

Resti Yudhawati <resti.yudhawati2021@gmail.com> Wed, Feb 2, 2022 at 9:26 PM
To: restiyudhawati@gmail.com

---------- Forwarded message ---------
Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Sab, 22 Jan 2022 pukul 05.58
Subject: Editor handles AMSU-D-21-01406R1
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Ms. Ref. No.: AMSU-D-21-01406R1
Title: Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study
Annals of Medicine and Surgery

Dear Mrs Yudhawati,

Your submission "Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study" will be handled by Editor in Chief Riaz Agha.

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

****
For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923
Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EM via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

_____
In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/amsu/login.asp?a=r). Please contact the publication office if you have any questions.

Gmail **Resti Yudhawati <restiyudhawati@gmail.com>**

Fwd: Your Submission

Resti Yudhawati <resti.yudhawati2021@gmail.com> Wed, Feb 2, 2022 at 9:25 PM
To: restiyudhawati@gmail.com

---------- Forwarded message ---------
Dari: **Annals of Medicine and Surgery** <em@editorialmanager.com>
Date: Jum, 7 Jan 2022 pukul 06.18
Subject: Your Submission
To: Resti Yudhawati <resti.yudhawati2021@gmail.com>

Ms. Ref. No.: AMSU-D-21-01406
Title: Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity: An Observational Study
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 Managing Editor (if applicable) and Reviewer(s) 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.

To submit your revision, please do the following:

1. Go to: https://www.editorialmanager.com/amsu/

2. Enter your login details

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Needing Revision]

I look forward to receiving your revised manuscript.

Yours sincerely,

Dr Riaz Agha

Editor-in-Chief
Annals of Medicine and Surgery

Reviewer(s) comments:

Managing Editor

Please can you make the following changes/checks:

1) Ensure your work is fully compliant with the STROCSS 2021 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:

Mathew G and Agha R, for the STROCSS Group. STROCSS 2021: Strengthening the Reporting of cohort, cross-sectional and case-control studies in Surgery. International Journal of Surgery 2021;96:106165.

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: Thank you for the opportunity to review the document. I am returning the manuscript with editing and proofreading revisions in accordance with the journal's guidelines. You will find margin comments, some of which only explain the reason for a change, but others ask to confirm that a change made does not alter the intended meaning. I indicated the missing parts requested in the journal's guidelines that you still need to insert. I found no major inconsistencies or points you should clarify. You will want to review the comments carefully. Please read all comments on the tracked file, and check all revisions throughout the document for the intended meaning. The article possessed all the essential details to allow a helpful conclusion. I believe the limitations section needs to be expanded. The author mentions there were limitations to the study, but it would be useful to know what the limitations were as this knowledge could lead to further research topics in the
future. The manuscript ensured that the patients' anonymity and confidentiality were protected. There were no apparent conflicts of interest. After the revisions have been accepted and comments/recommendations have been addressed, I recommend accepting the paper for publication.

****

Please note that the editorial process varies considerably from journal to journal. To view the submission-to-publication lifecycle, click here: http://help.elsevier.com/app/answers/detail/p/7923/a_id/160

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EM via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/amsu/login.asp?a=r). Please contact the publication office if you have any questions.

Annals of Medicine and Surgery

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity in Indonesian Adult: An Observational Study

--Manuscript Draft--

Manuscript Number:	
Article Type:	Cross-sectional Study
Keywords:	serum sRAGE; COVID-19 severity; infectious disease
Corresponding Author:	Resti Yudhawati Universitas Airlangga Fakultas Kedokteran Surabaya, East Java INDONESIA
First Author:	Gusti Noor Ramadany Saputra
Order of Authors:	Gusti Noor Ramadany Saputra
	Resti Yudhawati
	Munawaroh Fitriah
Abstract:	Background Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble Receptor for Advanced Glycation End-Products (sRAGE) is a biomarker that can be used to identify early lung damage. Objective Analyzing the association of serum sRAGE with COVID-19 severity in Indonesian adults. Methods This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020 – October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum with COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence t-test, Mann Withney test, and Spearman's rank test with p-value <0.05. Results The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL; z = 2.431; p = 0.015), lymphocytes (13.55 ± 8.48%; z = 2.256; p = 0.024), neutrophils (78.91 ± 10.50%; z = 2.464; p = 0.014), procalcitonin (0.92 ± 3.22 ng/mL; z = 3.323; p = 0.001), CRP (8.59 ± 7.62 mg/L; z = 2.114; p = 0.034), D-dimer (4,360.29 ± 7,797.81 ng/mL; z = 2.186; p = 0.029), and fibrinogen (474.58 ± 168.90 mg/dL; t = 0.383; p = 0.703). There was a significant difference in serum sRAGE values in the non-severe group (0.78 [0.63 – 1.00] ng/mL) and severe group (1.47 [0.97 – 2.25] ng/mL; r = 7.154; p <0.001). There was a significant relationship between serum sRAGE and COVID-19 severity (r = 0.598; p <0.001). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95% CI. Conclusion There is a significant relationship between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.
Suggested Reviewers:	Miriana d'Alessandro delassandro.miriana@gmail.com

	AN Frix an.frix@chuliege.be
	Xiaoping Tang tangxiaopinggz@163.com

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.

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: Health Research 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

To,

Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled **"Association of Soluble Receptor for Advanced Glycation End Products (sRAGE) Serum on COVID-19 Severity in Indonesian Adult: An Observational Study"** 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 the association of sRAGE serum on COVID-19 severity. This is significant because the process of interaction of sRAGE with its ligand becomes more due to an increase in HMGB1 which in the end increases the inflammatory response and can make tissue damage. The paper should be of interest to readers in the areas of respiratory and infectious disease.

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 for the research, authorship, and/or publication of this article.

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

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

Thanking you,

Yours' sincerely,

Resti Yudhawati
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Padjadjaran – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: resti.yudhawati2021@gmail.com
Phone: +6231-5501656
Orcid ID: 0000-0002-0808-8524

Highlights

1. Serum sRAGE can be used to identify COVID-19 severity.
2. The level of serum sRAGE in each COVID-19 patient is different.
3. The blood components of each COVID-19 severity are different.

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity in Indonesian Adult: An Observational Study

Running head: sRAGE on COVID-19 Severity

Gusti Noor Ramadany Saputra[1], Resti Yudhawati[1]*, Munawaroh Fitriah[2]

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

[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

***Corresponding author:** Resti Yudhawati

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia

Mail: restiyudhawati2021@gmail.com

Phone: +6231-5501656

Orcid ID: 0000-0002-0808-8524

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity in Indonesian Adult: An Observational Study

Abstract

Background: Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble Receptor for Advanced Glycation End-Products (sRAGE) is a biomarker that can be used to identify early lung damage. **Objective**: Analyzing the association of serum sRAGE with COVID-19 severity in Indonesian adults. **Methods**: This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020 – October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum with COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence t-test, Mann Withney test, and Spearman's rank test with *p*-value <0.05. **Results**: The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL; $z = 2.431$; $p = 0.015$), lymphocytes (13.55 ± 8.48%; $z = 2.256$; $p = 0.024$), neutrophils (78.91 ± 10.50%; $z = 2.464$; $p = 0.014$), procalcitonin (0.92 ± 3.22 ng/mL; $z = 3.323$; $p = 0.001$), CRP (8.59 ± 7.62 mg/L; $z = 2.114$; $p = 0.034$), D-dimer (4,360.29 ± 7,797.81 ng/mL; $z = 2.186$; $p = 0.029$), and fibrinogen (474.58 ± 168.90 mg/dL; $t = 0.383$; $p = 0.703$). There was a significant difference in serum sRAGE values in the non-severe group (0.78 [0.63 – 1.00] ng/mL) and severe group (1.47 [0.97 – 2.25] ng/mL; $r = 7.154$; $p < 0.001$). There was a significant relationship between serum sRAGE and COVID-19 severity ($r = 0.598$; $p < 0.001$). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95%

CI. **Conclusion**: There is a significant relationship between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.

Keywords: serum sRAGE, COVID-19 severity, infectious disease

Introduction

Coronavirus disease 2019 or better known as COVID-19 caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) became a worldwide pandemic at the end of 2019 with various systemic complaints but was more dominant in respiratory disorders. The worldwide mortality rate was 2.1% by February 12, 2020 [1]. The February 2020 data by Johns Hopkins University's Center for Systems Science and Engineering (CSSE) showed a total case of more than 60,331 patients, with a total death of more than 1,369 patients and an improvement of more than 6,061 patients [2]. On December 27, 2020, the total number of worldwide cases was more than 79 million, including 1,751,311 deaths. Incidents in Indonesia were 706,837 confirmed cases of COVID-19 and 20,994 cases of death [3].

The severity of COVID-19 according to WHO is divided into mild, moderate, severe, and critical [4, 5]. The most frequently encountered clinical symptoms are pneumonia symptoms. Biomarkers are frequently used to determine the severity of pneumonia such as procalcitonin, C-reactive protein (CRP), copeptin, pro-ANP (atrial natriuretic peptide), adrenomedullin, cortisol, and D-dimers [6]. These biomarkers are good in determining infection in pneumonia but have not been able to detect early tissue damage, as patients often go to the hospital with a more severe condition. Recent studies in immunology have examined soluble RAGE (sRAGE) as a biomarker of the severity of community pneumonia and can detect tissue damage in ARDS early [7].

Pathophysiology occurred in COVID-19 includes the inflammatory process. One of the inflammatory processes during pneumonia is characterized by an increase in Receptors for Advanced Glycation End-Products (RAGE). RAGE is one of the non-enzymatic receptors of Advanced Glycation End-Products (AGEs) which has a multi-ligand receptor, namely a V-type domain, two C-type domains, a transmembrane domain, and a cytoplasmic tail. RAGE has several ligands including AGEs, S100/calgranulins, and HMGB I which are present in different vascular cells such as endothelial cells, neuronal cells, smooth muscle cells, or inflammatory cells (monocytes). HMGB I is one of the RAGE ligands that play a role in the occurrence of sepsis which can stimulate the formation of cytokines along with TLRs in the immune system cells (B cells) [8]. The interaction between RAGE and its ligands will cause the formation of Reactive Oxygen Species (ROS) which will activate NADPH oxidation. The process will mediate the formation of inflammatory cells. Trianta et al. stated two processes of RAGE interaction with its ligands that are related to the inflammatory process, namely its interaction with leukocytes and on endothelial cells, RAGE is an adhesive receptor and directly forms inflammatory cells. The accumulation of RAGE ligands is predicted to cause chronic cell stimulation and tissue damage [9, 10].

RAGE is expressed in the membrane-bound form (fl-RAGE or mRAGE) and the soluble form in the transmembrane domain. Soluble RAGE is produced by proteolytic cleavage of fl-RAGE and alternative splicing mRNA [7]. The administration of sRAGE in experimental animals can also interact with the RAGE ligand [10]. Based on these studies, the role of sRAGE becomes very important in determining COVID-19 diagnosis based on the severity quickly, so that effective and adequate treatment planning can be carried out early to reduce the morbidity and mortality of COVID-19 patients. In addition, the level of sRAGE in serum can detect early tissue damage which in turn can affect the severity of COVID-19 patients as common biomarkers have not been able to detect the process of tissue damage

early. Research on sRAGE in the serum of COVID-19 patients is still limited and has never been carried out in Indonesia despite a few studies having been conducted in other countries. This biomarker is also easy to use and at a more affordable cost, so we are interested in analyzing the association of serum sRAGE on COVID-19 severity.

Methods

Participants

Participants in this study were COVID-19 patients diagnosed with real-time polymerase chain reaction (PCR) [5]. Participants' inclusion criteria included patients diagnosed with COVID-19 and aged >21 years. Participants' exclusion criteria included patients with a history of respiratory tract infection, myocardia infarct, cancer, and cerebral vascular attack. 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.

Study Design

This study used a cross-sectional design with a consecutive sampling method. It was carried out from May 2020 – October 2020. This study collected participant characteristics, serum sRAGE, and COVID-19 severity. This study reported the data based on the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 guideline [11]. The number of participants in this study was 145 participants that were divided into 2 groups (non-severe = 47 and severe = 98). The non-severe group consisted of participants identified as having COVID-19 in the mild and moderate category, while the severe group consisted of participants identified as having COVID-19 in the severe and critical categories [5].

Ethical Approval

We have conducted an ethical approval based 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).

Assessment of COVID-19 Severity

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 categories). Mild is a symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate include 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). Severe shows 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). Critical when patients have 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) [5].

sRAGE serum examination

The sRAGE is soluble forms in the transmembrane domain of RAGE which the serum levels of sRAGE are determined using a specific sandwich human ELISA kit BioAssay (MyBioSource Inc, San Diego, USA). The sRAGE measurement is in the range of 0.31 –

2.00 ng/mL. These results were obtained from taking 5 cc venous blood samples [12].

Statistical analysis

The analysis in this study used descriptive analysis and bivariate analysis. The descriptive analysis includes a descriptive presentation of the results using a distribution table, mean, median, standard deviation, maximum value, and minimum value. The analysis was conducted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA). Participants' characteristic data were analyzed using the chi-square test or Fisher's exact test. Meanwhile, the data from this study were first tested for normality using the Kolmogorov-Smirnov test. Analysis of the association of sRAGE serum with COVID-19 severity using the independence t-test or Mann Whitney test. The comparison between the two variables is significant if $p < 0.05$. In addition, Spearman's rank test was used to analyze the association between two variables.

Results

Characteristics of Participants

The demographic characteristics of participants included age and gender. The average age of participants was 50.54 ± 12.70 years (non-severe group = 49.11 ± 12.44 years and severe group = 51.23 ± 12.83 years). The median age of participants was 52.00 (43.00 – 59.00) years of which the youngest participant was 22.00 years old and the oldest participant was 80.00 years old. Most participants were in the age range of 35.00 – 55.00 years, consisting of 25 participants (53.2%) in the non-severe group and 51 participants (52.0%; $p = 0.705$) in the severe group. Most participants were male (90 participants; 62.1%), consisting of 25 participants (53.2%) in non-severe group and 65 participants in severe group (66.3%; OR = 0.577; $p = 0.179$; Table 1).

