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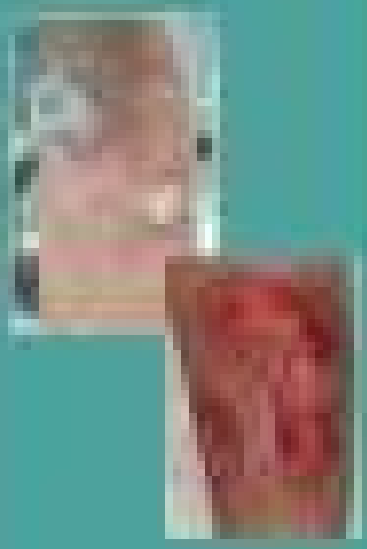

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Prioritizing Health Care Workers Safety: The International Year of Health and Care Workers 2021

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Healthcare workers pose a substantial risk of acquiring COVID-19 infection during their daily works. We have seen various conditions during the pandemic, such as limited adequate personal protective equipment (PPE), accurate diagnostic tests, lack of information regarding disease management, unsupportive work environment, and excessive workload, increased the number of HCWs-infected COVID-19. Compared to the general population, the risk of COVID-19 infection was several-fold higher in HCWs. HCWs with adequate PPE treating COVID-19 patients had a hazard ratio of 4.8 and those treating suspected COVID-19 had a hazard ratio of 2.4 compared to other HCWs that did not involve in COVID-19 management.[1] The non-prioritization of healthcare workers has led to detrimental effects. We have seen the lack of training and personal protective equipment (PPE) has led to a high number of physicians, nurses, and other health care workers' infection and mortality. The hesitation of authorities in early pandemics to heed the call of Indonesian medical professors for stricter rules and quarantine and prioritizing the economy might also play a role.

Up to September 2021, 730 Indonesian physicians, 670 Indonesian nurses, 398 Indonesian midwives died of COVID-19 infection. There were no available data of how many health care workers had been infected in Indonesia, but a total of 2066 HCWs were reported death of COVID-19.[2] The stressful working conditions of front-line health care workers and the dangers of becoming personally infected or infecting health care workers' families gave a substantial physical and emotional distress. Fear, anxiety, depression, posttraumatic stress disorder, leading to burnout for many health care workers. These conditions could eventually cause discontinuity of healthcare workloads to ensure the high-quality standard of care to the patients, or even fail to keep the health care system running.[3,4]

The condition was changed in January 2021 when the Indonesian government started the COVID-19 vaccination program with Sinovac. In its initial stage, the vaccination program is addressed only for a specific priority group, such as healthcare workers, including healthcare assistants and support workers who work at the healthcare facilities.[4] This was in line with the World Health Organization (WHO) call to ensure the world's health and care workers are prioritized for the COVID-19 vaccine in the first 100 days of 2021. World Health Organization (WHO) has even designated 2021 as the International Year of Health and Care Workers (YHCW).[5]

Santi, et al.[6] evaluated anti-SARS-CoV-2 antibodies after two doses of Sinovac vaccine in health care workers in the early COVID-19 vaccination period. The antibody titer did not decrease significantly after 3 months period. The observation period in this study might be too short because Chinese researchers later showed that Antibodies triggered by the

Sinovac COVID-19 vaccine declined below a key threshold from around six months after a second dose for most recipients.[7] Nevertheless, the coming of delta variant causing second wave increase in July made Indonesian government started to give all health care workers a third booster jab using Moderna vaccine.[8]

COVID-19 pandemic has raised the issue of health care workers' safety. Health care personnel or workers are defined as all paid and unpaid persons working in healthcare settings who have the potential for exposure to patients and/or to infectious materials, not only contaminated air but also body substances, contaminated medical supplies and equipment, and contaminated environmental surfaces.[9] Are health care workers only endangered by COVID-19? Certainly, many bloodborne or airborne infections could be transmitted to and from health care workers and patients. Globally, about one-third of health care workers are at risk of injury annually. More than 20 types of bloodborne pathogens can be transmitted following injury, including HIV, hepatitis B, and hepatitis C. The estimated transmission rates for HIV, hepatitis B, and hepatitis C after percutaneous injury are 0.2%, 1.8%, and 30%, but only HBV can be prevented by vaccination and post-exposure prophylaxis only available for HIV dan hepatitis B.[10] Our previous study reported the incidence of needle stick and sharp injury reported in a teaching hospital was 13.3 injuries per 1000 HCWs-years, though many were still underreported. More than 60% of the injuries reported involving sources with unknown HIV, hepatitis B, or hepatitis C status.[11]

Employers and health care workers both should share the responsibility to prevent occupationally acquired infections and avoid causing harm to patients by taking reasonable precautions to prevent vaccine-preventable disease transmission. Hepatitis B and influenza were the two most important vaccination for health care workers. Although many health care workers have taken hepatitis B vaccination, the question of how many health care workers have enough protection from hepatitis B infection remains. Annual influenza vaccination is recommended for three main reasons: reducing the risk of patients catching influenza from health care workers, protecting health care workers and their families against influenza, and reducing health care workers' absenteeism and presenteeism. Vaccine for mumps measles rubella (MMR), pertussis, and varicella were also highly recommended to be given for health care workers, while other vaccines might be indicated in certain circumstances.[9] However, there are no clear standard for vaccine requirement and who will cover vaccination and post-exposure prophylaxis.

This year, WHO has launched a year-long campaign under the theme -protect, invest, together'. It highlights the urgent need to invest in health care workers, not only during COVID-19. We need to ensure that all health care workers are supported, protected, motivated, and equipped to deliver safe health care at all times, to provide a high-quality standard of care to the patients.[5]

REFERENCES

1. Scherer MA, von Freyburg A, Brücher BDLM, Jamal IM, Schmidt A, Hagedom H. COVID-19: SARS-CoV-2 susceptibility in healthcare workers-cluster study at a German teaching hospital. Open. 2020;3:6.
2. Pusara digital tenaga kesehatan. 2066 tenaga kesehatan Indonesia gugur melawan COVID-19. Available online: https://nakes.laporcovid19.org/statistik
3. Koontalay A, Suksatan W, Prabsangob K, Sadang JM. Healthcare workers' burdens during the COVID-19 pandemic: A qualitative systematic review. J Multidiscip Health. 2021;14:3015-25.
4. The Jakarta Post. Top Indonesian doctors call for lockdown, say physical distancing not enough. https://www.thejakartapost.com/news/2020/03/27/top-indonesian-doctors-call-for-lockdown-say-physical-distancing-not-enough.html.
5. World Health Organization. Year of Health and Care Workers 2021. https://www.who.int/campaigns/annual-theme/year-of-health-and-care-workers-2021.
6. Santi T, De Samakto B, Kamarga L, et al. Factors associated with SARS-CoV-2 antibody titer after Sinovac vaccination among health care workers. Acta Med Indones. 2021;53(4):374-85.
7. Zeng G, Wu Q, Pan H, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. Lancet Infect Dis. 2021:S1473-3099(21)00681-2.
8. Office of Assistant to Deputy Cabinet Secretary for

State Documents & Translation. Gov't to Give Health Workers COVID-19 Booster Jab. Available online: https://setkab.go.id/en/govt-to-give-health-workers-covid-19-booster-jab/

9. Advisory Committee on Immunization Practices, et al. Immunization of health-care personnel: Recommendations of the Advisory Committee on Immunization Practices (ACIP) MMWR Recomm Rep. 2011;60(RR07):1-45.
10. Beltrami EM, Williams IT, Shapiro CN, Chamberland ME. Risk and management of blood-borne infections in health care workers. Clin Microbiol Rev. 2000;13:385–407.
11. Yunihastuti E, Ratih DM, Aisyah MR, et al. Needlestick and sharps injuries in an Indonesian tertiary teaching hospital from 2014 to 2017: a cohort study. BMJ Open. 2020;10:e041494.

Factors Associated with SARS-CoV-2 Antibody Titer After Sinovac Vaccination Among Health Care Workers

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ABSTRACT

Background: *One of the methods to record immunogenicity after vaccination is to measure antibody titer. This study aimed to get the value of antibody titer post Sinovac vaccination and to analyze factors that associate with it. The trend of titer changes within 3 months period and the incidence of COVID-19 were also observed.* ***Methods:*** *A prospective cohort study was conducted in March until May 2021 involving 250 health care workers of Siloam Hospitals Lippo-Cikarang who have completed two doses of Sinovac vaccination. We collected 3 titer data from each participant to observe the trend of changes. The incidence of COVID-19 among post-vaccinated subjects was also calculated.* ***Results:*** *From total of 250 participants, 88 (35.2%) were males and 162 (64.8%) were females. Fourteen days after vaccination, 248 subjects (99.2%) had seroconversion. The median antibody titer amounted to 63.58 U/ml (0.4->250 U/ml). The titer was higher in age group 26-39 years (85.1 U/ml, p=0.003) and in women (78.7 U/ml, p=0.007). Within 3 months period, 162 from 200 participants (81%) who completed 3 titer tests, had antibody titer reduction (p=0.231). In observation, 94 from 245 (38.3%) participants tested positive COVID-19, with only 5 out of 94 (5.3%) participants being hospitalized.* ***Conclusion:*** *The highest median titer was achieved 14 days after Sinovac vaccination (63.58 U/ml). Younger age group and women are associated with higher value. The reduction trend in titer within 3 months is insignificant. Among post-vaccinated infection subjects, the hospitalization rate is low, which shows that Sinovac vaccination still has a protective effect.*

Keywords**:** *Sinovac, SARS-CoV-2, COVID-19, antibody, vaccination, health care workers.*

INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is a novel corona virus that spreads Corona Virus Disease 2019 (COVID-19) worldwide, and vaccination to control the pandemic of COVID-19 has begun since December 2020.[1,2] Sinovac is an inactivated whole virus vaccine for COVID-19 developed by Sinovac Life Sciences which already completed its phase-3 clinical trials. Since January 11th 2021, Sinovac has already been approved by the Indonesian Food and Drugs Agency for emergency use of authorization in Indonesia, and the first target population was the health care workers as the front liners in fighting COVID-19.[3-9]

One of the methods to record immunogenicity after COVID-19 vaccination is the Anti-SARS-CoV-2 immunoassay which detects antibody titer of Spike protein (S-protein) from SARS-CoV-2.[10-12] There are many factors that affect the antibody titer after vaccination as stated in many journals and researches.[13] Age and gender has been already known to influence immune response after vaccination. Other factors such as body mass index, exercise, sleep duration had varied correlation with different types of vaccinations.[13-18] Correlation of those factors with Covid-19 vaccination is still unknown. In its clinical trial, Sinovac showed high immunogenicity among the subjects,[3-7] but the data about immune response post Sinovac vaccination among health care workers were still limited. It is important to have data on antibody titer, factors that influence the immunogenicity and incidence of infection after vaccination to monitor the impact of vaccination for health care workers and further get feedback for the next plan of their protection against COVID-19.

This study observed the data of quantitative antibody response 14 days after the second dose of Sinovac vaccination followed by 1 month and 2 months later, among the health care workers of Siloam Hospitals Lippo Cikarang. The main purpose of this study is to get the value of antibody titer post Sinovac vaccination and to analyze factors that might correlate with the titer (i.e age, gender, exercise, sleep and Body Mass Index). The trend of titer changes and the incidence of COVID-19 after vaccination were also observed as an additional objective.

METHODS

This study used a cohort design held at Siloam Hospitals Lippo Cikarang from March to May 2021. To improve quality of reporting, the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed.[19] The subjects of this study were health care workers who have already got two doses of Sinovac vaccines, administered intramuscularly with 14 days interval, with the dose of 30 µg in a volume of 0.5 mL of aluminium hydroxide diluent solution per dose. In our hospital, there was a policy that all employees must undergo post vaccination evaluation of antibody titer using the Elecsys anti-SARS-CoV-2 S-antibody assay at the hospital's laboratory. The tests were taken 3 times, beginning at 14 days after getting the second dose of Sinovac vaccine, followed by 2 consecutive months afterward. During and after the study period, participants were observed for COVID-19 infection by the hospital surveillance team for approximately 5 months (March-July 2021).

All of the antibody titer data were collected to observe trend of changes. For correlation analysis of antibody titer with age, sex, BMI, duration of sleep and exercise, we used the first antibody data taken 14 days after completed vaccination. We collected data from our laboratory as secondary data that has already been saved in its database.

We distributed google form questionnaires via Whatsapps application to collect demographic data and the health status of the participants. The questionnaires asked about age, sex, body weight, body height, sleep duration per day, and routine exercise. Before filling the questionnaires, all participants were given written explanations and informed consent forms were attached to the questionnaires.

Antigen-specific humoral immune response was analyzed using quantitative Elecsys anti-SARS-CoV-2 S-immunoassay (Roche Diagnostics, Mannheim, Germany).[20] It is an electrochemiluminescence immunoassay used to detect antibodies (including IgG) to the SARS-CoV-2 spike protein receptor-binding domain (RBD) on the Cobas e411 module (Roche Diagnostics, Mannheim, Germany). In this study, we used a commercial test that was usually used among the community, with the measurement ranges from 0.4 U/ml to 250 U/ml (values higher than 250 U/ml will be stated as >250 U/ml). A concentration of < 0.80 U/ml considered negative and > 0.80 U/ml considered positive. The WHO international standard for anti-SARS-CoV-2 immunoglobulin is BAU/ml (Binding Arbitrary Units per mL). The correlation between U/ml and BAU/ml was: U = 0.972 BAU. According to the manufacturer, the correlation test between Roche Elecsys Anti- SARS-CoV-2 S units per mL and WHO International Standards for anti-SARS-CoV-2 immunoglobulins was excellent

(r^2 = 0.9992, slope =0.972, intercept = 0.0072), thus allowing to consider specific Roche Elecsys anti-SARS-CoV-2 S U/mL units equivalent to WHO International Standard BAU/mL.[21]

We excluded the participants with a history of confirmed COVID-19 on the reverse transcriptase polymerase chain reaction test (RT-PCR) before and during antibody titer examination. We also excluded participants with a history of reactive serology or rapid screening tests. The regular qualitative serological screening had been conducted by our hospital since July 2020 (7 months before the vaccination program), using the Elecsys anti-SARS-CoV-2 assay (Roche Diagnostics) which detected antibodies to nucleocapsid (anti-N). All health care workers had this serology screening every 10 days. If the result turned reactive, they would have to continue for RT-PCR test. The history of confirmed COVID-19 and reactive serological tests were obtained from laboratory data confirmed by the confession of the participants in the questionnaires.

Ethical Approval

This research was approved by The Mochtar Riady Institute for Nanotechnology Ethics Committee (Protocol No: 2104011-03).

The Sample Size Calculation

The sample size was determined using the formula for mean difference for two different groups. From a previous study from hospital workers in Geneve, Switzerland,[22] the mean difference for two groups was 49 U/ml and the deviation standard was 156, so the minimum sample size calculated was 158 subjects.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS 26.*0 (Statistical Package for the Social Sciences, IBM Corp,. Armonk, NY, USA*). Normality test was done using Kolmogorov-Smirnov, if the p-value were more than 0.05 then the data would be considered normally distributed. According to the data distribution, numerical data would be presented in mean and deviation standard or median and interquartile range. Values lower than 0.4 U/ml were assumed as 0.4, and values higher than 250 U/ml were calculated as 251 U/ml. p values <0.05 were considered statistically significant.

We used bivariate analysis to predict the association between variables. If the data were normally distributed, the association would use an independent t-test (for two groups), or One-way ANOVA (for more than 2 groups). If the data were not normally distributed, the Mann-Whitney test (for two groups) or the Kruskal Wallis test (for more than two groups) would be applied. The statistical significance was set to $p<0.05$.

RESULTS

We invited 503 health care workers who eligible for vaccination from January to February 2021. Twenty-seven of them were excluded because of being confirmed with COVID-19 infection within 3 months prior. Thus, as many as 476 participants who were vaccinated twice and had their antibody titer tests were eligible, but only 440 of them returned the questionnaires. Sixty five out of 440 participants were excluded due to confirmed COVID-19 (positive RT-PCR swab test) and 125 participants were excluded due to reactive serological or rapid antibody screening before vaccination. Finally, we enrolled 250 eligible participants (in accordance with the minimum sample size requirement). A summary of the sampling process can be seen in **Figure 1**.

250 health care workers were not homogeneously distributed, with age grouping based on the age quartile <25 years: 60 people (24%), age 26-39 years: 131 people (52.4%), and age >40 years: 59 people (23.6%). The youngest age of participants was 21 years old (4 subjects) and the oldest age of participants was 60 years old (2 subjects). 248 out of 250 participants (99.2%) who have received Sinovac vaccination twice were being tested for their first-month antibody titer had antibody titer >0.8 U/ml (reactive), while 2 subjects had antibody titer: 0.4 and 0.7 U/ml. The median antibody titer of 250 participants amounted to 63.58 U/ml (minimum 0.4 U/ml, maximum >250 U/ml). The description of HCW who participated in the study can be seen in **Table 1**.

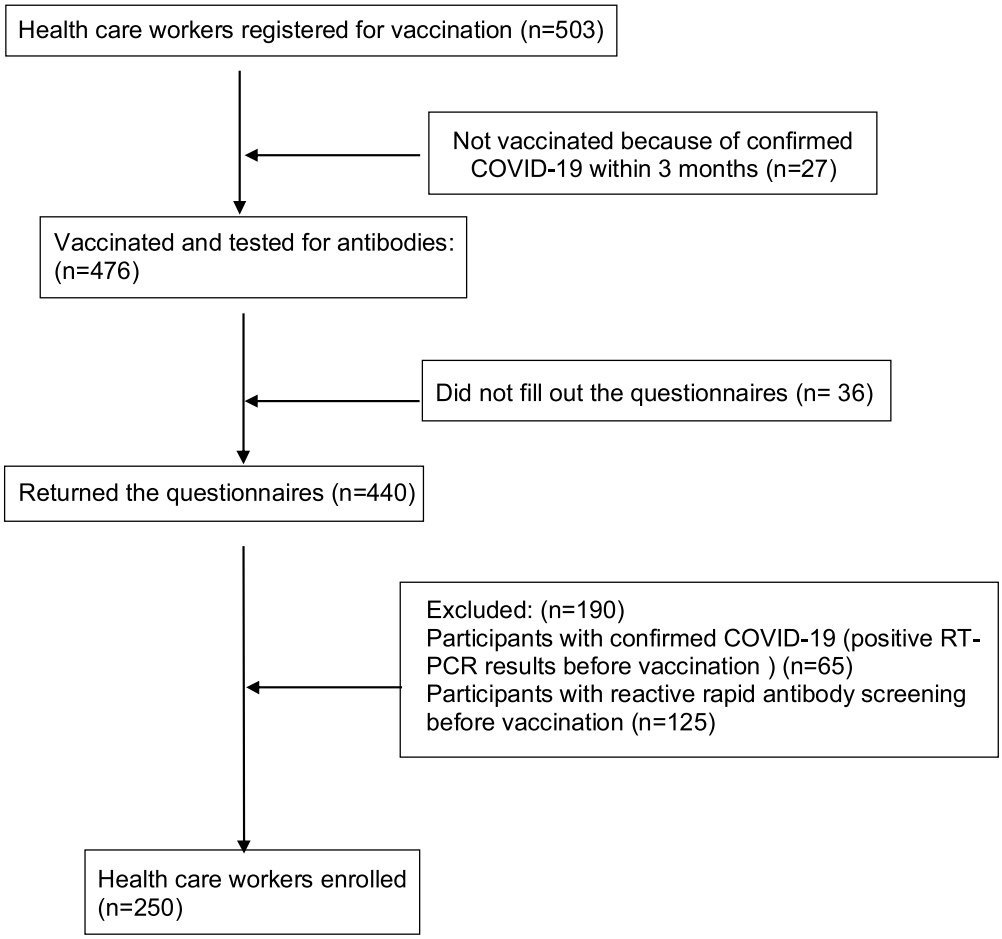

Figure 1. The overview of the research sampling process and participation rate

Table 1. Demographics of health care workers who participated in the study (N= 250).

Variables	n (%)
Age	
- ≤ 25 years old	60 (24.0)
- 26-39 years old	131 (52.4)
- ≥ 40 years old	59 (23.6)
Gender	
- Male	88 (35.2)
- Female	162 (64.8)
Body Mass Index	
- Underweight (≤18.5)	28 (11.2)
- Normoweight (18.51–22.99)	90 (36)
- Overweight (23–24.99)	48 (19.2)
- Obesity I (25–29.99)	65 (26)
- Obesity II (≥30)	19 (7.6)
Sleep Duration	
- <7 hours/day	144 (57.6)
- ≥7 hours/day	106 (42.4)
Physical Exercise	
- Not Routine	177 (70.8)
- Routine	73 (29.2)
1st Antibody titers, U/ml	63.58 (0.4 – >250)

Category variables: n(%)
Numeric variables: median (min-max)

When the bivariate analysis was performed to analyze the association between the first month antibody titer with age, there was a significant difference among antibody levels of the age groups ≤ 25 years, 26-39 years, and ≥ 40 years (64.1 U/ml, 85.1 U/ml, and 38.2 U/ml, respectively), Kruskal Wallis Test, p=0.003, <0.05). Antibody titer was also significantly different between men and women, which were higher in women (78.7 U/ml vs 49.6 U/ml respectively). Antibody titer was not significantly associated with BMI, sleep duration and exercise. (**Table 2**).

In addition, data about vitamin consumption of the participants were also collected. There was no association between vitamin D, C, and E consumption and antibody titer of the participants (p-value were 0.700, 0.270 and 0.223, respectively). We also collected the data of the participant's blood type and found no association between blood type and antibody

Table 2. Association of first month antibody titer with factors of age, sex, BMI, sleep duration and exercise (N=250).

	Antibody Titer (median, range, U/ml)	P Value
Age		
- ≤25 years old (n=60)	64.1 (0.7->250)	0.003 [a]
- 26-39 years old (n=131)	85.1 (1.02–>250)	
- ≥40 years old (n=59)	38.2 (0.4–>250)	
Gender		
- Male (n=88)	49.6 (0.4->250)	0.007 [b]
- Female (n=162)	78.1.02–>250)	
Body mass index		
- Underweight (≤18.5) (n=28)	44.1 (0.4–>250)	0.383 [c]
- Normoweight (18.5–22.99) (n=90)	58.29 (0.7–>250)	
- Overweight (23–24.99) (n=48)	70.65 (7.41->250)	
- Obesity I (25–29.9) (n=65)	90.9 (1.02–>250)	
- Obesity II (≥30) (n=19)	44.9 (7.73->250)	
Sleep duration		
- <7 hours/day (n=144)	62.2 (0.4–>250)	0.245 [b]
- ≥7hours/day (n=106)	64.5 (3.8–>250)	
Physical exercise		
- <3x/week (n=177)	64.7 (0.4–>250)	0.450 [b]
- ≥3x/week (n=73)	55.6 (0.7–>250)	

[a]Kruskall-Wallis test, Post-hoc Mann Whitney group <25 years vs group 26-39 years, p-value = 0.814, group 26-39 years vs. group >40 years, p-value = 0.001, group <25 years vs group >40 years , p value= 0.009
[b] Mann Whitney test
[c]Kruskall-Wallis test

titers of the participants (p=0.364). History of influenza vaccination also showed no significant association with the antibody titer (p=0.884).

In this study, the trend of antibody titer was observed in 3 months. The number of participants who were examined for the first antibody titer was 250, the second was 234 and the third was 200. Based on the results of 200 participants who completed the laboratory tests within 3 months, we identified that the antibody titer of 162 participants (81%) were gradually reduced. As many as 31 participants (15.5%) had increased antibodies, and 7 participants (3.5%) were stable. The median of the first antibody titer in 200 health care workers was 68.65 U/ml (1.02- >250 U/ml), the second one was 57.65 U/ml (6.13->250 U/ml), and the third one was 55.59 U/ml (4.80->250 U/ml). The reduction of median antibody titer from the first month until the third-month post-vaccination were 16% and 3.5%, respectively. However, the reduction was not statistically significant (Kruskal Wallis test, p=0.231). The trend of antibody titer reduction can be seen in **Figure 2**.

We observed the incidence of COVID-19 among participants within 4 months after completed vaccination, and after completing the monitoring of antibody titer trends (between

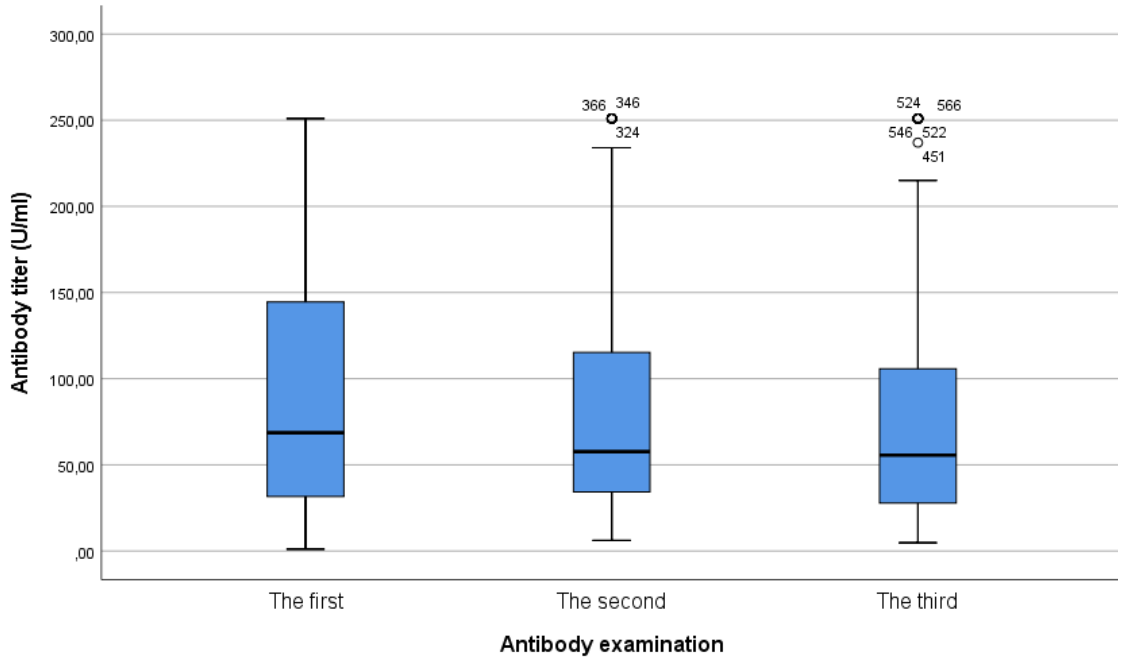

Figure 2. Reduction trend of antibody titer (n = 200).

June-July 2021). Five out of 250 participants were resigned from the hospital, leaving 245 remaining participants. The incidence of COVID-19 infections among the participants was 94 cases (38.3%): 9 cases (9.6%) were asymptomatic, 80 cases (85.1%) had mild symptoms, and 5 cases (5.3%) were admitted to the hospital due to moderate symptoms. We did not find any severe-symptom cases nor death cases among the infected participants. Thirty seven out of 94 infected participants (39.3%) had the first median antibody titer higher than baseline value (>63.58 U/ml), and 20 out of 37 participants still had high antibody titer 1 month before the infection.

DISCUSSION

The Anti-SARS-CoV-2 S-immunoassay is one the methods of serology testing which detects antibodies, including IgG antibody titers of Spike protein- Receptor Binding Domain (S-RBD) SARS-CoV-2.[20,23] Assessment of immunogenicity in clinical trials of COVID-19 vaccines were conducted using immunoassays that detect binding antibody titer, and also neutralizing antibody examination as the gold standard of functional antibody, which requires laboratory facilities with biosafety level 3.[7,10] The assays of S-RBD which detect antibodies against the S-protein of the SARS-CoV-2 virus have a strong correlation with neutralizing antibodies.[7,22,23] Due to the strong correlation between S-RBD and neutralizing antibody, the examination of antibody S-RBD titer is a good choice in further research on the immune response to vaccination, and may help to monitor the antibody response in patients after vaccination.

In Sinovac phase 1 and 2 clinical trials, seroconversion rates were defined as a change from seronegative at baseline to seropositive on day 14 after vaccination or a 4-fold increase antibody titer in those who were already seropositive.[8] The seroconversion rate of Sinovac vaccination in phase 2 clinical trial in China was 97%,[8] and in the interim report of phase 3 clinical trial in Indonesia was 99.7%.[6] In our study, we found that 99.2% of participants had detectable antibody on day 14 after the second Sinovac vaccination.

Previous studies have shown that antibodies to SARS-CoV-2 S-RBD would also increase after infection and could be detected until several months.[24,25] Although our study did not obtain antibody titer before the Sinovac vaccination, we tried to make sure that the antibody was not influenced by the past COVID-19 infection by excluding subjects who had been confirmed COVID-19 based on the history of RT-PCR results and subjects who had reactive rapid or serological tests before vaccination. Our hospital has already screened all health care workers routinely for early detection of COVID-19 with rapid serological tests and periodic RT-PCR examinations, so it was possible to exclude prior infection status of our participants.

Age has been shown to have a major influence on a person's response to vaccination. Vaccines response showed suboptimal effects in the elderly, who also have more rapid waning of antibodies.[13] Causes of a decreased immune response to vaccination include changes in cellular immunity, changes in humoral immunity, and the structure of lymphoid tissue.[26] Previous studies have shown a decrease in cellular and humoral immunity with age, thus affecting the response to vaccination.[13,26] Sinovac vaccine administration protocols are also different in those over 60 years of age, using a vaccination schedule of 0-28 days.[27] Research by Pellini et al[14] showed that the antibody titer after BNT162b2 (Pfizer-BioNTech) SARS-CoV-2 vaccination was related to age, i.e. young people had the highest antibody levels compared to other age groups. Research on health care workers in Israel after BNT162b2 (Pfizer-BioNTech) vaccination also showed that antibody levels decreased with age.[15] Our study showed similar results, that antibody titer post-Sinovac vaccination differed significantly according to age, which was higher in the age group 26-39 years.

Gender has been known to have different effects on immunity in general. Women tend to have antibody responses, basal immunoglobulin levels and B cell counts higher than men.[16] The causes of the different immune responses between women and men are related to the X chromosome which regulates more immune

functions, and the presence of hormonal factors that play a role in the regulation of the immune system. Gender has been known to produce differences in antibody titer after COVID-19 vaccination. Research on health care workers in Italy found that post-BNT162b2 vaccination, antibody levels in women were higher than in men.[14] Research by Jabal et al[15] on workers in Israel also showed significant differences in antibody levels post BNT162b2 vaccination in women and men. Our study results were in accordance with the two studies above that antibody titer in women was higher than men.

Systematic review from Zimmerman[13] stated that body mass index, sleep and exercise can be associated with immune response after immunization, but in our study, those factors were not associated with antibody titers after Sinovac vaccination. Body mass index has long been known to correlate with the immune response to vaccination due to some influences in the increased body fat and production of leptin.[13] According to Asia Pacific Criteria, body mass index is classified as underweight (<18.5), normoweight (18.5-22.9), overweight (23-24.9), and obese (>25).[28] Obesity is a product of biological and environmental influences that leads to an increase of excess adipose tissue, which correlates with an increase in debilitating conditions associated with increased morbidity and mortality.[29] The pro-inflammatory hormone leptin, which has many immunologic functions, has been shown to correlate with body fat mass since it is produced and secreted from adipocytes. Adipocytes also produce signaling molecules, such as TNFα, IL-6, and resistin, together with leptin induce a chronic state of inflammation.[29] The chronic inflammatory state has been shown to interfere with a proper vaccine-induced immune response through several mechanisms, including altered production of cytokines and T cells, diminished natural killer cell activity, and poor response to antigens, which leads to a dysregulated immune system.[29] So that, obesity may interfere with an obese individual's ability to mount an effective immune response to vaccination or infection due to increased body fat and increased leptin production.[29] Many kinds of researches show that there is a correlation between obesity and decreased vaccine-induced immune response for example hepatitis B vaccine, influenza, tetanus and rabies.[30,31] In our research, there was no correlation between antibody serum level and Body Mass Index. This finding is in accordance with the phase III trial of the SARS-CoV-2 vaccine from BNT162b2[32] and mRNA-1273[33], whereas no difference in immunogenicity between the normal BMI group and the obese group.