There were several clinical symptoms appeared, including shortness of breath in 122 participants (84.1%; 63.8% vs 93.9%; OR = 8,689; p <0.001), fever in 61 participants (42.1%; 46.8% vs 39.8%; OR = 0.751; p = 0.535), cough in 70 participants (70%; 59.6% vs. 42.9%; OR = 0.509; p = 0.088), painful swallowing in 4 participants (2.8%; 2.1% vs. 3.1%; OR = 1.453; p = 1,000), and diarrhoea in 7 participants (4.8%; 6.4% vs. 4.1%; OR = 0.624; p = 0.682). Based on the outcome of the COVID-19 treatment, most of non-severe participants recovered as many as 41 participants (87.2%) and most of severe participants were declared dead as many as 51 participants (52%; p <0.001). Overall, 88 participants (60.7%) were recovered. Several participants were declared to have comorbidities, including hypertension as many as 41 participants (28.3%; 27.7% vs 28.6%; OR = 1,046; p = 1,000), diabetes as many as 66 participants (45.5%; 42.6% vs 46.9%; OR = 1,194; p = 0.750), and obesity as many as 35 participants (24.1%; 19.1% vs. 26.5%; OR = 1.525; p = 0.444; Table 1).

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity

The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL), lymphocytes (13.55 ± 8.48%), neutrophils (78.91 ± 10.50%), procalcitonin (0.92 ± 3.22 ng/mL), CRP (8.59 ± 7.62 mg/ L), D-dimer (4,360.29 ± 7,797.81 ng/mL), and fibrinogen (474.58 ± 168.90 mg/dL). The average value of serum sRAGE was 1.48 ± 0.98 ng/mL, with a median value of 1.07 (0.85 – 1.84) ng/mL. The lowest and highest value of participants' serum sRAGE was 0.44 ng/mL and 5.14 ng/mL, respectively. The results of the COVID-19 severity measurement were divided into 4: mild as many as 2 participants (1.4%), moderate as many as 45 participants (31.0%), severe as many as 96 participants (66.2%), and critical as many as 2 participants (1.4%). Meanwhile, in this study,

COVID-19 severity was divided into 2 groups, namely the non-severe group with 47 participants (32.88%) and the severe group with 98 participants (68.53%).

There was a significant difference in blood component in the non-severe group and the severe group as follows: leukocyte value was 8.005.00 (6.157.50 – 9.687.50) vs 9.840.00 (7.420.00 – 12.830.00/µL; z = 2.431; p = 0.015), lymphocyte was 14.40 (8.83 – 21.65) vs 10.20 (6.60 – 16.80%; z = 2.256; p = 0.024), neutrophils was 77.40 (68.90 – 83.28) vs. 82.60 (76.00 – 87.10%; z = 2,464; p = 0.014), procalcitonin was 0.11 (0.07 – 0.22) vs 0.27 (0.13 – 0.46 ng/mL; z = 3.323; p = 0.001), CRP was 4.65 (0.80 – 11.35) vs. 8.70 (2.30 – 13.60 mg/L; z = 2.114; p = 0.034), and D-dimer was 810.00 (535.00 – 2,430.00) vs. 1,460.00 (740.00 – 4,025 ng/mL; z = 2.186; p = 0.029). Meanwhile, there was no significant difference in the levels of fibrinogen between participants in the two groups (465.50 ± 176.04 vs. 480.06 ± 165.92 mg/dL; t = 0.383; p = 0.703; Table 2).

There was a significant difference between serum sRAGE in the non-severe group and the severe group (0.78 (0.63 – 1.00) vs. 1.47 (0.97 – 2.25 ng/mL; r = 7.154; p <0.001; Table 2). There was a significant relationship between serum sRAGE and COVID-19 severity (r = 0.598; p <0.001). The cut-off value for serum sRAGE between the severe and non-severe group was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5%, OR of 8.077 and AUC 0.868 CI 95% (Figure 1).

Discussion

This study assessed serum sRAGE based on the severity of COVID-19. The results of this study are consistent with previous studies that examined sRAGE as a biomarker for COVID-19. A study examined the association of sRAGE with severity and as an indicator of mechanical ventilation requirements, ARDS, and mortality in COVID-19 patients. The results showed an increase in serum sRAGE concentrations in COVID-19 patients based on

severity [13]. These results are consistent with another study which stated a significant increase in serum sRAGE of ARDS patients admitted to non-isolated ICUs [14].

There is a significant relationship between serum sRAGE and COVID-19 severity. The serum sRAGE values in the severe group show a significant difference from serum sRAGE values in the non-severe group. The results are consistent with previous studies that showed an increase in serum sRAGE values in COVID-19 patients with a degree of severity. Increased sRAGE values can also help predict respiratory disorders that require mechanical ventilation and the mortality rate of COVID-19 patients [13]. Increased serum sRAGE is commonly found in ARDS patients admitted to the ICU [15]. As many as 20% of COVID-19 patients progress to the third phase called the involvement of the respiratory tract and progression to ARDS [16].

Increased serum sRAGE values can occur due to a viral infection process that will trigger an immune response, namely the innate immune system. Pattern-recognition receptors (PRR) recognize pathogen-associated molecular patterns (PAMPs) involving toll-like receptors (TLR) that detect components of infection and signaling tissue damage, one of which is HMGB1. Then it continues to the process of indirect lung tissue damage, namely damage-associated molecular patterns (DAMPs) that involve RAGE, NLR, TLR, and CLR which can exacerbate the occurrence of tissue damage that has occurred previously. The process of interaction of sRAGE with its ligand becomes more frequent due to an increase in HMGB1 that result in the increased inflammatory response in the form of IL-1 and TNF-Alpha activation [17, 18].

Other tissue damage processes can also occur when SARS-CoV2 invades AT2 cells located in the periphery and subpleural so that the patient begins to feel hypoxia. SARS-CoV2 replicates in AT2 lead to cell damage and death. Dead AT2 cells release toxins and damage surrounding cells. Infected cells send signals that are detected by the immune system

which then releases cytokines such as IL-1, IL-6, and TNF-α. These cytokine release aims to kill the virus, but it also causes damage to lung cells, namely diffuse alveolar damage, formation of hyaline membranes, and multinuclear giant cells. Abnormal wound healing leads to fibrosis [16, 19].

This study, however, has limitations, including the need for a future study that compares healthy individuals and pneumonia patients without COVID-19.

Conclusion

sRAGE is a biomarker that can be used to determine COVID-19 severity. The patients' COVID-19 severity in this study is categorized into 2, namely non-severe and severe. Based on blood component analysis, there are significant differences between the non-severe and severe groups. The differences consist of leukocytes, lymphocytes, neutrophils, procalcitonin, CRP, and D-dimer. The sRAGE values in the two groups also show a significant difference. In addition, there is a significant relationship between serum sRAGE and COVID-19 severity.

Conflict of interest

The authors declare they have no conflict of interest.

Funding

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Acknowledgment

We would like to thank the COVID-19 patients and Guardian. We would also thank Dr. Soetomo General Academic Hospital as the place of our research, and our editor “Fis Citra Ariyanto”.

References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020;109:102433. doi: 10.1016/j.jaut.2020.102433.
2. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. Journal of infection and public health. 2020;13(5):667-73. doi: 10.1016/j.jiph.2020.03.019.
3. Djalante R, Lassa J, Setiamarga D, Sudjatma A, Indrawan M, Haryanto B, et al. Review and analysis of current responses to COVID-19 in Indonesia: Period of January to March 2020. Progress in disaster science. 2020;6:100091. doi: 10.1016/j.pdisas.2020.100091.
4. Son KB, Lee TJ, Hwang SS. Disease severity classification and COVID-19 outcomes, Republic of Korea. Bulletin of the World Health Organization. 2021;99(1):62-6. doi: 10.2471/blt.20.257758.
5. Suryananda TD, Yudhawati R. Association of serum KL-6 levels on COVID-19 severity: A cross-sectional study design with purposive sampling. Annals of medicine and surgery (2012). 2021;69:102673. doi: 10.1016/j.amsu.2021.102673.
6. Seligman R, Ramos-Lima LF, Oliveira Vdo A, Sanvicente C, Pacheco EF, Dalla Rosa K. Biomarkers in community-acquired pneumonia: a state-of-the-art review. Clinics (Sao Paulo, Brazil). 2012;67(11):1321-5. doi: 10.6061/clinics/2012(11)17.
7. Narvaez-Rivera RM, Rendon A, Salinas-Carmona MC, Rosas-Taraco AG. Soluble RAGE as a severity marker in community acquired pneumonia associated sepsis. BMC infectious diseases. 2012;12:15. doi: 10.1186/1471-2334-12-15.
8. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. Journal of translational medicine. 2009;7:17. doi: 10.1186/1479-5876-7-17.
9. Chavakis T, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. Microbes and infection. 2004;6(13):1219-25. doi: 10.1016/j.micinf.2004.08.004.
10. Clynes R, Moser B, Yan SF, Ramasamy R, Herold K, Schmidt AM. Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. Current molecular medicine. 2007;7(8):743-51. doi: 10.2174/156652407783220714.
11. Agha R, Abdall-Razak A, Crossley E, Dowlut N, Iosifidis C, Mathew G. STROCSS 2019 Guideline: Strengthening the reporting of cohort studies in surgery. International journal of surgery (London, England). 2019;72:156-65. doi: 10.1016/j.ijsu.2019.11.002.
12. García-Salido A, Melen G, Gómez-Piña V, Oñoro-Otero G, Serrano-González A, Casado-Flores J, et al. Circulating soluble RAGE and cell surface RAGE on peripheral blood mononuclear cells in healthy children. Journal of pediatric endocrinology & metabolism : JPEM. 2018;31(6):649-54. doi: 10.1515/jpem-2017-0512.
13. Lim A, Radujkovic A, Weigand MA, Merle U. Soluble receptor for advanced glycation end products (sRAGE) as a biomarker of COVID-19 disease severity and indicator of the need for mechanical ventilation, ARDS and mortality. Annals of intensive care. 2021;11(1):50. doi: 10.1186/s13613-021-00836-2.
14. Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure JS, et al. Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study. Scientific reports. 2018;8(1):2603. doi: 10.1038/s41598-018-20994-x.

15. van Zoelen MA, Schouten M, de Vos AF, Florquin S, Meijers JC, Nawroth PP, et al. The receptor for advanced glycation end products impairs host defense in pneumococcal pneumonia. Journal of immunology (Baltimore, Md : 1950). 2009;182(7):4349-56. doi: 10.4049/jimmunol.0801199.
16. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. The European respiratory journal. 2020;55(4). doi: 10.1183/13993003.00607-2020.
17. Mondrinos MJ, Kennedy PA, Lyons M, Deutschman CS, Kilpatrick LE. Protein kinase C and acute respiratory distress syndrome. Shock (Augusta, Ga). 2013;39(6):467-79. doi: 10.1097/SHK.0b013e318294f85a.
18. Horiguchi H, Loftus TJ, Hawkins RB, Raymond SL, Stortz JA, Hollen MK, et al. Innate Immunity in the Persistent Inflammation, Immunosuppression, and Catabolism Syndrome and Its Implications for Therapy. Frontiers in immunology. 2018;9:595. doi: 10.3389/fimmu.2018.00595.
19. Carcaterra M, Caruso C. Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: A physio-pathological theory. Medical hypotheses. 2021;146:110412. doi: 10.1016/j.mehy.2020.110412.

Figure Legend

Figure 1. Cut-Off Serum sRAGE Level Based on Severe and Non-Severe Groups of COVID-19 Patients

Table and Legend

Table 1. Characteristics of Participants

Characteristics	COVID-19 Severity		*p*
	Non-severe	Severe	
Age (years)			
21-35	8 (17.0)	12 (12.2)	0.705
35-55	25 (53.2)	51 (52.0)	
55-65	8 (17.0)	24 (24.5)	
>65	6 (12.8)	11 (11.2)	
Gender			
Male	25 (53.2)	65 (66.3)	0.179
Female	22 (46.8)	33 (33.7)	
Clinical symptoms			
Shortness of breath	30 (63.8)	92 (93.9)	<0.001**
Fever	22 (46.8)	39 (39.8)	0.535
Cough	28 (59.6)	42 (42.9)	0.088
Painful swallowing	1 (2.1)	3 (3.1)	1.000
Diarrhea	3 (6.4)	4 (4.1)	0.682
Outcome			
Recovered	41 (87.2)	47 (48.0)	<0.001**
Died	6 (12.8)	51 (52.0)	
Comorbid			
Hypertension	13 (27.7)	28 (28.6)	1.000
Diabetes	20 (42.6)	46 (46.9)	0.750
Obesity	9 (19.1)	26 (26.5)	0.444

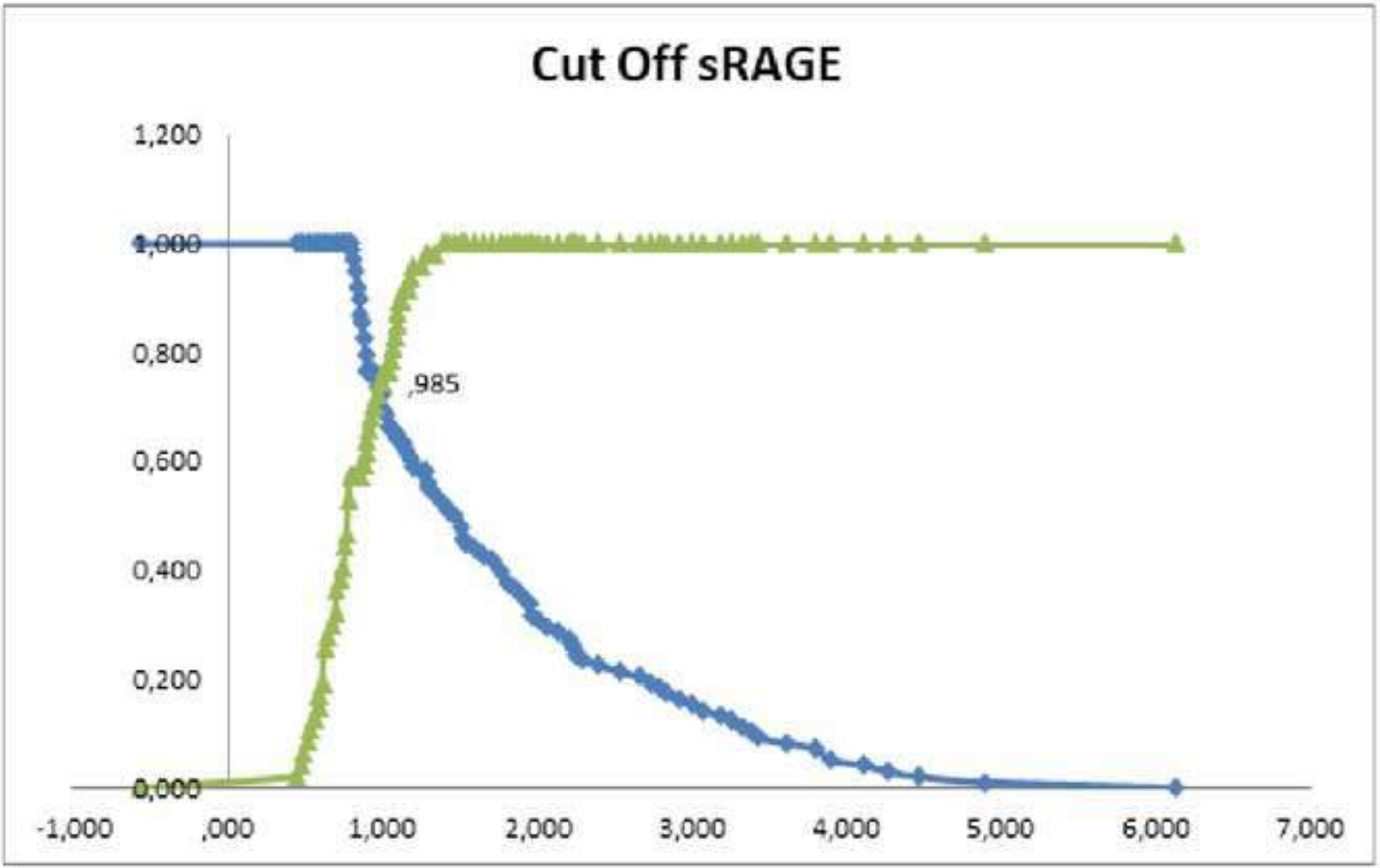
Note: *significant <0.05; **significant <0.01