Studies have found that shorter sleep duration (measured naturally) was related to reduced influenza and hepatitis B vaccine response in healthy young and middle-aged adults.[17] Sleep on the night after experimental vaccinations against hepatitis A produced a strong and persistent increase in the number of antigen-specific T helper cells and antibody titer.[34] The impact of sleep on immune response after COVID-19 vaccination so far is still unknown. Data from the phase 3 trial COVID-19 vaccine from BNT162b2 did not show a clear role of sleep in modulating vaccine efficacy.[35] In our study, we did not find an association between sleep duration and antibody titer after Sinovac vaccination. So far we have not yet found any studies about association between sleep and Sinovac vaccination. Further study to investigate association between certain duration and sleep quality around the time of vaccination with antibody titer needs to be explored for further insight.

Exercise has been identified as a behavioral factor that can increase immune function, possibly acting as an adjuvant for the immune response after vaccination. A meta-analysis from Chastin et al[18] from 6 studies about the interventional effect of physical activity to the result of H1N1, H3N3, influenza B, pneumococcus, and Varicella zoster vaccination, concluded that routine moderate to high-grade physical activity (3 times a week, 60 minutes duration, within 20 weeks before vaccination) related with increase vaccine potency. However, there was a different result between young adult and old age population. The young adult had a lesser effect of exercise on immune response after vaccination compared to the older age.[36] Our study did not find any significant correlation between regular exercise and antibody titer

after vaccination. It was probably affected by the greater percentage of young age subjects compared to the old ones and we also did not know about the intensity (mild/moderate/high) or duration of the exercise. In the future, we need a specific study with a certain type and duration of exercise on the homogenous population to better elucidate the association.

Concerns had been raised about the factors that affect the immunogenicity of vaccines against SARS-CoV-2. Studies from different platforms of SARS-CoV-2 vaccines showed several common influencing factors on humoral response, that were assessed by antibody titer or neutralizing antibody. The publications which showed correlation between age, gender and body mass index with immunogenicity after SARS-CoV2 vaccination were summarized in **Table 3**.

The observation of antibody titer trend within 3 months duration in our study showed reduction tendency. This finding is in line with the interim report of clinical trial phase 3 of Sinovac vaccine in Bandung which showed a reduction trend of IgG seropositive rate and neutralization antibody until 44.1% within 6 months after the second dose of Sinovac vaccination.[6] The interim report of phase 2 CoronaVac trial in Jiangsu, China, also showed that neutralizing antibody titer induced by the first two doses declined after 6-8 months to below the seropositive cutoff.[41] Prior shreds of evidences that showed waning antibody titers indicate that SARS-CoV-2 vaccines induced humoral immunity might not be as durable as that of other virus vaccines. But some studies showed that antigen-specific CD4 and CD8 T cells responses had an association with reduced disease severity while neutralizing antibody titer did not.[7,10-12]

Table 3. Summary of the previous studies about association between age, gender and body mass index with immunogenicity after SARS-CoV-2 vaccination.

Study	Methods/Samples	Results
Obesity May Hamper SARS-CoV-2 Vaccine Immunogenicity Pellini R et al, 2021.[14]	248 Health Care Workers (HCW) antibody titres ,7 days after second dose of BNT162b2 (Pfizer)	Higher antibody in female, young age and lean body weight.
Impact of Age,Ethnicity,Sex,and Prior Infection Status on Immunogenicity Following A Single Dose of The BNT162b2 mRNA COVID-19 vaccine: Real-World Evidence from Health Care Workers, Israel, December 2020 to January 2021 Jabbal et al, 2021.[15]	514 HCW antibody titres, 21 days after first dose of BNT162b2 (Pfizer)	Reduction of antibody titer with increasing age. No correlation of antibody titer with sex.
Assesment of Factors Affecting Inactivated COVID-19 (CORONAVAC) Vaccine Response and Antibody Response in Healthcare Proffesionals Ozdemir HO et al, 2021.[37]	264 HCW antibody titres, 28 days after second dose of CoronaVac	Lower immunogenicity in advanced age and male
Safety and Immunogenicity of An Inactivated SARS-CoV-2 Vaccine, BBIBP-CorV: A Randomized, Double-Blind, Placebo-Controlled, Phase 1/ 2 Trial.Xia S, et al.[38]	320 participants antibody titers, day 0, day 28, and day 56 after first dose of BBIBP-CorV (Sinopharm) a randomised, double-blind, placebo-controlled trial	People age 60 and above produced significantly fewer antibody than those aged 18-59.
Antibody Persistence Through 6 Months After The Second Dose of mRNA-1273 Vaccine for COVID-19 Doria-Rose N, et al.[39]	33 participants neutralizing antibodies at 180 days after second dose of mRNA-1273 (Moderna)	Age group 18 to 55 had higher antibody titer compared to older age groups
Safety and Immunogenicity of ChAdOx1 nCoV-19 Vaccine Administered in A Prime-Boost Regimen in Young and Old Adults (COV002) Ramasamy et al.[40]	560 participants neutralizing antibodies at 28 days after second dose of ChAdOx1 (Astra-Zeneca) A single-blind, randomized, controlled trial	The neutralizing Antibody titer did not differ significantly between the vaccinated population aged 18 to 55 years and those over 55 years of age.

In our study, we had 200 subjects who had 3 complete antibody titer data within 3 months period, 162 of them (81%) showed a reduction of antibody titer. The titer on the third examination showed a 19% reduction compared to the first one (55.59 U/ml vs 68.65 U/ml). Although we found the downward trend, it was statistically insignificant, probably due to the short period of observation. Thus, a study with a longer period of observation is needed policy makers about the timing of vaccine boosters in the community.

The observation about COVID-19 among our participants showed that vaccination protected 61.7% of participants from infections. This finding was similar to a prospective national cohort study in Chile, which also used Sinovac in a large number of participants, which had 65.9 % adjusted vaccine effectiveness.[42] Vaccination in our study also prevented 94.7% of infected participants from being hospitalized (only had mild or no symptoms at all), whereas the study in Chile had 87.5 % for the prevention of hospitalization and 90.3 % prevention of ICU admission. Data on the hospitalized participants in our study revealed no severe-symptom cases or death (0%) and no ICU admission. It shows that although there are many persons infected with COVID-19 after completed vaccination, it still has protective effects on morbidity and mortality, which is in line with the findings of a study in Chile.[42]

In order to reveal the variant of virus which infected participants, we did a whole-genome sequencing of 3 participants' samples (2 with moderate symptoms and 1 with mild symptoms). The results showed that three of them were infected by SARS-CoV-2 delta variants. Delta variants which were initially detected in India have been predominant in Indonesia, as the COVID-19 surged up since June 2021. Indonesian Ministry of Health reported that until 7 August 2021, there were 1477 cases of new SARS-CoV2 variants, whereas Delta variants were predominant with 1368 cases.[43] From our study, we thought that SARS-CoV-2 delta variants might reduce Sinovac vaccine efficacy which impacts on many infections among our participants.

The strengths of our study are that we used the Elecsys Anti-SARS-CoV-2 assay which its unit of measurement is equivalent to WHO International standard BAU (Binding Arbitrary Unit per mL) and our participants were selectively screened to be free from COVID-19. However, this study had some limitations. Firstly, that we used the assay without dilution (for commercial purposes, budget restriction) which could only detect the highest titer up to 250 U/ml. Other limitations are: the observation period of antibody titer trend is not long enough and we only did whole-genome sequencing to 3 of our subjects who got COVID-19 after completed vaccination due to access and time limitation.

This study showed the immune response of health care workers who have been vaccinated with 2 doses of Sinovac vaccine in the real world, outside clinical trials which have been done in Indonesia. To the authors' knowledge, there has not yet been any published Indonesian study about SARS-CoV2 antibody titer and incidence of COVID-19 after competed Sinovac vaccination. We got the basic data of antibody titer after vaccination which can be used as a comparison for other research in the future. Up till now, the optimal protective value of antibody against SARS-CoV-2 is still unknown, many studies with a bigger scale and longer duration are needed to reveal it.

CONCLUSION

The highest median antibody titer occurs 14 days after the Sinovac vaccination (63.58 U/ml). Age group 26-39 years and women are associated with the higher antibody titer. The antibody titer within 3 months after vaccination is not significantly reduced in all participants. COVID-19 incidence post-vaccination is quite high but the hospitalization rate is low with no mortality, which shows that Sinovac vaccination still has a protective effect.

CONFLICT OF INTEREST

The authors report no conflict of interest

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REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
2. World Health Organization. The COVID-19 candidate vaccine landscape. [Internet]. [Cited2021August6]. From: https://www.who.int/publications/m/item/draft-landscape-of-COVID-19-candidate-vaccines.
3. Palacios R, Patino E, Piorelli MD, et al. Double-blind, randomized, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of treating healthcare professionals with the adsorbed COVID-19 (inactivated) vaccine manufactured by Sinovac – PROVISCOV: A structured summary of a study protocol for a randomized controlled trial. Trials. 2020;21:853.
4. Tanriover MD, Dogonay HL, Akova M, et al. Efficacy and savety of an inactivated whole-virion SARS-CoV2 Vaccine (CoronaVac): interim results of a double-blind randomized, placebo-controlled, phase 3 trial in Turkey. Lancet. 2021;398:213-22.
5. Bueno SM, Abarca K Gonzales PA, et al. Interim report: safety and immunogenicity of an inactivated against SARS-CoV2 in healthy Chilean adults in a phase 3 clinical trial. [Internet]. MedRxiv [preprint] 2021. [Cited 2021 July 14]. Available from https://doi.org/10.1101/2021.03.31.21254494.
6. Rusmil K. Summary Final Report A Phase III, Observer-blind, Randomized, Placebo-controlled Study of the Efficacy, Safety and Immunogenicity of SARS-CoV2 inactivated vaccine in Healthy Adults Aged 18-59 Years Old in Indonesia. Interim report. In: Hadinegoro SR. Webinar Imunisasi Booster bagi Tenaga Kesehatan dan Tenaga Medis, July 14, 2021.
7. Chakraborty S, Mallajosyula V, Tato CM, Tan GS, Wang TT. SARS-CoV-2 vaccines in advanced clinical trials: Where do we stand? Adv Drug Deliv Rev. 2021;172: 314–38.
8. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21:181-92.
9. Satuan Tugas Penanganan COVID-19. Badan POM Keluarkan Ijin Penggunaan Darurat (EUA) untuk vaksin COVID-19 produksi Sinovac. 11 Januari 2021. [Internet] [Cited 2021 March 20]. Available from https://covid19.go.id.
10. Grigoryan L, Pulendran B. The immunology of SARS-CoV-2 infections and vaccines. Semin Immunol. 2020;50:101422. Available from: https://doi.org/10.1016/j.smim.2020.101422.
11. Lippi G, Sciacovelli L, Trenti T, Plebani M. Kinetics and biological characteristics of humoral response developing after SARS-CoV-2 infection: implications for vaccination. Clin Chem Lab Med. [internet] 2021 [cited 2021 Aug 20]. Available from: https://doi.org/1I0.1515/cclm-2021-0038.
12. He Q, Mao Q, Zhang J, et al. COVID-19 vaccines: Current understanding on immunogenicity, safety, and further considerations. Front Immunol. 2021;12:669339.
13. Zimmerman P, Curtis N. Factors that influence immune response to vaccination. Clin Microbiol Rev. [internet] 2019 [Cited 2021 Jul 8]; 32:e00084-18. Available from: https://doi.org/10.1128/CMR.00084-18.
14. Pellini R, Venuti A, Pimpinelli F, et al. Obesity may hamper SARS-CoV2 vaccine immunogenicity. [Internet]. medRxiv [preprint] 2021 [Cited 2021 Nov 25]. Available from: https://doi.org/10.1101/2021.02.24.21251664. Posted February 26, 2021.
15. Jabal KA, Ben-Amram H, Beiruti K, et al. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Euro Surveill. 2021;26(6):pii=2100096. Available from: https://doi.org/10.2807/1560-7917.ES.2021.26.6.2100096. Published February 11, 2021.
16. Klein SL, Flanagan K. Sex differences in immune respons. Nature. [Internet] [Cited 2021, August 12] 2016; 16:626-38. Available from: doi:10.1038/nri.2016.90. Published online 22 Aug 2016.
17. Taylor D, Kelly K, Kohut ML. Is insomnia a risk factor for decreased influenza vaccine response? Behav Sleep Med. 2017;15(4):270–87. doi:10.1080/15402002.2015.1126596.
18. Chastin SFM, Abaraougu U, Bourgois JG, et al. Effects of regular physical activity on the immune system, vaccination and risk of community-acquired infectious disease in the general population: Systematic review and meta-analysis. Sports Med. 2021;51(8):1673-86.
19. Von Elm E, Altman DG, Egger M, et al. The strengthening the eporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Medicine.

2007;4(10)e296.
20. Roche Diagnostic. Elecsys Roche. Anti-SARS-CoV2-S. [Internet]. [Cited 2021 August17]. Available from: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html.
21. World Health Organization, Expert Committee on Biological Standarization, 2020. Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV2 antibody. WHO/BS/2020-2403. [Internet]2020. [cited 2021 August 9] Available at: https://www.who.int/publications/m/item/WHO-BS-2020.2403.
22. Huilier AG, Meyer B, Andrey DO, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clin Microbiol Infect. 2021: 27:784e1-8.
23. Higgins V, Fabros A, Kulasingam V. Quantitative measurement of Anti-SARS-CoV-2 antibodies: analytical and clinical evaluation. J Clin Microbiol. 2021;59(4):e03149-20.
24. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science. 2020;370:1227–30.
25. Ni L, Ye F, Cheng M, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity. 2020;52: 971–7.
26. Crooke SN, Ovsyannikova IG, Polland GA, Kennedy NG. Immunosenescence and human vaccine immune responses. Immun ageing. 2019;16:25. Available from https://doi.org/10.1186/s12979-019-0164-9.
27. Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. [Internet] 2021 [cited 2021 August 9]; S1473-3099(20)30987-7. Available from https://doi.org/10.1016.
28. Annurad E, Shiwaku K, Nogi A. The new BMI criteria for Asians by the regional office for the western pacific region of WHO are suitable for screening of overweight to prevent metabolic syndrome in elder Japanese workers. J Occup Health. 2003;45:335-43.
29. Scott Painter SD, Ovsyannikova I, Polland GA. The weight of obesity on the human immune response to vaccination. Vaccine. 2015;33(36):4422–9. doi:10.1016/j.vaccine.2015.06.101.
30. Liu F, Guo Z, Dong C. Influences of obesity on the immunogenicity of Hepatitis B vaccine. Hum Vaccin Immunother. 2017;3(5):1014–7.
31. Sheridan PA, J Handy, Karlsson EA. Obesity is associated with impaired immune response to influenza vaccination in humans. Int J Obes. 2012;36:10727.
32. Pollack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. N engl J Med. 2020;383(27):2603-15. Doi:10.1056/NEJMoa2034577.
33. Baden LR, Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV2 vaccine. N Engl J Med. 2021;384(5):403-16.
34. Besedovsky L, Lange T, Bojn J. Sleep and immune function. Eur J Physiol. 2012;463:121-37.
35. Benedict C, Cadernaes J. Could a good night's sleep improve COVID-19 vaccine efficacy? Lancet. 2021;9:447-8.
36. Edward KM, Booy R. Effects of exercise on vaccine-induced immune responses. Hum Vaccin Immunother. 2013;9:907–10.
37. Ozdemir HO, Tosun S, Coskuner SA, Demir S. Assessment of factors affecting inactivated COVID-19 (CoronaVac) vaccine response and antibody response in healthcare professionals. [internet]. ReseachGate [Preprint]. 2021. [cited 2021 Nov 25]: 14 P. Available from: https://doi.org/10.21203/rs.3.rs-506854/v1.
38. Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomized, double-blind, placebo-controlled, phase 1/ 2 trial. Lancet Infect Dis. 2020;21:39-51.
39. Doria-Rose N, Suthar MS, Makowski M, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for COVID-19. N Engl J Med. 2021;384:2259-61.
40. Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): A single-blind, randomized, controlled, phase 2/ 3 trial. Lancet. 2021;396:1979-93.
41. Pan H, Wu Q, Zeng G, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. [Internet]. MedRxiv. [Cited 2021, August 9]. Available from https://doi.org/10.1101/2021.07.23.21261026.
42. Jara A, Undurraga EA, Gonzales C, et al. Inactivated SARS-CoV-2 vaccine effectively prevented Covid 19, including severe disease and death, supporting implementation of mass vaccination campaigns. N Engl J Med. 2021;385:875-884.
43. Kompas. Sebaran variant Alpha, Beta dan Delta di Indonesia hingga 7 Agustus 2021. [Internet] 2021 August 8; [cited 2021 August 9]. Available from: https://nasional.kompas.com/read/2021/08/09/16390581/sebaran-varian-alpha-beta-dan-delta-di-indonesia-hingga-7-agustus-2021?page=all.

Elevation of Cardiac Biomarkers in COVID-19 As a Major Determinant for Mortality: A Systematic Review

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ABSTRACT

Aim: *To summarize the prognosis of Corona Virus Disease 2019 (COVID-19) patients with elevated troponin and N-terminal pro brain natriuretic peptide (NT-proBNP) levels and demonstrate the involvement of myocardial injury as a complication in COVID-19.* ***Methods:*** *A systematic literature search was performed using several databases (PubMed, MEDLINE, PROQUEST and SCOPUS) for studies published up to August 2020. Observational studies about the mortality outcome of COVID-19 patients who experienced cardiac injury, as defined by the elevation of serum levels of troponin, brain natriuretic peptide (BNP), with NT-proBNP or only BNP or only NT-proBNP, were included. In addition, a critical appraisal was conducted for all included studies using the Critical Appraisal for Prognostic Studies checklist published by the Centre for Evidence-Based Medicine by the University of Oxford.* ***Results:*** *Seven retrospective observational studies fulfilled the inclusion criteria. This study found that there is a higher risk of death in COVID 19 patients with higher levels of troponin and NT-proBNP, indicating the importance of these biomarkers as determinant factors to predict in-hospital deaths.* ***Conclusion:*** *Based on the analysis, elevation of troponin and NT-proBNP levels plays an essential role in determining the patient prognosis because it is shown to be associated with in-hospital mortality. This also supports the involvement of myocardial injury as a prominent fatal complication in COVID-19.*

Keywords: *COVID-19, myocardial injury, troponin, BNP, NT-proBNP, prognostic factors.*

INTRODUCTION

In early 2020, a pandemic state was reported in relation to the coronavirus disease-2019 (COVID-19), a novel strand of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). [1,2] By August 21, 2020, the global number of confirmed cases had reached more than 4 million, with over 780,000 deaths having occurred in 213 different countries.[3]

Although the major complication of COVID-19 infection is respiratory failure, there have been reports of myocardial injury defined by elevated biomarker values in patients, with troponin I as the main biomarker used.[1,4-8] The use of troponin I is based on its unique regulatory protein encoded by a specific gene that is only found in the myocardium, making it a fundamental aspect to clinically define the diagnosis of myocardial injury.[9-10] Other biomarkers, such as troponin T (TnT), brain natriuretic peptide (BNP), and N-terminal brain natriuretic peptide (NT-proBNP), are also used to further support the determination of any myocardial damage.[11]

To date, the most plausible cause of the myocardial injury found in COVID-19 is the presence of ACE2 receptors in the myocardium; these receptors allow the binding of SARS-Cov2 structural protein, leading to a direct viral infection of the heart. Furthermore, infection-mediated vasculitis by COVID-19 may also contribute to causing direct myocardial injury because of the ACE2 receptor expressions in the arterial and venous endothelial cells. Both events may lead to an indirect immunological response resulting in a hypersensitivity reaction, which manifests as the myocardial injury and dysfunction seen in COVID-19. Such occurrences often lead to a fatal outcome.[1]

Many studies have found that intensive care unit (ICU) admissions and in-hospital mortality may also be associated with elevated biomarkers, further supporting the essential determinant factor of patients' prognosis in clinical settings.[12] Thus, many clinicians have drawn their attention to the role of troponin and NT-proBNP elevation in determining the prognosis of patients with COVID-19.

METHODS

As a foundation for performing an extensive literature search, this study chose a relevant clinical question using a patients/intervention/comparison/outcome (PICO) model to define suitable terms for use in the search. The chosen terms derived from the following question: *Could the elevation of troponin and BNP/NT-proBNP determine the prognosis of patients with COVID-19 myocardial injury?* The selected keywords comprised of P (patients), I (intervention) and O (outcome), whereas C (comparison) was not considered as the main focus and therefore, excluded (**Table 1**).

We used the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines for this review. A literature search was performed using four databases (PubMed, MEDLINE, PROQUEST and SCOPUS) from April 24 to August 26, 2020, using a combination of Medical Subject Headings (MeSH) terms based on the PICO keywords derived from the clinical question (**Table 2**). Only longitudinal cohort studies or randomized controlled trials that observed death as the main outcome for COVID-19 patients with elevated NT-proBNP and/or troponin were included in this study.

Table 1. PICO.

Patient (P)	Intervention (I)	Comparison (C)	Outcome (O)
COVID-19 Myocardial Injury Patients	Elevation of Troponin and BNP/NTpro-BNP	-	Mortality/Death Case

BNP/NTproBNP, Brain Natriuretic Protein/N-terminal pro brain natriuretic peptide ;COVID-19, Coronavirus Disease 2019;

Table 2. Literature search strategy.

Database	Search Terms	Hits
PubMed	(((((("COVID-19"[Title/Abstract]) OR (CORONAVIRUS[Title/Abstract])) AND (MYOCARDIAL INJURY [Title/Abstract]) AND ((TROPONIN[Title/Abstract]) OR (BNP [Title/Abstract]) OR (NT-PROBNP[Title/Abstract])) AND (MORTALITY [Title/Abstract])	178
PROQUEST	((COVID-19) OR (CORONAVIRUS)) AND (MYOCARDIAL INJURY) AND ((TROPONIN)) OR (BNP) OR (NT-PROBNP)) AND (MORTALITY)	45
SCOPUS	((COVID-19) OR (CORONAVIRUS)) AND (MYOCARDIAL INJURY) AND ((TROPONIN)) OR (BNP) OR (NT-PROBNP)) AND (MORTALITY)	17
Medline	((COVID-19) OR (CORONAVIRUS)) AND (MYOCARDIAL INJURY) AND ((TROPONIN)) OR (BNP) OR (NT-PROBNP)) AND (MORTALITY)	215
Total		455

Critical Appraisal

A critical appraisal was conducted thoroughly by the author for all included studies using the Critical Appraisal for Prognostic Studies checklist published by the University of Oxford (www.cebm.net). Any uncertainties for inappropriate topics was then consulted to the second reviewer. Study design, patient demographics, value changes in troponin and BNP and/or NT-proBNP value, mortality rate, risk ratio/odd ratio, hazard ratios and other outcomes from adjusted statistical (if stated) were extracted from the included studies for further analysis.

RESULTS

Search Selection

A total of 455 papers were identified from the four databases. After the deduplication process, 450 different papers were screened based on their titles and abstracts (**Figure 1**). As a result, 216 articles were excluded from the initial screening, leaving 234 articles to undergo a full review and further selection process. Seven articles were selected as the final sample for this study. A summary of the selection process according to the PRISMA statement can be seen in the PRISMA diagram on **Figure 1**.[13]

The characteristics of the baseline study can be seen in **Table 3**. All chosen studies varied from a single-center or multicenter observational cohort and observed the relationship between the elevation of myocardial biomarkers of COVID-19 patients and the mortality outcome. Zhou et al[14]., Chen et al.[15], and Stefanini et al.[16] focused on the association of patients' clinical outcomes with troponin and NT-proBNP as myocardial markers. Guo et al.[17], Gao et al.[18], Zhang et al.[19], and Shi et al.[20] included other myocardial injury biomarkers, such as Creatinine Kinase Myocardial Band (CKMB), myohemoglobin, or myoglobin. These studies underwent critical appraisal as presented in **Table 4**.

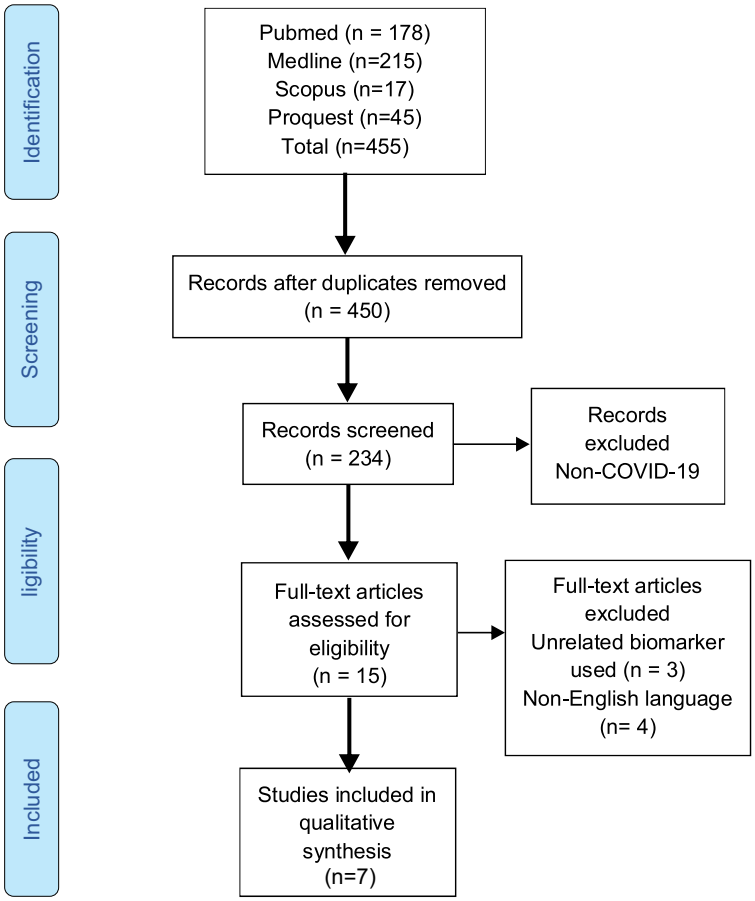

Figure 1. Prisma Flow Diagram of search and selection process

Table 3. Characteristic of included studies

Author	Institution/ Country Study Conducted	Design	Inclusion Criteria	Exclusion Criteria	Participants	Myocardial biomarker tested	Other biomarker tested	Primary endpoints	Secondary endpoint(s)	Results
Zhou et al	Jinyintan Hospital and Wuhan Pulmonary Hospital (Wuhan, China	Retrospective Cohort Study	Confirmed COVID-19 inpatients who died or discharged between 29th Dec 2019 and 31st Jan 2020	Non COVID-19 Inpatients, Patients without accessible medical records	191 Patients	Hs-cTnI	White blood cell, Lymphocyte count, Haemoglobin, Platelet count, Albumin, ALT, Creatinine, Lactate dehydrogenase, Creatinine kinase, serum ferritin, Il-6, Procalcitonin, , Prothrombin time, d-dimer	Death, Discharged	N/a	Hs-cTnI : 3.0 pg/ml survivor (54/191) vs 22.2pg/ml non survivor (137/191) OR Hs-cTnI 80.07 (CI 10.34 – 620.36)
Guo et al	Seventh Hospital of Wuhan City, China	Retrospective Cohort Study	Confirmed COVID-19 inpatients who died or discharged between 23rd Jan 2020 and 23rd Feb 2020	COVID-19 inpatients without a complete medical record	187 Patients	Troponin T, CKMB fraction, Myoglobin, NT-proBNP	White blood cell, Lymphocyte count, Neutrophil, Albumin, ALT, Creatinine, Aminotransferase (alanine, aspartate), hsCRP, Globulin, Procalcitonin, Prothrombin time, d-dimer, APTT, Cholesterol (total, triglyceride, HDL, LDL), Serum potassium, Serum Calcium	Death, Discharged	N/a	Mortality in Normal TnT (12[8.9%]) vs Elevated TnT (31[59.6%]) [p<0.001] Both TnT and NT-proBNP levels increased significantly during the course of hispitalization in those who ultimately died [p<0.001], but no such dygnamic changes in those biomarkers were evident in survivors
Shi et al	Renmin Hospital of Wuhan Universit	Retrospective Cohort Study	Confirmed COVID-19 inpatients between 20th Jan 2020 and 10th Feb 2020	COVID-19 inpatients without cardiac biomarkers data	416 Patients	Hs-cTnI, NT-proBNP, CKMB, NT pro-BNP, Myohemoglobin	Leukocyte, Lymphocyte, Platelet, Erythrocyte, Haemoglobin, C-reactive protein, Procalcitonin, Creatinine, Aminotransferase (alanine, aspartate), Serum potassium, Serum Calcium	Death, discharged, remained in hospital	N/a	Hs-cTnI : cardiac injury (0.19[0.08-1.12] p<0.001) vs without (<0.006) [<0.006-0.009] p<0.001] Death of Patient with cardiac injury (42/82) vs without (15/334) [p<0.001]

										Discharged patients with cardiac injury (2/82) vs without (38/334) [p<0.001] Patient remained in the hospital with cardiac injury (38/82) vs without (281/334) [p<0.001]
Chen et al	Tongji Hospital, Wuhan, China	Retrospective Cohort Study	Confirmed COVID-19 critically ill inpatients between 13th Jan 2020 and 28th Feb 2020	Not specified	274 Patients	Hs-TnI, NT-proBNP	White blood cell count, Neutrophil, Lymphocyte, Monocyte, Platelet, Haemoglobin, C-reactive protein, Procalcitonin, Creatinine, Aminotransferase (Alanine, Aspartate), Total Bilirubin, Alkaline phosphatase, gamma glutamyl transpeptidase, Triglycerides, Serum potassium, Serum Calcium, Blood Urea Nitrogen, Creatine Kinase, Lactate dehydrogenase, Prothrombin time, activated partial thromboplastine time, D-dimer, ferritin, erythrocyte, thyroid stimulating hormone, free triiodothyroxine, free thyroxin, Immunoglobulin (A,G,M), C3, C4, Il-1B, IL-1 receptor, Il-6, Il-8, Il-10, Tumor necrosis factor alpha, Urinary protein, Urinary Occult blood	Death, Recovered	N/a	Elevated Hs-TnI in deceased patients (68/94 [72%]) vs recovered patients 15/109[14%] Elevated NT-proBNP in deceased patients(68/80 [85%]) vs recovered patients (17/93 [18%])

Gao et al	Hubei General Hospital, China	Retrospective Observational Study	COVID-19 patients with severe conditions (respiratory rate ≥ 30/ min or rest oxyhemoglobin saturation (SPO2) ≤93% or oxygenation index (arterial oxygen tension/ inspired oxygen fraction, PaO2/ FiO2) ≤300 mmHg)	Patients lacking NT-proBNP results, Patients with stroke, acute myocardial infarction, malignant tumor, and pregnancy	54 participants	N-terminal pro-B-type natriuretic peptide (Nt-proBNP), High sensitive troponin I (Hs-TnI), Creatinine kinase-myocardial band (CKMB)	Myohaemoglobin, Urea, Creatinine, White blood cell count, Lymphocyte, CRP, Procalcitonin	Death, survived	N/a	NT-proBNP Area under the curve (AUC) for in-hospital mortality was 0.909 (95%CI 0.799–0.970, P < 0.001). using the cut-off 88.64 pg/mL	
Zhang et al	Wuhan No.1 Hospital, China	Retrospective Observational Study	Confirmed or Suspected COVID-19 inpatients admitted between 25th Dec 2019 and 15th Feb 2020, who underwent hs-cTni test within 48 hours after admission	Non COVID-19 Inpatients, did not undergo hs-cTnI within 48 hours after admission	48 patients	High sensitive troponin I (Hs-cTnI), Creatinine kinase-myocardial band (CKMB)	White blood cell count, Lymphocyte count, Neutrophil, Haemoglobin, Platelet, Thrombocyte Creatinine kinase, Aminotransferase (alanine, aspratate), Lactate dehydrogenase, serum creatinine, CRP, Fibrogen, d-dimer	Death, survived	Discharged, Transferred	Mortality: patients with elevated hs-cTnI 76.9% vs normal hs-cTnI 20% [p<0.001]	
Stefanini et al	Humanitas Clinical and Research Hospital (Rozzano-Milan, Lombardy, Italy)	Retrospective Observational Study	confirmed COVID-19 patients with available data on cardiac biomarkers	No exclusions were applied	397 patients	High-sensitivity troponin I (hs-TnI), B-type natriuretic peptide (BNP)	D-dimer, Fibrinogen, CRP, IL-6, Ferritin, Creatinine, White cell count, Neutrophils, Lymphocytes, Platelet baseline, Haemoglobin baseline, Procalcitonin, ALT, ALP, Total bilirubin baseline, PT ratio baseline, PTT ratio baseline, INR baseline	N/a	all-cause mortality	admission in intesive care unit (ICU), acute respiratory distress syndrome (ARDS) and shock	Mortality rate: elevated hs-TnI (22.5%, OR 4.35 [95% CI 1.72-11.04]) vs elevated BNP (33.9%, OR 7.37,

[95%CI 3.53 to 16.75]) vs both (55.6%, OR 18.75, [95%CI 9.32 to 37.71]) vs without elevated cardiac biomarkers (6.25%).

ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; ARDS, Acute Respiratory Distress Syndrome; CI, Confidence Interval; CKMB, Creatinine kinase-myocardial band; COVID-19, Coronavirus Disease 2019; CRP, C-Reactive Protein; hs-cTnI, high sensitivity cardiac troponin I; INR, International Normalized Ratio; ICU, Intensive Care Unit; IL, Interleukin; NT-proBNP, N-terminal pro brain natriuretic peptide; OR, Odds Ratio ; N/A, Not Applicable; PT, Ptothrombin Time; PTT, Partial prothrombin time; TnT, Troponin T.

Table 4. Critical Appraisal of the included studies using Prognosis Studies Questionnaire of Oxford CEEBM

	Criteria	Shi et al (2020)	Guo et al (2020)	Zhang et al (2020)	Zhou et al (2020)	Gao et al (2020)	Chen et al (2020)	Stefanini et al (2020)
Validity	**Was the defined representative sample of patients assembled at a common (usually early) point in the course of their disease**	Yes. Recruited participants were patients confirmed with COVID 19 according to WHO interim guidance and originated from Wuhan	Yes. Recruited participants were patients confirmed with COVID 19 according to WHO interim guidance and originated from Wuhan	Unclear. Recruited participants were patients suspected and confirmed with COVID 19 according to WHO interim guidance and originated from Wuhan. All patients who underwent hs-cTnI test within 48 hours after admission were included	Yes. Recruited participants were patients confirmed with COVID 19 according to WHO interim guidance and originated from Wuhan	Yes. Recruited participants were patients confirmed with COVID 19 according to WHO interim guidance and originated from Wuhan	Yes. Recruited participants were patients confirmed with COVID 19 according to Guidance for Corona Virus disease 2019 by the national health commission of China and originated from Wuhan	Yes. Yes. Recruited participants were patients confirmed with COVID 19 according to WHO interim guidance and originated from Italy
	Was patient follow-up sufficiently long and complete	Yes	Yes	Yes	Yes	No. biomarkers were only collected on a single test at admission	Yes	Yes
	Were outcome criteria either objective or applied in a 'blind' fashion?	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Unclear
	If subgroups with different prognoses are identified, did adjustment for important prognostic factors take place?	Yes. Age, comorbidities, creatinine levels and pro-BNP were taken account into the analysis	No	Yes. Age, Spo2, serum creatinine, and d-dimer value were taken account into the analysis	No	Yes. Sex, age, hypertension, coronary heart disease, myoglobin, CK-MB, troponin I, urea, creatinine, white blood cell, lymphocyte and procalcitonin is taken account into the analysis	No	Yes. Age, lymphocyte counts and D-dimer elevation were taken account into the analysis

Importance	**What were the results?**	Mortality rate was 51.2% among patients with cardiac injury.	Mortality rate was 59.6% among patients with cardiac injury.	Mortality rate was 76.9% among patients with cardiac injury.	Univariate OR of in-hospital death with elevation of hs-cTnI value 80.07	The AUC for in-hospital death was 0.909	Mortality rate was 72% and 85% in patients with increased troponin and Nt-proBNP concentrations consecutively	The AUC for all-cause mortality predictor is 0.938
	How precise are the prognostic estimates?	HR of death time from symptom onset was 4.26 [95% CI, 1.92-9.49] HR of death time from admission to study end point 3.41 [95% CI, 1.62-7.16]	Unclear (no CI was stated)	HR of death in elevation of hs-cTnI value 10.9 [95% CI, 1.28 -92.93]	95% CI, 10.34-620.36	95%CI, 0.79-0.97	Unclear (no CI was stated)	95%CI 1.06 to 9.93
Applicability	**Can I apply this valid, important evidence about prognosis to my patient?**	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Using a multivariable adjusted Cox proportional hazard regression model, Shi et al.[21] reported a hazard ratio of death of 3.4 in patients with cardiac injury (95% confidence interval [CI], 1.62–7.16). Zhang et al.[19] reported a hazard ratio of death among patients with high-sensitivity cardiac troponin I (hs-cTnI) elevation of 10.902 (95% CI, 1.279–92.927). Zhou et al.[14] calculated the odds ratio of death in COVID-19 patients with elevated hs-cTnI levels with a univariate regression analysis, and they reported a value of 80.07 (95% CI, 10.34–620.36).[19] Guo et al.[17] reported a higher mortality rate in patients with elevated serum troponin T levels (59.6%) than in those with normal levels (8.9%).

A single-center study conducted by Gao et al.[18] stated there is a significantly higher mortality rate in COVID-19 patients with high NT-proBNP compared with those with lower values (with a cut-off of 88.64 pg/ml); shown by the area under the curve (AUC) value of 0.909 (95% CI, 0.799–0.97) and hazard ratio of 1.373 (95% CI, 1.118–1.586, $p<0.001$) after adjusting for other risk factors (i.e sex, age, hypertension, coronary heart disease, myoglobin, CKMB, hs-TNI, urea and creatinine value. Both Chen et al.[15] and Stefanini et al.[16] reported higher concentrations of hs-cTnI (78% and 22.5%, respectively) and NT-proBNP markers (85% and 33.9%, respectively) in deceased patients compared with those who survived.

In deceased patients (85% and 33.9%, respectively) compared to those who survived.[15]

DISCUSSION

The World Health Organization (WHO) declared COVID-19 a global pandemic in early 2020.[2] This novel strain of coronavirus disease has various clinical manifestations: It can be asymptomatic in some people or result in a severe condition, with acute respiratory distress syndrome (ARDS) or other organ failures, in others.[3] Recent reports have shown evidence of myocardial injury involvement in COVID-19 patients, supported by the finding of elevated biomarkers, abnormal echocardiograph, and electrocardiograph in some hospitalized patients.[1-2,,6] Outcomes of patients with such conditions are often undesirable, and there is a high association with death.[1,6]

The finding of this study suggests that a higher risk of death can be found in COVID-19 patients with elevated cardiac biomarkers, consistent with previous reviews on the risk of mortality outcomes from COVID-19, especially for troponin I and BNP.[14,17,19-20.] Shi et al. reported a higher mortality rate in patients with higher levels of hs-cTnI (42/82) compared with those without such elevated levels (15/334).[11] This result was also in concordance with Zhang et al.'s finding, where the mortality rate was higher (up to 76.9%) among patients with hs-cTnI elevation compared with those without such biomarker elevation (20%).[20] Zhou et al. reported a similar finding of a higher chance of death in patients with elevated hs-cTnI levels, using a specific cut-off of 28 pg/ml.[14] This supports the hypothesis of myocardial injury involvement in COVID-19 related to SARS-CoV-2 structural S protein that binds to the ACE2 receptor of the myocardium, leading to possible cardiac injury and immune reactions throughout the body.

Hs-cTnI has mainly been used to determine troponin levels in many studies. Only Guo et al. used a slightly different biomarker component, TnT, to assess patients' troponin levels. Although this biomarker is less specific to define myocardial injury as a whole, other biomarkers of myocardial damage were also significantly elevated (CKMB, myoglobin, NT-proBNP), providing supporting evidence of myocardial injury in patients.[17] Moreover, this study showed similar results to other studies that used hs-cTnI, underlining that the elevation of any troponin increases patients' risk of death.

The complex mechanism of the clinical manifestation and limited information regarding disease progression indicate the need for other biomarkers to be considered when assessing patients' prognoses. Gao et al.[18] reported that procalcitonin and white blood cells also make a significant contribution to predicting in-hospital deaths. Stefanini et al.[15] and Chen et al.[16] reported that the values of Nt-proBNP and BNP were also shown to be increased in accordance with the elevation of troponin levels. This highlighted the essential need for further investigation of biomarkers' roles in myocardial

injury in COVID-19 patients and the associated prognoses, which should be conducted with better quality controls.

Only Gao et al.[18] included a bias control for the outcome analysis in the studies in this review, but an insufficient patient follow-up process was employed. All the other studies did not clearly state whether they were using blind assessment for the patients outcome or not. Blinding is crucial because unblinded investigators may search more aggressively for outcomes in people with known elevated cardiac biomarkers. Deciding on the underlying factors of mortality is a bit more complicated in patients with systematic diseases and requires blinding of the risk factors to ensure that it is unbiased. Moreover, Guo et al[17], Chen et al[15], Zhou et al[14] did not make any adjustment for other prognostic factors so it may pose a significant threat to the validity of the study.

Limitation the Study

The sources we used in this systematic review have potential bias and flaws due to the limited time and resources in this pandemic condition. Pre-prints articles were also used due to the as-yet limited information regarding COVID-19. However, the author only included studies with relevant information. Under current pandemic circumstances, we believe our study may be beneficial to the medical society and general public.

CONCLUSION

Although limited, there is evidence of higher mortality rate in COVID-19 patients with elevated troponin and NT-proBNP levels. Our findings highlight the importance of evaluating myocardial injury biomarkers, especially in terms of the early analysis of troponin and NT-proBNP levels. This may guide clinicians in considering the required preventive measures against further deterioration in patients' condition and avoiding fatal outcomes.

REFERENCES

1. Atri D, Siddiqi HK, Lang J, Nauffal V, Morrow DA, Bohula EA. COVID-19 for the Cardiologist: A current review of the virology, clinical epidemiology, cardiac and other clinical manifestations and potential therapeutic strategies. JACC: Basic to Translational Science. 2020. doi: https://doi.org/10.1016/j.jacbts.2020.04.002.
2. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J. 2020;55(4):2000607. Published 2020 Apr 16. doi:10.1183/13993003.00607-2020
3. WHO. Coronavirus disease 2019 (COVID-19) Situation Report – 97. Geneva, Switzerland. WHO; 2020. Accessed at 26/04/2020. Cited from https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200426-sitrep-97-COVID-19.pdf?sfvrsn=d1c3e800_6
4. Zeng JH, Liu YX, Yuan J, et al. First case of COVID-19 complicated with fulminant myocarditis: a case report and insights [published online ahead of print, 2020 Apr 10]. Infection. 2020;1-5. doi:10.1007/s15010-020-01424-5
5. Rizzo P, Vieceli Dalla Sega F, Fortini F, Marracino L, Rapezzi C, Ferrari R. COVID-19 in the heart and the lungs: could we «Notch» the inflammatory storm?. Basic Res Cardiol. 2020;115(3):31. Published 2020 Apr 9. doi:10.1007/s00395-020-0791-5
6. Zheng Y., Ma Y., Zhang J, et al. COVID-19 and the cardiovascular system. Nat Rev Cardiol. 2020;17:259–60 (2020). https://doi.org/10.1038/s41569-020-0360-5
7. Hua A, O'Gallagher K, Sado D, Byrne J. Life-threatening cardiac tamponade complicating myo-pericarditis in COVID-19 [published online ahead of print, 2020 Mar 30]. Eur Heart J. 2020;ehaa253. doi:10.1093/eurheartj/ehaa253
8. Hu H, Ma F,Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin, European Heart Journal. 2020, ehaa190. https://doi.org/10.1093/eurheartj/ehaa190
9. Gao et al. Role of Troponin I Proteolysis in the Pathogenesis of Stunned Myocardium. AHA J. 2020;80(3):393-9. https://doi.org/10.1161/01.res.0000435855.49359.47
10. Sharma S, Jackson PG, Makan J. Cardiac troponins. J Clin Pathol. 2004;57(10):1025-6. doi:10.1136/jcp.2003.015420
11. Babapoor-Farrokhran S, Gill D, Walker J, Rasekhi RT, Bozorgnia B, Amanullah A. Myocardial injury and COVID-19: Possible mechanisms. Life Sci. 2020;253:117723. doi:10.1016/j.lfs.2020.117723
12. Clerkin KJ, Fried JA, Raikhelkar J, et al. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease [published online ahead of print, 2020 Mar 21]. Circulation. 2020;10.1161/CIRCULATIONAHA.120.046941.
13. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6(7):e1000100. doi:10.1371/journal.pmed.1000100

14. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study [published correction appears in Lancet. 2020;395(10229):1038] [published correction appears in Lancet. 2020 Mar
15. Chen Tao, Wu Di, Chen Huilong, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020; 368
16. Stefanini, et al. Early detection of elevated cardiac biomarkers to optimise risk stratification in patients with COVID-19. Heart. 2020;106:1512–8.
17. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with Coronavirus Disease 2019 (COVID-19) [published online ahead of print, 2020 Mar 27]. JAMA Cardiol. 2020;e201017. doi:10.1001/jamacardio.2020.1017
18. Gao L., Jiang D., Wen X, et al. Prognostic value of NT-proBNP in patients with severe COVID-19. Respir Res. 2020;21:83. https://doi.org/10.1186/s12931-020-01352-w
19. Zhang, et al. Myocardial injury is associated with in-hospital mortality of confirmed or suspected COVID-19 in Wuhan, China: A single center retrospective cohort study
20. Preprint medRxiv. 2020. doi: https://doi.org/10.1101/2020.03.21.20040121.
21. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol. Published online March 25, 2020. doi:10.1001/jamacardio.2020.0950

Positive Deviance: Frequent Blood Pressure Monitoring Among Non-hypertensive Middle-aged Women in Rural Indonesia

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ABSTRACT

Background: *In Indonesia, as in many low and middle-income countries, hypertension is a significant health issue. Community health nurses need to identify those with early onset of hypertension by promoting frequent blood pressure (BP) checks, even among those with normal BP. Positive deviance approaches focus on identifying people who undertake uncommon preventive actions. Among middle-aged women in rural West Java, Indonesia, we aimed to identify covariates of the positive deviant practice of having one's BP checked at least once every three months even when having normal BP.* ***Methods:*** *We conducted a cross-sectional survey recruiting participants at health centers. Our structured questionnaire measured socio-demographic characteristics, frequency of BP checks, BMI, beliefs and practices. We used binomial logistic regression to identify covariates.* ***Results:*** *Among 520 participants, 265 had normal BP, and of those 156 had obtained frequent BP checks, making them positive deviants. For women with normal BP, significant covariates of obtaining frequent BP checks were: 1) having BMI ≥25.0 (adjusted odds ratio (AOR) =2.57, 95% confidence interval (CI)=1.39–4.78), 2) greater tendency to seek health information (AOR=1.13, 95% CI=1.03–1.24), 3) receiving less support from family members (AOR=0.87, 95% CI=0.77–0.97), and 4) receiving greater support from health volunteers (AOR=1.12, 95% CI=1.01–1.23).* ***Conclusion:*** *Positive deviants were more likely to be proactive because of the convergence of their own individual-level tendencies to learn about their health, family-level conditions that allowed for greater autonomy, and community-level capacity of health volunteers to provide them with support. Community health nurses should focus simultaneously on activating individual-level, family-level, and community-level capacity to prevent hypertension.*

Keywords**:** *Blood pressure screening, middle-aged, women, rural, positive deviance.*

INTRODUCTION

In low- and middle-income countries (LMICs), large proportions of populations have undiagnosed hypertension.[1] Frequent blood pressure (BP) checks are an important modifiable health practice for preventing and controlling hypertension, and thus reducing subsequent complications. In high-income countries, the importance of home-based self-monitoring of BP to reduce and control BP is well established in the literature.[2,3] Frequent home BP monitoring is associated with decreased cardiovascular events and mortality.[4-6] In LMICs, many people in rural communities have limited access to preventative screening through home BP monitoring devices.[7,8] In limited-resource settings in LMICs, here is an urgent need to facilitate frequent BP checks, particularly in at-risk populations.[9]

Worldwide, several factors have been found to be associated with frequency of BP checks. Less frequent BP checks have been associated with socio-demographic characteristics such as lower education,[10] lower income,[11,12] not having hypertension,[11,13] and living in a rural area.[12] Indonesia has an extensive network of *pusat kesehatan masyarakat: puskesmas* (community health centers) and *pos pelayanan terpadu: posyandu* (health posts) that provide monthly preventive healthcare services, including checking BP.[14] However, in Indonesia as in many LMICs, little is known about factors that effectively promote frequent BP checks.

In community health, researchers have recently taken more interest in studying positive deviance.[15,16] Positive deviants are individuals who deviate substantially from the norm in that they engage in uncommon favorable behaviors, practices or habits despite living in the same group as the majority or facing the same difficult conditions as the majority.[17] The positive deviance approach to health promotion focuses on studying and highlighting people who successfully discover solutions based on assets in their communities.[17] The approach of studying positive deviants can be applied even in limited resource settings because in some cases the solutions are within the community. The first step in this approach is to identify positive deviants, and then study what they are doing and why.

In this study, in rural communities in West Java, Indonesia we sought locate and study middle-aged women who were positive deviants in that they had obtained frequent BP checks even though they had normal BP. Hypertension prevention in middle-aged women is important because they may experience increased arterial stiffness due to estrogen decrease, which leads to increased blood pressure.[18,19] The purpose of this study was to identify covariates of practicing frequent BP checks among these non-hypertensive women.

METHODS

Study Design, Sample, and Operational Definition of Positive Deviants

In a rural district of West Java, Indonesia, in December 2016, we conducted a cross-sectional survey. We recruited 530 middle-aged female participants from two community health centers. Inclusion criteria were: 1) self-identifying as female, 2) ages 40-64, and 3) living in one of the communities in the district. Exclusion criterion was: not agreeing with participation in the study. Having a healthcare professional check one's BP once or more every three months is recommended by the Indonesian Ministry of Health. We developed and administered a structured paper-and-pencil questionnaire in Bahasa Indonesia language. We collected data by having research assistants ask a participant face-to-face each questionnaire item and then recording the participant's answers on the questionnaires because some of the participants had somewhat low levels of formal education.[20] Research assistants who were nursing students or public health students in a bachelor's degree program in the district collected all of the data. The researchers trained the research assistants on the study aims, methods, ethical considerations, and personal safety. The two research assistants worked as a pair to double-check that they were following the protocol correctly. During data collection, whenever the research assistants had questions they contacted the investigators for clarification.

We measured the outcome variable based on the intersection of two variables. First, we

assessed current BP based on the medical record where normal BP was defined as systolic below 140 mmHg and diastolic BP below 90 mmHg based on the current Indonesian guidelines for management of hypertension in cardiovascular disease.[21] Second, we measured frequency of BP checks using a five-point Likert-type scale of 1 = never, 2 = have ever, 3 = once a year, 4 = once in three months, and 5 = once a month. We classified cases as positive deviants if they had normal BP and had checked their BP at least once in three months.[22]

Questionnaire items measured participants' age, level of educational attainment, occupation, monthly family income, and whether or not they had health insurance. We also asked participants to respond to 47 statements using either a four-point Likert scale (1 = never to 4 = routinely) or a five-point Likert scale (1 = strongly disagree to 5 = strongly agree). We grouped the 47 statements for form composite measures of 1) seeking health information, 2) receiving support from family members, 3) receiving support from health volunteers, 4) receiving support from health professionals, 5) caring about others, 6) practices based on prior experiences, 7) motivated to practice healthful behaviors, 8) sense of competence, 9) devout spiritual practice, and 10) belief in Allah's gifts (*see specific items and grouping in supplementary table*).

For logistic regression modeling, a standard recommendation is to have at least 10 observations for each variable entered in a model.[23-25] Our instrument contains 17 explanatory variables, thus requiring a sample size of 170 for each group (hypertensive and non-hypertensive).

Ethics Approvals and Consent to Participate

Informed consent was obtained from the all participants prior to the collection of the data. We explained all the eligible participants about the purpose and procedures of the study, voluntary participation, confidentiality, and right to withdrawal. We submitted our research protocol and a request for permission to conduct this study to *Badan Kesatuan Bangsa, Politik dan Perlindungan Masyarakat* (the Agency for National Unity, Politics, and Community Protection) and the *Dinas Kesehatan Kabupaten* (the District Health Office), and permissions were obtained in 2016 (No.37/070/Rekomlit/Kesbangpol/2016, No.070/1727/Um.Peg, respectively). Ethical approval for this study was received from the Research Ethics Committee of Shiga University of Medical Science, Japan in 2016 (No.28-068).

Data Analysis

We excluded respondents from our analysis if data was missing for frequency of BP checks. We first calculated descriptive statistics for all variables. Then, we conducted bivariate analyses using chi-square tests and unpaired t-tests by comparing participants of each group (normal BP, elevated BP, and total) based on their status (frequent BP checks vs. less-frequent BP checks) against the socio-demographic characteristics and other covariates. We then conducted univariate and multivariate analyses by specifying logistic regression models to identify covariates of frequent BP checks for each group (normal BP, elevated BP, and total) while controlling for potential confounding variables. We calculated the crude and adjusted odds ratios (OR and AOR) for each variable. Statistical analyses were performed using IBM SPSS Statistics 26.0 for Windows. Significance levels were set at $p < 0.05$ for all tests. For logistic regression models, significance levels were assessed by 95% confidence interval (CI).

RESULTS

Participants' Characteristics

Of the 530 people we attempted to recruit, 100% agreed to participate in the study. We excluded 10 from analysis because of missing data of blood pressure check frequency. Among the 520 participants, 265 had normal BP, and 255 had elevated BP. Of those with normal BP, 156 were positive deviants (30% of the total sample). The mean age of the sample was 51.2 years (SD = 7.3 years). More than half of the participants had not completed primary school (58.1%) and had limited monthly family income (56.7%). The proportion of frequent BP checks was higher among participants with elevated BP status (69.8%) than among those with normal BP status (58.9%) (**Table 1**). Among those with normal BP, univariate analysis shows that

the mean of aggregated scores for those who checked their BP frequently was significantly greater than for those who checked their BP less frequently for measures of seeking health information (16.4 vs. 14.9), receiving support from health volunteers (19.2 vs. 17.8), receiving support from health professionals (16.3 vs. 15.4) and caring about others (9.5 vs. 8.9) (**Table 2**). For those with elevated BP, the only significant difference was for measures of practices based on prior experiences (15.7 vs. 14.9).

Covariates of Frequent BP Checks

A preliminary univariate logistic regression revealed that middle-aged women with elevated BP were more likely to obtain frequently BP checks (OR=1.62, 95% CI =1.12–2.32) (**Table 3**). To run multivariate logistic regression models, we created an outcome variable of frequency of BP checks (0 = less than once in 3 months, 1 = once or more in 3 months), and then we ran fully specified models to identify covariates. Among women with normal BP, models revealed that after controlling for age, BMI (which was a significant covariate with BMI ≥ 25 having an AOR=2.57, 95% CI=1.39–4.78), educational level, occupation, income, and health insurance status, participants with normal BP were significantly more likely to have had their BP checked every 3 months if they had higher aggregate scores on seeking health information (AOR =1.13, 95% CI=1.03–1.24), receiving a lot of support from health volunteers (AOR=1.12, 95% CI=1.01–1.23) and less likely if they had received a lot of support from family members (AOR=0.87, 95% CI=0.77–0.97).

We conducted analyses using data from women who had elevated BP (n = 255). Fully specified logistic regression models revealed that for women with elevated BP, the only statistically significant covariate of women with elevated BP getting a BP check once or more every 3 months was having not received a lot of support from family members (AOR=0.89, 95% CI=0.81–0.98). BMI was not a significant covariate. Additional logistic regression analysis of the total sample (n = 520) revealed that BMI ≥25.0 and moderate household income were significant covariates of having frequent BP checks, along with seeking health information (AOR=1.07, 95% CI=1.01–1.14), not receiving support from family members (AOR=0.90, 95% CI=0.84–0.96), and receiving a lot of support from health volunteers (AOR=1.10, 95% CI=1.03–1.18).

DISCUSSION

This study is the first to apply the concept of positive deviance to examine frequency of BP checks in an LMIC. In among middle-aged women living a rural district in Indonesia, we found that 30.0% of participants met our definition of being positive deviants i.e., those with normal BP who obtained BP checks at least once or more every three months. This proportion was similar to proportions identified in other public health studies on positive deviants.[26-28] Our study showed that those women with normal BP who had high BMI scores (≥25.0) were about two and a half times more likely to have their BP checked frequently. This suggests that many women in our study who had normal BP but were overweight realized that their excess weight was a substantial risk factor for developing hypertension.

Previous studies have shown that self-care for BP checks is associated with having relevant knowledge and health awareness.[29,30] Our study shows that middle-aged rural Indonesian women with normal BP who obtained BP checks frequently had a stronger tendency to seek health information from family, friends, health volunteers, health centers, and health posts. It is important for health professionals not only to provide knowledge, but also to support people seeking and obtaining health information by themselves. Further study is necessary to explore deeply what kind of information, which sources, and how people seek information.

Our study identified another covariate for being a positive deviant – receiving support from health volunteers in the forms of expressing concern and encouragement, giving suggestions, and accompanying women when they needed to visit healthcare facilities. In rural Indonesia, a community health volunteer is a person chosen by community members who takes on the role of mobilizing their community to use basic health services like those provided at community health

Table 1. Participant characteristics

	Normal BP (n = 265)					Elevated BP (n = 255)					Total (N = 520)				
	Checked BP frequently (n = 156, 58.9%)		Less frequent (n = 109, 41.1%)			Checked BP frequently (n = 178, 69.8%)		Less frequent (n = 77, 30.2%)			Checked BP frequently (n = 334, 64.2%)		Less frequent (n = 186, 35.8%)		
	n	%	n	%	P	n	%	n	%	P	n	%	n	%	P
BP status															0.009
Normal BP	–	–	–	–		–	–	–	–		156	58.9	109	41.1	
Elevated BP	–	–	–	–		–	–	–	–		178	69.8	77	30.2	
Age group					0.986					0.933					0.939
40–49 years	74	58.7	52	41.3		55	71.4	22	28.6		129	63.5	74	36.5	
50–59 years	60	59.4	41	40.6		69	69.0	31	31.0		129	64.2	72	35.8	
60–64 years	22	57.9	16	42.1		54	69.2	24	30.8		76	65.5	40	34.5	
BMI					0.003					0.565					0.004
<18.5	9	56.3	7	43.8		4	80.0	1	20.0		13	61.9	8	38.1	
18.5–24.9	52	47.3	58	52.7		62	66.0	32	34.0		114	55.9	90	44.1	
≥25.0	93	68.9	42	31.1		111	71.6	44	28.4		204	70.3	86	29.7	
Education					0.727					0.031					0.249
Not completed primary school	77	56.6	59	43.4		108	65.1	58	34.9		185	61.3	117	38.7	
Completed primary school	50	61.0	32	39.0		40	71.4	16	28.6		90	65.2	48	34.8	
Completed middle school	14	56.0	11	44.0		19	95.0	1	5.0		33	73.3	12	26.7	
Completed high school or above	15	68.2	7	31.8		10	83.3	2	16.7		25	73.5	9	26.5	
Occupation					0.340					0.075					0.059
Housewife	102	61.1	65	38.9		118	73.8	42	26.3		220	67.3	107	32.7	
Other work	54	55.1	44	44.9		60	63.2	35	36.8		114	59.1	79	40.9	
Income (Indonesian rupiah)					0.132					0.061					0.016
< 1 million	86	60.6	56	39.4		114	74.5	39	25.5		200	67.8	95	32.2	
≥1 million – <2 million	51	53.1	45	46.9		56	60.9	36	39.1		107	56.9	81	43.1	
≥ 2 million	18	75.0	6	25.0		8	80.0	2	20.0		26	76.5	8	23.5	
Having health insurance					0.198					0.702					0.172
No	70	54.7	58	45.3		74	68.5	34	31.5		144	61.0	92	39.0	
Yes	85	62.5	51	37.5		104	70.7	43	29.3		189	66.8	94	33.2	

Chi-square test

Table 2. Univariate associations with frequent BP check-ups

	Normal BP (n = 265)					Elevated BP (n = 255)					Total (N = 520)				
	Checked BP frequently (n = 156, 58.9%)		Less frequent (n = 109, 41.1%)			Checked BP frequently (n = 178, 69.8%)		Less frequent (n = 77, 30.2%)			Checked BP frequently (n = 334, 64.2%)		Less frequent (n = 186, 35.8%)		
	mean	SD	mean	SD	P	mean	SD	mean	SD	P	mean	SD	mean	SD	P
Seeking health information (range 7–28)	16.4	3.8	14.9	4.0	0.001	15.5	4.0	15.6	3.8	0.860	15.9	3.9	15.2	3.9	0.032
Support from family members (range 7–35)	28.3	3.0	28.7	3.2	0.261	27.0	4.5	27.7	5.0	0.236	27.6	4.0	28.3	4.1	0.047
Support from health volunteers (range 5–25)	19.2	3.1	17.8	4.1	0.002	18.2	3.4	17.5	3.8	0.139	18.7	3.3	17.7	4.0	0.003
Support from health professionals (range 4–20)	16.3	2.1	15.4	2.9	0.004	15.6	2.7	15.7	2.7	0.738	15.9	2.5	15.5	2.8	0.099
Caring about others (range 4–16)	9.5	2.0	8.9	2.1	0.015	8.8	1.9	9.1	2.4	0.304	9.1	2.0	8.9	2.2	0.457
Practices based on prior experiences (range 4–20)	15.2	3.0	15.0	2.7	0.527	15.7	2.1	14.9	2.6	0.010	15.5	2.6	14.9	2.7	0.017
Motivated to practice healthful behaviors (range 5–25)	21.7	1.9	21.5	2.0	0.256	21.4	2.0	21.1	2.5	0.337	21.6	1.9	21.3	2.2	0.178
Sense of competence (range 3–15)	12.4	1.2	12.4	1.3	0.878	12.2	1.5	12.0	1.7	0.384	12.3	1.3	12.2	1.5	0.658
Devout spiritual practice (range 4–16)	10.7	2.8	10.1	2.8	0.093	10.7	3.1	10.4	2.9	0.414	10.7	3.0	10.2	2.8	0.066
Belief in Allah's gifts (range 4–20)	18.1	1.8	18.1	1.8	0.963	18.1	1.9	18.3	1.7	0.435	18.1	1.9	18.2	1.7	0.606

Unpaired t-test

Table 3. Logistic regression model identifying predictors of frequent BP check-ups
Outcome variable: frequency of BP check-ups (0 = less than once in 3 months, 1 = once or more in 3 months)

	Normal BP (n = 265), Nagelkerke R^2 = 0.232								Elevated BP (n = 255), Nagelkerke R^2 = 0.199								Total (N = 520), Nagelkerke R^2 = 0.169							
	Unadjusted				Adjusted				Unadjusted				Adjusted				Unadjusted				Adjusted			
	OR	95%CI		P	AOR	95%CI		P	OR	95%CI		P	AOR	95%CI		P	OR	95%CI		P	AOR	95%CI		P
BP status																								
Normal BP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00				1.00			
Elevated BP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.62	1.12 –	2.32	0.010	1.54	1.01 –	2.36	0.047
Age group																								
40–49 years	1.00				1.00				1.00				1.00				1.00				1.00			
50–59 years	1.03	0.50 –	2.16	0.927	0.93	0.36 –	2.40	0.877	1.11	0.56 –	2.21	0.765	0.76	0.28 –	2.10	0.597	1.03	0.68 –	1.54	0.895	1.30	0.76 –	2.22	0.335
60–64 years	1.06	0.50 –	2.27	0.872	1.27	0.51 –	3.16	0.604	0.99	0.52 –	1.88	0.974	1.01	0.47 –	2.17	0.986	1.09	0.68 –	1.76	0.724	1.31	0.69 –	2.50	0.406
BMI																								
< 18.5	1.43	0.50 –	4.12	0.504	1.99	0.61 –	6.49	0.256	2.06	0.22 –	19.25	0.525	2.83	0.23 –	34.43	0.414	1.28	0.51 –	3.23	0.597	1.82	0.67 –	4.95	0.238
18.5–24.9	1.00				1.00				1.00				1.00				1.00				1.00			
≥25.0	2.47	1.46 –	4.16	0.001	2.57	1.39 –	4.78	0.003	1.30	0.75 –	2.26	0.348	1.02	0.54 –	1.95	0.945	1.87	1.29 –	2.72	0.001	1.67	1.10 –	2.55	0.017
Education																								
Not completed PS	1.00				1.00				1.00				1.00				1.00				1.00			
Completed PS	0.61	0.23 –	1.59	0.311	0.49	0.14 –	1.74	0.271	0.37	0.08 –	1.76	0.212	0.22	0.04 –	1.25	0.088	1.19	0.78 –	1.80	0.426	1.38	0.80 –	2.36	0.247
Completed JHS	0.73	0.27 –	1.98	0.536	0.53	0.15 –	1.84	0.318	0.50	0.10 –	2.54	0.403	0.48	0.09 –	2.71	0.407	1.74	0.86 –	3.50	0.121	2.24	0.95 –	5.30	0.067
Completed HS or above	0.59	0.18 –	1.96	0.393	0.52	0.12 –	2.28	0.384	3.80	0.31 –	47.21	0.299	3.53	0.24 –	50.87	0.354	1.76	0.79 –	3.90	0.165	2.45	0.95 –	6.30	0.063
Occupation																								
Other works	1.00				1.00				1.00				1.00				1.00				1.00			
Housewife	1.28	0.77 –	2.12	0.340	0.99	0.53 –	1.83	0.962	1.64	0.95 –	2.83	0.076	1.29	0.68 –	2.46	0.435	1.42	0.99 –	2.06	0.060	1.12	0.73 –	1.71	0.604
Income																								
< 1 million	1.00				1.00				1.00				1.00				1.00				1.00			
≥1 million – <2 million	0.51	0.19 –	1.37	0.182	0.77	0.23 –	2.53	0.665	0.73	0.15 –	3.59	0.699	1.78	0.28 –	11.21	0.541	0.63	0.43 –	0.92	0.016	0.57	0.36 –	0.89	0.014
≥ 2 million	0.38	0.14 –	1.03	0.058	0.47	0.14 –	1.56	0.219	0.39	0.08 –	1.94	0.249	0.90	0.14 –	5.63	0.908	1.54	0.67 –	3.54	0.305	1.16	0.45 –	2.99	0.760
Having health insurance																								
No	1.00				1.00				1.00				1.00				1.00				1.00			
Yes	1.38	0.84 –	2.26	0.198	1.69	0.93 –	3.05	0.085	1.11	0.65 –	1.91	0.702	1.11	0.59 –	2.06	0.749	1.28	0.90 –	1.84	0.173	1.40	0.93 –	2.11	0.110
Seeking health information (range 7–28)	1.11	1.04 –	1.19	0.002	1.13	1.03 –	1.24	0.013	0.99	0.93 –	1.06	0.860	1.05	0.96 –	1.15	0.323	1.05	1.00 –	1.10	0.032	1.07	1.01 –	1.14	0.024
Support from family members (range 7–35)	0.95	0.88 –	1.03	0.261	0.87	0.77 –	0.97	0.012	0.96	0.90 –	1.03	0.238	0.89	0.81 –	0.98	0.015	0.95	0.91 –	1.00	0.050	0.90	0.84 –	0.96	0.001
Support from health volunteers (range 5–25)	1.12	1.04 –	1.20	0.002	1.12	1.01 –	1.23	0.031	1.06	0.98 –	1.14	0.140	1.11	0.99 –	1.23	0.075	1.08	1.03 –	1.14	0.002	1.10	1.03 –	1.18	0.007
Support from health professionals (range 4–20)	1.17	1.05 –	1.31	0.004	1.04	0.90 –	1.22	0.576	0.98	0.89 –	1.09	0.737	1.00	0.86 –	1.16	0.999	1.06	0.99 –	1.13	0.090	1.01	0.92 –	1.12	0.768
Caring about others (range 4–16)	1.16	1.03 –	1.31	0.017	1.02	0.86 –	1.22	0.805	0.93	0.81 –	1.06	0.262	0.93	0.78 –	1.10	0.393	1.03	0.95 –	1.13	0.440	1.02	0.91 –	1.14	0.728
Practices based on prior experiences (range 4–20)	1.03	0.94 –	1.12	0.525	0.99	0.88 –	1.11	0.875	1.18	1.05 –	1.32	0.006	1.18	1.00 –	1.40	0.054	1.09	1.01 –	1.16	0.018	1.05	0.95 –	1.15	0.332
Motivated to practice healthful behaviors (range 5–25)	1.08	0.95 –	1.23	0.255	1.12	0.92 –	1.37	0.270	1.07	0.94 –	1.21	0.294	1.03	0.87 –	1.21	0.722	1.06	0.97 –	1.16	0.178	1.07	0.95 –	1.20	0.272
Sense of competence (range 3–15)	1.02	0.83 –	1.25	0.877	0.97	0.73 –	1.30	0.860	1.08	0.91 –	1.28	0.384	1.02	0.81 –	1.29	0.864	1.03	0.90 –	1.17	0.657	1.05	0.88 –	1.25	0.584
Belief in Allah's gifts (range 4–20)	1.00	0.87 –	1.14	0.963	0.96	0.78 –	1.19	0.709	0.94	0.81 –	1.10	0.454	0.88	0.73 –	1.07	0.201	0.97	0.88 –	1.08	0.612	0.91	0.80 –	1.03	0.142
Devout spiritual practice (range 4–16)	1.08	0.99 –	1.18	0.094	1.06	0.96 –	1.18	0.267	1.04	0.95 –	1.13	0.413	1.06	0.95 –	1.18	0.319	1.06	1.00 –	1.13	0.067	1.07	1.00 –	1.15	0.054

posts.[31] In Indonesia, there is a wide network of community-level health centers and health posts that emphasize prevention, early detection, and control of NCDs where community members can receive BP checks as well as counseling and health education.[14] The benefits communities receive from the work of non-professional lay health workers have been shown in Indonesia and other LMICs.[32] A randomized controlled trial in LMICs showed an effect of lifestyle intervention by female community health volunteers on controlling BP.[33] Health professionals need to work more closely with health volunteers to support community members' health practices. Further study is also needed in Indonesia to learn more about how health volunteers support community members.

For women with normal BP, those who received higher levels of family support were less likely to have their BP checked frequently. In rural West Java, middle-aged women's lives are often embedded within a context of strong family bonds. Providing care for older female family members is an important family value rooted in culture and religion.[34] Younger family members, particularly younger women, are highly involved in taking care of their older female family members by attending to their physical symptoms and spiritual needs.[35,36] When middle-aged women live in such a highly supportive family environments, they may feel reluctant to have their BP checked frequently at health centers, health posts, or clinics because they may assume that their family members who take care of them know what is best and are doing what is necessary to care for their health. Conversely, those middle-aged women who need to be more self-reliant in terms of looking out for their own health may be more likely to seek care outside of their family circles consistently, and therefore may have a greater tendency to have their BP checked more frequently. This is one possible explanation for the association between middle-aged women with normal BP obtaining frequent BP checks and receiving less support from family members.

In our additional analysis of covariates of frequent BP checks among middle-aged women with elevated BP, we also found the association between receiving less family support and obtaining frequent BP checks, suggesting that this may be a universal factor. Our analysis showed some indication that women with elevated BP who had practices based on their own prior experiences or those of others they knew were more likely to obtain frequent BP checks. If women with elevated BP heard about someone whose health status deteriorated for example because of a stroke due to uncontrolled hypertension, they might be able to imagine their health could deteriorate similarly and have fear about not knowing their BP levels. Community people have a right over their healthcare decisions.[37] In LMICs that lack health check systems based on the law, community members have to make decisions by themselves about whether and when to go have health checks. Our findings suggest that it is important to provide health information based on peers' experiences. Other studies have mentioned the importance of narratives or storytelling by peers to motivate community members.[38] Further study is needed to find out what types of information about peers' experiences will motivate middle-aged Indonesian women in rural areas to have their BP checked more frequently.

There were some limitations to this study. Because the data are cross-sectional, we cannot assert that there were causal relationships between the frequency of a woman getting her BP checked and the covariates identified in our analysis. Longitudinal studies are necessary to determine whether the associations we identified are causal. In addition, it is important to explore how positive deviants get their BP checked frequently. Future research using qualitative methods should be conducted to examine the processes middle-aged women in these communities engage in to have their BP checked frequently. Another limitation is about selection bias because we conducted a study in one district. We need to conduct further study to examine the situation of BP checks and their covariates in rural Indonesia.

CONCLUSION

In rural West Java of Indonesia, as is likely to be the case in many rural places in LMICs,

middle-aged women who have not developed hypertension appear to benefit in monitoring their BP frequently when they tend to seek health information in a context when they have somewhat greater health autonomy within their families, and when they can receive support from health volunteers. The convergence of these three factors shows the importance of understanding what needs to happen simultaneously at the individual level, the family level, and the community level so that preventive positive deviant practices become the norm. Our findings can be used to inform health promotion on BP screening. Activating factors simultaneously at the individual, family, and community levels is the first step for developing community health nursing approaches for primary hypertension prevention.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Hussain MA, Mamun AA, Reid C, et al. Prevalence, awareness, treatment and control of hypertension in Indonesian adults aged ≥40 years: findings from the Indonesia Family Life Survey (IFLS). Plos One. 2016;11(8):e0160922-e.
2. Glynn LG, Murphy AW, Smith SM, et al. Interventions used to improve control of blood pressure in patients with hypertension. Cochrane Database Syst Rev. 2010(3):CD005182.
3. Crabtree MM, Stuart-Shor E, McAllister M. Home blood pressure monitoring: an integrated review of the literature. J Nurse Pract. 2013;9(6):356-61.
4. Fuchs SC, Mello RGBd, Fuchs FC. Home blood pressure monitoring is better predictor of cardiovascular disease and target organ damage than office blood pressure: a systematic review and meta-analysis. Curr Cardiol Rep. 2013;15(11):413.
5. Sheikh S, Sinha AD, Agarwal R. Home blood pressure monitoring: how good a predictor of long-term risk? Curr Hypertens Rep. 2011;13(3):192-9.
6. George J, MacDonald T. Home blood pressure monitoring. Eur Cardiol. 2015;10(2):95-101.
7. Guenter D, Angeles R, Kaczorowski J, et al. Choosing the optimal method of blood pressure measurement for limited-resource rural communities in the "Community Health Assessment Program-Philippines". J Clin Hypertens. 2017;19(9):899-903.
8. Park S, Buranakitjaroen P, Chen C-H, et al. Expert panel consensus recommendations for home blood pressure monitoring in Asia: the Hope Asia Network. J Hum Hypertens. 2018;32(4):249-58.
9. World Health Organization. A global brief on hypertension 2013. Accessed September 2, 2021 http://apps.who.int/iris/bitstream/10665/79059/1/WHO_DCO_WHD_2013.2_eng.pdf?.
10. Wang Y, Wang Y, Gu H, et al. Use of home blood pressure monitoring among hypertensive adults in primary care: Minhang community survey. Blood Press Monit. 2014;19(3):140-4.
11. Ostchega Y, Berman L, Hughes JP, et al. Home blood pressure monitoring and hypertension status among US adults: the National Health and Nutrition Examination Survey (NHANES), 2009-2010. Am J Hypertens. 2013;26(9):1086-92.
12. Wang Q, Xu L, Sun L, et al. Rural-urban difference in blood pressure measurement frequency among elderly with hypertension: a cross-sectional study in Shandong, China. J Health Popul Nutr. 2018;37(1):25-.
13. Ostchega Y, Zhang G, Kit BK, et al. Factors associated with home blood pressure monitoring among US adults: National Health and Nutrition Examination Survey, 2011-2014. Am J Hypertens. 2017;30(11):1126-32.
14. Kementerian Kesehatan Republik Indonesia [Indonesian Ministry of Health]. Petunjuk teknis pos pembinaan terpadu penyakit tidak menular (POSBINDU PTM) [Technical instructions for integrated health post of noncommunicable diseases]. 2012. Accessed August 28, 2018. http://www.p2ptm.kemkes.go.id/dokumen-p2ptm/petunjuk-teknis-pos-pembinaan-terpadu-penyakit-tidak-menular-posbindu-ptm.
15. Pascale R, Sternin J, Sternin M. The power of positive deviance: how unlikely innovators solve the world's toughest problems. Boston, MA: Harvard Business School Publishing. 2010.
16. Banerjee ES, Herring SJ, Hurley KE, et al. Overcoming obesity: a mixed methods study of the impact of primary care physician counseling on low-income African American women who successfully lost weight. Am J Health Promot. 2018;32(2):374-80.
17. Positive Deviance Collaborative. What is positive

deviance? n.d. Accessed May 4, 2019. https://positivedeviance.org/.
18. Staessen JA, van der Heijden-Spek JJ, Safar ME, et al. Menopause and the characteristics of the large arteries in a population study. J Hum Hypertens. 2001;15(8):511-8.
19. Coylewright M, Reckelhoff JF, Ouyang P. Menopause and hypertension: an age-old debate. Hypertension. 2008;51(4):952-9.
20. Mizutani M, Tashiro J, Maftuhah, et al. Model development of healthy-lifestyle behaviors for rural Muslim Indonesians with hypertension: a qualitative study. Nurs Health Sci. 2016;18(1):15-22.
21. Perhimpunan Dokter Spesialis Kardiovaskular Indonesia [Indonesian Heart Association]. Pedoman tatalaksana hipertensi pada penyakit kardiovaskular [Guidelines for management of hypertension in cardiovascular disease]. 2015. Accessed September 2, 2021 https://inaheart.org/guideline/
22. Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan Kementerian Kesehatan Republik Indonesia [Directorate General of Disease Control and Environmental Health Indonesian Ministry of Health]. Profil pengendalian penyakit dan penyehatan lingkungan tahun 2011 [Profile of disease control and environmental health 2011]. 2012. Accessed May 2, 2014. http://pppl.depkes.go.id/upt?id=85.
23. Hair JF, Black WC, Babin BJ, et al. Multivariate data analysis: a global perspective. 7th ed. Upper Saddle River NJ: Pearson Education; 2010.
24. Peduzzi P, Concato J, Kemper E, et al. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49(12):1373-9.
25. Long JS. Regression models for categorical and limited dependent variables. Thousand Oaks, CA: Sage Publications 1997.
26. Ma P, Magnus JH. Exploring the concept of positive deviance related to breastfeeding initiation in black and white WIC enrolled first time mothers. Matern Child Health J. 2012;16(8):1583-93.
27. Wallace ME, Harville EW. Predictors of healthy birth outcome in adolescents: a positive deviance approach. J Pediatr Adolesc Gynecol. 2012;25(5):314-21.
28. Marty L, Dubois C, Gaubard MS, et al. Higher nutritional quality at no additional cost among low-income households: insights from food purchases of "positive deviants". Am J Clin Nutr. 2015;102(1):190-8.
29. Peters RM, Templin TN. Measuring blood pressure knowledge and self-care behaviors of African Americans. Res Nurs Health. 2008;31(6):543-52.
30. Diederichs C, Neuhauser H. The frequency and determinants of blood pressure measurement by a health professional in Germany: a cross-sectional study. Medicine (Baltimore). 2019;98(16):e15093.
31. Kementerian Kesehatan Republik Indonesia [Indonesian Ministry of Health]. Peraturan Menteri Kesehatan Republik Indonesia nomor 8 tahun 2019 tentang pemberdayaan masyarakat bidang kesehatan [Regulation of the Minister of Health of the Republic of Indonesia number 8 of 2019 concerning community empowerment in the field of health] 2019. Accessed December 19, 2019. http://promkes.kemkes.go.id/permenkes-no8-th-2019-tentang-pemberdayaan-masyarakat-bidang-kesehatan.
32. Lewin S, Munabi-Babigumira S, Glenton C, et al. Lay health workers in primary and community health care for maternal and child health and the management of infectious diseases. Cochrane Database Syst Rev. 2010(3):CD004015.
33. Neupane D, McLachlan CS, Mishra SR, et al. Effectiveness of a lifestyle intervention led by female community health volunteers versus usual care in blood pressure reduction (COBIN): an open-label, cluster-randomised trial. Lancet Glob Health. 2018;6(1):e66-e73.
34. Kristanti MS, Effendy C, Utarini A, et al. The experience of family caregivers of patients with cancer in an Asian country: a grounded theory approach. Palliat Med. 2019;33(6):676-84.
35. Effendy C, Vissers K, Tejawinata S, et al. Dealing with symptoms and issues of hospitalized patients with cancer in Indonesia: the role of families, nurses, and physicians. Pain Pract. 2015;15(5):441-6.
36. Lukman NA, Leibing A, Merry L. Self-care experiences of adults with chronic disease in Indonesia: an integrative review. Int J Chronic Dis. 2020;2020:1379547.
37. Kruk ME, Gage AD, Arsenault C, et al. High-quality health systems in the Sustainable Development Goals era: time for a revolution. Lancet Glob health. 2018;6(11):e1196-e252.
38. Perrier MJ, Martin Ginis KA. Changing health-promoting behaviours through narrative interventions: a systematic review. J Health Psychol. 2018;23(11):1499-517.

Prognostic Scoring System for Mortality of Hospitalized COVID-19 Patients in Resource-Limited Settings: A Multicenter Study from COVID-19 Referral Hospitals

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ABSTRACT

Background: *Many studies identified the risk factors and prognostic factors related to in-hospital COVID-19 mortality using sophisticated laboratory tests. Cost and the availability of supporting blood tests may be problematic in resource-limited settings. This multicenter cohort study was conducted to assess the factors associated with mortality of COVID-19 patients aged 18 years and older, based on history taking, physical examination, and simple blood tests to be used in resource-limited settings.* ***Methods:*** *The study was conducted between July 2020 and January 2021 in five COVID-19 referral hospitals in Indonesia. Among 1048 confirmed cases of COVID-19, 160 (15%) died during hospitalization.* ***Results:*** *Multivariate analysis showed eight predictors of in-hospital mortality, namely increased age, chronic kidney disease, chronic obstructive pulmonary disease, fatigue, dyspnea, altered mental status, neutrophil-lymphocyte ratio (NLR) ≥ 5.8, and severe-critical condition. This scoring system had an Area-under-the-curve (AUC) of 84.7%. With cut-off score of 6, the sensitivity was*

76.3% and the specificity was 78.2%. ***Conclusion:*** *The result of this practical prognostic scoring system may be a guide to decision making of physicians and help in the education of family members related to the possible outcome.*

Keywords*: COVID-19, prognostic, predictive score, mortality, resource-limited settings.*

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection has become a pandemic, also known as coronavirus disease 2019 (COVID-19) pandemic.[1] To date, most of the available prognostic scoring systems for mortality of COVID-19 patients were based on studies in upper-middle-income countries.[2–5] In reality, countries with the highest COVID-19 case fatality rates mainly were developing countries, including Yemen, Peru, Mexico, Sudan, Syria, Ecuador[6], and Indonesia[6,7], and the risk factors related to COVID-19 mortality in lower-middle-income countries may differ.

The established COVID-19 prognostic scoring system has been found to have a high risk of bias. Risk stratification scoring methods have also been developed by incorporating costly supporting tests.[8] In fact, cost and the availability of supporting blood tests may be problematic in resource-limited settings, especially in Indonesia where the epicenter of the pandemic recently showed signs of shifting to the outside of country's capital city. A physician should preferably try to estimate the prognosis of each patient based on the combination of limited number of nonpatient-burdening and easily measurable variables.[9] Therefore, a feasible prognostication method should be developed to predict mortality of COVID-19 patients based on empirical prognostic research.

Our aim was to identify the prognostic factors as well as to establish and validate a prognostic scoring system for mortality of hospitalized COVID-19 patients using available information on hospital admission. The source of information would be history taking, physical examination and simple blood tests. The prognostic model may help detect high-risk patients as early as possible. The multicenter study included wider range of population groups to increase the generalizability of the study.

METHODS

This study is reported as per TRIPOD Checklist: Prediction Model Development and Validation guidelines.[10] The design of this multicenter study was both retrospective and prospective cohort study to assess the factors associated with in-hospital mortality of COVID-19 patients. The inclusion criteria were all patients aged 18 years old and older hospitalized with confirmed COVID-19 marked by positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test.[11] Patients with incomplete data were excluded. The sample was collected from nasal and/or oropharyngeal swab. Data were taken between July 2020 and January 2021 from five COVID-19 referral hospitals in Indonesia, namely Cipto Mangunkusumo National Hospital as the national referral hospital, Cengkareng District Hospital, Tarakan District Hospital, Jakarta Islamic Hospital Cempaka Putih, and Pasar Minggu District Hospital. The patients were followed from the first day of hospital admission until discharge or in-hospital death.

Ethics Approval

This study was approved by the Faculty of Medicine Universitas Indonesia Ethical Committee (KET-419/UN2.F1/ETIK/PPM.00.02/2020). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Data Collection

We used consecutive sampling technique until it reached the minimum sample size, which was 1000 subjects obtained with the rule of thumb. Data, collected from medical records, included the demographic data (age and sex), chronic medical conditions or comorbidities (hypertension,

diabetes mellitus, cardiovascular diseases, pulmonary tuberculosis, chronic obstructive lung disease [COPD], asthma, chronic kidney disease [CKD], chronic liver disease [CLD], autoimmune disease, immunocompromised condition, and malignancy), clinical symptoms (fever, cough, dyspnea or shortness of breath, fatigue, nausea with/without vomiting, diarrhea, and abdominal pain), level of consciousness, neutrophil-lymphocyte ratio (NLR), and radiological findings (pneumonia based on X-ray reading). Blood analysis and radiological findings were routinely obtained during admission.

Statistical Analysis

The dependent variable in this study was discharge status of confirmed COVID-19. The independent variables include demographic data, chronic medical conditions, clinical symptoms, vital signs, blood analysis, and radiological findings. For the statistical analysis, age was categorized into: (1) 18-49 years old, (2) 50-69 years old or above, (3) ≥70 years old. The subjects were categorized based on their gender into male and female. Chronic medical conditions or comorbidities (hypertension, diabetes mellitus, cardiovascular diseases, pulmonary tuberculosis, COPD, asthma, CKD, CLD, autoimmune disease, immunocompromised condition, and malignancy), clinical symptoms (fever, cough, dyspnea or shortness of breath, fatigue, nausea and/or vomiting, diarrhea, and abdominal pain) were recorded as (1) yes, (2) no. Categories for level of consciousness were: (1) alert (Glasgow Coma Scale 15), (2) altered mental status (Glasgow Coma Scale less than 15). Based on the Indonesian National Guideline on COVID-19, the severity of the COVID-19 was categorized into (1) mild, (2) moderate, (3) severe, or (4) critical. Patients were categorized as mild COVID-19 cases in the presence of symptom(s) such as fever, cough, fatigue, shortness of breath, myalgia, sore throat, headache, diarrhea, nausea/vomiting, and abdominal pain, but without the evidence of pneumonia. Moderate COVID-19 cases had symptoms and evidence of pneumonia without hypoxemia. Patients with severe COVID-19 have the symptoms, evidence of pneumonia, and one of the following criteria: (1) respiratory rate more than 30 breaths per minute, (2) severe respiratory distress, or (3) peripheral oxygen saturation < 90%. Patients were considered to have critical COVID-19 if the patients have respiratory distress syndrome (ARDS) and/or respiratory failure, sepsis, septic shock, or other conditions that require the provision of life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy.[12] Categories for NLR ratio were (1) ≥5.8 and (2) <5.8 [13]. Radiological findings of pneumonia on X-ray was recorded as (1) yes or (2) no. The primary outcome of interest was discharge status during hospitalization: (1) died, (2) discharge from COVID-19 isolation ward.

We performed statistical analysis with SPSS Version 26 (IBM, Armonk, New York, USA). Descriptive analysis was done to explore the characteristics of subjects. Categorical variables were presented as numbers and percentages. The numerical or continuous variables were presented as median (Interquartile Range/IQR). Bivariate and multivariate cox regression analyses were done to determine the mortality risk, using a hazard ratio with a 95% confidence interval (95% CI). All independent variables with a p-value <0.25 in the bivariate analysis were included in the multivariate analysis. A Chi-square test was used in the bivariate analysis to assess the association between the independent variables and mortality of COVID-19 patients. During the model development, patient's demographic, clinical characteristics, signs and symptoms, vital signs, blood analysis, and radiological findings were analyzed for the possible association. Factors with two-sided p-value <0.05 were categorized as significant predictors of mortality. During the model development, patients' demographic, clinical characteristics, sign and symptoms, vital signs, and blood analysis were analyzed for possible association. The final model was established with using the Area-under-the-curve (AUC) value and Hosmer-Lemeshow test. Model calibration was performed to ensure the robustness. Internal validation was performed by bootstrapping technique. We repeated the entire modeling process, including variable selection in 1000 samples drawn with replacement from the original sample. Since we only calculated the

cases with complete data in this study, missing data were analyzed using the missing value analysis to know whether the missing value may affect the results.

RESULTS

A total of 1194 hospitalized patients tested positive for COVID-19 between July 2020 and January 2021 in selected hospitals, of whom 1048 met the inclusion criteria. We excluded 146 subjects due to the incomplete data. Of 1048 patients, 160 (15%) died during hospitalization. The flow of subjects throughout the study can be seen in **Figure 1**. The baseline characteristics of the patients were presented in **Table 1**. Bivariate cox regression analysis was performed (**Table 2**). All independent variables with a p-value <0.25 in the bivariate analysis were included in the multivariate analysis.

In the final model, age 50-69 years old (Hazard Ratio [HR] 1.58, 95% CI 1.07-2.32), age 70 years old and older (HR 2.64, 95% CI 1.56-4.45), CKD (HR 1.61, 95% CI 1.02-2.54), COPD (HR 3.11, 95% CI 1.00-9.86), fatigue (HR 2.12, 95% CI 1.50-3.00), dyspnea (HR 1.55, 95% CI 1.06-2.27), altered mental status (HR 2.46, 95% CI 1.28-4.76), NLR ≥ 5.8 (HR 3.38, 95% CI 2.35-4.85), and severe-critical condition (HR 2.31, 95% CI 1.48-3.60) were included to create the scoring system (**Table 3**). We developed the scoring system with variable score given for the presence of the variables (**Table 4**) with the AUC was 84.7% (95% CI 0.82-0.88) (**Figure 2)**.

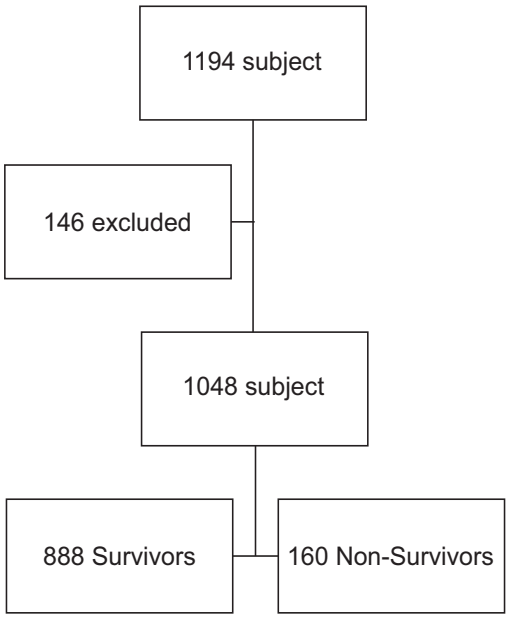

Figure 1. Flowchart of patients.

The optimal cut-off point with the highest combined sensitivity and specificity was a score of ≥ 6, which has a sensitivity of 76.3 % and specificity 78.2% to predict mortality of hospitalized COVID-19 patient. A total score of ≥ 6 had an HR of 1.54 (95% CI 1.41-1.69) compared to a score below 6. Based on internal validation with bootstrapping technique, the result of Hosmer-Lemeshow test was p = 0.784. The result of the missing data compared with the total patients was not statistically significant.

DISCUSSION

Age, as one of the risk factors, is in line with several studies showing that increased age was one of the determining factors for COVID-19 mortality.[8,14] There is an increased risk of having multimorbidity with increasing age. In addition, immunosenescence in older adults may result in impairment of immune response.[15–17]

Several comorbidities are already known to be related to COVID-19 mortality. In this study, we found that CKD and COPD were significant predictors. Previous studies suggested that COVID-19 patient with CKD had nearly twofold higher risk for mortality, compared to patients without CKD, which was similar to our multivariate cox-regression result.[18] CKD patients were hypothesized to have low-grade inflammation causing baseline lymphopenia. Furthermore, abundant reactive oxygen species (ROS) in CKD patients were enhanced by the uremic toxins and angiotensin-mediated activation of Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, promoting endothelial dysfunction leading to the progression of COVID-19.[19] CKD together with COVID-19 may result in several complications, such as myocarditis, acute myocardial injury, heart failure, and arrhythmias. CKD is one of the major complications of both hypertension and diabetes in the disease course. Contrary to the significance of CKD, the role of hypertension and diabetes mellitus as the predictors of mortality in this study was not statistically significant after

Table 2. Cox regression analysis of variables that were significantly different between survivors and non-survivors.

Variables	Discharge Status		HR (95% CI)	p-value
	Survivor, n (%)	Non-Survivor, n (%)		
Age				
- 18-49 years old	504 (92.8)	39 (7.2)		
- 50-69 years old	342 (77.9)	97 (22.1)	2.81 (1.94-4.08)	<0.001
- ≥ 70 years old	42 (63.6)	24 (36.4)	4.97 (2.99-8.27)	<0.001
Sex				
- Female	502 (88.7)	64 (11.3)		
- Male	386 (80.1)	96 (19.9)	1.65 (1.20-2.27)	<0.001
Hypertension				
- No	546 (88.2)	73 (11.8)		
- Yes	342 (79.7)	87 (20.3)	1.53 (1.12-2.09)	0.01
Diabetes Mellitus				
- No	666 (87.1)	99 (12.9)		
- Yes	222 (78.4)	61 (21.6)	1.48 (1.08-2.04)	0.02
Heart Disease				
- No	761 (86.2)	122 (13.8)		
- Yes	127 (77.0)	38 (23.0)	1.42 (0.99-2.05)	0.06
COPD				
- No	885 (84.9)	157 (15.1)		
- Yes	3 (50.0)	3 (50.0)	4.64 (1.48-14.57)	0.01
Pulmonary tuberculosis				
- No	860 (85.5)	146 (14.5)		
- Yes	28 (66.7)	14 (33.3)	1.85 (1.07-3.21)	0.03
CKD				
- No	839 (85.9)	138 (14.1)		
- Yes	49 (69.0)	22 (31.0)	2.20 (1.40-3.44)	<0.001
Nausea-Vomiting				
- No	564 (87.6)	80 (12.4)		
- Yes	324 (80.2)	80 (19.8)	1.40 (1.03-1.91)	0.03
Fatigue				
- No	501 (91.4)	47 (8.6)		
- Yes	387 (77.4)	113 (22.6)	2.72 (1.94-3.83)	<0.001
Dyspnea				
- No	555 (92.7)	42 (7.3)		
- Yes	353 (74.9)	118 (25.1)	3.32 (2.34-4.73)	<0.001
Altered Mental Status				
- No	884 (85.5)	150 (14.5)		
- Yes	4 (28.6)	10 (71.4)	7.74 (4.07-14.71)	<0.001
NLR ≥ 5.8				
- No	655 (93.6)	45 (6.4)		
- Yes	233 (67.0)	115 (33.0)	5.65 (4.00-7.80)	<0.001
Severe-Critical condition**				
- No	535 (94.9)	29 (2.1)		
- Yes	353 (72.9)	131 (27.1)	4.93 (3.30-7.37)	<0.001

*Variables with p<0.25 were selected for the multivariate analysis, HR: hazard risk, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, NLR: Neutrophil-lymphocyte ratio.
**Severe-Critical condition criteria were based on the COVID-19 severity upon admission, we categorized mild-moderate COVID-19 as no, while severe-critical COVID-19 as yes.

after adjustment.