Table 2. Comparison of Blood Component Based on COVID-19 Severity

Blood Analysis	COVID-19 Severity		*p*
	Non-severe	Severe	
Leukocytes (n = 139)	8,622.10 ± 4,204.47	10,526.86 ± 5,185.37	0.015*
Lymphocyte (n = 139)	15.50 ± 8.22	12.58 ± 8.49	0.024*
Neutrophile (n = 139)	76.06 ± 10.36	80.32 ± 10.34	0.014*
Procalcitonin (n = 143)	1.01 ± 4.67	0.88 ± 2.22	0.001*
CRP (n = 90)	6.52 ± 6.71	9.53 ± 7.87	0.034*
Fibrinogen (n = 85)	465.50 ± 176.04	480.06 ± 165.92	0.703
D-Dimer (n = 139)	2,790.64 ± 5,558.74	5,162.17 ± 8,641.11	0.029*
s-RAGE (n = 143)	0.82 ± 0.23	1.80 ± 1.04	<0.001**

Note: CRP = C-reactive protein; s-RAGE = soluble receptor for advanced glycation end products; *significant <0.05; **significant <0.001

Figure 1



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
INTRODUCTION		
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
METHODS		
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)	4
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	4
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	3
5d	Subgroup Analysis: the following areas are described comprehensively - Planned subgroup analyses - Methods used to examine subgroups and their interactions	3
6a	Participants: the following areas are described comprehensively - Eligibility criteria - Recruitment sources - Length and methods of follow-up	3
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
INTERVENTION AND CONSIDERATIONS		
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)	4-5
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	4-5
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
RESULTS		
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)	6
10b	Participant Comparison: the following areas are described comprehensively - Table comparing demographics included - Differences, with statistical relevance - Any group matching, with methods	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	6-7
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	6-7
11b	Tolerance: the following areas are described comprehensively - Assessment of tolerance - Loss to follow up, with reasons (percentage and fraction) - Cross-over with explanation	6-7
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	7

	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	7
DISCUSSION		
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	7-9
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
CONCLUSION		
16	Conclusions: - Key conclusions are summarised - Key directions for future research are summarised	10
DECLARATIONS		
17a	Conflicts of interest - Conflicts of interest, if any, are described	10
17b	Funding - Sources of funding (e.g. grant details), if any, are clearly stated	10

Annals of Medicine and Surgery

Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study

--Manuscript Draft--

Manuscript Number:	AMSU-D-21-01406R1
Article Type:	Cross-sectional Study
Keywords:	serum sRAGE; COVID-19 severity; Infectious disease
Corresponding Author:	Resti Yudhawati Universitas Airlangga Fakultas Kedokteran Surabaya, East Java INDONESIA
First Author:	Gusti Noor Ramadany Saputra
Order of Authors:	Gusti Noor Ramadany Saputra
	Resti Yudhawati
	Munawaroh Fitriah
Abstract:	Background Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble Receptor for Advanced Glycation End-Products (sRAGE) is a biomarker that can be used to identify early lung damage. Objective Analyzing the association of serum sRAGE with COVID-19 severity. Methods This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020 – October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum with COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence t-test, Mann Withney test, and Spearman's rank test with p-value <0.05. Results The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL; z = 2.431; p = 0.015), lymphocytes (13.55 ± 8.48%; z = 2.256; p = 0.024), neutrophils (78.91 ± 10.50%; z = 2.464; p = 0.014), procalcitonin (0.92 ± 3.22 ng/mL; z = 3.323; p = 0.001), CRP (8.59 ± 7.62 mg/L; z = 2.114; p = 0.034), D-dimer (4,360.29 ± 7,797.81 ng/mL; z = 2.186; p = 0.029), and fibrinogen (474.58 ± 168.90 mg/dL; t = 0.383; p = 0.703). There was a significant difference in serum sRAGE values in the non-severe group (0.78 [0.63 – 1.00] ng/mL) and severe group (1.47 [0.97 – 2.25] ng/mL; r = 7.154; p <0.001). There was a significant association between serum sRAGE and COVID-19 severity (r = 0.598; p <0.001). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95% CI. Conclusion There is a significant association between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.
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 according to reviewer comment.

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.

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: Health Research Ethics Coommitee in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

2. Unique Identifying number or registration ID: 1954/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

To, Covering Letter

The Editor

Sub: Submission of Manuscript for publication

Dear sir,

We intend to publish an article entitled **"Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 Severity: A cross-sectional study"** 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 the association of sRAGE serum on COVID-19 severity. This is significant because the process of interaction of sRAGE with its ligand becomes more due to an increase in HMGB1 which in the end increases the inflammatory response and can make tissue damage. The paper should be of interest to readers in the areas of respiratory and infectious disease.

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 for the research, authorship, and/or publication of this article.

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

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

Thanking you,

Yours' sincerely,

Resti Yudhawati
Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Padjadjaran – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: resti.yudhawati2021@gmail.com
Phone: +6231-5501656
Orcid ID: 0000-0002-0808-8524

Response to Reviewer

Annals of Medicine and Surgery

Title: Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study

Dear Mrs Yudhawati,

Thank you for your recent submission to Annals of Medicine and Surgery.

Registration of Research mandatory

Apologies but research registration is not completed as requested, there is no url provided and no confirmation that it is a publicly accessible data base. Please see the requirements 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

*Researchregistry.com - for all human studies - charge

*ISRCTN.com - for all human studies – charge

*Prospero - for systematic reviews - free

*There are many national registries approved by the UN that can be found, please refer to the Guide for Authors.

Elsevier does not support or endorse any registry.

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 a hyperlink to the registration site to the Author Form.

Author response: we have added in our manuscript and author form. We have conducted a research register based on the Declaration of Helsinki at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia with certificate number "1954/KEPK/IV/2020".

Provenance and peer review

Please add the following statement to your manuscript above the references.

Provenance and peer review

Not commissioned, externally peer reviewed.

Author response: we have added in our manuscript.

Highlights

1. Serum sRAGE can be used to identify COVID-19 severity.
2. The level of serum sRAGE in each COVID-19 patient is different.
3. The blood components of each COVID-19 severity are different.

Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 Severity: A cross-sectional study

Running head: sRAGE on COVID-19 Severity

Gusti Noor Ramadany Saputra[1], Resti Yudhawati[1]*, Munawaroh Fitriah[2]

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

[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

***Corresponding author:** Resti Yudhawati

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia

Mail: restiyudhawati2021@gmail.com

Phone: +6231-5501656

Orcid ID: 0000-0002-0808-8524

Association of soluble receptor for advanced glycation end-products (sRAGE) serum ~~with~~ on COVID-19 Severity: A~~n Observational~~ cross-sectional Study

Abstract

Background: Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble Receptor for Advanced Glycation End-Products (sRAGE) is a biomarker that can be used to identify early lung damage. **Objective**: Analyzing the association of serum sRAGE with COVID-19 severity. **Methods**: This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020 – October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum with COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence t-test, Mann Withney test, and Spearman's rank test with *p*-value <0.05. **Results**: The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL; $z = 2.431$; $p = 0.015$), lymphocytes (13.55 ± 8.48%; $z = 2.256$; $p = 0.024$), neutrophils (78.91 ± 10.50%; $z = 2.464$; $p = 0.014$), procalcitonin (0.92 ± 3.22 ng/mL; $z = 3.323$; $p = 0.001$), CRP (8.59 ± 7.62 mg/L; $z = 2.114$; $p = 0.034$), D-dimer (4,360.29 ± 7,797.81 ng/mL; $z = 2.186$; $p = 0.029$), and fibrinogen (474.58 ± 168.90 mg/dL; $t = 0.383$; $p = 0.703$). There was a significant difference in serum sRAGE values in the non-severe group (0.78 [0.63 – 1.00] ng/mL) and severe group (1.47 [0.97 – 2.25] ng/mL; $r = 7.154$; $p < 0.001$). There was a significant association between serum sRAGE and COVID-19 severity ($r = 0.598$; $p < 0.001$). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95% CI. **Conclusion**:

There is a significant association between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.

Keywords: serum sRAGE, COVID-19 severity, infectious disease

Introduction

Coronavirus disease 2019 or better known as COVID-19 caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) became a worldwide pandemic at the end of 2019 with various systemic complaints but was more dominant in respiratory disorders. The worldwide mortality rate was 2.1% by February 12, 2020 [1]. The February 2020 data by Johns Hopkins University's Center for Systems Science and Engineering (CSSE) showed a total case of more than 60,331 patients, with a total death of more than 1,369 patients and an improvement of more than 6,061 patients [2]. On December 27, 2020, the total number of worldwide cases was more than 79 million, including 1,751,311 deaths. Incidents in Indonesia were 706,837 confirmed cases of COVID-19 and 20,994 cases of death [3].

The severity of COVID-19 according to WHO is divided into mild, moderate, severe, and critical [4, 5]. The most frequently encountered clinical symptoms are pneumonia symptoms. Biomarkers are frequently used to determine the severity of pneumonia such as procalcitonin, C-reactive protein (CRP), copeptin, pro-ANP (atrial natriuretic peptide), adrenomedullin, cortisol, and D-dimers [6]. These biomarkers are good in determining infection in pneumonia but have not been able to detect early tissue damage, as patients often go to the hospital with a more severe condition. Recent studies in immunology have examined soluble RAGE (sRAGE) as a biomarker of the severity of community pneumonia and can detect tissue damage in ARDS early [7].

Pathophysiology occurred in COVID-19 includes the inflammatory process. One of the inflammatory processes during pneumonia is characterized by an increase in Receptors for Advanced Glycation End-Products (RAGE). RAGE is one of the non-enzymatic receptors of Advanced Glycation End-Products (AGEs) which has a multi-ligand receptor, namely a V-type domain, two C-type domains, a transmembrane domain, and a cytoplasmic tail. RAGE has several ligands including AGEs, S100/calgranulins, and HMGB I which are present in different vascular cells such as endothelial cells, neuronal cells, smooth muscle cells, or inflammatory cells (monocytes). HMGB I is one of the RAGE ligands that play a role in the occurrence of sepsis which can stimulate the formation of cytokines along with TLRs in the immune system cells (B cells) [8]. The interaction between RAGE and its ligands will cause the formation of Reactive Oxygen Species (ROS) which will activate NADPH oxidation. The process will mediate the formation of inflammatory cells. Trianta et al. stated two processes of RAGE interaction with its ligands that are related to the inflammatory process, namely its interaction with leukocytes and on endothelial cells, RAGE is an adhesive receptor and directly forms inflammatory cells. The accumulation of RAGE ligands is predicted to cause chronic cell stimulation and tissue damage [9, 10].

RAGE is expressed in the membrane-bound form (fl-RAGE or mRAGE) and the soluble form in the transmembrane domain. Soluble RAGE is produced by proteolytic cleavage of fl-RAGE and alternative splicing mRNA [7]. The administration of sRAGE in experimental animals can also interact with the RAGE ligand [10]. Based on these studies, the role of sRAGE becomes very important in determining COVID-19 diagnosis based on the severity quickly, so that effective and adequate treatment planning can be carried out early to reduce the morbidity and mortality of COVID-19 patients. In addition, the level of sRAGE in serum can detect early tissue damage which in turn can affect the severity of COVID-19 patients as common biomarkers have not been able to detect the process of tissue damage

early. Research on sRAGE in the serum of COVID-19 patients is still limited and has never been carried out in Indonesia despite a few studies having been conducted in other countries. This biomarker is also easy to use and at a more affordable cost, so we are interested in analyzing the association of serum sRAGE on COVID-19 severity.

Methods

Participants

Participants in this study were COVID-19 patients diagnosed with real-time polymerase chain reaction (PCR) [5]. Participants' inclusion criteria included patients diagnosed with COVID-19 and aged >21 years. Participants' exclusion criteria included patients with a history of respiratory tract infection, myocardia infarct, cancer, and cerebral vascular attack. 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.

Study Design

This study used a cross-sectional design with a consecutive sampling method. It was carried out from May 2020 – October 2020. This study collected participant characteristics, serum sRAGE, and COVID-19 severity. This study reported the data based on the strengthening the reporting of cohort studies in surgery (STROCSS) 20219 guideline [11] [11]. The number of participants in this study was 145 participants that were divided into 2 groups (non-severe = 47 and severe = 98). The non-severe group consisted of participants identified as having COVID-19 in the mild and moderate category, while the severe group consisted of participants identified as having COVID-19 in the severe and critical categories [5].

Ethical Approval

We have conducted an ethical approval based on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Hospital.

Assessment of COVID-19 Severity

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 categories). Mild is a symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate include 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). Severe shows 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). Critical when patients have 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) [5].

sRAGE serum examination

The sRAGE is soluble forms in the transmembrane domain of RAGE which the serum levels of sRAGE are determined using a specific sandwich human ELISA kit BioAssay (MyBioSource Inc, San Diego, USA). The sRAGE measurement is in the range of 0.31 –

2.00 ng/mL. These results were obtained from taking 5 cc venous blood samples [12].