COPD was also already known to be related to COVID-19 mortality, which was possibly linked to the underlying disruption in the bronchial epithelial cells resulting in increased expression of Angiotensin-converting enzyme-2 (ACE-2). When the spike protein of SARS-CoV-2 binds to the ACE-2, it increases the tissue damage due to an increase in pro-inflammatory effects.[20,21]

Fatigue and dyspnea were other predictors of mortality in our study. The information can

Table 3. Multivariate cox regression analysis of variables that were significantly different between survivors and non-survivors.

	Hazard Ratio	95% CI	p-value
Age ≥ 70 years old	2.64	1.56-4.45	<0.001
Age 50-69 years old	1.58	1.07-2.32	<0.001
CKD	1.61	1.02-2.54	0.04
COPD	3.11	1.00-9.86	0.05
Fatigue	2.12	1.50-3.00	<0.001
Dyspnea	1.55	1.06-2.27	0.02
Altered mental status	2.46	1.28-4.76	0.01
NLR ≥ 5.8	3.38	2.35-4.85	<0.001
Severity: Severe-Critical condition	2.31	1.48-3.60	<0.001

*HR: hazard risk, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, NLR: Neutrophil-lymphocyte ratio.

Table 4. The COVID-19 mortality score for predicting mortality in hospitalized patients.

Components	Category	Points
Age	≥ 70 years old	2
	50-69 years old	1
	18-49 years old	0
CKD	Yes	1
	No	0
COPD	Yes	1
	No	0
Fatigue	Yes	2
	No	0
Dyspnea	Yes	1
	No	0
Altered mental status	Yes	1
	No	0
NLR ≥ 5.8	Yes	3
	No	0
Severity: Severe-Critical condition	Yes	2
	No	0

*COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, NLR: Neutrophil-lymphocyte ratio

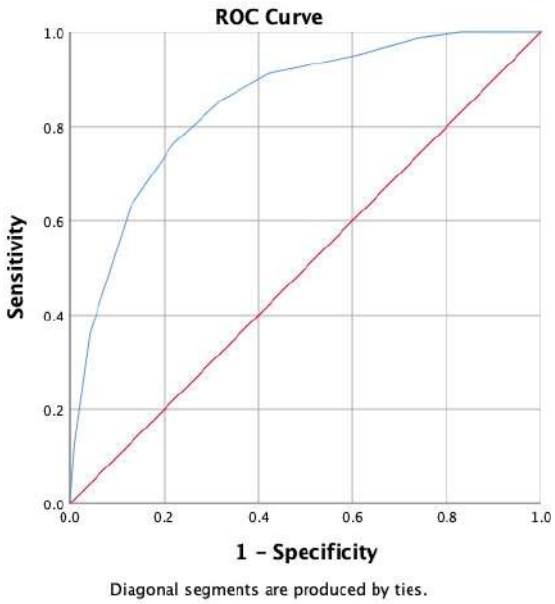

Figure 2. Receiver operating characteristic curve (ROC) for prediction of mortality in hospitalized COVID-19 patients

be easily obtained from history taking. A meta-analysis study done by He et al, presented that dyspnea (Odds Ratio [OR] 6.2, 95% CI 3.6–10.6) and fatigue (OR 2.1, 95% CI 1.3–3.3) were the main difference between mild and severe COVID-19.[22] Previous study also suggested that dyspnea (OR 4.52, 95% CI 3.15, 6.48) and fatigue (OR 1.27, 95% CI 1.09, 1.48) were significantly related to increase in mortality of COVID-19 patients.[23] Not only does the virus affect lungs, but it is also hypothesized that the virus can invade the skeletal muscle leading to fatigue. In addition, SARS-CoV-2 is known to affect the central nervous system, including decreasing neurotransmitters such as dopamine and serotonin.[24]

Overall score of Glasgow Coma Scale (GCS) of less than 15 is also one of the predictors of mortality in our study, a finding that is supported by a previous study.[25] The most common neurological symptom of COVID-19 patients is altered mental status. Several hypotheses stated that it may be related to the severity of the disease itself or due to the direct injury of the brain.[25,26] After adjustment of the comorbidities and severity in our study, altered mental status was still an independent predictor of mortality during hospitalization.

Two meta-analyses showed that NLR was a significant predictor of disease severity and mortality.[27,28] Increase in NLR on admission is known to be independently related to COVID-19 mortality. Prior to COVID-19 pandemic, NLR was known to be a predictor of systemic inflammation in several diseases, including cardiovascular diseases and acute pancreatitis.[27] It is hypothesized that in COVID-19, there was more excessive immune response, consequently decreasing the circulating lymphocyte and increasing the gap between neutrophil and

lymphocytes count. In addition, viral infection may also increase the neutrophil by increasing pro-inflammatory markers, such as interleukin-6 (IL-6).[28] Compared to another inflammatory marker, such as C-reactive protein (CRP), NLR may be a more accessible biomarker in low-middle-income countries.

Severe and critical conditions of COVID-19 patients were also related to mortality. Patients who required oxygen supplementation during hospitalization were associated with respiratory distress, indicating pulmonary inflammation. The immune system tries to eliminate the virus resulting in damage of the epithelial cells of the lungs and can lead to prolonged inflammation.[29] The overdrive immune system also contributes to cytokine storm of which there were a large number of proinflammatory cytokines, especially IL-6, leading to more injury to the lungs.[30] Prolonged inflammation occurs in the lung and other organs, such as blood vessels, heart, and kidney, therefore leading to many complications and increased mortality rate.

The AUC of this prognostic scoring system suggested that the model will be able to predict mortality during hospitalization of COVID-19 patients with 84.7% certainty. A patient with score of 7 or higher had 55% 7-day mortality rate. Moreover, the 14-day mortality rate was higher (73%). Despite having several missing data, the result of the missing data compared with the total patients was not statistically significant.

Due to the growing number of new confirmed case of COVID-19 and potential intensive care bed shortage, we proposed an algorithm for healthcare worker's use. First, physicians may follow local guideline related to patient's criteria for Intensive Care Unit (ICU) transfer. Second, physicians may stratify the mortality risk of each patient using the established prognostic scoring system. Patients with lower mortality risk may be prioritized to be transferred to the ICU. Identifying the population at risk of mortality during hospitalization since their first admission is important to allocate the healthcare resources and educate the family members regarding the prognosis of the patients.

The strength of this scoring system is that it has an AUC of 0.85, which was higher than that of other prognostic scoring system.[8,31] In addition, this prognostic scoring system was based on history taking, physical examination, and easily measurable blood test. Thus, it can be widely applicable in low-middle income countries as these countries are still trying to catch up with the COVID-19 vaccination program. The data in this study were collected from five COVID-19 referral centers in Indonesia. Therefore, there is possibility of inclusion of a wider range of population groups, which may subsequently increase the generalizability of the study. This prognostic scoring system may be used in clinical practice depending on the relevant clinical condition of cases with viral variants spread at certain point of time. An external validation study is still needed to ensure the performance of this scoring system.

CONCLUSION

Components of history taking, physical examination, and simple blood tests can be used to predict the risk for mortality of hospitalized COVID-19 patients. The components include age, underlying CKD, COPD, fatigue, dyspnea, altered mental status, NLR ≥ 5.8, and severe-critical COVID-19. The result of this practical prognostic scoring system may be a guide to decision making of physicians and help in the education of family members related to the possible outcome of the patient.

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CONFLICT OF INTERESTS

All authors declare that there is no conflict of interests.

REFERENCES

1. Centers for Disease Control and Prevention. 2019 Novel Coronavirus, Wuhan, China, https://www.cdc.gov/coronavirus/2019-ncov/about/index.html. (2020, accessed 17 June 2021).
2. Mostaza JM, García-Iglesias F, González-Alegre T, et al. Clinical course and prognostic factors of COVID-19 infection in an elderly hospitalized population. Arch Gerontol Geriatr. 2020;91:104204.
3. Gupta RK, Marks M, Samuels THA, et al. Systematic evaluation and external validation of 22 prognostic models among hospitalised adults with COVID-19: An observational cohort study. Eur Respir J. 2020; 56. Epub ahead of print 1 December 2020. DOI: 10.1183/13993003.03498-2020.
4. Gue YX, Tennyson M, Gao J, et al. Development of a novel risk score to predict mortality in patients admitted to hospital with COVID-19. Sci Rep. 2020; 10:21379.
5. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020;584:430–6.
6. Johns Hopkins Coronavirus Resource Center. Mortality Analyses, https://coronavirus.jhu.edu/data/mortality (2021, accessed 25 August 2021).
7. Setiati S, Azwar MK. COVID-19 and Indonesia. Acta Med Indones. 2020;52:84–9.
8. Knight SR, Ho A, Pius R, et al. Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: development and validation of the 4C Mortality Score. BMJ. 2020;m3339.
9. Grobbee D, Hoes A. Clinical Epidemiology: Principles, Methods, and Applications for Clinical Research. 2nd ed. Burlington, MA: Jones & Bartlett Learning; 2014.
10. Collins GS, Reitsma JB, Altman DG, et al. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMJ. 2015;350:g7594.
11. World Health Organization. WHO Information Notice for IVD Users 2020/05, https://www.who.int/news/item/20-01-2021-who-information-notice-for-ivd-users-2020-05 (2021, accessed 5 March 2021).
12. World Health Organization. Clinical management of COVID-19: living guidance, https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1 (2021).
13. Song C, Xu J, He J, et al. COVID-19 early warning score: a multi-parameter screening tool to identify highly suspected patients. medRxiv Prepr. Epub ahead of print 2020. DOI: 10.1101/2020.03.05.20031906.
14. Bradley P, Frost F, Tharmaratnam K, et al. Utility of established prognostic scores in COVID-19 hospital admissions: multicentre prospective evaluation of CURB-65, NEWS2 and qSOFA. BMJ Open Respir Res. 2020;7:e000729.
15. Booth A, Reed AB, Ponzo S, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PLoS One. 2021;16:e0247461.
16. Franceschi C, Garagnani P, Parini P, et al. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol. 2018;14:576–90.
17. Azwar MK, Setiati S, Rizka A, et al. Clinical Profile of Elderly Patients with COVID-19 hospitalised in Indonesia's National General Hospital. Acta Med Indones. 2020;52:199–205.
18. Pakhchanian H, Raiker R, Mukherjee A, et al. Outcomes of COVID-19 in CKD Patients. Clin J Am Soc Nephrol. 2021;CJN.13820820.
19. Podestà MA, Valli F, Galassi A, et al. COVID-19 in Chronic Kidney Disease: The Impact of Old and Novel Cardiovascular Risk Factors. Blood Purif. 2021;1–10.
20. Leung JM, Niikura M, Yang CWT, et al. COVID-19 and COPD. Eur Respir J. 2020;56:2002108.
21. Lee SC, Son KJ, Han CH, et al. Impact of COPD on COVID-19 prognosis: A nationwide population-based study in South Korea. Sci Rep. 2021;11:3735.
22. He X, Cheng X, Feng X, et al. Clinical Symptom Differences Between Mild and Severe COVID-19 Patients in China: A Meta-Analysis. Front Public Health; 8. Epub ahead of print 14 January 2021. DOI: 10.3389/fpubh.2020.561264.
23. Yang L, Jin J, Luo W, et al. Risk factors for predicting mortality of COVID-19 patients: A systematic review and meta-analysis. PLoS One. 2020;15:e0243124.
24. Rudroff T, Fietsam AC, Deters JR, et al. Post-COVID-19 fatigue: Potential contributing factors. Brain Sci. 2020;10:1012.
25. Kenerly MJ, Shah P, Patel H, et al. Altered mental status is an independent predictor of mortality in hospitalized COVID-19 patients. Irish J Med Sci. 2021;1971. Epub ahead of print 10 February 2021. DOI: 10.1007/s11845-021-02515-4.
26. Pezzini A, Padovani A. Lifting the mask on neurological

manifestations of COVID-19. Nat Rev Neurol. 2020; 16:636–44.
27. Simadibrata DM, Calvin J, Wijaya AD, et al. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: A meta-analysis. Am J Emerg Med. 2021;42:60–9.
28. Li X, Liu C, Mao Z, et al. Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. Crit Care. 2020;24:647.
29. Torres Acosta MA, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. Eur Respir J. 2020;56:2002049.
30. Hojyo S, Uchida M, Tanaka K, et al. How COVID-19 induces cytokine storm with high mortality. Inflamm Regen. 2020;40:37.
31. Mei Q, Wang AY, Bryant A, et al. Development and validation of prognostic model for predicting mortality of COVID-19 patients in Wuhan, China. Sci Rep. 2020;10:1–12.

The Validation of Drug Resistance in Pneumonia (DRIP) Score in Predicting Infections due to Drug-Resistant Pathogens in Community-acquired Pneumonia at Cipto Mangunkusumo Hospital, Jakarta, Indonesia

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ABSTRACT

Background: *The emergence of drug-resistant pathogens (DRP) in recent years possibly contributes to the common problems associated with community-acquired pneumonia. However, to predict the risk of the ailment, the DRIP score is mainly applied, although no validation study has been reported in Indonesia. Therefore, the score prediction accuracy in the population, patient characteristics and germ patterns appears indefinite, particularly for Cipto Mangunkusumo Hospital, Jakarta. The purpose of this study is to determine the DRIP performance as an instrument in predicting infections due to drug-resistant pathogens (DRP) in community-acquired pneumonia at Cipto Mangunkusumo Hospital.* ***Methods:*** *This research employed a cross-sectional design, where the subjects were community-acquired pneumonia patients treated between January 2019 and June 2020. In addition, adequate medical records of the participants were obtained. The condition is defined as DRP when the sputum culture results show resistance to non-pseudomonal β-lactam antibiotics, macrolides, and respiratory fluoroquinolones. Furthermore, the score performance was analyzed by determining the calibration and discrimination values, using the Hosmer-Lemeshow test and AUROC, respectively.* ***Results:*** *A total of 254 subjects were known to have satisfied the selection criteria. These participants were categorized into DRP and non-DRP groups, with 103 (40.6%) and 151 (59.4%) patients, correspondingly. The DRIP calibration analysis using the Hosmer-Lemeshow test obtained p-value = 0.001 (p <0.05), while an AUC value of 0.759 (CI 95%,*

0.702-0.810) was derived from the ROC curve. However, at a score of ≥ 4, the DRIP showed sensitivity, specificity, positive and negative predictive values of 70.9, 92.7, 86.9, and 82.3%, respectively. ***Conclusion:*** *The DRIP score demonstrated a significant performance in predicting infections due to DRP in community-acquired pneumonia.*

Keywords**:** *DRIP score, resistant pathogens, community-acquired pneumonia.*

INTRODUCTION

In recent years, frequent community-acquired pneumonia problems have been linked to the emergence of drug-resistant causative pathogens.[1,2] Drug-resistant pathogens (DRP) require different antibiotics compared to the initial empiric antibiotics recommended in the guidelines for community-acquired pneumonia. Subsequently, for DRP pathogens, the initial empiric antibiotics administered include antipseudomonal and anti-MRSA.[1,3] However, there are a total of 14 alternative score models in addition to the HCAP criteria that are used for the prediction of infection by DRP pathogens in community-acquired pneumonia that has been published, from 2004 till date.

The DRIP (Drug resistance in Pneumonia) score published in the United States by Webb in 2016 is a predictive model with the most external validation tests and implementation research compared to other prediction models.[4] According to research conducted by Webb in 2016, the DRIP score has better predictive accuracy of DRP pathogens compared to several other alternative scores, such as Schreiber, Schorr, Niedermann, Shindo, Aliberti, Park, PES, and HCAP scores, with an AUC value of 0, 88 (95% CI 0.82-0.93).[5] The DRIP score consists of ten risk factors associated with DRP pathogens, including the history of antibiotic use, long-term hospital stay, enteral nutrition, history of infection with previous PRO pathogens, history of previous treatment, chronic lung disease, poor functional status, gastric acid suppression, wound care, and history of MRSA colonization.[5]

Several institutions have performed validation tests and implemented the DRIP score as a new predictive model to replace the HCAP criteria. Based on various research on the implementation of the DRIP score in patients suffering from community-acquired pneumonia, it was concluded that the use of the DRIP score was more effective than the HCAP criteria in helping clinicians avoid the unnecessary use of broad-spectrum antibiotics.[6,7,8] However, this research also recommends that each institution validate it prior to regular use, as the predictive value of this DRIP score will be relatively different depending on the population, region, patient characteristics, and bacterial pattern.

To date, there has been no research in Indonesia that validates or uses a predictive model to predict infection by DRP pathogens in community-acquired pneumonia. Therefore, this research aims to determine the performance of the DRIP score as an instrument to predict infection due to DRP pathogens in patients suffering from community-acquired pneumonia. Furthermore, by knowing the performance of the DRIP score, it is hoped that it will help clinicians in selecting the initial empiric antibiotics in community-acquired pneumonia if they are to use broad-spectrum antibiotics that include antipseudomonal or not in such a way that the patient outcome will be better.

METHODS

The cross-sectional method was used in this research at the Dr. Cipto Mangunkusumo National Central General Hospital from April to May 2021. Furthermore, secondary data in the form of medical records of the patients with community-acquired pneumonia that were hospitalized from January 2019 to June 2020 were used. The population is community-acquired pneumonia patients that are hospitalized at Dr. Cipto Mangunkusumo Hospital. The study has been approved by the Ethics Committee of Faculty of Medicine Universitas Indonesia (Reference No. 226/UN2.F!/ETIK/PPM.00.02/2021).

A sequential sampling method was used and the inclusion criteria include: 1) patients hospitalized with community-acquired pneumonia; 2) age ≥18 years; 3) initial empiric

antibiotics were administered at the time of admission, and 4) sputum specimens were collected for culture and antibiotic sensitivity testing within 2x24 hours after admission. An example of an exclusion criterion was that the inadequate quality of the sputum culture samples, and incomplete data.

Data on DRIP score variables or risk factors are collected from medical records, with a score greater than 4, corresponding to a high risk group with DRP, while a score less than 4 corresponds to a low risk group with DRP. The definition of a drug-resistant pathogen (DRP) is given when the results of sputum cultures and antibiotic susceptibility tests show that a pathogen is resistant to non-pseudomonal beta-lactam antibiotics (Ceftriaxone, Cefotaxime, Ampicillin Sulbactam) and respiratory fluoroquinolones (Levofloxacin, Moxifloxacin).

Statistical Analysis

The data analysis was performed using SPSS 22.0. The basic and clinical characteristics of the research subjects are presented in tabular form. The validation test or the performance of the DRIP score for predicting infection due to DRP pathogens in community-acquired pneumonia was assessed by determining the calibration value with the Hosmer-Lemeshow test and the discrimination value with the area under the curve (AUC) of the ROC curve. The calibration is adequate if the p-value of the Hosmer-Lemeshow test is greater than 0.05. Furthermore, the discrimination value is good if the AUC value is greater than or equal to the minimum expected AUC value (greater than 80%).

RESULTS

In this research, out of 694 patients with community-acquired pneumonia that had sputum culture results from medical records, 440 were excluded because the culture specimens were invalid, and 254 were included as subjects. However, the infection due to DRP pathogens in community-acquired pneumonia was less than non-DRP. There were 103 patients (40.6%) with DRP pathogenic infections, while 151 patients (59.4%) were classified as non-DRP. The demographic and clinical characteristics of the subjects, as well as the proportion of research subjects in both DRP and non-DRP groups based on risk class DRIP scores, can be seen in full in **Table 1**.

Table 2. shows that a total of 323 isolates were obtained, indicating that 131 (40.6%) were classified as DRP and 192 (59.4%) were classified as non-DRP.

The performance of the DRIP score was determined from the calibration and discrimination values. As shown in **Table 3** and **Figure 1**. The value of $p=0.001$ ($p<0.05$) was obtained from the Hosmer-Lemeshow test. This shows that the calibration value of the DRIP score was not good. Meanwhile, the discriminatory ability of the DRIP score toward DRP pathogens was quite good with an AUC value of 0.759 (95% CI; 0.702-0.810) on the ROC curve, as shown in **Figure 2**.

DISCUSSION

The prevalence of patients with community-acquired pneumonia caused by DRP pathogens was 40.6%. The prevalence of DRP Pathogens in community-acquired pneumonia was found to vary in various regions and areas, namely 7.2-36.0% in the Asia Pacific region, 20.0-45.2% in America, and 5.9-33.0% in Europe.[9] For Southeast Asia, the data in the Philippines is 29.7%.[6]

In this research, it was found that the incidence of DRP pathogen infection increased along with the higher risk class according to the DRIP score. Based on these data, it is shown that the DRIP score can predict infection with DRP pathogens in community-acquired pneumonia. This is in line with 2016 Webb research that compared DRIP scores with HCAP criteria. Furthermore, it was stated that the DRIP score ≥ 4 can effectively distinguish low and high risks in community pneumonia due to infection with DRP pathogens, with a sensitivity of 0.82, specificity of 0.81, and a positive predictive value (PPV) of 0.68, and a negative predictive value (NPV) of 0.90, compared to the HCAP criteria, which had a lower predictive value with a sensitivity of 0.79, specificity of 0.65, PPV 0.53 and NPV 0.86.[5]

In this research, the calibration of the DRIP score based on the Hosmer-Lemeshow test has

Table 1. Demographic and clinical characteristics of community-acquired pneumonia patients due to DRP and non-DRP pathogens.

Variables	All Subjects n=254 (%)	DRP Pathogens n=103 (%)	Non DRP Pathogens n=151 (%)
Gender, n (%)			
- Male	143 (56,3)	63 (61,2)	80 (53,0)
- Female	111 (43,7)	40 (38,8)	71 (47,0)
Age, n (%)			
- 18-60 years old	165 (65,0)	60 (58,3)	105 (69,5)
- >60 years old	89 (35,0)	43 (41,7)	46 (30,5)
Comorbidities, n (%)			
- Malignancy	70 (27,5)	28 (27,2)	42 (27,8)
- Diabetes Mellitus	57 (22,4)	25 (24,3)	32 (21,1)
- Cerebrovascular disease	43 (16,9)	23 (22,3)	20 (13,2)
- Chronic Kidney Disease	42 (16,5)	16 (15,5)	26 (17,2)
- Chronic Heart Disease	30 (11,8)	9 (8,7)	21 (13,9)
- Chronic Lung Disease	12 (4,7)	6 (5,8)	6 (3,9)
- Chronic Liver disease	12 (4,7)	6 (5,8)	6 (3,9)
- Other Comorbidities	14 (5,5)	3 (2,9)	11 (7,3)
- No Comorbiditities	7 (2,7)	2 (1,9)	5 (3,3)
Severity			
- No Sepsis	169 (66,5)	70 (68,6)	99 (65,1)
- Sepsis	30 (11,8)	9 (8,8)	21 (13,8)
- Sepsis Shock	55 (21,7)	23 (22,6)	32 (21,1)
Use of Ventilators, n (%)			
- Using a ventilator	49 (19,3)	23 (22,3)	26 (17,2)
- No ventilators	205 (80,7)	80 (77,7)	125 (82,8)
Initial Empirical Antibiotics, n (%)			
- Ceftriaxone-Azitromisin	75 (30,2)	22 (22.5)	53 (35.6)
- Cefotaxime-Levofloxacin	46 (18,6)	24 (24.5)	22 (14.8)
- Ceftriaxone	45 (18,1)	14 (14.3)	31 (20.8)
- Cefepime	21 (8,5)	7 (7.1)	14 (9.4)
- Meropenem	12 (4,9)	8 (8.2)	4 (2.7)
- Ampicillin Sulbactam	11 (4,4)	6 (6.1)	5 (3.4)
- Levofloxacin	9 (3,6)	5 (5.1)	4 (2.7)
- Meropenem-Levofloxacin	9 (3,6)	5 (5.1)	4 (2.7)
- Cefepime-Levofloxacin	9 (3,6)	5 (5.1)	4 (2.7)
- Cefoperazone	3 (1,2)	0 (0.0)	3 (2.0)
- Cefotaxime-Azitromisin	3 (1,2)	0 (0.0)	3 (2.0)
- Ceftazidime	3 (1,2)	2 (2.0)	1 (0.6)
- Cefotaxime	1 (0.4)	0 (0.0)	1 (0.6)
- Moxifloxacin	0 (0,0)	0 (0.0)	0 (0.0)
Antibiotic escalation, n (%)			
- Yes	83 (32.7)	50 (48.1)	33 (21.8)
- No	171 (67.3)	53 (51.9)	118 (78.2)
DRIP Score, n (%)			
- DRIP Score of ≥ 4 (High Risk of DRP)	84 (33.1)	73 (70.9)	11 (7.3)
- DRIP Score of < 4 (Low Risk of DRP)	170 (66.9)	30 (29.1)	140 (92.7)

a p-value of 0.001. The p-value <0.05 indicates that the DRIP score shows a weak correlation between the expected infection by the pathogenic DRP or the expected DRP with the observed results (**Figure 3**). In the Hosmer-Lemeshow test, there was a statistically significant difference ($p<0.05$) between the predicted results of infection due to pathogenic DRP of the DRIP scores and the actual observations obtained from this research. Furthermore, it was also found that the incidence of DRP pathogen infection in community-acquired pneumonia did not increase along with the increase in the total DRIP score. Meanwhile, in the DRIP score discrimination

Table 2. Gram-positive and negative bacteria as the cause of community-acquired pneumonia.

	All Isolates n=323 (%)	DRP Isolate n=131 (%)	Non-DRP isolate n=192 (%)
Gram Positive	52 (16.1)	21 (16.1)	31 (16.1)
- S. epidermidis	17 (5.3)	9 (6.9)	8 (4.2)
- S. aureus	15 (4.6)	2 (1.5)	13 (6.8)
- S. saprophyticus	10 (3.1)	5 (3.8)	5 (2.6)
- E. faecalis	9 (2.8)	5 (3.8)	4 (2.1)
- S. sciuri	1 (0.3)	0 (0.0)	1 (0.5)
Gram Negative	271 (83.9)	110 (83.9)	161 (83.9)
- K. pneumonia	99 (30.7)	41 (31.3)	58 (30.2)
- P. aeuruginosa	53 (16.4)	14 (10.7)	39 (20.3)
- Acinetobacter sp.	51 (15.8)	23 (17.6)	28 (14.6)
- E. coli	27 (8.4)	16 (12.2)	11 (5.7)
- A. baumannii	13 (4.0)	5 (3.8)	8 (4.2)
- E. cloacae	11 (3.4)	4 (3.1)	7 (3.6)
- Enterobacter sp.	9 (2.8)	2 (1.5)	7 (3.6)
- K. oxytoca	6 (1.9)	4 (3.0)	2 (1.1)
- A. xylosoxidants	1 (0.3)	1 (0.8)	0 (0.0)
- S. maltophilia	1 (0.3)	0 (0.0)	1 (0.5)

Table 3. Calibration of DRIP score in the expected and observed groups (n=254).

		DRP Pathogen (%)	
Score	**n**	**Observed**	**Expected**
1	111	30.00	19.620
2	17	0.00	5.255
3	42	0.00	20.263
4	67	59.00	44.230
5	17	14.00	13.632

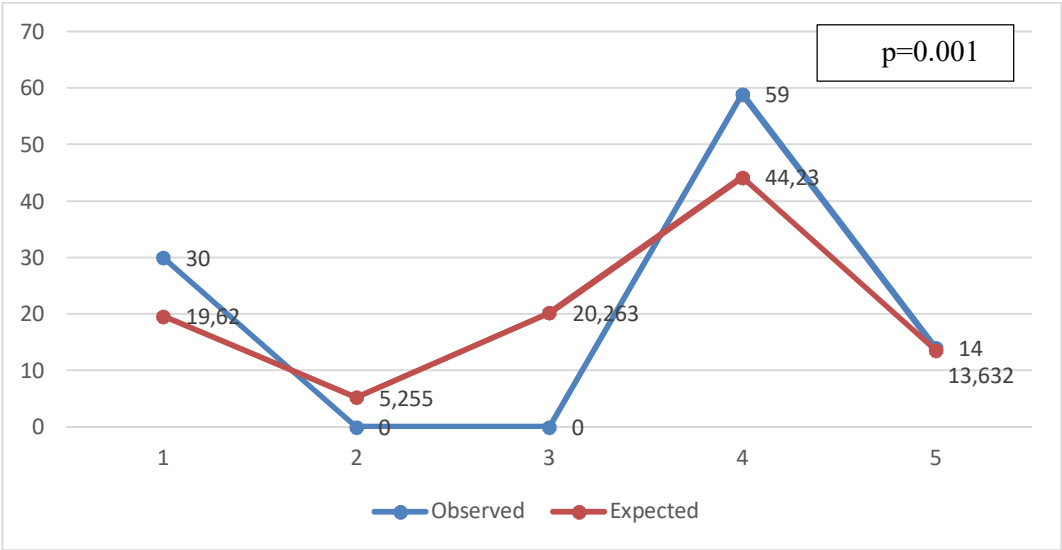

Figure 1. Graph of DRIP score calibration in the expected and observed groups.

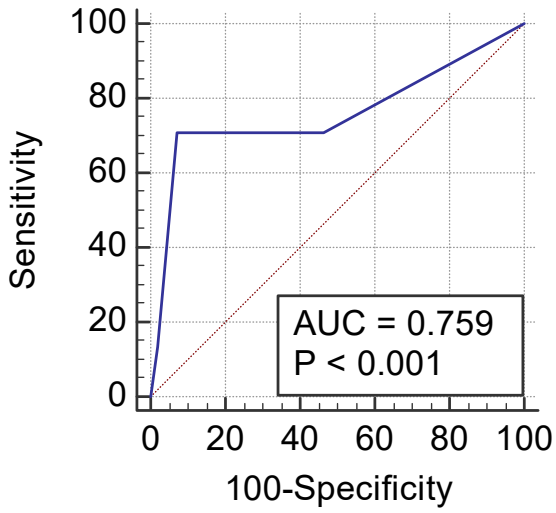

Figure 2. ROC curve of DRIP score model validation.

test, discrimination performance was obtained with an AUC value of 0.759 (95% CI; 0.702-0.810) on the ROC curve. The AUC value of 0.7-0.8 indicates that the DRIP score has a good value in predicting infection due to DRP pathogens in community pneumonia. With a cut-off value of ≥ 4, the DRIP score can differentiate high-risk groups with a low risk of being infected with DRP pathogens with a sensitivity value of 70.9%, specificity of 92.7%, the positive predictive value of 86.9%, the negative predictive value of 82.3%, the positive likelihood ratio of 9.73 and negative likelihood ratio of 0.31.

The calibration value was found to be poor, while the discrimination value with an AUC of 0.759 was good enough to predict infection by DRP pathogen in community-acquired pneumonia. The value of sensitivity, specificity, positive predictive value, and negative predictive value was also obtained quite well. This research aims to determine the ability of the DRIP score to predict infections of community-acquired pneumonia was caused by a DRP pathogen or not. In other words, this study acts as a diagnostic predictive research of DRIP scores in predicting DRP in community-acquired pneumonia. Therefore, the discriminated value of the ROC curve is more suitable to be guided than the calibration value, before the score is applied in daily clinical practice.[10]

Based on the value of AUC, sensitivity, specificity, positive prediction, and negative prediction obtained, it can be concluded that the DRIP score has a good value in predicting community-acquired pneumonia due to a DRP pathogen. This is not different from several other research that validates and implement the DRIP score. Furthermore, Farkas et al stated that the DRIP score was used as a tool in predicting the incidence of infection due to DRP pathogens in community-acquired pneumonia. In this research, the coefficient value was 5.62 (95% CI; 1.07-21.54), and the Hosmer Lemeshow test obtained p<0.0001. The coefficient value indicates that the trend of intervention with broad-spectrum antibiotics in the DRIP score ≥ 4 group was 5.62 times higher than the DRIP score <4 group. It was concluded that the application of the DRIP score for patients suffering from community-acquired pneumonia succeeded in reducing the use of broad-spectrum antibiotics.[11] In another cohort research conducted by Webb et al that implemented the DRIP score in 2,169 patients, the AUC value was 0.79 (95% CI; 0.65-0.93) on the ROC curve. This value indicates that the DRIP score has a good value in predicting infection due to DRP pathogens in community-acquired pneumonia.[12]

In retrospective research carried out by Babbel et al, it was stated that the DRIP score is a new predictive tool developed to identify patients suffering from community-acquired pneumonia with an increased risk of DRP pathogens. Furthermore, DRIP scores have better performance characteristics than other available predictive tools that have been associated with the increased use of broad-spectrum antibiotics.[13] This was also stated in prospective research

Supplementary Table. DRIP score.