Statistical analysis

The analysis in this study used descriptive analysis and bivariate analysis. The descriptive analysis includes a descriptive presentation of the results using a distribution table, mean, median, standard deviation, maximum value, and minimum value. The analysis was conducted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA). Participants' characteristic data were analyzed using the chi-square test or Fisher's exact test. Meanwhile, the data from this study were first tested for normality using the Kolmogorov-Smirnov test. Analysis of the association of sRAGE serum with COVID-19 severity using the independence t-test or Mann Whitney test. The comparison between the two variables is significant if $p < 0.05$. In addition, Spearman's rank test was used to analyze the association between two variables.

Results

Characteristics of Participants

The demographic characteristics of participants included age and gender. The average age of participants was 50.54 ± 12.70 years (non-severe group = 49.11 ± 12.44 years and severe group = 51.23 ± 12.83 years). The median age of participants was 52.00 (43.00 – 59.00) years of which the youngest participant was 22.00 years old and the oldest participant was 80.00 years old. Most participants were in the age range of 35.00 – 55.00 years, consisting of 25 participants (53.2%) in the non-severe group and 51 participants (52.0%; p = 0.705) in the severe group. Most participants were male (90 participants; 62.1%), consisting of 25 participants (53.2%) in non-severe group and 65 participants in severe group (66.3%; OR = 0.577; *p* = 0.179; Table 1).

There were several clinical symptoms appeared, including shortness of breath in 122 participants (84.1%; 63.8% vs 93.9%; OR = 8,689; p <0.001), fever in 61 participants (42.1%; 46.8% vs 39.8%; OR = 0.751; p = 0.535), cough in 70 participants (70%; 59.6% vs. 42.9%; OR = 0.509; p = 0.088), painful swallowing in 4 participants (2.8%; 2.1% vs. 3.1%; OR = 1.453; p = 1,000), and diarrhoea in 7 participants (4.8%; 6.4% vs. 4.1%; OR = 0.624; p = 0.682). Based on the outcome of the COVID-19 treatment, most of non-severe participants recovered as many as 41 participants (87.2%) and most of severe participants were declared dead as many as 51 participants (52%; p <0.001). Overall, 88 participants (60.7%) were recovered. Several participants were declared to have comorbidities, including hypertension as many as 41 participants (28.3%; 27.7% vs 28.6%; OR = 1,046; p = 1,000), diabetes as many as 66 participants (45.5%; 42.6% vs 46.9%; OR = 1,194; p = 0.750), and obesity as many as 35 participants (24.1%; 19.1% vs. 26.5%; OR = 1.525; p = 0.444; Table 1).

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity

The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL), lymphocytes (13.55 ± 8.48%), neutrophils (78.91 ± 10.50%), procalcitonin (0.92 ± 3.22 ng/mL), CRP (8.59 ± 7.62 mg/ L), D-dimer (4,360.29 ± 7,797.81 ng/mL), and fibrinogen (474.58 ± 168.90 mg/dL). The average value of serum sRAGE was 1.48 ± 0.98 ng/mL, with a median value of 1.07 (0.85 – 1.84) ng/mL. The lowest and highest value of participants' serum sRAGE was 0.44 ng/mL and 5.14 ng/mL, respectively. The results of the COVID-19 severity measurement were divided into 4: mild as many as 2 participants (1.4%), moderate as many as 45 participants (31.0%), severe as many as 96 participants (66.2%), and critical as many as 2 participants (1.4%). Meanwhile, in this study,

COVID-19 severity was divided into 2 groups, namely the non-severe group with 47 participants (32.88%) and the severe group with 98 participants (68.53%).

There was a significant difference in blood component in the non-severe group and the severe group as follows: leukocyte value was 8.005.00 (6.157.50 – 9.687.50) vs 9.840.00 (7.420.00 – 12.830.00/µL; z = 2.431; p = 0.015), lymphocyte was 14.40 (8.83 – 21.65) vs 10.20 (6.60 – 16.80%; z = 2.256; p = 0.024), neutrophils was 77.40 (68.90 – 83.28) vs. 82.60 (76.00 – 87.10%; z = 2,464; p = 0.014), procalcitonin was 0.11 (0.07 – 0.22) vs 0.27 (0.13 – 0.46 ng/mL; z = 3.323; p = 0.001), CRP was 4.65 (0.80 – 11.35) vs. 8.70 (2.30 – 13.60 mg/L; z = 2.114; p = 0.034), and D-dimer was 810.00 (535.00 – 2,430.00) vs. 1,460.00 (740.00 – 4,025 ng/mL; z = 2.186; p = 0.029). Meanwhile, there was no significant difference in the levels of fibrinogen between participants in the two groups (465.50 ± 176.04 vs. 480.06 ± 165.92 mg/dL; t = 0.383; p = 0.703; Table 2).

There was a significant difference between serum sRAGE in the non-severe group and the severe group (0.78 (0.63 – 1.00) vs. 1.47 (0.97 – 2.25 ng/mL; r = 7.154; p <0.001; Table 2). There was a significant association between serum sRAGE and COVID-19 severity (r = 0.598; p <0.001). The cut-off value for serum sRAGE between the severe and non-severe group was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5%, OR of 8.077 and AUC 0.868 CI 95% (Figure 1).

Discussion

This study assessed serum sRAGE based on the severity of COVID-19. The results of this study are consistent with previous studies that examined sRAGE as a biomarker for COVID-19. A study examined the association of sRAGE with severity and as an indicator of mechanical ventilation requirements, ARDS, and mortality in COVID-19 patients. The results showed an increase in serum sRAGE concentrations in COVID-19 patients based on

severity [13]. These results are consistent with another study which stated a significant increase in serum sRAGE of ARDS patients admitted to non-isolated ICUs [14].

There is a significant association between serum sRAGE and COVID-19 severity. The serum sRAGE values in the severe group show a significant difference from serum sRAGE values in the non-severe group. The results are consistent with previous studies that showed an increase in serum sRAGE values in COVID-19 patients with a degree of severity. Increased sRAGE values can also help predict respiratory disorders that require mechanical ventilation and the mortality rate of COVID-19 patients [13]. Increased serum sRAGE is commonly found in ARDS patients admitted to the ICU [15]. As many as 20% of COVID-19 patients progress to the third phase called the involvement of the respiratory tract and progression to ARDS [16].

Increased serum sRAGE values can occur due to a viral infection process that will trigger an immune response, namely the innate immune system. Pattern-recognition receptors (PRR) recognize pathogen-associated molecular patterns (PAMPs) involving toll-like receptors (TLR) that detect components of infection and signaling tissue damage, one of which is HMGB1. Then it continues to the process of indirect lung tissue damage, namely damage-associated molecular patterns (DAMPs) that involve RAGE, NLR, TLR, and CLR which can exacerbate the occurrence of tissue damage that has occurred previously. The process of interaction of sRAGE with its ligand becomes more frequent due to an increase in HMGB1 that result in the increased inflammatory response in the form of IL-1 and TNF-Alpha activation [17, 18].

Other tissue damage processes can also occur when SARS-CoV2 invades AT2 cells located in the periphery and subpleural so that the patient begins to feel hypoxia. SARS-CoV2 replicates in AT2 lead to cell damage and death. Dead AT2 cells release toxins and damage surrounding cells. Infected cells send signals that are detected by the immune system

which then releases cytokines such as IL-1, IL-6, and TNF-α. These cytokine release aims to kill the virus, but it also causes damage to lung cells, namely diffuse alveolar damage, formation of hyaline membranes, and multinuclear giant cells. Abnormal wound healing leads to fibrosis [16, 19].

This study, however, has limitations, including the need for a future study that compares healthy individuals and pneumonia patients without COVID-19.

Conclusion

sRAGE is a biomarker that can be used to determine COVID-19 severity. The patients' COVID-19 severity in this study is categorized into 2, namely non-severe and severe. Based on blood component analysis, there are significant differences between the non-severe and severe groups. The differences consist of leukocytes, lymphocytes, neutrophils, procalcitonin, CRP, and D-dimer. The sRAGE values in the two groups also show a significant difference. In addition, there is a significant relationship between serum sRAGE and COVID-19 severity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We would like to thank the COVID-19 patients and Guardian. We would also thank Dr. Soetomo General Academic Hospital as the place of our research, and our editor "Fis Citra Ariyanto".

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 (1954/KEPK/IV/2020).

Funding

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Registration of research studies

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

Unique identifying number or registration ID: 1954/KEPK/IV/2020.

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

Guarantor

Resti Yudhawati.

Author 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.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020;109:102433. doi: 10.1016/j.jaut.2020.102433.
2. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. Journal of infection and public health. 2020;13(5):667-73. doi: 10.1016/j.jiph.2020.03.019.
3. Djalante R, Lassa J, Setiamarga D, Sudjatma A, Indrawan M, Haryanto B, et al. Review and analysis of current responses to COVID-19 in Indonesia: Period of January to March 2020. Progress in disaster science. 2020;6:100091. doi: 10.1016/j.pdisas.2020.100091.
4. Son KB, Lee TJ, Hwang SS. Disease severity classification and COVID-19 outcomes, Republic of Korea. Bulletin of the World Health Organization. 2021;99(1):62-6. doi: 10.2471/blt.20.257758.
5. Suryananda TD, Yudhawati R. Association of serum KL-6 levels on COVID-19 severity: A cross-sectional study design with purposive sampling. Annals of medicine and surgery (2012). 2021;69:102673. doi: 10.1016/j.amsu.2021.102673.
6. Seligman R, Ramos-Lima LF, Oliveira Vdo A, Sanvicente C, Pacheco EF, Dalla Rosa K. Biomarkers in community-acquired pneumonia: a state-of-the-art review. Clinics (Sao Paulo, Brazil). 2012;67(11):1321-5. doi: 10.6061/clinics/2012(11)17.
7. Narvaez-Rivera RM, Rendon A, Salinas-Carmona MC, Rosas-Taraco AG. Soluble RAGE as a severity marker in community acquired pneumonia associated sepsis. BMC infectious diseases. 2012;12:15. doi: 10.1186/1471-2334-12-15.
8. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. Journal of translational medicine. 2009;7:17. doi: 10.1186/1479-5876-7-17.
9. Chavakis T, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. Microbes and infection. 2004;6(13):1219-25. doi: 10.1016/j.micinf.2004.08.004.
10. Clynes R, Moser B, Yan SF, Ramasamy R, Herold K, Schmidt AM. Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. Current molecular medicine. 2007;7(8):743-51. doi: 10.2174/156652407783220714.
11. Mathew G, Agha R. STROCSS 2021: Strengthening the reporting of cohort, cross-sectional and case-control studies in surgery. International journal of surgery (London, England). 2021;96:106165. doi: 10.1016/j.ijsu.2021.106165.
12. García-Salido A, Melen G, Gómez-Piña V, Oñoro-Otero G, Serrano-González A, Casado-Flores J, et al. Circulating soluble RAGE and cell surface RAGE on peripheral blood mononuclear cells in healthy children. Journal of pediatric endocrinology & metabolism : JPEM. 2018;31(6):649-54. doi: 10.1515/jpem-2017-0512.
13. Lim A, Radujkovic A, Weigand MA, Merle U. Soluble receptor for advanced glycation end products (sRAGE) as a biomarker of COVID-19 disease severity and indicator of the need for mechanical ventilation, ARDS and mortality. Annals of intensive care. 2021;11(1):50. doi: 10.1186/s13613-021-00836-2.
14. Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure JS, et al. Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study. Scientific reports. 2018;8(1):2603. doi: 10.1038/s41598-018-20994-x.
15. van Zoelen MA, Schouten M, de Vos AF, Florquin S, Meijers JC, Nawroth PP, et al. The receptor for advanced glycation end products impairs host defense in pneumococcal pneumonia. Journal of immunology (Baltimore, Md : 1950). 2009;182(7):4349-56. doi: 10.4049/jimmunol.0801199.

Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Indent: Left: 0", Hanging: 0.28"
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt
Formatted: Font: (Default) Times New Roman, 12 pt

16. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. The European respiratory journal. 2020;55(4). doi: 10.1183/13993003.00607-2020.
17. Mondrinos MJ, Kennedy PA, Lyons M, Deutschman CS, Kilpatrick LE. Protein kinase C and acute respiratory distress syndrome. Shock (Augusta, Ga). 2013;39(6):467-79. doi: 10.1097/SHK.0b013e318294f85a.
18. Horiguchi H, Loftus TJ, Hawkins RB, Raymond SL, Stortz JA, Hollen MK, et al. Innate Immunity in the Persistent Inflammation, Immunosuppression, and Catabolism Syndrome and Its Implications for Therapy. Frontiers in immunology. 2018;9:595. doi: 10.3389/fimmu.2018.00595.
19. Carcaterra M, Caruso C. Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: A physio-pathological theory. Medical hypotheses. 2021;146:110412. doi: 10.1016/j.mehy.2020.110412.

Formatted: Indent: Left: 0", Hanging: 0.28", Space After: 0 pt

Figure Legend

Figure 1. Cut-off Serum sRAGE level based on severe and non-severe groups of COVID-19 patients

Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 Severity: A cross-sectional Study

Abstract

Background: Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble Receptor for Advanced Glycation End-Products (sRAGE) is a biomarker that can be used to identify early lung damage. **Objective**: Analyzing the association of serum sRAGE with COVID-19 severity. **Methods**: This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020 – October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum with COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence t-test, Mann Withney test, and Spearman's rank test with *p*-value <0.05. **Results**: The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL; $z = 2.431$; $p = 0.015$), lymphocytes (13.55 ± 8.48%; $z = 2.256$; $p = 0.024$), neutrophils (78.91 ± 10.50%; $z = 2.464$; $p = 0.014$), procalcitonin (0.92 ± 3.22 ng/mL; $z = 3.323$; $p = 0.001$), CRP (8.59 ± 7.62 mg/L; $z = 2.114$; $p = 0.034$), D-dimer (4,360.29 ± 7,797.81 ng/mL; $z = 2.186$; $p = 0.029$), and fibrinogen (474.58 ± 168.90 mg/dL; $t = 0.383$; $p = 0.703$). There was a significant difference in serum sRAGE values in the non-severe group (0.78 [0.63 – 1.00] ng/mL) and severe group (1.47 [0.97 – 2.25] ng/mL; $r = 7.154$; $p <0.001$). There was a significant association between serum sRAGE and COVID-19 severity ($r = 0.598$; $p <0.001$). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95% CI. **Conclusion**:

There is a significant association between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.

Keywords: serum sRAGE, COVID-19 severity, infectious disease

Introduction

Coronavirus disease 2019 or better known as COVID-19 caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) became a worldwide pandemic at the end of 2019 with various systemic complaints but was more dominant in respiratory disorders. The worldwide mortality rate was 2.1% by February 12, 2020 [1]. The February 2020 data by Johns Hopkins University's Center for Systems Science and Engineering (CSSE) showed a total case of more than 60,331 patients, with a total death of more than 1,369 patients and an improvement of more than 6,061 patients [2]. On December 27, 2020, the total number of worldwide cases was more than 79 million, including 1,751,311 deaths. Incidents in Indonesia were 706,837 confirmed cases of COVID-19 and 20,994 cases of death [3].

The severity of COVID-19 according to WHO is divided into mild, moderate, severe, and critical [4, 5]. The most frequently encountered clinical symptoms are pneumonia symptoms. Biomarkers are frequently used to determine the severity of pneumonia such as procalcitonin, C-reactive protein (CRP), copeptin, pro-ANP (atrial natriuretic peptide), adrenomedullin, cortisol, and D-dimers [6]. These biomarkers are good in determining infection in pneumonia but have not been able to detect early tissue damage, as patients often go to the hospital with a more severe condition. Recent studies in immunology have examined soluble RAGE (sRAGE) as a biomarker of the severity of community pneumonia and can detect tissue damage in ARDS early [7].

Pathophysiology occurred in COVID-19 includes the inflammatory process. One of the inflammatory processes during pneumonia is characterized by an increase in Receptors for Advanced Glycation End-Products (RAGE). RAGE is one of the non-enzymatic receptors of Advanced Glycation End-Products (AGEs) which has a multi-ligand receptor, namely a V-type domain, two C-type domains, a transmembrane domain, and a cytoplasmic tail. RAGE has several ligands including AGEs, S100/calgranulins, and HMGB I which are present in different vascular cells such as endothelial cells, neuronal cells, smooth muscle cells, or inflammatory cells (monocytes). HMGB I is one of the RAGE ligands that play a role in the occurrence of sepsis which can stimulate the formation of cytokines along with TLRs in the immune system cells (B cells) [8]. The interaction between RAGE and its ligands will cause the formation of Reactive Oxygen Species (ROS) which will activate NADPH oxidation. The process will mediate the formation of inflammatory cells. Trianta et al. stated two processes of RAGE interaction with its ligands that are related to the inflammatory process, namely its interaction with leukocytes and on endothelial cells, RAGE is an adhesive receptor and directly forms inflammatory cells. The accumulation of RAGE ligands is predicted to cause chronic cell stimulation and tissue damage [9, 10].

RAGE is expressed in the membrane-bound form (fl-RAGE or mRAGE) and the soluble form in the transmembrane domain. Soluble RAGE is produced by proteolytic cleavage of fl-RAGE and alternative splicing mRNA [7]. The administration of sRAGE in experimental animals can also interact with the RAGE ligand [10]. Based on these studies, the role of sRAGE becomes very important in determining COVID-19 diagnosis based on the severity quickly, so that effective and adequate treatment planning can be carried out early to reduce the morbidity and mortality of COVID-19 patients. In addition, the level of sRAGE in serum can detect early tissue damage which in turn can affect the severity of COVID-19 patients as common biomarkers have not been able to detect the process of tissue damage

early. Research on sRAGE in the serum of COVID-19 patients is still limited and has never been carried out in Indonesia despite a few studies having been conducted in other countries. This biomarker is also easy to use and at a more affordable cost, so we are interested in analyzing the association of serum sRAGE on COVID-19 severity.

Methods

Participants

Participants in this study were COVID-19 patients diagnosed with real-time polymerase chain reaction (PCR) [5]. Participants' inclusion criteria included patients diagnosed with COVID-19 and aged >21 years. Participants' exclusion criteria included patients with a history of respiratory tract infection, myocardia infarct, cancer, and cerebral vascular attack. 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.

Study Design

This study used a cross-sectional design with a consecutive sampling method. It was carried out from May 2020 – October 2020. This study collected participant characteristics, serum sRAGE, and COVID-19 severity. This study reported the data based on the strengthening the reporting of cohort studies in surgery (STROCSS) 2021 guideline [11]. The number of participants in this study was 145 participants that were divided into 2 groups (non-severe = 47 and severe = 98). The non-severe group consisted of participants identified as having COVID-19 in the mild and moderate category, while the severe group consisted of participants identified as having COVID-19 in the severe and critical categories [5].

Ethical Approval

We have conducted an ethical approval based on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Hospital.

Assessment of COVID-19 Severity

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 categories). Mild is a symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate include 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). Severe shows 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). Critical when patients have 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) [5].

sRAGE serum examination

The sRAGE is soluble forms in the transmembrane domain of RAGE which the serum levels of sRAGE are determined using a specific sandwich human ELISA kit BioAssay (MyBioSource Inc, San Diego, USA). The sRAGE measurement is in the range of 0.31 – 2.00 ng/mL. These results were obtained from taking 5 cc venous blood samples [12].

Statistical analysis

The analysis in this study used descriptive analysis and bivariate analysis. The descriptive analysis includes a descriptive presentation of the results using a distribution table, mean, median, standard deviation, maximum value, and minimum value. The analysis was conducted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA). Participants' characteristic data were analyzed using the chi-square test or Fisher's exact test. Meanwhile, the data from this study were first tested for normality using the Kolmogorov-Smirnov test. Analysis of the association of sRAGE serum with COVID-19 severity using the independence t-test or Mann Whitney test. The comparison between the two variables is significant if $p < 0.05$. In addition, Spearman's rank test was used to analyze the association between two variables.

Results

Characteristics of Participants

The demographic characteristics of participants included age and gender. The average age of participants was 50.54 ± 12.70 years (non-severe group = 49.11 ± 12.44 years and severe group = 51.23 ± 12.83 years). The median age of participants was 52.00 (43.00 – 59.00) years of which the youngest participant was 22.00 years old and the oldest participant was 80.00 years old. Most participants were in the age range of 35.00 – 55.00 years, consisting of 25 participants (53.2%) in the non-severe group and 51 participants (52.0%; p = 0.705) in the severe group. Most participants were male (90 participants; 62.1%), consisting of 25 participants (53.2%) in non-severe group and 65 participants in severe group (66.3%; OR = 0.577; $p = 0.179$; Table 1).

There were several clinical symptoms appeared, including shortness of breath in 122 participants (84.1%; 63.8% vs 93.9%; OR = 8,689; p <0.001), fever in 61 participants (42.1%; 46.8% vs 39.8%; OR = 0.751; p = 0.535), cough in 70 participants (70%; 59.6% vs. 42.9%; OR = 0.509; p = 0.088), painful swallowing in 4 participants (2.8%; 2.1% vs. 3.1%; OR = 1.453; p = 1,000), and diarrhoea in 7 participants (4.8%; 6.4% vs. 4.1%; OR = 0.624; p = 0.682). Based on the outcome of the COVID-19 treatment, most of non-severe participants recovered as many as 41 participants (87.2%) and most of severe participants were declared dead as many as 51 participants (52%; p <0.001). Overall, 88 participants (60.7%) were recovered. Several participants were declared to have comorbidities, including hypertension as many as 41 participants (28.3%; 27.7% vs 28.6%; OR = 1,046; p = 1,000), diabetes as many as 66 participants (45.5%; 42.6% vs 46.9%; OR = 1,194; p = 0.750), and obesity as many as 35 participants (24.1%; 19.1% vs. 26.5%; OR = 1.525; p = 0.444; Table 1).

Association of Soluble Receptor for Advanced Glycation End-Products (sRAGE) Serum with COVID-19 Severity

The results of blood analysis showed several blood components such as leukocytes (9,896.51 ± 4,949.64/µL), lymphocytes (13.55 ± 8.48%), neutrophils (78.91 ± 10.50%), procalcitonin (0.92 ± 3.22 ng/mL), CRP (8.59 ± 7.62 mg/ L), D-dimer (4,360.29 ± 7,797.81 ng/mL), and fibrinogen (474.58 ± 168.90 mg/dL). The average value of serum sRAGE was 1.48 ± 0.98 ng/mL, with a median value of 1.07 (0.85 – 1.84) ng/mL. The lowest and highest value of participants' serum sRAGE was 0.44 ng/mL and 5.14 ng/mL, respectively. The results of the COVID-19 severity measurement were divided into 4: mild as many as 2 participants (1.4%), moderate as many as 45 participants (31.0%), severe as many as 96 participants (66.2%), and critical as many as 2 participants (1.4%). Meanwhile, in this study,

COVID-19 severity was divided into 2 groups, namely the non-severe group with 47 participants (32.88%) and the severe group with 98 participants (68.53%).

There was a significant difference in blood component in the non-severe group and the severe group as follows: leukocyte value was 8.005.00 (6.157.50 – 9.687.50) vs 9.840.00 (7.420.00 – 12.830.00/µL; z = 2.431; p = 0.015), lymphocyte was 14.40 (8.83 – 21.65) vs 10.20 (6.60 – 16.80%; z = 2.256; p = 0.024), neutrophils was 77.40 (68.90 – 83.28) vs. 82.60 (76.00 – 87.10%; z = 2,464; p = 0.014), procalcitonin was 0.11 (0.07 – 0.22) vs 0.27 (0.13 – 0.46 ng/mL; z = 3.323; p = 0.001), CRP was 4.65 (0.80 – 11.35) vs. 8.70 (2.30 – 13.60 mg/L; z = 2.114; p = 0.034), and D-dimer was 810.00 (535.00 – 2,430.00) vs. 1,460.00 (740.00 – 4,025 ng/mL; z = 2.186; p = 0.029). Meanwhile, there was no significant difference in the levels of fibrinogen between participants in the two groups (465.50 ± 176.04 vs. 480.06 ± 165.92 mg/dL; t = 0.383; p = 0.703; Table 2).

There was a significant difference between serum sRAGE in the non-severe group and the severe group (0.78 (0.63 – 1.00) vs. 1.47 (0.97 – 2.25 ng/mL; r = 7.154; p <0.001; Table 2). There was a significant association between serum sRAGE and COVID-19 severity (r = 0.598; p <0.001). The cut-off value for serum sRAGE between the severe and non-severe group was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5%, OR of 8.077 and AUC 0.868 CI 95% (Figure 1).

Discussion

This study assessed serum sRAGE based on the severity of COVID-19. The results of this study are consistent with previous studies that examined sRAGE as a biomarker for COVID-19. A study examined the association of sRAGE with severity and as an indicator of mechanical ventilation requirements, ARDS, and mortality in COVID-19 patients. The results showed an increase in serum sRAGE concentrations in COVID-19 patients based on

severity [13]. These results are consistent with another study which stated a significant increase in serum sRAGE of ARDS patients admitted to non-isolated ICUs [14].

There is a significant association between serum sRAGE and COVID-19 severity. The serum sRAGE values in the severe group show a significant difference from serum sRAGE values in the non-severe group. The results are consistent with previous studies that showed an increase in serum sRAGE values in COVID-19 patients with a degree of severity. Increased sRAGE values can also help predict respiratory disorders that require mechanical ventilation and the mortality rate of COVID-19 patients [13]. Increased serum sRAGE is commonly found in ARDS patients admitted to the ICU [15]. As many as 20% of COVID-19 patients progress to the third phase called the involvement of the respiratory tract and progression to ARDS [16].

Increased serum sRAGE values can occur due to a viral infection process that will trigger an immune response, namely the innate immune system. Pattern-recognition receptors (PRR) recognize pathogen-associated molecular patterns (PAMPs) involving toll-like receptors (TLR) that detect components of infection and signaling tissue damage, one of which is HMGB1. Then it continues to the process of indirect lung tissue damage, namely damage-associated molecular patterns (DAMPs) that involve RAGE, NLR, TLR, and CLR which can exacerbate the occurrence of tissue damage that has occurred previously. The process of interaction of sRAGE with its ligand becomes more frequent due to an increase in HMGB1 that result in the increased inflammatory response in the form of IL-1 and TNF-Alpha activation [17, 18].

Other tissue damage processes can also occur when SARS-CoV2 invades AT2 cells located in the periphery and subpleural so that the patient begins to feel hypoxia. SARS-CoV2 replicates in AT2 lead to cell damage and death. Dead AT2 cells release toxins and damage surrounding cells. Infected cells send signals that are detected by the immune system

which then releases cytokines such as IL-1, IL-6, and TNF-α. These cytokine release aims to kill the virus, but it also causes damage to lung cells, namely diffuse alveolar damage, formation of hyaline membranes, and multinuclear giant cells. Abnormal wound healing leads to fibrosis [16, 19].

This study, however, has limitations, including the need for a future study that compares healthy individuals and pneumonia patients without COVID-19.

Conclusion

sRAGE is a biomarker that can be used to determine COVID-19 severity. The patients' COVID-19 severity in this study is categorized into 2, namely non-severe and severe. Based on blood component analysis, there are significant differences between the non-severe and severe groups. The differences consist of leukocytes, lymphocytes, neutrophils, procalcitonin, CRP, and D-dimer. The sRAGE values in the two groups also show a significant difference. In addition, there is a significant relationship between serum sRAGE and COVID-19 severity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We would like to thank the COVID-19 patients and Guardian. We would also thank Dr. Soetomo General Academic Hospital as the place of our research, and our editor “Fis Citra Ariyanto”.

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 (1954/KEPK/IV/2020).

Funding

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Registration of research studies

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

Unique identifying number or registration ID: 1954/KEPK/IV/2020.

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

Guarantor

Resti Yudhawati.