Risk Factors	Score
Major	
Antibiotic use within previous 60 days	2
Residence in a long-term-care facility	2
Tube feeding	2
Prior infection with a DRP (1 year)	2
Minor	
Hospitalization within previous 60 days	1
Chronic pulmonary disease	1
Poor functional status	1
Gastric acid suppression	1
Wound care	1
MRSA Colonization (1 year)	1
Total score	14

carried out by Villalobos et al, that showed the performance of the DRIP score in predicting pneumonia due to DRP pathogens. Furthermore, the DRIP score is a good predictive model, which can be used to reduce the irrational use of broad-spectrum antibiotics among low-risk patients.[6]

With the good performance of this DRIP score, this score can be used in patients suffering from community-acquired pneumonia as an instrument for predicting infection due to DRP pathogens, therefore the initial empiric antibiotic selection can be more appropriate.

CONCLUSION

The DRIP score has good performance in predicting infection due to drug-resistant pathogens in community-acquired pneumonia in the population, patient characteristics, and germ or bacterial patterns at the National Central General Hospital of dr. Cipto Mangunkusumo Jakarta.

REFERENCES

1. Webb BJ, Jones B, Dean NC. Empiric antibiotic selection and risk prediction of drug-resistant pathogens in community-onset pneumonia. Curr opin Infect Dis. 2016;29(2):167-77.
2. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45-67.
3. Prina E, Ranzani OT, Polverino E, et al. Risk factors associated with potentially antibiotic-resistant pathogens in community-acquired pneumonia. Ann Am Thorac Soc. 2015;12(2):153-60.
4. Gil R, Webb BJ. Strategies for prediction of drug-resistant pathogens and empiric antibiotic selection in community-acquired pneumonia. Curr Opin Pulm Med. 2020;26(3):249-59.
5. Webb BJ, Dascomb K, Stenehjem E, et al. Derivation and multicenter validation of the drug resistance in pneumonia clinical prediction score. Antimicrobial agents and chemotherapy. 2016;60(5):2652-63.
6. Villalobos MJ, Zotomayor R, Gerodias A, et al. Validation study of the drug resistance in pneumonia (DRIP) score in predicting the risk of drug-resistant pathogens among patients with pneumonia: A single center cross-sectional study. Clinical Diagnosis, Prediction and Outcomes of Lung Infections. Am J respir crit care med. 2020;A2130-A2130).
7. Korobey M, Harris L. Drug resistance in pneumonia scoring system for risk of drug-resistant pathogens in sepsis. Critical Care Medicine. 2020;48(1):782.
8. Purewal J, Kanagala K, King E, Anghel S. Validation of the DRIP score at a community-teaching hospital. Chest. 2019;156(4):A351.
9. Shindo Y, Hasegawa Y. Regional differences in antibiotic-resistant pathogens in patients with pneumonia: Implications for clinicians. Respirology. 2017;22(8):1536-46.
10. Cook NR. Statistical evaluation of prognostic versus diagnostic models: Beyond the ROC curve. Clin Chem. 2008;54(1):17-23.
11. Farkas A, Sassine J, Mathew JP, et al. Outcomes associated with the use of a revised risk assessment strategy to predict antibiotic resistance in community-onset pneumonia: a stewardship perspective. J Antimicrobial Chemother. 2018;73(9):2555-8.
12. Webb BJ, Sorensen J, Mecham I, Buckel W, Ooi L, Jephson A, Dean NC. Antibiotic use and outcomes after implementation of the drug resistance in pneumonia score in ED patients with community-onset pneumonia. Chest. 2019;156(5):843-51.
13. Babbel D, Sutton J, Rose R, et al. Potential impact of the DRIP score on antibiotic use: a retrospective single-center study. Open Forum Inf Dis. 2017;4(Suppl 1): S497.

The Effect of Physical Activity on Social Isolation in Elderly

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ABSTRACT

Background: *Elderly people who have poor social relationships have a higher risk of death than those who have strong social networks. Loneliness and social isolation are associated with an increased risk of coronary heart disease and stroke. Physical activity can reduce social isolation, diverting feelings of loneliness by socializing with other people and expanding social networks by participating in the community. This study aimed to determine the effect of physical activity on social isolation in the elderly.* ***Methods:*** *A Cross-sectional study was conducted to 181 respondents. The data is collected through interviewing respondents with the International Physical Activity Questionnaire (IPAQ) and social isolation questionnaires. The data was then analyzed descriptively and calculated using Fisher's exact test. Setting: Ardirejo and Panggungrejo villages, Kepanjen District, Malang Regency.* ***Results:*** *Based on the Fisher's exact between physical activity and social isolation results were obtained p-value 0.000 (PR = 23.407; 95% CI = 3,117-175,800).* ***Conclusion:*** *There is a significant relationship between physical activity and social isolation in the elderly in the community.*

Keywords*: Social isolation, the elderly, physical activity, social interaction.*

INTRODUCTION

The aging process causes various problems. One of the health problems experienced by elderly persons is a psychological problem, loneliness.[1] *The National Council on Aging and Older People* reports that the prevalence of elderly people in America who feel loneliness is 62%.[2]. In Indonesia, the percentage of elderly who experience slight loneliness is 69%, there were 11% having moderate loneliness and 2% having severe loneliness, and 16% others did not experience loneliness. The loneliness felt by the elderly is generally caused by a lack of social interaction and attention from the surrounding environment.[3] This is a triggering factor for depression and other related symptoms, especially in the geriatric population.[4] Social isolation refers to situations when an individual does not have a sense of belonging to socialize, has no involvement with other people, has a minimal number of social contacts, and unable to have quality relationships.[5] The occurrence of social isolation can be a potential issue of emotional or psychological symptoms.[6]

Elderly who have poor social relationships have a higher risk of death compared to elderly who have strong social networks. It was also found that loneliness and social isolation are associated with an increased risk of coronary heart disease and stroke.[7]

Interventions to overcome social isolation in the elderly should focus on improving social relationships and interactions. Physical activity is known to reduce the social isolation in adults.[9] Based on a qualitative study conducted by Robins [2016], elderly people believe that physical activity interventions conducted in groups can help them meet other people, expand their social networks, and help maintain their health by participating in the community. Thus avoiding social isolation being one of the most effective ways to improve psychosocial health.[8] Currently, there are various physical training programs and exercises for the elderly.[9] These programs include; daily active life program, physical fitness improvement program, strength improvement, and walking activities.[10] These activities are low-cost alternatives to physical activities that are easy to implement in the elderly community. This can also be done in urban and rural areas.[4] Based on Arahaf's study [2017], shown 64.2% of the elderly in Malang City can do physical activity independently and 35.8% feel dependence on family members in doing physical activities in daily life[11]. Other data revealed that elder people at Malang city have low level physical activity or inactive (35,4%), minimally activity (52,8%), and high activity (11,8%).[12]

Physical activity, especially regular exercise, is a non-pharmacological therapy that can be used as an intervention in preventing social isolation and managing loneliness in the elderly.[13] Based on this information, the researcher wanted to know the association of the physical activity to the prevention of social isolation in the geriatric population.

METHODS

This is a cross-sectional study among elderly people who came to Posyandu lansia (community-based integrated service center for older adults) in Ardirejo and Panggungrejo villages, Kepanjen District, Malang Regency.

Measurement

The research instrument used was the physical activity questionnaire from IPAQ (International Physical Activity Questionnaire), which categorized physical activity into two levels: light and moderate to vigorous physical activity. IPAQ questionnaires have been validated in 14 centers in 12 countries that have been internationally standardized with more than adequate level of validity (r=0.40) and reliability (0.70-0.87).[14] Result from Indonesian version of the IPAQ questionnaires had small but significant correlation with physical activity recall (r=0.28).[15] Social isolation questionnaire was taken from the Handbook of Geriatric Assessment 5th Edition. The questionnaire consists of 10 questions, with the answer "YES" or "NO" to each question. If the answer "YES" $\geq$ 5, then the participant is declared positive to experiencing social isolation.[16] The author examined the validity and reability for social isolation questionaire. Validity and reliability were measured using the product moment technique. The criteria for selecting items are based on the total item correlation value, generally using a total item coefficient 0.02 because the number of respondents is 50 (r=0.2). In the social isolation measurement questionnaire, the validity coefficient moves from 0.258 to 0.0727, except for item no. 7 which has a validity coefficient of -0.053 and a Cornbach's alpha value of 0.775. Item no. 7 will still be used for data collection by researchers, because it is considered not to affect other items. The Standardized protocols for data collection, including validity and reliability, can minimize inter-observer variability.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was reviewed and approved by the research Ethics Universitas Brawijaya Malang No.100/EC/KEPK/04/2020.

Statistical Analysis

Data analysis includes descriptive analysis and hypothesis testing. In the descriptive analysis, categorical scale data, such as the level of physical activity (light and moderate to vigorous), were expressed as a frequency distribution. Hypothesis testing in data analysis uses an association test to determine the relationship between the independent and dependent variables. The association test used is *Fisher's exact* test. The confidence interval states the magnitude of the independent variable's influence on the dependent variable, and the p-value is considered significant if the p-value is <0.05. Data analysis was performed using SPSS 25.

RESULTS

Based on **Table 1**, it is known that from 181 respondents, the most respondents were elderly aged 60-70 years, which was 126 elderly (69.61%). Proportion of males and females in the elderly respondents were 21.54% (n=39) and 78.46% (n=142), respectively. The characteristics of respondents based on occupation were mostly as housewives, 93 elderly (51.4%). The highest level of education was primary school, with 75 elderly (41.4%).

According to **Table 2**, it can be seen that most of the elderly have a moderate level of physical activity, which was 93 elderly (51.38%). In **Table 3**, it is known that social isolation occurred in 34 elderly (18.78%).

Table 4 shows the results of the *Fisher's exact* test are obtained with significance or *p value* = 0.000. So that if the *p value* <0.05, there is a significant correlation between physical activity and social isolation in the elderly.

Regarding to the calculations obtained from **Table 5**, it is known that the *odds ratio* between the level of light and moderate physical activity on the social isolation in the elderly is 23.407 (OR = 23.407; 95% CI = 3.117-175.800). It showed that the elderly with light physical activity levels are 23 times more likely to experience social isolation than the elderly who have moderate and vigorous physical activity levels.

DISCUSSION

In this study, it was found the most respondents who did physical activity was elderly aged 60-70 years old and the least was the elderly aged 91-100 years. According to data from Badan Pusat Statistik (BPS), the percentage of elderly in Indonesia is dominated by young elderly (aged 60-69 years) 63.82%, the rest are middle elderly and the older elderly people.[14] According to Sharkey (2011) a person at the age of 60 and has retired, has more time to increase activity, although it can decrease with age.[15] The

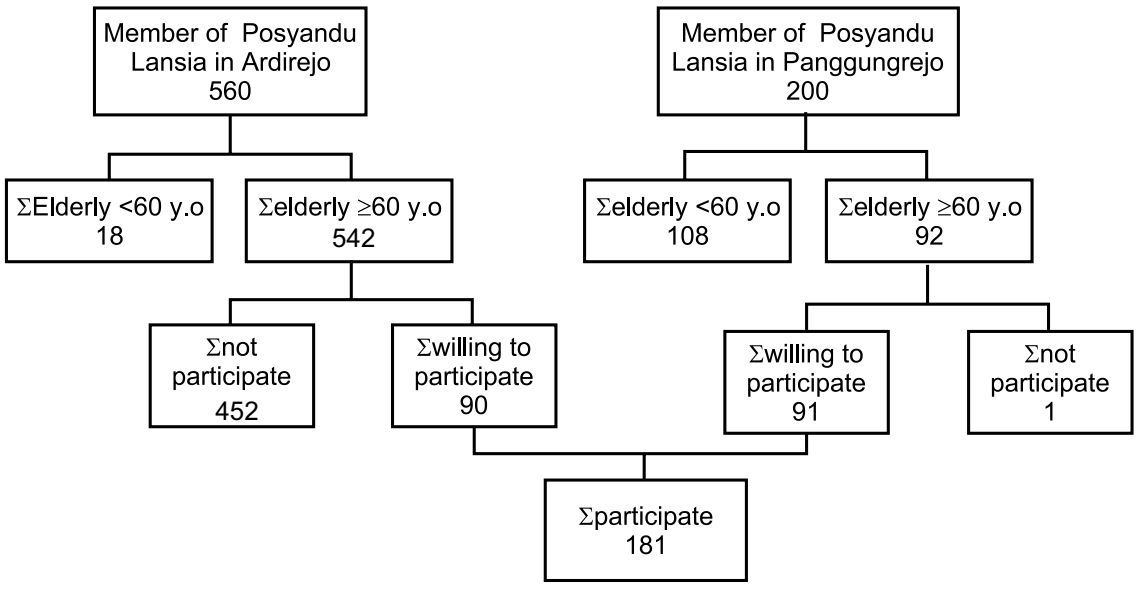

Figure 1. Flowchart participant recruitment

Table 1. Distribution of respondents' frequency characteristics (N=181).

Respondents' Characteristics	Frequency [f]	Percentages [%]
Age		
- 60-70	126	69.61%
- 71-80	48	26.52%
- 81-90	6	3.32%
- 91-100	1	0.55%
Gender		
- Male	39	21,54%
- Female	142	78,46%
Occupation		
- Housewife	93	51,4%
- Retired employee	36	19,9%
- Private employee	9	5%
- Entrepreneur	14	7,7%
- Driver/Taxibiker/ Pedicab driver	6	3,3%
- Labor	5	2,8%
- Farmer	8	4,4%
- Stockbreeder	1	0,5%
- Freelancer	4	2,2%
- Unemployed	5	2,8%
Level of Education		
- Unschoolers	4	2,2%
- Had not finished primary education	14	7,8%
- Elementary School	75	41,4%
- Junior High School	33	18,3%
- Senior High School	37	20,4%
- Bachelor	18	9,9%

(Source: Primary Data, 2020)

Table 2. Distribution of elderly physical activity frequency.

No.	Level of Physical Activity	Frequency [f]	Percentage [%]
1	Light	26	14.37%
2	Moderate to vigorous	155	85.63%
	Total	181	100%

(Source: Primary Data, 2020)

Table 3. Distribution of elderly sosial isolation frequency

No.	Incidence of Social Isolation	Frequency [f]	Percentage [%]
1	Social Isolation	34	18.78
2	Normal	147	81.22
	Total	181	100

(Source: Primary Data, 2020)

older they get, the lower the physical activity will be performed, this is due to a decrease in the strength level caused by comorbidities suffered by the elderly, thus it prevented the elderly to perform physical activity.[16]

Table 4. Cross tab between light and moderate to vigorous physical activity levels on the incidence of social isolation in the elderly

	Social Isolation	
Physical Activity	**Normal**	**Social Isolation**
Light	61	1
Moderate - Vigorous	87	32
Total	148	33

Table 5. Fisher's exact test, prevalence ratio calculation between light, moderate to vigorous physical activity levels on social isolation in the elderly.

No.	Incidence of Social Isolation	Frequency [f]	Percentage [%]
1	Social Isolation	34	18.78%
2	Normal	147	81.22%
	Total	181	100%

(Source: Primary Data, 2020)

The gender of respondents was dominated by women, which was 142 (78.46%) elderly, and the largest proportion had an occupation of being a housewife with 93 (51.4%) elderly. Demographically, women have a higher life expectancy[20] and women who are mother figures for their children and have maternal innate responsibilities where they are fully responsible for their family's condition and themselves, especially in the health aspect (housewives).[18]

In addition, the other respondents' occupations involves vigorous physical activity, such as 5 labourers, 8 farmers, 6 drivers, and 1 stockbreeder. Only 5 out of 181 respondents are jobless. This is in line with research conducted by Noni EJW and Katrin R (2008) which states that having job as farmers/laborers, tea pickers, and factory workers require vigorous physical activity.[19] Workers who use skills over physical strength tend to involve lower levels of physical activity.[20] The heavier the activity, the more energy required to carry out these activities.[21] In this study, the elderly who work as housewives generally have moderate to vigorous physical activity levels. This is in accordance with the previous theory which states that in general women have the responsibility to cook, clean

the house, doing errands, and do all activities that require a lot of walking, bending, standing, and lifting, thus when accumulated, the activity level of housewives are included in the category of moderate to vigorous physical activity levels. Since housewives do not accrue occupational physical activity or work-related commuting activity, housewives does engage in domestic activities, and may have additional child care or home care activity compared to employed women.[22] By engaging in light to vigorous intensity physical activity for many hours during the day, housewives may achieve the same volume of activity as employed woman who engage in more short-term moderate to vigorous physical activity, but also spend more time sedentary.[23,24]

Based on educational background, it can be seen that the majority of the elderly in Ardirejo and Panggungrejo village have a fairly low level of education, which is elementary school education. This was shown by 75 (41.4%) of 181 respondents only completed having their primary education. From these data, it was found that the physical activity of the elderly in Panggungrejo and Ardirejo villages had more moderate to vigorous physical activities than light physical activities. The results of this data are in accordance with research conducted by Cheah YK and Poh BK (2014) which states that the higher a person's education level, the lower the level of their physical activity.[23]

Physical Activities of the Elderly in Ardirejo and Panggungrejo Villages, Kepanjen District, Malang Regency

According to the level of physical activity, 155 (88.05%) elderly had moderate to vigorous physical activity levels and 26 (14.37%) elderly had low activity levels. A *systematic review* of research articles on physical activity in elderly population around the world in 2000-2012 showed that physical activity is consistently associated with functional capacity, overall quality of life, autonomy, past, present, and future activities, death, relationship intimacy, mental health, vitality, and psychological conditions.[24] A high level of physical activity reduces the risk of mortality in the elderly. The elderly who were physically active at a moderate level of 150 minutes per week experienced a mortality reduction by 30% compared to those who were less active. The greatest benefit from physical activity is obtained by those aged 60 years and above.[25] Previous studies have shown that subjects with moderate to vigorous levels of physical activity are associated with good quality of life and good mental health.[30–32]

Social Isolation of the Elderly in Ardirejo and Panggungrejo Villages, Kepanjen District, Malang Regency

In this study, it was found that social isolation only occurred in 34 (18.78%) of the elderly in Panggungrejo and Ardirejo villages. Social isolation is defined as the termination of real relationship with society, groups and communities. In fact, it is defined as the weakness of social relationship and friendship, as well as correlation with formal and informal groups.[26] In Tehran of Iran, the proportion of elderly people in the population was 7.5% and it is known that 62% of the elderly of that proportion experienced social isolation. It shows the expansion of extensive social isolation in Tehran.[27]

If it is reviewed with frequency distribution of physical activity data, it can be seen that the physical activity of the elderly in the two villages has moderate to vigorous activity levels. In accordance with the research hypothesis that the higher the physical activity performed by a person, the higher the reduction of the occurrence of social isolation. This is also in line with research conducted by Robins (2016) which explains group physical activity is known to reduce the occurrence of social isolation in adults.[8] Elderly who live in a supportive environment for physical activity has lower risk of social isolation.[28]

The Effect of Physical Activity on Social Isolation in the Elderly

In this study, it was found that the elderly in Ardirejo and Panggungrejo villages had a fairly high level of activity. It can be seen from the data that there are more elderly who have moderate to vigorous physical activity than light physical activity. Physical activity level was related to the level of social isolation that occurs in the two villages. The percentage of elderly with social isolation was only 18.78%

in the two villages. It could be due to the high level of physical activity of the elderly in the two villages. After doing the *fisher's exact test*, there was a significant correlation (p value <0.05) with the p value between the level of physical activity and social isolation in the elderly being 0.000 (PR = 23.407; 95% CI = 3.117-175.800). The results of this study is in line with research conducted by Robins that physical activity was significantly associated with social isolation with an odds ratio of 1.03 (CI 1.01-1.04, *p-value* 0.002).[9] Intervals that are very wide (3.117-175.800) indicate that we have little knowledge about the result, and that further information is needed.

It shows that there is an effect of physical activity on the occurrence of social isolation in the elderly where the higher the level of physical activity, the lower the incidence of social isolation. This is in line with one of previous studies which states that social isolation is associated with sedentary behaviour, mild, and moderate levels of physical activity. These findings are consistent with the possible role of physical activity in health risks toward social isolation. Although not large, there still seem to be difference in physical activity between the daily life of more isolated individuals and those who are not. These differences will accumulate as the time goes by and contribute to an increased likelihood of chronic disease and disability in the elderly.[29]

Physical activity is one of the most effective ways to improve health in populations and psychosocial health.[30] Old elderly can avoid the occurrence of health problems associated with social isolation by participating in social interactions that are held regularly by communities or institutions.[31] Voluntary activities such as social service performed by older adults provide better mental health and physical function also reduce the risk of death.[32–34] Regular exercise can improve cardiovascular, metabolic, endocrine, and psychological health. Support from others is an effective way to reduce the negative effects of loneliness for the elderly to stay active.[13] Currently, there are various physical exercise programs and sports for the elderly with the aim of reducing social isolation and its various consequences.[9] These programs include; daily active life program, physical fitness improvement, strengthening exercises, and walking activities.[10]

In line with the research explored by,[35] compared to other forms of treatment therapy of social isolation (e.g mindfulness therapy, art, and craft therapy), physical activity intervention, especially in small groups (up to eight to nine people), can assist in building friendly and trusting relationships between participants. Some intervention consist of aerobic exercise training in small group, that allow the participants to interact each other.[35]

Mechanism of physical activity can affect social isolation through loneliness reduction models, stress reduction and increased social support during activities. Physical activities can increase peripheral social networking during friendly conversation between participants.[36] Beside that, enjoyable forms of physical activities generate happiness and bring positive emotions, which in turn could be related to loneliness reduction as shown in longitudinal study by Newall.[37] In the group, elderlies can share interest and goals, which in turn strengthening the social interaction and promote well-being.[38]

Confounding Factors Affecting Association Between Social Isolation and Physical Activity

Firstly, poor physical health, limits capacity for physical activity and is associated with social isolation and loneliness (Coyle and Dugan, 2012). Second, problem with modality and impairment in activities of daily living (ADLs) may restrict social interaction and associated with depression in elderlies.[39] Socioeconomic status partly explains links between social isolation/ loneliness, disease risk, and mortality.[40,41] An association between social isolation and physical activity could be secondary to any of these factors.

The relation between physical activity and social isolation is a vicious cycle. Poor physical activity will reduce physical fitness and limit social interaction. In the other side, lack of social interaction can pomote loneliness and induce depression, which interferes with motivation to be physically active.

There are limitations of this study. In this research the willingness elderly to participate was low. This study has not performed multivariate analysis, because the data did not fulfill the basic requirements for independent variables of at least having 0.05 the principle of factor analysis is the correlation between variables. This may lead future research to evaluate deeply.

In addition, the better way to estimate the level of physical activity is with qualitative measures, such as accelerometer-based measurements rather than self-reported results.[29] Changes in norms with age and disability of what constitutes vigorous activity, cognitive and recall problem can limit the accuracy of self-reports in older age.

Future research may need to address the limitations above for better elucidation of the association.

Implications for the Field of Medicine

This research is expected to be an alternative method to prevent and reduce the occurrence of social isolation in the elderly through increased physical activity, especially physical activity in groups or in a community. We also hope that this research can give insight into the effect of physical activity on the management of loneliness, physical health and psychological health improvement in the elderly.

CONCLUSION

There is a significant correlation between physical activity and social isolation in the elderly. The vigorous the physical activity, the lower the occurrence of social isolation in the elderly.

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REFERENCES

1. Narulita. Manajemen sehat lansia. Jakarta: Pustaka Belajar; 2013.
2. Damayanti Y, Sukmono AC. Perbedaan tingkat kesepian lansia yang tinggal di panti werdha dan di rumah bersama keluarga. Jurnal Ilmiah Keperawatan. 2013;8(1):1–10.
3. Kementrian Kesehatan RI. Buletin jendela data dan informasi kesehatan. Kementrian Kesehatan RI. 2013.
4. Ogundiran OO. Social isolation in the elderly: Psysical activity to the rescue. Degenerative Intellect Dev Disabil. 2017;1(1):1–3.
5. Nicholson NR. Social isolation in older adults: An evolutionary concept analysis. J Advan Nurs. 2009;65(6):1342–52.
6. Stephanie Desmon. Mouse research links adolescent stress and severe adult mental illness [Internet]. John Hopkins Medicine. 2013. Available from: https://www.hopkinsmedicine.org/news/media/releases/mouse_research_links_adolescent_stress_and_severe_adult_mental_illness
7. Valtorta NK, Kanaan M, Gilbody S, Ronzi S, Hanratty B. Loneliness and social isolation as risk factors for coronary heart disease and stroke: Systematic review and meta-analysis of longitudinal observational studies. Heart. 2016;102:1009–16.
8. Robins LM, Jansons P, Haines T. The impact of physical activity interventions on social isolation among community-dwelling older adults: A systematic review. Research & Reviews: J Nurs Health Sci. 2016;2(1):62–71.
9. Robins LM, Hill KD, Finch CF, Clemson L, Haines T. The association between physical activity and social isolation in community-dwelling older adults. Aging and Mental Health. 2018.
10. Coyle CE, Dugan E. Social isolation, loneliness and health among older adults. J Aging Health. 2012;24(8):1346–63.
11. National Prevention Council. Healthy aging in action. Healthy aging in action: Advancing the National Prevention Strategy. 2016.
12. Abdi A, Hariyanto T, Ardiyani VM. Aktivitas lansia berhubungan dengan status kesehatan lansia di Posyandu Permadi Kelurahan Tlogomas Kecamatan Lowokwaru Kota Malang. Nursing News. 2017;2(3):272–80.
13. Sunarti S, Hariyanti T, Chriswantara RT. Physical activities, sitting time and frailty syndrome in elderly (A cross sectional study among elder person by Fried criteria). MNJ (Malang Neurol J). 2018;4(2):59–64.

14. Harris Country Psychiatric Centre. Schizophrenia. University of Texas Health County Psychiatric Centre. 2008.
15. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise. 2003;35(8):1381–95.
16. Suyoto PST, Huriyati E, Susilowati R, Julia M. Relative validity of administered Indonesian version of the short-form international physical activity questionnaire (IPAQ-SF) among obese adolescent girl population. Pakistan J Nutr. 2016;15(9):816–20.
17. Auerbach J, DeSalvo K. The social determinants of health. Handbook of geriatric assessment. 5th Edition. Burlington, Massachusetts: Jones & Bartlett; 2019. p. 89–101.
18. Badan Pusat Statistik. Statistik penduduk usia lanjut 2019. Badan Pusat Statistik. 2019.
19. BJ S. Fitness and health. 2nd ed. In: Nasution TED, editor. Jakarta: Raja Grafindo Persada; 2011.
20. Ismayanti N, Solikhah. Hubungan antara pola konsumsi dan aktivitas fisik dengan status gizi pada lansia di panti sosial tresna werdha unit Abiyoso Yogyakarta. J Kes Masy. 2013;6(3):162–73.
21. Vaupel JW, Villavicencio F, Bergeron-Boucher M-P. Demographic perspectives on the rise of longevity. Proceedings of the National Academy of Sciences [Internet]. 2021;118(9). Available from: https://www.pnas.org/content/118/9/e2019536118
22. Mindianata P. Faktor-faktor yang berpengaruh terhadap niat keaktifan lansia dalam mengikuti Posyandu lansia. Jurnal PROMKES. 2018;6(2):2013–225.
23. Wardani NEJ, Roosita K. Aktivitas fisik, asupan energi, dan produktivitas kerja pria dewasa: studi kasus di perkebunan teh Malabar PTPN VIII Bandung, Jawa Barat. Jurnal Gizi dan Pangan. 2008.
24. FAO/WHO/UNU. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation: Rome, 17–24 October 2001. AO food and nutrition technical report series. 2004;
25. Khumaidi M. Gizi Masyarakat. Bogor: Departemen Pendidikan dan Kebudayaan, Direktorat Jenderal Pendidikan Tinggi, Pusat antar Universitas Pangan dan Gizi IPB; 1989.
26. Steeves JA, Murphy RA, Zipunnikov V, Strath SJ, Harris TB. Women workers and women at home are equally inactive: NHANES 2003-2006. Medicine and science in sports and exercise [Internet]. 2015 Aug;47(8):1635–42. Available from: https://pubmed.ncbi.nlm.nih.gov/25412296
27. Dunlop DD, Song J, Semanik PA, et al. Relation of physical activity time to incident disability in community dwelling adults with or at risk of knee arthritis: prospective cohort study. BMJ (Clinical research ed). 2014;348:g2472.
28. Thivel D, Tremblay A, Genin PM, Panahi S, Rivière D, Duclos M. Physical activity, inactivity, and sedentary behaviors: Definitions and implications in occupational health. Frontiers Public Health. 2018;6:288.
29. Cheah YK, Poh BK. The determinants of participation in physical activity in Malaysia. Osong Public Health and Research Perspectives. 2014;5(1):20–7.
30. Vagetti GC, Filho VCB, Moreira NB, De Oliveira V, Mazzardo O, De Campos W. The association between physical activity and quality of life domains among older women. J Aging Physic Act. 2015;23(4):524–33.
31. Arem H, Moore SC, Patel A, et al. Leisure time physical activity and mortality: A detailed pooled analysis of the dose-response relationship. JAMA Intern Med. 2015;175(6):959–67.
32. McKee G, Kearney PM, Kenny RA. The factors associated with self-reported physical activity in older adults living in the community. Age and ageing. 2015;44(4):586–92.
33. Donoghue O, Connell M, Kenny R. Walking to wellbeing: Physical activity, social participation and psychological health in Irish adults aged 50 years and older. The Irish Longitudinal Study on Ageing 2016. 2016;
34. Seyfzadeh A, Haghighatian M, Mohajerani A. Social isolation in the elderly: The neglected issue. Iranian J Publ Health. 2019;48(2):365–6.
35. Seyfzadeh A, Hagighatian M, Mohajerani A. The relationship between social isolation and health among the tehranian elderly. J Edu Comm Health. 2017;4(3):19–25.
36. Keysor JJ, Jette AM, Lavalley MP, et al. Community environmental factors are associated with disability in older adults with functional limitations: The (MOST) study. J Gerontol - Series A Biological Sci Med Sci. 2010;65(4):393–9.
37. Schrempft S, Jackowska M, Hamer M, Steptoe A. Associations between social isolation, loneliness, and objective physical activity in older men and women. BMC Public Health. 2019;19(74):1–10.
38. Paterson DH, Jones GR, Rice CL. Ageing and physical activity: Evidence to develop exercise recommendations for older adults. Applied Physiol Nutr Metabolism. 2007;98:S69-108.
39. Hinterlong JE. Race disparities in health among older adults: Examining the role of productive engagement. Health Social Work. 2006;31(4):275–88.
40. Brown SL, Nesse RM, Vinokur AD, Smith DM. Providing social support may be more beneficial than receiving it: Results from a prospective study of mortality. Psychol Sci. 2003;14(4):320–7.
41. Gottlieb BH, Gillespie AA. Volunteerism, health, and civic engagement among older adults. Canadian J Aging/La Revue canadienne du vieillissement. 2008;27(4):399–406.
42. Hao Y. Productive activities and psychological well-being among older adults. J Gerontol - Series B Psychol Sci Soc Sci. 2008;63(2):s64-72.
43. Shvedko A V., Thompson JL, Greig CA, Whittaker

AC. Physical activity intervention for loneliness (PAIL) in community-dwelling older adults: Protocol for a feasibility study. Pilot and Feasibility Studies. 2018;6(72):1–15.
44. Stevens N. Combating loneliness: A friendship enrichment programme for older women. Ageing and Society. 2001;21(2):183–102.
45. Newall NEG, Chipperfield JG, Bailis DS, Stewart TL. Consequences of loneliness on physical activity and mortality in older adults and the power of positive emotions. Health Psychol. 2013;32(8):921–4.
46. Henry KB, Arrow H, Carini B. A tripartite model of group identification: Theory and measurement. Small Group Research. 1999;30(5):558–81.
47. Blazer DG, Hybels CF. Origins of depression in later life. Psychol Med. 2005;35:1241–52.
48. Elovainio M, Hakulinen C, Pulkki-Råback L, et al. Contribution of risk factors to excess mortality in isolated and lonely individuals: an analysis of data from the UK Biobank cohort study. Lancet Publ Health. 2017;2(6):e260–6.
49. Hakulinen C, Pulkki-Råback L, Virtanen M, Jokela M, Kivimäki M, Elovainio M. Social isolation and loneliness as risk factors for myocardial infarction, stroke and mortality: UK Biobank cohort study of 479 054 men and women. Heart. 2018;104(18):1536–42.

The Association between Plasma miRNA-21 Levels with Overall 1-year Survival Rate of Breast Cancer Patients at Various Stages

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ABSTRACT

Background: *miRNA 21 exhibits an increased expression in breast cancer (BC). However, its relationship with the 1-year survival of breast cancer patients is still disputable and under serious discussion.* ***Methods:*** *Cohort prospective study involving 49 breast cancer patients was done, comprising 26 in early stage and 23 in end-stage. We evaluated miRNA-21 values and observed its association with mortality within 1 year.* ***Results:*** *In general, there was a correlation between the increase in miRNA-21 levels and the mortality rate of breast cancer patients (r = 0.651; p <0.05). In the early stages, the increase in miRNA-21 values was not associated with breast cancer mortality (r= 0.25; p=0.218), but in the later stages, we found that the increase in miRNA-21 had a correlation with mortality (r=0.866; p=<0, 05). In advanced stage, high level of miRNA-21 had high asscociation with mortality rate (HR 17,27 95%CI 7,37-40,69).* ***Conclusion:*** *The increase in miRNA-21 values is associated with the 1-year survival of breast cancer patients.*

Keywords**:** *miRNA-21, breast cancer, mortality rate, survival, health.*

INTRODUCTION

Breast cancer has been noticed as a worldwide health issue with an increasing prevalence in the world with a high mortality rate. Based on GLOBOCAN data (Global Cancer Statistics) 2018, breast cancer ranks first in the incidence of cancer in women with the number of new cases worldwide of 2,088,849 people (24.2%) with a mortality rate of 626,679 people (15%), of which half of the new cases and mortality cases found in Asia.[1] Indonesia came with the data revealing that breast cancer is the second most common malignancy after cervical cancer with a prevalence of 0.5 percent and an estimated number of cases of 61,682 patients, where the highest incidence is found in the Central Java, followed by East Java, West Java and DKI Jakarta.[2] Data on one-year, five-year and ten-year survival rates in breast cancer patients aged 15 to 99 years during the period 2013-2017 in the UK were found to be 95.8%, 85% and 75.9%, respectively, where plans of action are somewhat still needed in increasing breast cancer survival rates.[3]

The examination of minimally invasive circulating biomarkers offers new hope to diagnose breast cancer, monitor progression,

determine prognosis and predict response to treatment, which is a vital part of strategies to reduce breast cancer morbidity and mortality. Several conventional circulating biomarkers that are known and used in monitoring and assessing breast cancer progression are carcino-embryonic antigen (CEA) and carbohydrate antigens (CA) such as (CA15-3). However, the sensitivity values of the biomarkers CEA and CA 15-3 are relatively low, around 22% and 15%, respectively. Due to the low sensitivity of existing biomarkers, new minimally invasive biomarkers with better sensitivity and specificity are required to monitor breast cancer progression.[4-6]

The discovery of microRNA (miRNA) in the last decade has unfolded new opportunities for the detection and prognostication of breast cancer. The advantages of miRNA in the oncogenesis process include various expression patterns associated with the type of cancer, easy detection in biopsies, fine stability in blood and other body fluids, especially in blood, plasma, serum, and saliva so that miRNAs can be used as promising biomarkers.[7,8] Dysregulation in miRNA genes in the form of mutations, deletions, translocations, increased expression, or decreased expression can trigger biological changes in cells, and is believed to have an important role in cancer development.[9] Several literature studies have shown that miRNA-21 has increased expression in breast cancer. MiRNA-21 targets that have been identified are tumor suppressor tropomyosin 1 (TPM1), TIMP3, RECK Cdc25A network of p53, PDCD4, PTEN, and Maspin. MiRNA-21 overexpression correlates with specific breast cancer bio-pathological features, such as advanced tumor stage, lymph node metastases, and poor patient survival, suggesting that miRNA-21 may serve as a molecular prognostic biomarker for breast cancer progression. High miRNA-21 expression had a negative impact on overall survival and recurrence-free survival in breast cancer.[10]

Several studies that have been conducted regarding the relationship of miRNA-21 with breast cancer prognosis had contradictory results. The meta-analysis by Wang et al.[11] on the association of miRNA-21 expression with breast cancer prognosis identified 10 studies involving 1,439 cases, showing that high miRNA-21 expression predicted poor overall survival and disease-free survival and low relapse-free survival in breast cancer patients.[11] Study of Khanbashi et al. (2015) on 27 locally-advanced breast cancer patients, Radojicic et al. (2011) on 49 triple-negative breast cancer patients, and Qian et al. (2009) on 344 breast cancer patients, showed that the results of the analysis of serum and tissue miRNA-21 expression were not correlated with overall survival and disease-free survival.[12-14]

According to the fact that there are contradicting results among several previous studies, further analysis is considered necessary to apply miRNA-21 as a prognostic factor for breast cancer. This study aims to determine the relationship between circulating miRNA 21 levels and overall 1-year survival in breast cancer, where the circulating miRNA-21 parameter used is blood plasma which is more stable and different from previous studies using serum or tissue.

METHODS

This is a cohort-retrospective study which was conducted at the Oncology Hematology Outpatient Clinic, Airlangga University Hospital, Surabaya, starting from November 2019 through November 2020. Participants taking a part in the study were those who met all of the following criteria: female patients aged 18 and above; breast cancer patients who have not received chemotherapy and radiotherapy; and are willingly involved in the study by signing an informed consent form.

Participants who were excluded from the study were patients who met one or more of the following criteria: breast cancer patients with terminal conditions (ECOG2, Karnofsky, score 50%), patients with a history of other malignancy in the last 5 years, and patients with comorbid diseases such as COPD, heart failure, chronic kidney disease, liver cirrhosis and diabetes mellitus that are not under control. Data were obtained from history taking, physical examination and additional examination.

The expressed value of circulating plasma miRNA-21 in the blood plasma of breast cancer

patients is measured using the quantitative Real Time Polymerase Chain Reaction (qRT-PCR) method. The Hsa-miR-16-5p LNA PCR primer set was used as an endogenous control and a qRT-PCR process was used to calculate the expression value of circulating miRNA-21. Blood samples withdrawal were carried out by the Surabaya Prodia Laboratories and then sent for storage and processing at the Jakarta Prodia Laboratory.

Samples for MiRNA21 examination was gained from 10 cc venous blood of deceased and healthy patients. Centrifugation was carried out at 3000 RPM for 5 minutes to separate the serum and then sent and stored in a container with a temperature of -80C until RNA was able to be extracted. Total RNA extraction, including small RNA, was performed by the Qiagen miRNeasy serum/plasma kit according to the protocol of the product supplier.

The miRNA-21 reverse transcription technique used Taqman TM microRNA reverse transcription kit (Applied Biosystem) and miRNA-21 specific stem-loop primer (Applied Biosystem, assay) for miRNA-21 reverse transcription (RT) reaction. RT primers for small nuclear miRNA-16 (Applied Biosystem) were used as endogenous controls.

Ethics Approval

This study has been approved by the research ethics committee of Universitas Airlangga Hospital (No: 164/KEP/2019).

Statistical Analysis

The sample size in this study was calculated by the formula of correlative analytic sample. Furthermore, the number of samples required was approximately 37 patients, but our study obtained 49, consisting of 26 patients with early-stage breast cancer and 23 advanced-stage patients. Statistical analysis was carried out using SPSS version 24.0. (Chicago, IL, USA). Statistical analysis to determine the correlation of the two variables used the Pearson statistical test if the data distribution was normal, otherwise, Spearman test was used. The correlation test results are interpreted based on the p value, the strength of the correlation and the direction of the correlation.

RESULTS

The characteristics of 49 breast cancer patients are presented in **Table 1**. Of the 49 breast cancer patients, 26 patients were in the early stages, consisting of 5 at stage 1 and 21 at stage 2. Meanwhile, of 23 patients with breast cancer in the advanced stage, 11 at stage 3 while 12 at stage 4.

Table 1. Characteristics of breast cancer patients.

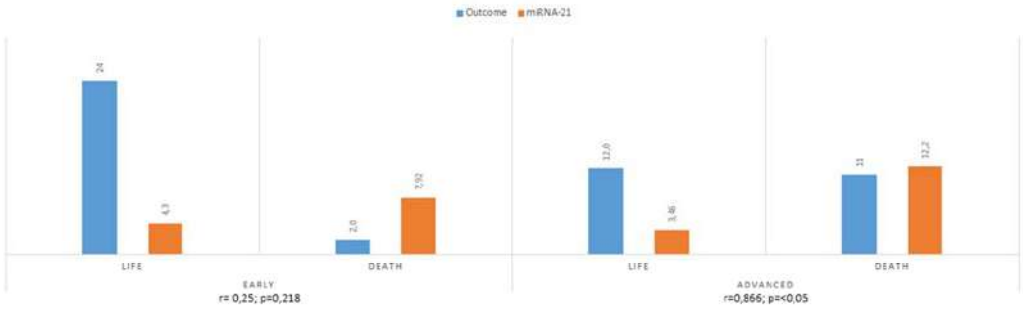
Variables	n	%
Age (Mean/SD)	51.95	10.21
Stage		
Early (n,%):	26	53.06
- 1A	4	8.16
- 1B	1	2.04
- 2A	9	18.37
- 2B	12	24.49
Advanced (n,%):	23	46.94
- 3A	3	6.12
- 3B	5	10.20
- 3C	3	6.12
- 4	12	24.49
Menarche		
- Premenopause	25	51.02
- Menopause	24	48.98
Comorbid		
- HT	13	26.53
- DM	5	10.20
- Thallasemia	1	2.04
Chemotherapy		
- Adjuvan	23	46.94
- Neoadjuvan	14	28.57
- Palliative	12	24.49
Performance status (ECOG)		
- 0	32	65.31
- 1	15	30.61
- 2	2	4.08
Estrogen receptor		
- Neg	14	28.57
- Pos	23	46.94
Progesteron receptor		
- Neg	19	38.78
- Pos	18	36.73
Human Epidermal Growth Factor Receptor (HER-2)		
- Neg	17	34.69
- Pos 1	4	8.16
- Pos 2	4	8.16
- Pos 3	11	22.45
Ki67 (mean)	50%	

Vital sign		
- Systolic Pressure (mean, SD)	128.38	15.09
- Diastolic Pressure (mean,SD)	78.14	9.59
- Pulse (mean, SD)	85.88	8.64
- Respiration Rate (mean, SD)	19.08	1
- Temperature (mean,SD)	36.19	0.91
Physical status:		
- Height (cm)	152.84	4.88
- Weight (kg)	57.03	11.18
- Body Surface Area (BSA/m^2)	1.51	0.16
Laboratory		
- Hb (g/dl) (mean, SD)	12.24	1.28
- WBC (10^3/L) (mean, SD)	7.75	2.62
- PLT (10^3/L) (mean, SD)	344.16	100.01
- AST (u/L) (mean, SD)	26.57	16.7
- ALT (u/L) (mean, SD)	28.35	29.83
- Basal Urea Nitrogen (mg/L) (mean, SD)	10.77	4.12
- Creatinine (mg/L)(mean, SD)	0.69	0.17
- miRNA-21	6	5.35
- CEA	5.63	6.74
- CA 15-3	68.62	141.95
Outcome:		
- Life (n,%)	36	73.47
- Death (n,%)	13	26.53

Based on the breast cancer stages, the number of pre-menopausal patients were dominantly found (57.69%) in the early stages compared to the menopausal ones, while at an advanced stage, menopausal patients were more frequent than premenopausal ones (56.52%). Hypertension seemed to be the first-ranked comorbidity both in the early and advanced stage patients. MiRNA, CEA, and CA 15-3 values in advanced stages had higher mean values. There were 2 deaths in the early stage, while in the late stage there were 11 deaths. Eleven cases of death at an advanced stage consisted of 3 cases of death at stage 3 (1 in case 3A and 2 in case 3B) and 8 cases of death occurred at stage 4. Overall the increase in miRNA-21 values correlated with the death of breast cancer patients ($r = 0.651$; $p<0.05$). Nonetheless, in the early stages, the increase in miRNA-21 did not show any correlation ($r = 0.25$; $p = 0.218$). Otherwise, miRNA-21 was correlated with death in the advanced stages ($r = 0.866$; $p = <0.05$). (**Table 2**).

Tabel 2. The comparison between early and advanced stage and the association between death and miRNA-21 in breast cancer patients.

	Stage				Overall (n=49)	
	Early (n=26)		Advance (n=23)			
Menarche						
- Menopause	11	42.31	13	56.52	24	48.98
- Premenopause	15	57.69	10	43.48	25	51.02
Comorbid						
- HT	9	34.62	4	17.39	13	26.53
- DM	2	7.69	3	13.04	5	10.2
- Thalasemia	1	3.85	0	0.00	1	2.04
Ca Mammae marker						
- miRNA-21 (mean,SD)	4.57	2.83	7.62	6.94	6	5.35
- CEA (mean, SD)	3.43	3.09	8.12	8.73	5.63	6.74
- CA 15-3 (mean, SD)	17.60	4.66	128.91	3.52	68.62	142
Outcome						
- Life (n,%)	24	92.31	12	52.17	36	73.47
- Death (n,%)	2	7.69	11	47.83	13	26.53
Correlation with Mortality rate						
- miRNA-21 (r; pvalue)	0.25	0.218	0.866	<0.05	0.651	<0.05



	Stage			
	Early		Advanced	
	Life	Death	Life	Death
Outcome	24	2,0	12,0	11
miRNA-21	4,3	7,92	3,46	12,2

Figure 1.

As seen on **Figure 1**, it appears that in the advanced and early stages, the mean miRNA values were lower in the surviving patients and higher in the deceased patients.

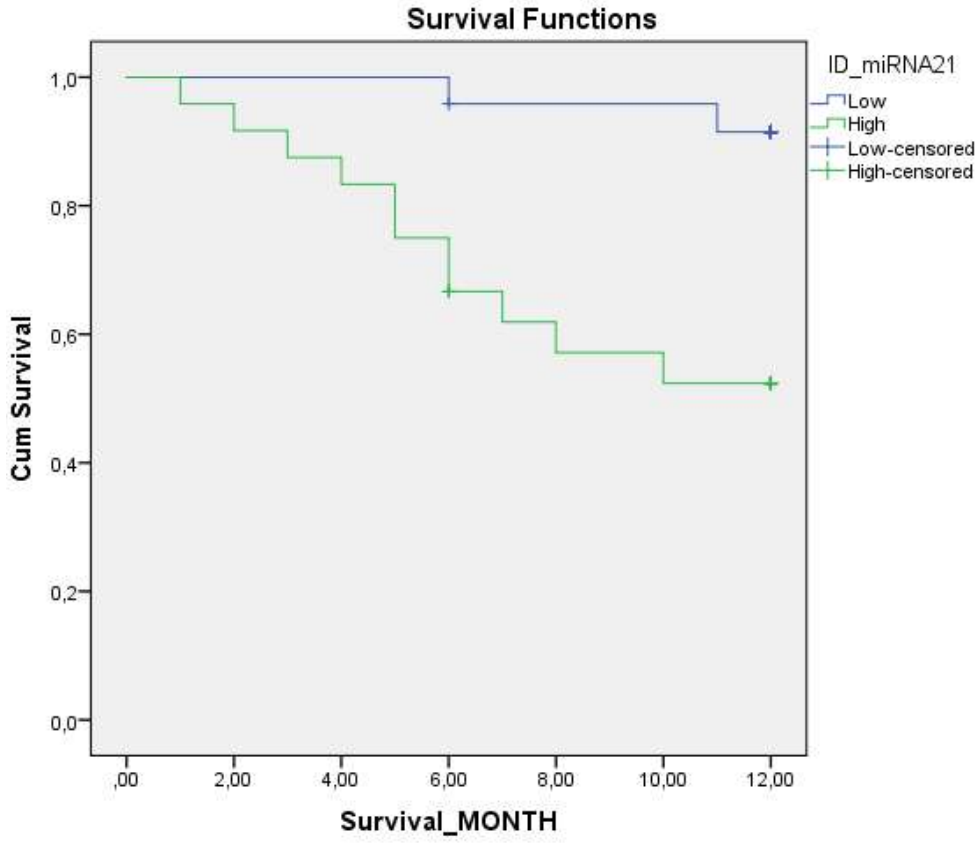


Figure 2. Graphic survival mRNA-21 level in 1 year

Table 3. One year survival breast cancer according miRNA21 level

mRNA21	One year survival			
	Low mRNA		High miRNA	
	HR	95% CI	HR	95% CI
Overall	0.18	0.11-0.31	5.5	3.2-9.46
Early stadium	0.766	0.33-1.8	1.31	0.55-3.07
Advance stdium	0.058	0.03-0.14	17.27	7.33-40.69

According to **Figure 2**, it appears that low level of miRNA21 indicates better survival which is statistically significant ($p<0.05$). Meanwhile, high levels of miRNA21 represented a statistically significant acceleration of death within 1 year in all cases, particularly in advanced stages (HR 5.5; 95%CI (3.2-9.46); HR 17.27; 95% CI : 7.33-40.69).

DISCUSSION

The mean age of the subjects was 51.95 ± 10.21 years, with the youngest being 29 years and the oldest being 73. This is in line with a study conducted by Elghourory et al., (2018) on the evaluation of miRNA-21 as a prognostic marker in breast cancer in 50 breast cancer patients with a mean age of 53.4 ± 9.9 years, also with a study by Cuk et al. (2013) who examined circulating plasma miRNA-21 as a marker for early detection of breast cancer in 127 breast cancer patients with a mean age of 56.6 years.[15, 16]

Age somewhat is the major risk of breast cancer, in addition to reproductive factors, heredity and lifestyle factors, because the incidence of breast cancer is strongly associated with the increasing age. Data in 2016 in the United States about 99% of breast cancers were reported at the age of over 40 years, so it is highly recommended to screen women aged 40 and over.[17, 18] Recent research data showed that in East Asian and Southeast Asian, the breast cancer was more commonly found in the younger women than in the older ones. Factors that influence this trend are "westernization" which includes dietary changes (high fat, low vegetables) and environmental factors (physical inactivity, smoking, alcoholism) in the last two decades in Asian countries.[19]

In this study, according to the menarche status, 25 patients were the premenopausal women (51.02%), while 24 were the at the menopause stage (48.97%). This was supported by several previous studies where the percentage of the pre-menopausal group was higher than the menopausal one, such as by Dong et al., (2014) (54.16% and 45.84%), Elghourory et al. (2018) (64% and 36%). Meanwhile, another study by Papadaki et al. (2018) on circulating miRNA-21 as a predictive factor for recurrence in 133 breast cancer patients, stated that the proportion of those being pre-menopausal subjects were 42.9% (n=57), while menopausal ones were 57.1% (n= 76).[15, 20]

Reproductive factors such as menstrual history (early menarche, late menopause) are one of the risk factors for breast cancer. Every one year delay in menopause has been shown to elevate the risk of breast cancer by 3%, while every one year delaying menarche has been shown to reduce the it by 5%. [17, 18] In this study, the percentage of pre-menopause patients was slightly higher than the menopause group, which differred from the European study. This condition is in accordance with the age factor of breast cancer patients in Asia who tend to be younger than European and American countries. Reproductive factors also play a role because Asian women today tend to delay the birth of children, limit the number of children and rarely breastfeed. All of those factors elevate the tendency of breast cancer incidence at a younger age. Menarche status in the two groups of subjects in this study was homogeneous, so the relative risk for breast cancer did not differ.

Of all 49 research subjects, 5 were at the stage 1 (10,2%), 21 were at the stage 2 (42,85%), 10 were at the stage 3 (20,40%), and 13 were at the stage 4 (26,53%). This is congruent with a study by Gao et al. (2013) comparing miRNA-21 with CEA and CA 15-3 in 89 breast cancer patients, which obtained 23.59% stage 1 (n=21), 31.46% stage 2 (n=28), 10.11% stage 3 (n=9), and 34.83% stage 4 (n=31) patients.[5] Research by Zhang et al. (2016) revealed that stage 1 was 10.4% (n=11), stage 2 was 40.5% (n=43), stage 3 was 30.2% (n=32), and stage 4 was 18, 9% (n=20).[8] While Fattah et al. (2018) stated that 20% (n=6) were at, 30% at stage 2 (n=9), 23.33%

at stage 3 (n=7), and 26.66% at stage 4 (n=8).[21] The subjects at stage 1 of breast cancer were at the smallest proportion, while the stage 4 group is second largest proportion. In contrast to other studies, this was due to the fact that breast cancer patients in Indonesia often come at an advanced stage and rarely check themselves routinely for early detection.

The results of this study obtained circulating plasma miRNA-21 expression values using the 2-ΔΔCt formula to calculate relative gene expression in samples using the quantitative Real Time polymerase chain reaction (qRT-PCR). The calculation of Ct (cycle threshold of the fluorescence cycle sample from the qRT-PCR product) was obtained from the difference between Ct of the breast cancer group and Ct of the control group. The calculation of Ct was obtained from the qRT-PCR value results of miRNA-21 as the expression of the gene under study minus the value of the qRT-PCR miRNA-16 (Hsa-miR-16-5p LNA PCR primer set) as the expression of endogenous control genes that were not affected in the study.

The mean expression of circulating plasma miRNA-21 in the breast cancer group was 6.00±5.35 with a median of 4.43 (min-max:1.11-32.22). According to the clinical stage in the breast cancer group, the mean was 4.31 ±0.97 and the median was 4.75 (min-max: 2.88-5.35) at stage 1, 4.62 ±3.13 and the median was 3.68. (min-max: 1.18-11.79) at stage 2, 5.40 ± 4.27 and median 3.83 (min-max: 1.11-14.12) at stage 3, and 9.32 ± 8.21 and median 5.77 (min-max: 3.18-32.22) at stage 4. In line with Fattah et al. (2018), it was found that the expression of circulating miRNA-21 in the stage 1 breast cancer group was 1.6 (1.2-1.9), at stage 2 it was 2.6 (2.3-3.0), at stage 2 it was 2.6 (2.3-3.0). 3 was 3.5 (3.2-4.8), and in stage 4 it was 7.4 (5.9-8.7) (p= < 0.001).[22] This result was also in accordance with the research of Abdulhussain et al. (2017) who examined the relationship between serum and tissue miRNA-21 levels with PDCD4 expression in breast cancer patients in Iraq, where circulating miRNA-21 in the stage 1 breast cancer group was 0.3±0.29, stage 2 was 3.4±0.35, stage 3 was 3.57±0.44 and stage 4 was 4.26.[23]

The measurement of circulating plasma miRNA-21 levels was performed using the qRT-PCR technique. The sample was obtained from venous blood since the levels were more stable than those from breast tumor tissue. Blood plasma was more preferred than serum due to the occurrence of erythrocyte hemolysis during the blood coagulation process which releases miRNA. Blood plasma was stable in an RNAse-rich environment in the bloodstream, in high and low temperature changes, in high and low pH conditions, in repeated freezing-thawing processes, and in long-term storage.[24, 25] MiRNAs were protected by microparticles such as exosomes or bound to the AGO2 protein which was part of the RNA-induced silencing complex. This study used the Hsa-miRNA-16-5p LNA PCR primer set as an endogenous control and carried out a duplicate process in the qRT-PCR process to further improve the accuracy of circulating miRNA-21 expression values. This study used miRNA-16 as an endogenous control, which was consistently expressed in all study groups and was not affected by breast cancer status, and this is also consistent with several studies using miRNA-16 as a cancer normalizer.[21]

In the breast cancer group, higher miRNA-21 levels are detected due to over-expression (up regulation) of the miR-21 gene which further escalates the release of miR-21 in the circulation and acts on several tumor suppressor genes such as P53 Network and Cdc25A which manage cell proliferation; TPM1, TIMP, RECK which have roles in cell invasion and metastases; and PTEN, PDCD4, FasL which regulate apoptosis. The whole process of increasing miRNA-21 expression and inhibition of tumor suppressor genes would trigger breast cancer. miRNAs expression in the breast cancer group significantly increased as the stage got more advanced. The positive interrelation of miRNA-21 and 155 exosomal plasma and tissue miRNAs signify the role of these miRNA cargoes in tumor metastasis and explain the invasiveness of cancer cells through the activation of TIMP3/miRNA-21 expression.

Survival is one of the benchmarks in assessing the mortality and morbidity rate of a

disease in an area, and the survival rate is related to risk factors as well as treatment and prevention plans. In recent years, the mortality rate for breast cancer had down-trended in developed countries. In Indonesia, despite relatively-low survival rate of breast cancer patients, early diagnostic tool and more advanced treatment modalities are currently under the development.

The results of the 1-year overall survival evaluation in 49 breast cancer patients in this study showed that 36 patients lived (73.46%) while 13 patients (26.54%) died. Based on the clinical stage, in stage 1 all (100%) survived, 19 (90.47%) did at stage 2, 7 (70%) at stage 3, and 5 at stage 4 (38.46%).

The overall 1-year survival rate for breast cancer patients based on research data in England, Denmark and China was 95.8%, 94.4% and 94.91%, respectively. Research data in China also showed that the overall 1-year survival rate when assessed based on clinical stage, the survival rate will decrease in more advanced stage. In this study, the overall 1-survival rate were lower than the data in England, Denmark and China, possibly because breast cancer patients in Indonesia often come at an advanced stage so that the therapy might not be optimal, then affected the survival rate. This was supported by the data from the Indonesian Ministry of Health where the cancer survival rate in Indonesia was still low, and research data from Noorwati et al. (2020) on cancer patients at Dharmais Cancer Hospital stating 77.9% of patients were in stage 3 and stage 4.[2]

Enhanced expression of miRNA-21 in breast tissue is significantly associated with tumor size, histopathological grade, lymph node involvement, tumor invasion to the vasculature and lymphatic flow, visceral metastases, and advanced stage. Increased miRNA-21 expression is also associated with a lower response to therapy and a poorer breast cancer prognosis. This fact is consistent with the oncogenic role of miRNA-21 during proliferation, invasion, metastasis, and inhibition of apoptosis of cancer cells. miRNA-21 targets that have been identified were tumor suppressor tropomyosin 1 (TPM1), TIMP3, RECK Cdc25A network of p53, PDCD4, PTEN, and Maspin. miRNA-21 overexpression correlates with specific breast cancer bio-pathological features, such as advanced tumor stage, lymph node metastases, and poor patient survival, suggesting that miRNA-21 may act as a molecular prognostic biomarker for breast cancer progression.

The relationship between circulating miRNA-21 plasma expression levels and overall 1-year survival of breast cancer in this study was found to have a strong, negative correlation between circulating miRNA-21 plasma expression and overall 1-year survival of breast cancer (r of 0,-651, p-value <0.05). The negative or opposite direction of the correlation means that the higher the expression of circulating miRNA-21 plasma, the lower the overall 1-year survival. Further statistical analysis shown in the Kaplan-Meier graph revealed that high miRNA-21 expression when compared to low miRNA-21 expression had a hazard ratio value of 5.5 with p <0.05. The research of Yan et al., (2008) showed that miRNA-21 expression was significantly associated with poor prognosis in breast cancer patients (p<0.001), with a Hazard Ratio value of 5.476.[10] The research of Papadaki et al., (2018) stated that circulating miRNA-21 was significantly correlated with overall survival (p=0.033), with a Hazard Ratio value of 2.884.[26] While the research by Dong et al., (2014) showed that miRNA-21 was significantly associated with overall survival (p=0.0107), with a Hazard Ratio value of 2.32.[20]

Nevertheless, this study had a number of weaknesses. The research subjects were not homogeneously distributed among stages, that might cause bias. The follow-up monitoring time took only a year; thus it was not able to conclude patients' survival in a longer period. In addition, this study also did not observe other survival metrics such as recurrence period or disease-free survival. This study also did not explore other factors that influence survival such as tumor histopathological profile or hormone receptor status, and only one type of miRNA was assessed.

CONCLUSION

Our principal finding is that overexpression of miR-21, one of the most significantly altered

miRNAs in BC, is associated with progression and poor prognosis of the patients. Although the precise molecular mechanism surrounding miRNA-21 up- regulation requires further clarification, our data indicate that miRNA-21 may be a good candidate as a molecular prognostic marker. Future study on the role of miRNA-21 in BC progression will no doubt add to the knowledge of this nascent field.

CONFLICT OF INTERESTS

The authors have no relevant conflict of interest.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J Clin. 2018;68(6):394-424.
2. Kemenkes. Kementrian Kesehatan RI: 'Situasi Penyakit Kanker Indonesia'2015.
3. UK. Office of National Statistics: Cancer survival by stage at diagnosis for England 2019. Available from: https://www.gov.uk/government/statistics/cancer-survival-in-england-for-patients-diagnosed-between-2014-and-2018-and-followed-up-until-2019/cancer-survival-in-england-for-patients-diagnosed-between-2014-and-2018-and-followed-up-to-2019.
4. Uehara M, Kinoshita T, Hojo T, Akashi-Tanaka S, Iwamoto E, Fukutomi T. Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer. International J Clin Oncol. 2008;13(5):447-51.
5. Gao J, Zhang Q. Clinical significance of serum miR-21 in breast cancer compared with CA153 and CEA. 2013.
6. Stieber P, Nagel D, Blankenburg I, et al. Diagnostic efficacy of CA 15-3 and CEA in the early detection of metastatic breast cancer—A retrospective analysis of kinetics on 743 breast cancer patients. Clin Chim Acta. 2015;448:228-31.
7. Mar-Aguilar F, Mendoza-Ramírez JA, Malagón-Santiago I, et al. Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. Dis Markers. 2013;34(3):163-9.
8. Zhang J, Jiang C, Shi X, Yu H, Lin H, Peng Y. Diagnostic value of circulating miR-155, miR-21, and miR-10b as promising biomarkers in human breast cancer. Int J Clin Experiment Pathol. 2016;9(10):10258-65.
9. Kayani M, Kayani MA, Malik FA, Faryal R. Role of miRNAs in breast cancer. Asian Pac J Cancer Prev. 2011;12(12):3175-80.
10. Yan LX, Huang XF, Shao Q, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. Rna. 2008;14(11):2348-60.
11. Wang G, Wang L, Sun S, Wu J, Wang Q. Quantitative Measurement of Serum MicroRNA-21 Expression in Relation to Breast Cancer Metastasis in Chinese Females. Annals Lab Med. 2015;35(2):226-32.
12. Qian B, Katsaros D, Lu L, et al. High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-β1. Breast Cancer Res Treat. 2008;117(1):131-40.
13. Radojicic J, Zaravinos A, Vrekoussis T, Kafousi M, Spandidos DA, Stathopoulos EN. MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. Cell Cycle. 2011;10(3):507-17.
14. Aboussekhra A, Al-Khanbashi M, Caramuta S, et al. Tissue and Serum miRNA Profile in Locally Advanced Breast Cancer (LABC) in Response to Neo-Adjuvant Chemotherapy (NAC) Treatment. Plos One. 2016;11(4):e0152032.
15. Elghoroury EA, ElDine HG, Kamel SA, et al. Evaluation of miRNA-21 and miRNA Let-7 as prognostic markers in patients with breast cancer. Clin Breast Cancer. 2018;18(4):e721-e6.
16. Cuk K, Zucknick M, Heil J, et al. Circulating microRNAs in plasma as early detection markers for breast cancer. Int J Cancer. 2013;132(7):1602-12.
17. Shah R. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol. 2014;5(3):283.
18. Sun Y-S, Zhao Z, Yang Z-N, et al. Risk factors and preventions of breast cancer. Int J Biol Sci. 2017;13(11):1387-97.
19. Lin C-H, Yap YS, Lee K-H, et al. Contrasting epidemiology and clinicopathology of female breast cancer in Asians vs the US population. JNCI: Journal of the National Cancer Institute. 2019;111(12):1298-306.
20. Dong G, Liang X, Wang D, et al. High expression of miR-21 in triple-negative breast cancers was correlated with a poor prognosis and promoted tumor cell in vitro proliferation. Med Oncol. 2014;31(7).
21. Fattah H, Mahmoud N, Elzoghby D, Matar M, El Shaer I. Clinical utility of circulating microRNA-21 in breast cancer. Egyptian J Hosp Med. 2018;71(4):2950-5.
22. Sakr HI, Khowailed AA, Al-Fakharany RS, Abdel-Fattah DS, Taha AA. Serum uric acid level as a predictive biomarker of gestational hypertension severity; A prospective observational case-control study. Reviews on recent clinical trials. 2020;15(3):227-39.
23. Abdulhussain MM, Hasan NA, Hussain AG. Interrelation of the circulating and tissue MicroRNA-21

with tissue PDCD4 expression and the invasiveness of Iraqi female breast tumors. Indian J Clin Biochem. 2017.
24. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences. 2008;105(30):10513-8.
25. Kim Y-K. Extracellular microRNAs as biomarkers in human disease. Chonnam Med J. 2015;51(2):51.
26. Papadaki C, Stratigos M, Markakis G, et al. Circulating microRNAs in the early prediction of disease recurrence in primary breast cancer. Breast Cancer Res. 2018;20(1).