Author 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.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

1. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020;109:102433. doi: 10.1016/j.jaut.2020.102433.
2. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. Journal of infection and public health. 2020;13(5):667-73. doi: 10.1016/j.jiph.2020.03.019.
3. Djalante R, Lassa J, Setiamarga D, Sudjatma A, Indrawan M, Haryanto B, et al. Review and analysis of current responses to COVID-19 in Indonesia: Period of January to March 2020. Progress in disaster science. 2020;6:100091. doi: 10.1016/j.pdisas.2020.100091.
4. Son KB, Lee TJ, Hwang SS. Disease severity classification and COVID-19 outcomes, Republic of Korea. Bulletin of the World Health Organization. 2021;99(1):62-6. doi: 10.2471/blt.20.257758.
5. Suryananda TD, Yudhawati R. Association of serum KL-6 levels on COVID-19 severity: A cross-sectional study design with purposive sampling. Annals of medicine and surgery (2012). 2021;69:102673. doi: 10.1016/j.amsu.2021.102673.
6. Seligman R, Ramos-Lima LF, Oliveira Vdo A, Sanvicente C, Pacheco EF, Dalla Rosa K. Biomarkers in community-acquired pneumonia: a state-of-the-art review. Clinics (Sao Paulo, Brazil). 2012;67(11):1321-5. doi: 10.6061/clinics/2012(11)17.
7. Narvaez-Rivera RM, Rendon A, Salinas-Carmona MC, Rosas-Taraco AG. Soluble RAGE as a severity marker in community acquired pneumonia associated sepsis. BMC infectious diseases. 2012;12:15. doi: 10.1186/1471-2334-12-15.
8. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. Journal of translational medicine. 2009;7:17. doi: 10.1186/1479-5876-7-17.
9. Chavakis T, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. Microbes and infection. 2004;6(13):1219-25. doi: 10.1016/j.micinf.2004.08.004.
10. Clynes R, Moser B, Yan SF, Ramasamy R, Herold K, Schmidt AM. Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response. Current molecular medicine. 2007;7(8):743-51. doi: 10.2174/156652407783220714.
11. Mathew G, Agha R. STROCSS 2021: Strengthening the reporting of cohort, cross-sectional and case-control studies in surgery. International journal of surgery (London, England). 2021;96:106165. doi: 10.1016/j.ijsu.2021.106165.
12. García-Salido A, Melen G, Gómez-Piña V, Oñoro-Otero G, Serrano-González A, Casado-Flores J, et al. Circulating soluble RAGE and cell surface RAGE on peripheral blood mononuclear cells in healthy children. Journal of pediatric endocrinology & metabolism : JPEM. 2018;31(6):649-54. doi: 10.1515/jpem-2017-0512.
13. Lim A, Radujkovic A, Weigand MA, Merle U. Soluble receptor for advanced glycation end products (sRAGE) as a biomarker of COVID-19 disease severity and indicator of the need for mechanical ventilation, ARDS and mortality. Annals of intensive care. 2021;11(1):50. doi: 10.1186/s13613-021-00836-2.
14. Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure JS, et al. Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study. Scientific reports. 2018;8(1):2603. doi: 10.1038/s41598-018-20994-x.
15. van Zoelen MA, Schouten M, de Vos AF, Florquin S, Meijers JC, Nawroth PP, et al. The receptor for advanced glycation end products impairs host defense in pneumococcal pneumonia. Journal of immunology (Baltimore, Md : 1950). 2009;182(7):4349-56. doi: 10.4049/jimmunol.0801199.

16. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. The European respiratory journal. 2020;55(4). doi: 10.1183/13993003.00607-2020.
17. Mondrinos MJ, Kennedy PA, Lyons M, Deutschman CS, Kilpatrick LE. Protein kinase C and acute respiratory distress syndrome. Shock (Augusta, Ga). 2013;39(6):467-79. doi: 10.1097/SHK.0b013e318294f85a.
18. Horiguchi H, Loftus TJ, Hawkins RB, Raymond SL, Stortz JA, Hollen MK, et al. Innate Immunity in the Persistent Inflammation, Immunosuppression, and Catabolism Syndrome and Its Implications for Therapy. Frontiers in immunology. 2018;9:595. doi: 10.3389/fimmu.2018.00595.
19. Carcaterra M, Caruso C. Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: A physio-pathological theory. Medical hypotheses. 2021;146:110412. doi: 10.1016/j.mehy.2020.110412.

Figure Legend

Figure 1. Cut-off Serum sRAGE level based on severe and non-severe groups of COVID-19 patients

Table and Legend

Table 1. Characteristics of Participants

Characteristics	COVID-19 Severity		*p*
	Non-severe	Severe	
Age (years)			
21-35	8 (17.0)	12 (12.2)	0.705
35-55	25 (53.2)	51 (52.0)	
55-65	8 (17.0)	24 (24.5)	
>65	6 (12.8)	11 (11.2)	
Gender			
Male	25 (53.2)	65 (66.3)	0.179
Female	22 (46.8)	33 (33.7)	
Clinical symptoms			
Shortness of breath	30 (63.8)	92 (93.9)	<0.001**
Fever	22 (46.8)	39 (39.8)	0.535
Cough	28 (59.6)	42 (42.9)	0.088
Painful swallowing	1 (2.1)	3 (3.1)	1.000
Diarrhea	3 (6.4)	4 (4.1)	0.682
Outcome			
Recovered	41 (87.2)	47 (48.0)	<0.001**
Died	6 (12.8)	51 (52.0)	
Comorbid			
Hypertension	13 (27.7)	28 (28.6)	1.000
Diabetes	20 (42.6)	46 (46.9)	0.750
Obesity	9 (19.1)	26 (26.5)	0.444

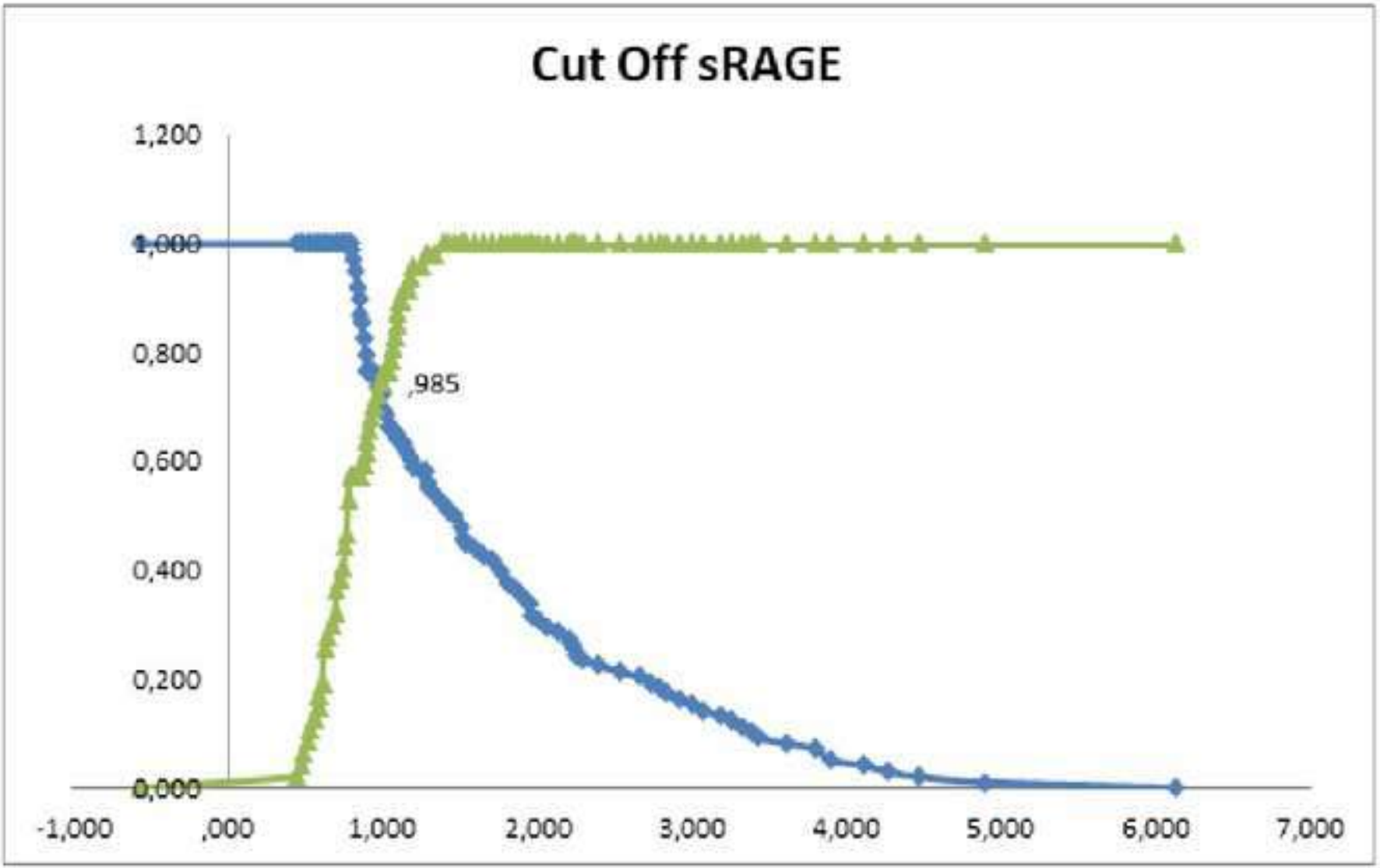
Note: *significant <0.05; **significant <0.01

Table 2. Comparison of Blood Component Based on COVID-19 Severity

Blood Analysis	COVID-19 Severity		*p*
	Non-severe	Severe	
Leukocytes (n = 139)	8,622.10 ± 4,204.47	10,526.86 ± 5,185.37	0.015*
Lymphocyte (n = 139)	15.50 ± 8.22	12.58 ± 8.49	0.024*
Neutrophile (n = 139)	76.06 ± 10.36	80.32 ± 10.34	0.014*
Procalcitonin (n = 143)	1.01 ± 4.67	0.88 ± 2.22	0.001*
CRP (n = 90)	6.52 ± 6.71	9.53 ± 7.87	0.034*
Fibrinogen (n = 85)	465.50 ± 176.04	480.06 ± 165.92	0.703
D-Dimer (n = 139)	2,790.64 ± 5,558.74	5,162.17 ± 8,641.11	0.029*
s-RAGE (n = 143)	0.82 ± 0.23	1.80 ± 1.04	<0.001**

Note: CRP = C-reactive protein; s-RAGE = soluble receptor for advanced glycation end products; *significant <0.05; **significant <0.001

Figure 1



The STROCSS 2021 Guideline		
Item no.	**Item description**	**Page**
TITLE		
1	**Title** • The word cohort or cross-sectional or case-control is included* • Temporal design of study is stated (e.g. retrospective or prospective) • The focus of the research study is mentioned (e.g. population, setting, disease, exposure/intervention, outcome etc.) *STROCSS 2021 guidelines apply to cohort studies as well as other observational studies (e.g. cross-sectional, case-control etc.)	1
ABSTRACT		
2a	**Introduction** – briefly describe: • Background • Scientific rationale for this study • Aims and objectives	1
2b	**Methods** - briefly describe: • Type of study design (e.g. cohort, case-control, cross-sectional etc.) • Other key elements of study design (e.g. retro-/prospective, single/multi-centred etc.) • Patient populations and/or groups, including control group, if applicable • Exposure/interventions (e.g. type, operators, recipients, timeframes etc.) • Outcome measures – state primary and secondary outcome(s)	1
2c	**Results** - briefly describe: • Summary data with qualitative descriptions and statistical relevance, where appropriate	1
2d	**Conclusion** - briefly describe: • Key conclusions • Implications for clinical practice • Need for and direction of future research	2
INTRODUCTION		
3	**Introduction** – comprehensively describe: • Relevant background and scientific rationale for study with reference to key literature • Research question and hypotheses, where appropriate • Aims and objectives	2-4
METHODS		
4a	**Registration** • In accordance with the Declaration of Helsinki*, state the research registration number and where it was registered, with a hyperlink to the registry entry (this can be obtained from ResearchRegistry.com, ClinicalTrials.gov, ISRCTN etc.) • All retrospective studies should be registered before submission; it should be stated that the research was retrospectively registered ** "Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject"*	5
4b	**Ethical approval** • Reason(s) why ethical approval was needed • Name of body giving ethical approval and approval number • Where ethical approval wasn't necessary, reason(s) are provided	5

4c	**Protocol** • Give details of protocol (*a priori* or otherwise) including how to access it (e.g. web address, protocol registration number etc.) • If published in a journal, cite and provide full reference	4
4d	**Patient and public involvement in research** • Declare any patient and public involvement in research • State the stages of the research process where patients and the public were involved (e.g. patient recruitment, defining research outcomes, dissemination of results etc.) and describe the extent to which they were involved.	4
5a	**Study design** • State type of study design used (e.g. cohort, cross-sectional, case-control etc.) • Describe other key elements of study design (e.g. retro-/prospective, single/multi-centred etc.)	4
5b	**Setting and timeframe of research** – comprehensively describe: • Geographical location • Nature of institution (e.g. primary/secondary/tertiary care setting, district general hospital/teaching hospital, public/private, low-resource setting etc.) • Dates (e.g. recruitment, exposure, follow-up, data collection etc.)	4
5c	**Study groups** • Total number of participants • Number of groups • Detail exposure/intervention allocated to each group • Number of participants in each group	4
5d	**Subgroup analysis** – comprehensively describe: • Planned subgroup analyses • Methods used to examine subgroups and their interactions	4
6a	**Participants** – comprehensively describe: • Inclusion and exclusion criteria with clear definitions • Sources of recruitment (e.g. physician referral, study website, social media, posters etc.) • Length, frequency and methods of follow-up (e.g. mail, telephone etc.)	4
6b	**Recruitment** – comprehensively describe: • Methods of recruitment to each patient group (e.g. all at once, in batches, continuously till desired sample size is reached etc.) • Any monetary incentivisation of patients for recruitment and retention should be declared; clarify the nature of any incentives provided • Nature of informed consent (e.g. written, verbal etc.) • Period of recruitment	4
6c	**Sample size** – comprehensively describe: • Analysis to determine optimal sample size for study accounting for population/effect size • Power calculations, where appropriate • Margin of error calculation	4
METHODS - INTERVENTION AND CONSIDERATIONS		
7a	**Pre-intervention considerations** – comprehensively describe: • Preoperative patient optimisation (e.g. weight loss, smoking cessation, glycaemic control etc.) • Pre-intervention treatment (e.g. medication review, bowel preparation, correcting hypothermia/-volemia/-tension, mitigating bleeding risk, ICU care etc.)	5

7b	**Intervention** – comprehensively describe: • Type of intervention and reasoning (e.g. pharmacological, surgical, physiotherapy, psychological etc.) • Aim of intervention (preventative/therapeutic) • Concurrent treatments (e.g. antibiotics, analgesia, anti-emetics, VTE prophylaxis etc.) • Manufacturer and model details, where applicable	5
7c	**Intra-intervention considerations** – comprehensively describe: • Details pertaining to administration of intervention (e.g. anaesthetic, positioning, location, preparation, equipment needed, devices, sutures, operative techniques, operative time etc.) • Details of pharmacological therapies used, including formulation, dosages, routes, and durations • Figures and other media are used to illustrate	5
7d	**Operator details** – comprehensively describe: • Requirement for additional training • Learning curve for technique • Relevant training, specialisation and operator's experience (e.g. average number of the relevant procedures performed annually)	5
7e	**Quality control** – comprehensively describe: • Measures taken to reduce inter-operator variability • Measures taken to ensure consistency in other aspects of intervention delivery • Measures taken to ensure quality in intervention delivery	5
7f	**Post-intervention considerations** – comprehensively describe: • Post-operative instructions (e.g. avoid heavy lifting) and care • Follow-up measures • Future surveillance requirements (e.g. blood tests, imaging etc.)	5
8	**Outcomes** – comprehensively describe: • Primary outcomes, including validation, where applicable • Secondary outcomes, where appropriate • Definition of outcomes • If any validated outcome measurement tools are used, give full reference • Follow-up period for outcome assessment, divided by group	5
9	**Statistics** – comprehensively describe: • Statistical tests and statistical package(s)/software used • Confounders and their control, if known • Analysis approach (e.g. intention to treat/per protocol) • Any sub-group analyses • Level of statistical significance	6
RESULTS		
10a	**Participants** – comprehensively describe: • Flow of participants (recruitment, non-participation, cross-over and withdrawal, with reasons). Use figure to illustrate. • Population demographics (e.g. age, gender, relevant socioeconomic features, prognostic features etc.) • Any significant numerical differences should be highlighted	6
10b	**Participant comparison** • Include table comparing baseline characteristics of cohort groups • Give differences, with statistical relevance • Describe any group matching, with methods	6
10c	**Intervention** – comprehensively describe:	7

	• Degree of novelty of intervention • Learning required for interventions • Any changes to interventions, with rationale and diagram, if appropriate	
11a	**Outcomes** – comprehensively describe: • Clinician-assessed and patient-reported outcomes for each group • Relevant photographs and imaging are desirable • Any confounding factors and state which ones are adjusted	7
11b	**Tolerance** – comprehensively describe: • Assessment of tolerability of exposure/intervention • Cross-over with explanation • Loss to follow-up (fraction and percentage), with reasons	7
11c	**Complications** – comprehensively describe: • Adverse events and classify according to Clavien-Dindo classification* • Timing of adverse events • Mitigation for adverse events (e.g. blood transfusion, wound care, revision surgery etc.) *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	7
12	**Key results** – comprehensively describe: • Key results with relevant raw data • Statistical analyses with significance • Include table showing research findings and statistical analyses with significance	7-8
DISCUSSION		
13	**Discussion** – comprehensively describe: • Conclusions and rationale • Reference to relevant literature • Implications for clinical practice • Comparison to current gold standard of care • Relevant hypothesis generation	8-10
14	**Strengths and limitations** – comprehensively describe: • Strengths of the study • Weaknesses and limitations of the study and potential impact on results and their interpretation • Assessment and management of bias • Deviations from protocol, with reasons	10
15	**Relevance and implications** – comprehensively describe: • Relevance of findings and potential implications for clinical practice • Need for and direction of future research, with optimal study designs mentioned	-
CONCLUSION		
16	**Conclusions** • Summarise key conclusions • Outline key directions for future research	10
DECLARATIONS		
17a	**Conflicts of interest** • Conflicts of interest, if any, are described	-
17b	**Funding** • Sources of funding (e.g. grant details), if any, are clearly stated • Role of funder	-

17c	**Contributorship** • Acknowledge patient and public involvement in research; report the extent of involvement of each contributor	-

Table 2: The full revised STROCSS 2021 checklist

Cross-sectional Study

Association of soluble receptor for advanced glycation end-products (sRAGE) serum on COVID-19 severity: A cross-sectional study

Q1

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

Gusti Noor Ramadany Saputra[a], Resti Yudhawati[a,*], restiyudhawati2021@gmail.com, Munawaroh Fitriah[b]

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

[b]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Q3

~~*Corresponding author.~~

*Corresponding author: Resti Yudhawati, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jl. Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia

Abstract

***Background*:** Coronavirus disease 2019 (COVID-19) is a new health problem discovered in 2019 thus requires biomarkers that can detect early tissue damage. Soluble ~~R~~receptor for a~~A~~dvanced ~~G~~glycation ~~E~~end-p~~P~~roducts (sRAGE) is a biomarker that can be used to identify early lung damage.

***Objective*:** Analyzing the association of serum sRAGE ~~with~~on COVID-19 severity.

***Methods*:** This study employed a cross-sectional design with a consecutive sampling method. It was conducted from May 2020–October 2021. The number of participants in this study was 145 participants which were divided into 2 groups (non-severe = 47 and severe = 98). Association of sRAGE serum ~~with~~on COVID-19 severity was analyzed using the chi-square test, Fisher's exact test, independence *t*~~-~~test, Mann Withney test, and Spearman's rank test with *p*-value <0.05.

***Results*:** The results of blood analysis showed several blood components such as leukocytes (9896.51 ± 4949.64/µL; $z = 2.431$; $p = 0.015$), lymphocytes (13.55 ± 8.48%; $z = 2.256$; $p = 0.024$), neutrophils (78.91 ± 10.50%; $z = 2.464$; $p = 0.014$), procalcitonin (0.92 ± 3.22 ng/mL; $z = 3.323$; $p = 0.001$), CRP (8.59 ± 7.62 mg/L; $z = 2.114$; $p = 0.034$), D-dimer (4360.29 ± 7797.81 ng/mL; $z = 2.186$; $p = 0.029$), and fibrinogen (474.58 ± 168.90 mg/dL; $t = 0.383$; $p = 0.703$). There was a significant ~~difference~~comparison in serum sRAGE values in the non-severe group (0.78 [0.63–1.00] ng/mL) and severe group (1.47 [0.97–2.25] ng/mL; $r = 7.154$; $p <$ 0.001). There was a significant association between serum sRAGE and COVID-19 severity ($r = 0.598$; $p <$ 0.001). The cut-off value for serum sRAGE between the severe and non-severe groups was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5% OR 8.077 and AUC 0.868 95% CI.

***Conclusion*:** There is a significant association between serum sRAGE and COVID-19 severity and there is also a significant difference in serum sRAGE in the two groups.

Keywords:

Serum sRAGE, COVID-19 severity, Infectious disease

Abbreviations

No keyword abbreviations are available

1 Introduction

Coronavirus disease 2019 or better known as COVID-19 caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) became a worldwide pandemic at the end of 2019 with various systemic complaints but was more dominant in respiratory disorders. The worldwide mortality rate was 2.1% by February 12, 2020 [1]. The February 2020 data by Johns Hopkins University's Center for Systems Science and Engineering (CSSE) showed a total case of more than 60,331 patients, with a total death of more than 1369 patients and an improvement of more than 6061 patients [2]. On December 27, 2020, the total number of worldwide cases was more than 79 million, including 1,751,311 deaths. Incidents in Indonesia were 706,837 confirmed cases of COVID-19 and 20,994 cases of death [3].

The severity of COVID-19 according to WHO is divided into mild, moderate, severe, and critical [4,5]. The most frequently encountered clinical symptoms are pneumonia symptoms. Biomarkers are frequently used to determine the severity of pneumonia such as procalcitonin, C-reactive protein (CRP), copeptin, pro-ANP (atrial natriuretic peptide), adrenomedullin, cortisol, and D-dimers [6]. These biomarkers are good in determining infection in pneumonia but have not been able to detect early tissue damage, as patients often go to the hospital with a more severe condition. Recent studies in immunology have examined soluble ~~RAGE~~receptors for advanced glycation end-products (sRAGE) as a biomarker of the severity of community pneumonia and can detect tissue damage in ARDS early [7].

Pathophysiology occurred in COVID-19 includes the inflammatory process. One of the inflammatory processes during pneumonia is characterized by an increase in ~~R~~receptors for a~~A~~dvanced ~~G~~glycation ~~E~~end-~~P~~products (RAGE). RAGE is one of the non-enzymatic receptors of Advanced Glycation End-Products (AGEs) which has a multi-ligand receptor, namely a V-type domain, two C-type domains, a transmembrane domain, and a cytoplasmic tail. RAGE has several ligands including AGEs, S100/calgranulins, and HMGB I which are present in different vascular cells such as endothelial cells, neuronal cells, smooth muscle cells, or inflammatory cells (monocytes). HMGB I is one of the RAGE ligands that play a role in the occurrence of sepsis which can stimulate the formation of cytokines along with TLRs in the immune system cells (B cells) [8]. The interaction between RAGE and its ligands will cause the formation of Reactive Oxygen Species (ROS) which will activate NADPH oxidation. The process will mediate the formation of inflammatory cells. Trianta et al. stated two processes of RAGE interaction with its ligands that are related to the inflammatory process, namely its interaction with leukocytes and on endothelial cells, RAGE is an adhesive receptor and directly forms inflammatory cells. The accumulation of RAGE ligands is predicted to cause chronic cell stimulation and tissue damage [9,10].

RAGE is expressed in the membrane-bound form (fl-RAGE or mRAGE) and the soluble form in the transmembrane domain. Soluble RAGE is produced by proteolytic cleavage of fl-RAGE and alternative splicing mRNA [7]. The administration of sRAGE in experimental animals can also interact with the RAGE ligand [10]. Based on these studies, the role of sRAGE becomes very important in determining COVID-19 diagnosis based on the severity quickly, so that effective and adequate treatment planning can be carried out early to reduce the morbidity and mortality of COVID-19 patients. In addition, the level of sRAGE in serum can detect early tissue damage which in turn can affect the severity of COVID-19 patients as common biomarkers have not been able to detect the process of tissue damage early. Research on sRAGE in the serum of COVID-19 patients is still limited and has never been carried out in Indonesia despite a few studies having been conducted in other countries. This biomarker is also easy to use and at a more affordable cost, so we are interested in analyzing the association of serum sRAGE on COVID-19 severity.

2 Methods

2.1 Participants

Participants in this study were COVID-19 patients diagnosed with real-time polymerase chain reaction (PCR) [5]. Participants' inclusion criteria included patients diagnosed with COVID-19 and aged >21 years. Participants' exclusion criteria included patients with a history of respiratory tract infection, myocardia infarct, cancer, and cerebral vascular attack. 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.

2.2 Study design

This study used a cross-sectional design with a consecutive sampling method. It was carried out from May 2020–October 2020. This study collected participant characteristics, serum sRAGE, and COVID-19 severity. This study reported the data based on the strengthening the reporting of cohort studies in surgery (STROCSS) 2021 guideline [11]. The number of participants in this study was 145 participants that were divided into 2 groups (non-severe = 47 and

severe = 98). The non-severe group consisted of participants identified as having COVID-19 in the mild and moderate category, while the severe group consisted of participants identified as having COVID-19 in the severe and critical categories [5].

~~**Ethical approval**~~

~~We have conducted an ethical approval based on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Hospital.~~

2.3 Assessment of COVID-19 severity

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 categories). Mild is a symptomatic patient who meets the COVID-19 case definition without evidence of viral pneumonia or hypoxia. Moderate include 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). Severe shows 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). Critical when patients have 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) [5].

2.4 sRAGE serum examination

The sRAGE is soluble forms in the transmembrane domain of RAGE which the serum levels of sRAGE are determined using a specific sandwich human ELISA kit BioAssay (MyBioSource Inc, San Diego, USA). The sRAGE measurement is in the range of 0.31–2.00 ng/mL. These results were obtained from taking 5 cc venous blood samples [12].

2.5 Statistical analysis

The analysis in this study used descriptive analysis and bivariate analysis. The descriptive analysis includes a descriptive presentation of the results using a distribution table, mean, median, standard deviation, maximum value, and minimum value. The analysis was conducted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA). Participants' characteristic data were analyzed using the chi-square test or Fisher's exact test. Meanwhile, the data from this study were first tested for normality using the Kolmogorov-Smirnov test. Analysis of the association of sRAGE serum with COVID-19 severity using the independence *t*-test or Mann Whitney test. The comparison between the two variables is significant if $p < 0.05$. In addition, Spearman's rank test was used to analyze the association between two variables.

3 Results

3.1 Characteristics of participants

The demographic characteristics of participants included age and gender. The average age of participants was 50.54 ± 12.70 years (non-severe group = 49.11 ± 12.44 years and severe group = 51.23 ± 12.83 years). The median age of participants was 52.00 (43.00–59.00) years of which the youngest participant was 22.00 years old and the oldest participant was 80.00 years old. Most participants were in the age range of 35.00–55.00 years, consisting of 25 participants (53.2%) in the non-severe group and 51 participants (52.0%; $p = 0.705$) in the severe group. Most participants were male (90 participants; 62.1%), consisting of 25 participants (53.2%) in non-severe group and 65 participants in severe group (66.3%; OR = 0.577; $p = 0.179$; 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 participants.

Characteristics	COVID-19 Severity		*p*
	Non-severe	Severe	
Age (years)			0.705

21-35	8 (17.0)	12 (12.2)	
35-55	25 (53.2)	51 (52.0)	
55-65	8 (17.0)	24 (24.5)	
>65	6 (12.8)	11 (11.2)	
Gender			
Male	25 (53.2)	65 (66.3)	0.179
Female	22 (46.8)	33 (33.7)	
Clinical symptoms			
Shortness of breath	30 (63.8)	92 (93.9)	<0.001**
Fever	22 (46.8)	39 (39.8)	0.535
Cough	28 (59.6)	42 (42.9)	0.088
Painful swallowing	1 (2.1)	3 (3.1)	1.000
Diarrhea	3 (6.4)	4 (4.1)	0.682
Outcome			
Recovered	41 (87.2)	47 (48.0)	<0.001**
Died	6 (12.8)	51 (52.0)	
Comorbid			
Hypertension	13 (27.7)	28 (28.6)	1.000
Diabetes	20 (42.6)	46 (46.9)	0.750
Obesity	9 (19.1)	26 (26.5)	0.444

Note: *significant <0.05; **significant <0.01.

There were several clinical symptoms appeared, including shortness of breath in 122 participants (84.1%; 63.8% vs 93.9%; OR = 8689; p <0.001), fever in 61 participants (42.1%; 46.8% vs 39.8%; OR = 0.751; p = 0.535), cough in 70 participants (70%; 59.6% vs. 42.9%; OR = 0.509; p = 0.088), painful swallowing in 4 participants (2.8%; 2.1% vs 3.1%; OR = 1.453; p = 1.000), and diarrhea in 7 participants (4.8%; 6.4% vs. 4.1%; OR = 0.624; p = 0.682). Based on the outcome of the COVID-19 treatment, most of non-severe participants recovered as many as 41 participants (87.2%) and most of severe participants were declared dead as many as 51 participants (52%; p <0.001). Overall, 88 participants (60.7%) were recovered. Several participants were declared to have comorbidities, including hypertension as many as 41 participants (28.3%; 27.7% vs 28.6%; OR = 1.046; p = 1.000), diabetes as many as 66 participants (45.5%; 42.6% vs 46.9%; OR = 1.194; p = 0.750), and obesity as many as 35 participants (24.1%; 19.1% vs. 26.5%; OR = 1.525; p = 0.444; Table 1).

3.2 Association of soluble receptor for Advanced Glycation End-Products (sRAGE) serum with COVID-19 severity

The results of blood analysis showed several blood components such as leukocytes (9896.51 ± 4949.64/µL), lymphocytes (13.55 ± 8.48%), neutrophils (78.91 ± 10.50%), procalcitonin (0.92 ± 3.22 ng/mL), CRP (8.59 ± 7.62 mg/L), D-dimer (4360.29 ± 7797.81 ng/mL), and fibrinogen (474.58 ± 168.90 mg/dL). The average value of serum sRAGE was 1.48 ± 0.98 ng/mL, with a median value of 1.07 (0.85–1.84) ng/mL. The lowest and highest value of participants' serum sRAGE was 0.44 ng/mL and 5.14 ng/mL, respectively. The results of the COVID-19 severity measurement were divided into 4: mild as many as 2 participants (1.4%), moderate as many as 45 participants (31.0%), severe as many as 96 participants (66.2%), and critical as many as 2 participants (1.4%). Meanwhile, in this study, COVID-19 severity was divided into 2 groups, namely the non-severe group with 47 participants (32.88%) and the severe group with 98 participants (68.53%).

There was a significant difference in blood component in the non-severe group and the severe group as follows: leukocyte value was 8005.00 (6157.50–9687.50) vs 9840.00 (7420.00–12,830.00/µL; z = 2.431; p = 0.015), lymphocyte was 14.40 (8.83–21.65) vs 10.20 (6.60–16.80%; z = 2.256; p = 0.024), neutrophils was 77.40 (68.90–83.28) vs. 82.60 (76.00–87.10%; z = 2464; p = 0.014), procalcitonin was 0.11 (0.07–0.22) vs 0.27 (0.13–0.46 ng/mL; z = 3.323; p = 0.001), CRP was 4.65 (0.80–11.35) vs. 8.70 (2.30–13.60 mg/L; z = 2.114; p = 0.034), and D-dimer was 810.00 (535.00–2430.00) vs. 1460.00 (740.00–4025 ng/mL; z = 2.186; p = 0.029). Meanwhile, there was no significant difference in the levels of fibrinogen between participants in the two groups (465.50 ± 176.04 vs. 480.06 ± 165.92 mg/dL; t = 0.383; p = 0.703; 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.

Comparison of blood component based on COVID-19 severity.

Blood Analysis	COVID-19 Severity		*p*
	Non-severe	Severe	
Leukocytes (n = 139)	8622.10 ± 4204.47	10,526.86 ± 5185.37	0.015*
Lymphocyte (n = 139)	15.50 ± 8.22	12.58 ± 8.49	0.024*
Neutrophile (n = 139)	76.06 ± 10.36	80.32 ± 10.34	0.014*
Procalcitonin (n = 143)	1.01 ± 4.67	0.88 ± 2.22	0.001*
CRP (n = 90)	6.52 ± 6.71	9.53 ± 7.87	0.034*
Fibrinogen (n = 85)	465.50 ± 176.04	480.06 ± 165.92	0.703
D-Dimer (n = 139)	2790.64 ± 5558.74	5162.17 ± 8641.11	0.029*
s-RAGE (n = 143)	0.82 ± 0.23	1.80 ± 1.04	<0.001**

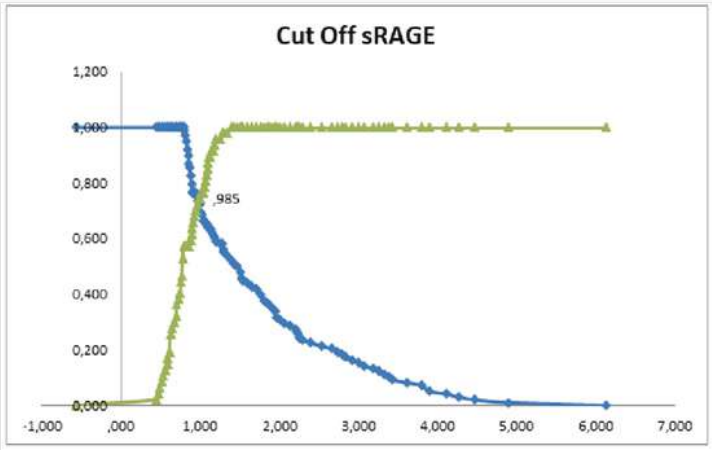
Note: CRP = C-reactive protein; s-RAGE = soluble receptor for advanced glycation end products; *significant <0.05; **significant <0.001.

There was a significant difference between serum sRAGE in the non-severe group and the severe group of 0.78 (0.63–1.00) vs 1.47 (0.97–2.25 ng/mL; $r = 7.154$; $p < 0.001$; Table 2). There was a significant association between serum sRAGE and COVID-19 severity ($r = 0.598$; $p <$ 0.001). The cut-off value for serum sRAGE between the severe and non-severe group was 0.985 ng/mL. This study obtained sensitivity of 73.5%, specificity of 74.5%, OR of 8.077 and AUC 0.868 CI 95% (Fig. 1).

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 1

Fig. 1



Cut-off Serum sRAGE level based on severe and non-severe groups of COVID-19 patients.

4 Discussion

This study assessed serum sRAGE based on the severity of COVID-19. The results of this study are consistent with previous studies that examined sRAGE as a biomarker for COVID-19. A study examined the association of sRAGE with severity and as an indicator of mechanical ventilation requirements, ARDS, and mortality in COVID-19 patients. The results showed an increase in serum sRAGE concentrations in COVID-19 patients based on severity [13]. These results are consistent with another study which stated a significant increase in serum sRAGE of ARDS patients admitted to non-isolated ICUs [14].

There is a significant association between serum sRAGE and COVID-19 severity. The serum sRAGE values in the severe group show a significant difference from serum sRAGE values in the non-severe group. The results are consistent with previous studies that showed an increase in serum sRAGE values in COVID-19 patients with a degree of severity. Increased sRAGE values can also help predict respiratory disorders that require mechanical ventilation and the mortality rate of COVID-19 patients [13]. Increased serum sRAGE is commonly found in ARDS patients admitted to the ICU [15]. As many as 20% of COVID-19 patients progress to the third phase called the involvement of the respiratory tract and progression to ARDS [16].

Increased serum sRAGE values can occur due to a viral infection process that will trigger an immune response, namely the innate immune system. Pattern-recognition receptors (PRR) recognize pathogen-associated molecular patterns (PAMPs) involving toll-like receptors (TLR) that detect components of infection and signaling tissue damage, one of which is HMGB1. Then it continues to the process of indirect lung tissue damage, namely damage-associated molecular patterns (DAMPs) that involve RAGE, NLR, TLR, and CLR which can exacerbate the occurrence of tissue damage that has occurred previously. The process of interaction of sRAGE with its ligand becomes more frequent due to an increase in HMGB1 that result in the increased inflammatory response in the form of IL-1 and TNF-Alpha activation [17,18].

Other tissue damage processes can also occur when SARS-CoV2 invades AT2 cells located in the periphery and subpleural so that the patient begins to feel hypoxia. SARS-CoV2 replicates in AT2 lead to cell damage and death. Dead AT2 cells release toxins and damage surrounding cells. Infected cells send signals that are detected by the immune system which then releases cytokines such as IL-1, IL-6, and TNF-α. These cytokine release aims to kill the virus, but it also causes damage to lung cells, namely diffuse alveolar damage, formation of hyaline membranes, and multinuclear giant cells. Abnormal wound healing leads to fibrosis [16,19].

This study, however, has limitations, including the need for a future study that compares healthy individuals and pneumonia patients without COVID-19.

5 Conclusion

sRAGE is a biomarker that can be used to determine COVID-19 severity. The patients' COVID-19 severity in this study is categorized into 2, namely non-severe and severe. Based on blood component analysis, there are significant differences between the non-severe and severe groups. The differences consist of leukocytes, lymphocytes, neutrophils, procalcitonin, CRP, and D-dimer. The sRAGE values in the two groups also show a significant difference. In addition, there is a significant association between serum sRAGE and COVID-19 severity.

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~~. (1954/KEPK/IV/2020).~~

Funding

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

~~Registration of research studies~~

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

~~Unique identifying number or registration ID: 1954/KEPK/IV/2020.~~

~~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.~~

Guarantor

Resti Yudhawati.

Author 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.

Provenance and peer review

Not commissioned, externally peer-reviewed.

~~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 contributions

~~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: Health Research Ethics Committee~~Coommitee~~ in the Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
2. Unique Identifying number or registration ID: 1954/KEPK/IV/2020.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): -.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgment

We would like to thank the COVID-19 patients and Guardian. We would also thank Dr. Soetomo General Academic Hospital as the place of our research, and our editor “Fis Citra Ariyanto”.

Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103303.

References

The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

[1] H.A. Rothan, S.N. Byrareddy, The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak, J. Autoimmun. 109 (2020) 102433, doi:10.1016/j.jaut.2020.102433.

[2] H. Harapan, N. Itoh, A. Yufika, W. Winardi, S. Keam, H. Te, et al., Coronavirus disease 2019 (COVID-19): a literature review, J.of infection and public health 13 (5) (2020) 667–673, doi:10.1016/j.jiph.2020.03.019.

[3] R. Djalante, J. Lassa, D. Setiamarga, A. Sudjatma, M. Indrawan, B. Haryanto, et al., Review and analysis of current responses to COVID-19 in Indonesia: period of january to march 2020, Progress in disaster science 6 (2020) 100091, doi:10.1016/j.pdisas.2020.100091.

[4] K.B. Son, T.J. Lee, S.S. Hwang, Disease severity classification and COVID-19 outcomes, Republic of Korea, Bull. World Health Organ. 99 (1) (2021) 62–66, doi:10.2471/blt.20.257758.

[5] T.D. Suryananda, R. Yudhawati, Association of serum KL-6 levels on COVID-19 severity: a cross-sectional study design with purposive sampling, Ann. Med. Surg. 69 (2021) 2012 102673, doi:10.1016/j.amsu.2021.102673.

[6] R. Seligman, L.F. Ramos-Lima, A. Oliveira Vdo, C. Sanvicente, E.F. Pacheco, K. Dalla Rosa, Biomarkers in community-acquired pneumonia: a state-of-the-art review, Clinics 67 (11) (2012) 1321–1325, doi:10.6061/clinics/2012(11)17.

[7] R.M. Narvaez-Rivera, A. Rendon, M.C. Salinas-Carmona, A.G. Rosas-Taraco, Soluble RAGE as a severity marker in community acquired pneumonia associated sepsis, BMC Infect. Dis. 12 (2012) 15, doi:10.1186/1471-2334-12-15.

[8] L.J. Sparvero, D. Asafu-Adjei, R. Kang, D. Tang, N. Amin, J. Im, et al., RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation, J. Transl. Med. 7 (2009) 17, doi:10.1186/1479-5876-7-17.

[9] T. Chavakis, A. Bierhaus, P.P. Nawroth, RAGE (receptor for advanced glycation end products): a central player in the inflammatory response, Microb. Infect. 6 (13) (2004) 1219–1225, doi:10.1016/j.micinf.2004.08.004.

[10] R. Clynes, B. Moser, S.F. Yan, R. Ramasamy, K. Herold, A.M. Schmidt, Receptor for AGE (RAGE): weaving tangled webs within the inflammatory response, Curr. Mol. Med. 7 (8) (2007) 743–751, doi:10.2174/156652407783220714.

[11] G. Mathew, R. Agha, Strocss 2021: strengthening the reporting of cohort, cross-sectional and case-control studies in surgery, Int. J. Surg. 96 (2021) 106165, doi:10.1016/j.ijsu.2021.106165.

[12] A. García-Salido, G. Melen, V. Gómez-Piña, G. Oñoro-Otero, A. Serrano-González, J. Casado-Flores, et al., Circulating soluble RAGE and cell surface RAGE on peripheral blood mononuclear cells in healthy children, J. Pediatr. Endocrinol. Metab. : JPEM (J. Pediatr. Endocrinol. Metab.) 31 (6) (2018) 649–654, doi:10.1515/jpem-2017-0512.

[13] A. Lim, A. Radujkovic, M.A. Weigand, U. Merle, Soluble receptor for advanced glycation end products (sRAGE) as a biomarker of COVID-19 disease severity and indicator of the need for mechanical ventilation, ARDS and mortality, Ann. Intensive Care 11 (1) (2021) 50, doi:10.1186/s13613-021-00836-2.

[14] M. Jabaudon, P. Berthelin, T. Pranal, L. Roszyk, T. Godet, J.S. Faure, et al., Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study, Sci. Rep. 8 (1) (2018) 2603, doi:10.1038/s41598-018-20994-x.

[15] M.A. van Zoelen, M. Schouten, A.F. de Vos, S. Florquin, J.C. Meijers, P.P. Nawroth, et al., The receptor for advanced glycation end products impairs host defense in pneumococcal pneumonia, J. Immunol. (Baltimore, Md : 1950 182 (7) (2009) 4349–4356, doi:10.4049/jimmunol.0801199.

[16] R.J. Mason, Pathogenesis of COVID-19 from a cell biology perspective, Eur. Respir. J. 55 (4) (2020), doi:10.1183/13993003.00607-2020.

[17] M.J. Mondrinos, P.A. Kennedy, M. Lyons, C.S. Deutschman, L.E. Kilpatrick, Protein kinase C and acute respiratory distress syndrome, Shock 39 (6) (2013) 467–479, doi:10.1097/SHK.0b013e318294f85a.

[18] H. Horiguchi, T.J. Loftus, R.B. Hawkins, S.L. Raymond, J.A. Stortz, M.K. Hollen, et al., Innate immunity in the persistent inflammation, immunosuppression, and catabolism syndrome and its implications for therapy, Front. Immunol. 9 (2018) 595, doi:10.3389/fimmu.2018.00595.

[19] M. Carcaterra, C. Caruso, Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: a physio-pathological theory, Med. Hypotheses 146 (2021) 110412, doi:10.1016/j.mehy.2020.110412.

Highlights

- Serum sRAGE can be used to identify COVID-19 severity.
- The level of serum sRAGE in each COVID-19 patient is different.
- The blood components of each COVID-19 severity are different.

Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

alt-text: Multimedia component 1

Queries and Answers

Q1

Query: Correctly acknowledging the primary **funders and grant IDs** of your research is important to ensure compliance with funder policies. Please make sure that funders are mentioned accordingly.

Answer: Reviewed

Q2

Query: Please confirm that the provided **email** "restiyudhawati2021@gmail.com" is the correct address for official communication, else provide an alternate e-mail address to replace the existing one, because private e-mail addresses should not be used in articles as the address for communication.

Answer: Reviewed

Q3

Query: Note: The author's **telephone/fax number** has been removed as these are not published in journal articles.

Answer: Done

Q4

Query: Please confirm that **given names and surnames** have been identified correctly and are presented in the desired order and please carefully verify the spelling of all authors' names.

Answer: Reviewed