Palliative Prognostic Index Validation in Hospitalized Advanced Cancer Patients in Indonesia Tertiary Hospitals

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ABSTRACT

Background: *Accurate prediction of survival is important for advanced cancer patients such as this to determine medical interventions, to plan the patient's lives and prepare end of life care. The palliative prognostic index (PPI) is most popular scores used worldwide to predict life expectancy in advanced cancer palliative patients. The purpose of this study was to test validity and the performance of PPI in Cipto Mangunkusumo Hospital as a Tertiary Referral Nasional Hospital.* ***Methods:*** *This retrospective cohort study, uses total subject during study with consecutive sampling. Palliative prognostic index was assessed by a palliative care team (PCT). Demographic data were summarized as n (%) and Chi square for categorical variables and median or mean for continuous variables. Overall survival was calculated using the Kaplan-Meier method with hazard ratios. The performance of PPI analyzed using SPSS version 20.0, includes for Receiving Operator Characteristics (ROC) and Hosmer-Lemeshow calibration test.* ***Results:*** *160 patients were included in the PPI study. The subjects have an average age of 50.08 years and are mostly women 68.10%. 28 (17.50%) had symptoms of dyspnoea, 22 (14.60%) pneumonia, and 19 (11.90%) had pain. The number of patients who died during hospitalisation was 83 (51.90%). PPI sum score >6 109 (68,10%). Calibration performance PPI score reached x2 = 8.915 (p = 0.259), and showed correlation r 0.799 (p 0.000). The accuracy of PPI scores in predicting survival in advanced cancer patients in studies for survival <3 weeks 81%, with a sensitivity of 85%, specificity 70%, PPV 86%, and NPV 67%. Predictive accuracy of survival within 3-6 weeks had 76%, sensitivity 66%, specificity 88%, PPV 85% and NPV 70%. PPI score discrimination performance is had a AUC value of 0.822 (95% CI 0.749-0.895).* ***Conclusion:*** *Palliative Prognostic Index (PPI) is valid and has good performance in predicting the survival of advanced cancer patients and may be used to help clinicians in palliative care consultation.*

Keywords*: Cancer, palliative prognostic index (PPI), validation, tertiary hospital.*

INTRODUCTION

Malignancies are increasing globally, especially in developing countries.[1-4] According to the Riskesdas data of 2013[5], the prevalence of cancer patients in Indonesia is 1.4 per 1,000. Of the 240,000 new cases per year, 65% of them seek health assistance at an advanced stage where curative management is no longer effective.[1-5] There are several models created to assist clinicians in predicting prognosis in palliative patients.[6-13] Palliative prognostic index (PPI) is a prognostic score that is popular and commonly used for patients in palliative care units,[14-18] and is recommended by the European Association of Palliative Care.[19]

Palliative prognostic index is a score that has been validated in various medical centres in the word [20-24] was developed in 1999 by Morita[25] et al. in Japan to predict the probability of survival in patients with terminal cancer, by making and summarizing various prognostic factors.

Palliative prognostic index relies on the assessment of five clinical variables which includes performance status using the palliative performance scale, oral intake, absence or presence of dyspnea, edema, and delirium, without requiring blood tests or any clinical prediction of survival.[26-28]

The palliative performance scale is a modification of the Karnofsky Performance Scale Index, which grades a patient's general condition on a scale from 0 (death) to 100 (normal).[29] Palliative performance scale 10-20, 30-50 and >60 with partial score 4, 2.5 and 0. Clinical symptoms of oral intake, divided to mouthfuls or less**,** reduced but more than mouthfuls, and normal with partial score 4, 2.5 and 0. Clinical symptoms of oedema absent or present with partial score 0 or 1, symptoms of dyspnoea at rest absent or present its partial score 0 or 3.5, symptoms of delirium absent or present with partial score 0 or 4. The total PPI score is calculated from the sum of the partial scores of those five variables, range from 0 to 15 points which predicts survival time.[20,24]

The resulting PPI total score puts the patient into one of three groups, predicting survival of more than 6 weeks (PPI score ≤ to 4), 3-6 weeks (PPI score >4-6), or shorter than 3 weeks (PPI score > 6). Palliative prognostic index has been validated in patients with the end stage cancer in a number of settings: hospital, hospice, and home. In different populations there can be differences in the course of the disease, so it is important to assess the prognostic system in the local population. [21,26-28,30,31] Palliative prognostic index has been routinely assessed in advanced cancer patients who have consulted to palliative care team (PCT) at the Cipto Mangunkusumo General Hospital since 2017, but it has not been evaluated yet. The purpose of this study was to test the performance of predicted survival of PPI in hospitalised advanced cancer patients who have consulted to the PCT at Cipto Mangunkusumo Hospital as a tertiary referral Nasional Hospital.

METHODS

This is a retrospective cohort study to assess the performance of the PPI in advanced cancer patients. The sample selection was advanced cancer patients who were consulted to the PCT Cipto Mangunkusumo Referral Hospital from July 2017-December 2018. Palliative prognostic index was assessed by the PCT at first palliative consultation. Secondary data were taken from patients' medical records. The number of samples involved in this study was the total sample who met the inclusion criteria. Sampling technique used is consecutive sampling method from July 2017 to December 2018. Patients who met the inclusion criteria and were not exclude will be followed and the outcome will be observed (died or not) to know the survival. Inclusion criteria includes adult patients over 18 years of age with advanced cancer proven with histopathological examination who are consulted to the PCT. The exclusion criteria in this study includes patients who are in life-support supportive invasive therapy e.g., mechanical ventilation, having unavailable research data and censored patient who are lost to follow up.

Ethics Approval

The study was reviewed and approved by the Institutional Review Board, the Ethics Committee of the Faculty of Medicine of Universitas Indonesia, Cipto Mangunkusumo National Referral Hospital, Jakarta, Indonesia (Ref. Number 235/UN2.F1/ETIK/IPM 00.02/2017).

Statistical Analysis

Statistical analyses were performed using SPSS 20.0 statistics. Basic demographic data were summarized as n (%) and Chi square for categorical variables and median with the interquartile range for continuous variables or mean, respectively. Overall survival was calculated using the Kaplan-Meier method. Hazard ratios were estimated for severe and intermediate PPI sum scores relative to good PPI sum scores (<4) using unstratified Cox regression. The calibration performance of the PPI score system was assessed by the calibration plot and the Hosmer-Lemeshow test. Discrimination performance was assessed by the area under the receiver operating characteristic curve (AUC) which was made based on the predicted mortality of each subject. To investigate the predictive accuracy of prognostic scores, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of each prognostic score were calculated. Accuracy was calculated by dividing the sum of true positive and true negative cases by the total number. We adopted cut off points according to the original studies. All statistical assessments were considered significant when $p<0.05$.

RESULTS

During the period of July 2017 to December 2018, there were 257 advance stage cancer patients consulted to PCT. Based on the inclusion criteria of the study as well as the availability of medical record data. The number of subjects who were successfully recruited were 160 patients.

The characteristic of the subjects are shown in **Table 1**. Subjects have an average age of 50.08 years, ranging in from 20-83 years. The proportion of women is 68.1%. Data on the clinical characteristics of the subjects showed that the majority knew they had terminal cancer (52.5%). The three main diagnoses at entry were cancer with symptoms of dyspnoea in 28 subjects (17.5%), pneumonia in 22 (14.6%) followed by cancer with symptoms of pain in 19 subjects (11.9%). The number of patients who died during hospitalisation was 83 (51.9%). The time interval between the patient entering and being consulted to the PCT was mostly less than 1 week (63.9%). Patient performance status and clinical symptoms by PPI are summarized in **Table 2**.

Table 1. Patient characteristics.

Patient Characteristics	N (%)
Age (mean, years)	50.08
- ≥60 years	41 (24.30)
- <60 years	119 (70.40)
Gender	
- Female	109 (68.10)
- Male	51 (31.90)
Referral Department	
- Internal Medicine	101 (63.00)
- Onco-Genecology	31 (19.40)
- Surgery	18 (11.03)
- Ear Nose and Throat	5 (3.01)
- Neurology	5 (3.01)
Origin of primary tumour	
- Genecology	43 (26.90)
- Breast	27 (16,90)
- Head and Neck	27 (16.90)
- Digestive	16 (10.00)
- HCC	13 (8.10)
- Lung	11 (6.90)
- Skin and soft tissue	6 (3.80)
- Urology	3 (1.90)
- Bone	3 (1.90)
- Haematology	2 (1.30)
Stage at diagnosis of Cancer	
- Stage IV	84 (52.50)
- Stage III	37 (23.10)
- Stage II	20 (12.50)
- Stage I	7 (4.40)
- No data	12 (7,5)
Reason for Admission	
Symptom's control:	
- Dyspnoea	28 (17.50)
- Pain	19 (11.90)
- Consciousness loss	18 (11.30)
- Appetite loss	17 (10.60)
- Bleeding	6 (3.80)
Pneumonia	22 (14.60)
Anaemia	7 (4.40)
Electrolyte imbalance	6 (3.80)
Gastrointestinal bleeding	4 (2.50)
Diarrheal	3 (1.90)
Sepsis	2 (1.30)
Hospital Outcome	
- In hospital death	83 (51,9)
- Live discharge	77 (48,1)

Hospital Outcome	
- In hospital death	83 (51,9)
- Live discharge	77 (48,1)
Cause of death	
- Respiratory failure	49 (58.30)
- Multiple organ failure	19 (22.60)
- Sepsis shock	13 (15.80)
- Liver failure	3 (1,9)
Interval from hospital admission to palliative consultation	
- < 1 week	108 (63.90)
- 1 – 2 weeks	30 (17.80)
- >2 – 4 weeks	19 (11.20)
- >4 weeks	3 (1.80)

Table 2. Patient performance status and clinical symptoms base on palliative performance index (PPI)

Clinical Performance	Value	Score	Number (%)
Palliative	≥ 60	0.0	9 (5.6)
Performance	30-50	2.5	84 (52.5)
Scale	10-20	4.0	67 (41.6)
Oral intake	Normal	0.0	83 (51.9)
	Reduced but more than mouthfuls	1.0	45 (28.1)
	Mouthfuls or less	2.5	32 (20)
Oedema	Absent	0.0	87 (44.4)
	Present	1.0	73 (45.6)
Dyspnea at rest	Absent	0.0	81 (51.9)
	Present	3.5	79 (49.1)
Delirium	Absent	0.0	97 (60.90)
	Present	4.0	63 (39.10)

The PPI sum score was ≤ 4 in 30 (18.8%) subjects, 4 - ≤6 in 21 (13.1%) subjects and >6 in 109 (68.1%) subjects.

A Kaplan Meier curve was constructed for each of the groups (Figure 1). The overall median survival was 8 days and for groups 1, 2, and 3 was 68 (47 to 87), 22 (15 to 29), and 5 (3 to 7) days. **Table 3** shows median survival of each category.

From the Kaplan Meier survival curve between the Palliative Prognostic score group and then Cox regression, the value of the PPI Hazard ratio (HR) > 6 was 4.22 (95% CI.2.67-6.66) with a p value of 0.001, and PPI score> 4 and ≤ 6 with the HR 2.02 (95% CI.1.11-3.68) with a p value < 0.02.

The accuracy of PPI score in predicting survivability <3 weeks was 81%, while accuracy in predicting survivability of 3-6 weeks was 76%. Discrimination PPI score expressed by the AUC score was 0.822 (IK95% 0.749-0.895). (**Figure 2**)

The PPI performance in predicting mortality in advanced cancer patients based on the Hosmer-Lemeshow test calibration performance reached x2 = 8.915 (p= 0.259), and showed correlation r 0.799 (p 0.000). (**Figure 3**)

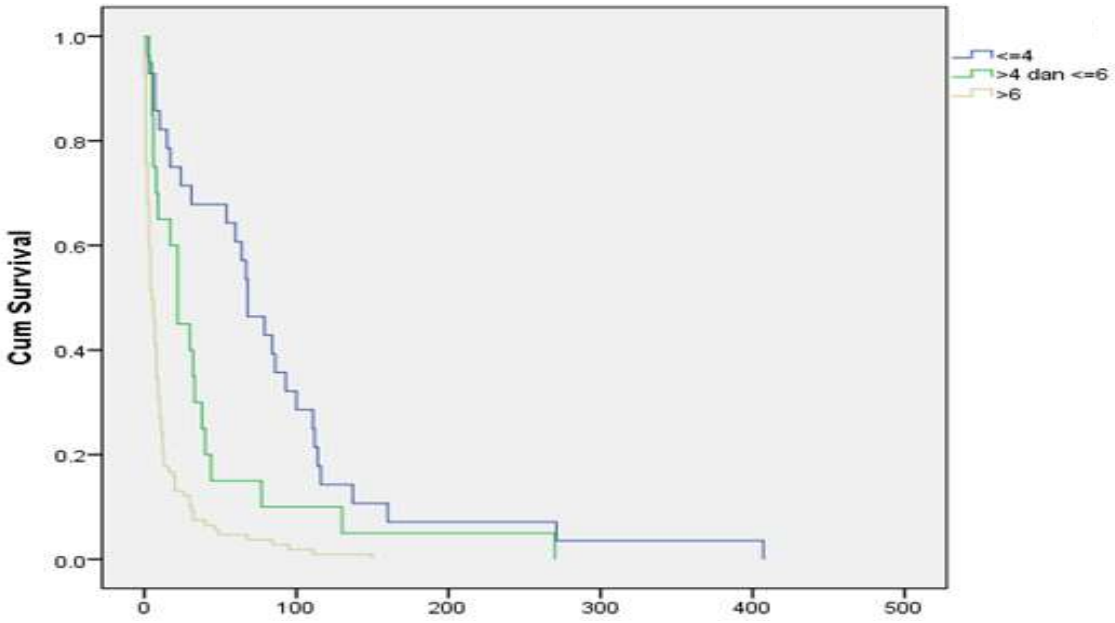

Figure 1. Kaplan-Meier survival curves for each PPI group.

Table 3. Characteristic of the three groups as defined by PPI.

Group	PPI score	N (%)	Median survival Days (95%CI)	HR (95% CI)
1	≤4	30 (18.8%)	68 (46.90-87.09)	reference
2	>4 - ≤ 6	21 (13.1 %)	22 (14.84-29.16)	2.02 (1.11-3.68)*
3	>6	109 (68.1%)	5 (2.98-7.02)	4.22 (2.67-6.66)*
Overall		160 (100%)	8 (6.04-9.95)	

Chi-square analysis of the distribution of survival (Mantel-Cox) *p value of 0.05

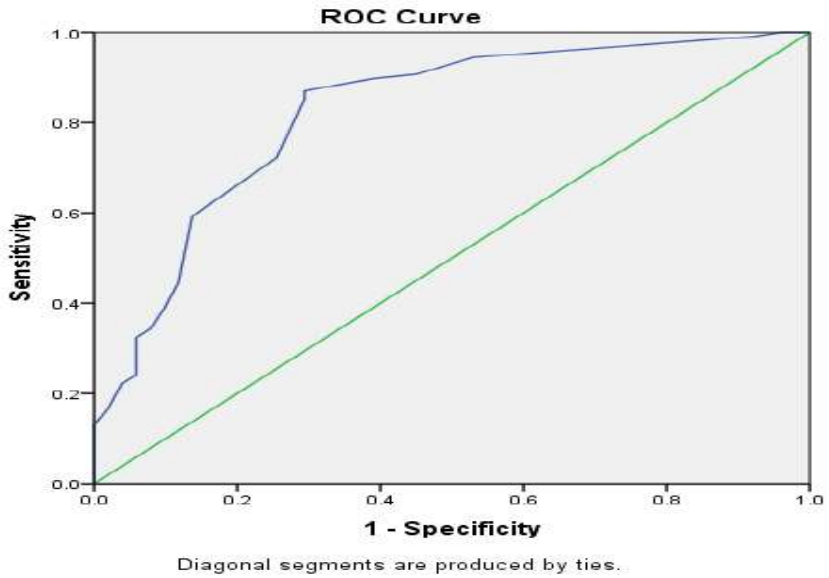

Figure 2. ROC curve of the PPI score.

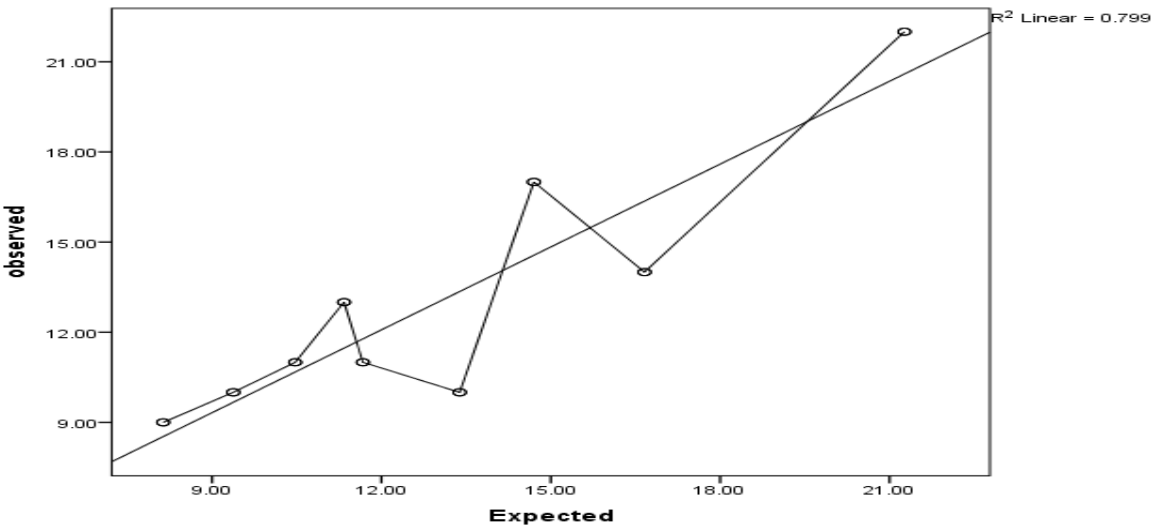

Figure 3. Diagram plot calibrations palliative prognostic index (PPI) scores.

The accuracy of the PPI model in predicting mortality in advanced cancer patients in this study was 81% for survival <3 weeks with a sensitivity of 85%, specificity 70%, PPV 86% and NPV 67%. Accuracy for prediction of survival <6 weeks was 76 %, sensitivity 66% and specificity 88%, with PPV 85% and NPV 70%. (**Table 4**)

Table 4. Accuracy of predictions using PPI

Accuracy	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Overall accuracy (%)
<3 Weeks (PPI > 6)	85	70	86	67	81
<6 Weeks (PPI > 4)	66	88	85	70	76

DISCUSSION

Subjects in this study had an average age of 50 years old. The study by Morita et al.[25] in a population of patients with terminal cancer in hospice in Japan had an average age of 66 years old. Meanwhile, Stone et al.[15] found that in cancer patients who received palliative care at the hospital, as well as in hospice and at home in Ireland showed the average age of subjects 69.9 years old. This study had subjects with a younger average age than in other studies. This illustrates the occurrence of cancer in a younger age group in developing countries. This is thought due to the high rates of chronic infection, genetic factors, an increasing number of smokers, especially those who are young, as well as a lack of screening and access to health services.[30]

There were more female subjects than male subjects (68.1% vs 31.9%) which differed from the study of Morita et al.[25] in which there were more men than women. This is because breast cancer and cervical cancer having the highest prevalence in Indonesia, different from Japan and other developed countries where lung and gastric cancers have the highest prevalence.[6,25]

Delirium was found in 39.1% of the study subjects. This is consistent with the research by Bush et al.[31] where the prevalence of delirium in patients in palliative care ranged from 13-14% at admission and 88% in the last week of life. Proportion of subjects with PPS 30-50 were 52.5% and those with PPS 10-20 were 41.6%.[31,32] This study shows that the majority (94.1%) of advanced cancer patients were consulted to a PCT with poor performance status and clinical symptoms which includes delirium, dyspnoea, oedema and mouthfuls with PPI score > 6 (68%) that results shows poor prognosis or short survival.[31-41]

Patients in different PPI groups had significantly different lengths of survival in this study. The results showed in group 1 with PPI score <4, median survival was 68 days, in group 2 with PPI score > 4 - ≤ 6 median survival was 22 days, while in group 3 with PPI score >6, median survival was the shortest with only 5 days. The hazard ratio value obtained from the group with a PPI score> 6 compared to the group with a score of ≤ 4 was 4.22 (95% CI, 2.67 - 6.66) with a p value of 0.001, and a PPI score> 4 and ≤ 6 compared with a group with a score of ≤ 4 of 2.02 (95% CI, 1.11 - 3.68) with a p value of 0.021. Discrimination of the PPI score in the study subjects was expressed by the AUC score of 0.822. A good discrimination performance is if the AUC value above 0.8; if the AUC value is 0.7 and above, then it is still acceptable for a prognostic model, and the performance is considered weak if the AUC value is below 0.7. This study shows good calibration performance of PPI scores with the Hosmer-Lemeshow test showing a value of p > 0.05, which means PPI was well-calibrated. In this study, predictions of survival of less than three weeks using a PPI greater than 6 and of less than 6 weeks using a PPI greater than 4 were accurate, with the accuracy of PPV and NPV almost similar to the others include the original study.[15,20,22,26.]

PPI has been shown to improve the clinical predictions of survival by doctors in palliative care. the European association for palliative care working group on prognosis recommended the use of prognostic tools in combination with the clinical prediction of survival to estimate survival of patients with advanced cancer.[19.]

The Palliative Prognostic Index is easy to use and does not require blood testing. It has been validated in patients with the final stage cancer in a number of settings: hospital, hospice, and home.[20-24] This study has shown that it is valid for use in patients with advanced cancer who were consulted to the PCT in Tertiary Referral Hospital.

The limitations of this study include: having a retrospective study design, so that some bias may have existed in the way in which the data were obtained. This study was conducted at the single centre; however, this is the first study validation of the PPI score in Indonesia in advanced cancer patients receiving palliative care. A prospective study to valid PPI scores for all patient's referral to palliative consultation care should be conducted in the future and conducted with a multi-centre setting. Adding variables of PCT clinical judgment and consideration of acute conditions may increase prognostic performance

CONCLUSION

Palliative Prognostic Index scores is valid and has good performance including adequate accuracy and discrimination in predicting the survival of advanced cancer patients. It's can be used to help clinicians predict survival in advanced cancer patients who were consulted to the palliative care team.

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REFERENCES

1. Kanavos P. The rising burden of cancer in the developing world. Ann Oncol. 2006;17(Suppl.8): 15–23.
2. Reeler AV, Mellstedt H. Cancer in developing countries: Challenges and solutions. Ann Oncol. 2006:17(Suppl.8):7–8.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. ACS Journals. 2015.https://doi.org/10.3322/caac.21262
4. Connor SR, Sepulveda Bermedo MC. Global atlas of palliative care at the end of life. World Health Organization. 2014. p. 111.
5. Kementrian Kesehatan RI Pusat Data dan Informasi Kesehatan. Stop Kanker. Jakarta: Pusat Data dan Informasi Kementrian Kesehatan RI; 2015. p. 1–8.
6. Morita T, Tsunoda J, Inoue S, Chihara S. Survival prediction of terminally ill cancer patients by clinical symptoms: Development of a Simple Indicator. Jpn J Clin Onco. 1999;29:156-9.
7. Teunissen SC, Graeff A de, de Haes HC, Voest EE. Prognostic significance of symptoms of hospitalized advanced cancer patients. Eur J Cancer. 2006;42(15):2510-6.
8. Stiel S, Bertram L, Neuhaus S, Nauck F. Evaluation and comparison of two prognostic scores and the physicians ' estimate of survival in terminally ill patients. Support Care Cancer. 2010: 43-9.
9. Hyodo I, Morita T, Adachi I, Shima Y, Yoshizawa A, Hiraga K. Development of a predicting tool for survival of terminally ill cancer patients. Jpn J Clin Oncol. 2010;40(5):442-8.
10. Zeng L, Zhang L, Culleton S, et al. Edmonton symptom assessment scale as a prognosticative indicator in patients with advanced cancer. J Palliat Med. 2011;14(3):337–42, 55.
11. Hui D, Parkb M, Liub D, Paivac CE, Suhd SY, Moritae T. Clinician prediction of survival versus the palliative prognostic score: Which approach is more accurate? Published in final edited form as. Eur J Cancer. 2016; 64: 89–95. doi:10.1016/j.ejca.2016.05.009
12. Simmons CPL, McMillan DC, McWilliams K, et al. Prognostic tools in patients with advanced cancer: a systematic review. J Pain Symptom Manage. 2017; 53(5):962-70.
13. Yoon SJ, Suh SY, Hui D, et al. Accuracy of the palliative prognostic score with or without clinicians' prediction of survival in patients with far advanced cancer. J Pain Symptom Manage.2020;21;S0885-3924(20)30825-3.
14. Hamano J, Morita T, Ozawa T, Shishido H. Validation of the simplified palliative prognostic index using a single item from the communication capacity scale. J Pain Symptom Manage. 2015;50(4):542-7.
15. Stone CA, Tiernan E, Dooley BA. Prospective validation of the palliative prognostic index in patients with cancer. 2008;35(6):617–22.
16. Hamano J, Maeno T. Usefulness of palliative prognostic index for patient with advanced cancer in home care setting. American J Hospice Palliative Med. 2012;30(3):264–7.
17. Buckingham VM, Bruera J, Suarez-Almazor S. Survival prediction in terminal cancer patients: a systematic review of the medical literature. Palliat Med. 2000;14(5):363–74.
18. Baba M , Maeda I, Morita T, et al. Survival prediction for advanced cancer patients in the real world: A comparison of the Palliative Prognostic Score, Delirium-Palliative Prognostic Score, Palliative Prognostic Index and modified Prognosis in Palliative Care Study predictor model. Eur J Cancer. 2015; 51(12):1618-29.
19. Maltoni M, Caraceni A, Brunelli C, et al. Prognostic factors in advanced cancer patients: Evidence-based clinical recommendations - A study by the steering committee of the european association for palliative care. J Clin Oncol. 2005;23:6240–8.
20. Cheng WH, Kao CY, Hung YS, et al. Validation of a palliative prognostic index to predict life expectancy

for terminally ill cancer patients in a hospice consultation setting in Taiwan. Asian Pacific J Cancer Prev. 2012; 13: 2861- 6

21. Alshemmari S, Ezzat H, Samir Z, Refaat S, Alsirafy SA. The palliative prognostic index for the prediction of survival and in-hospital mortality of patients with advanced cancer in Kuwait. J Palliat Med. 2012; 15(2):200-4.
22. Hung YS, Su PJ, Hsieh CH, Chen JS, et al. Validation of a palliative prognostic index to predict life expectancy for terminally ill cancer patients in a hospice consultation setting in Taiwan. Asian Pacific J Cancer Prev. 2012;13:2861- 6.
23. Sonoda H, Yamaguchi T, Matsumoto M, Hisahara K. Validation of the palliative prognostic index and palliative prognostic score in a palliative care consultation team setting for patients with advanced cancers in an acute care hospital in Japan. Am J Hosp Palliat Care. 2014;31(7):730-4.
24. Baba M, Maeda I, Morita T, et al. Prognostic tools in patients with advanced cancer: a systematic review. Eur J Cancer. 2015;51(12):1618-29.
25. Morita T, Tsunoda J, Inoue S, Chihara S. The palliative prognostic index: A scoring system for survival prediction of terminally ill cancer patients. Support Care Cancer. 1999;7:128-33.
26. Subramaniam S, Thorns A, Ridout M, Thirukkumaran T, Osborne TR. Accuracy of prognosis prediction by PPI in hospice inpatients with cancer: a multi-centre prospective study. BMJ Supportive & Palliative Care. 2013: 324–9.
27. Morita T, Tsunoda J, Inoue S, Chihara S. Improved accuracy of physicians' survival prediction for terminally ill cancer patients using the Palliative Prognostic Index. Palliat Med. 2001;15(5):419-24.
28. Morita T, Tsunoda J, Inoue S. Prediction of survival of terminally ill cancer patients-a prospective study. Jpn J Cancer Chemotherapy. 1998;25:1203-11.
29. Anderson F, Downing GM, Hill J, Casorso L, Lerch N. Palliative performance scale (PPS): a new tool. J Palliat Care. 1996;12:5-11.
30. Hadi AM, Al Madani R, Arida LA, Al Ghareeb B. Breast cancer age in developing countries: The narrowing gap. Clinics in surgery. 2018;3:2017–9.
31. Bush SH, Tierney S, Lawlor PG. Clinical assessment and management of delirium in the Palliative Care Setting. Drugs. 2017;77(15):1623-43.
32. Centeno C, Sanz Á, Bruera E. Delirium in advanced cancer patients. Palliat Med. 2004;18(3):184–94.
33. Caraceni A, Nanni O, Maltoni M, et al. Impact of delirium on the short-term prognosis of advanced cancer patients. Italian Multicenter Study Group on Palliative Care. Cancer. 2000;89(5):1145–9.
34. Centeno C, Sanz Á, Bruera E. Delirium in advanced cancer patients. Palliat Med. 2004;18(3):184–94.
35. Muliawati Y, Haroen H, Rotty LW a. Cancer anorexia - cachexia syndrome. Acta Med Indones. 2012;44(2):154–62.
36. Berger A, Shuster J, Von Roenn J. Anorexia and weight loss. Palliative care and supportive care. 2005. 203–208.
37. Kloke M, Cherny N. Treatment of dyspnea in advanced cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol. 2015;26(August):169–73, 75.
38. Keeley V. Pathophysiology and presentation of oedema in advanced cancer and the end of life. Manag Lymphoedema Adv Cancer Oedema End Life [Internet]. 2010;4–7.
39. Woelk, C and Harlos M. Prognostic scoring guideline for estimating length of survival in palliative patients. GSF. 2005;1–5.
40. Inomata M, Hayashi R, Tokui K, et al. Usefulness of the palliative prognostic index in patients with lung cancer. Med Oncol. 2014;31(9):1–4.
41. Llobera J, Esteva M, Rifà J, et al. Terminal cancer duration and prediction of survival time. Eur J Cancer. 2000;36(16):2036–43.

Herpes Zoster After Tocilizumab Therapy in COVID-19 Survivor: A Case Report

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ABSTRACT

The COVID-19 pandemic has become a concern for the world community. However, despite abundant attempts taking place, there is currently no definitive therapy for COVID-19 yet. The clinical approaches recently adopted are the provision of antiviral therapy and immunomodulators. One of the immunomodulators that are currently being researched is tocilizumab, an IL-6 receptor antagonist monoclonal antibody. Many case studies and retrospective observational studies have shown that there is a chance that tocilizumab could diminish death rates in COVID-19 patients with severe or critical symptoms. Along with the growing use of tocilizumab in the COVID-19 cases that are emergent in nature, the occurrence of unpredictable adverse effects of the drugs have also been raising. This case report describes a COVID-19 confirmed patient with severe symptoms who was given tocilizumab in addition to standard therapy. The patient developed Herpes Zoster infection which was suspected to be related to the adverse effects of using tocilizumab, which is known for its likelihood to increase the risk of new infections and also probably reactivate latent infections. Tocilizumab use seems to be effective in combating cytokine storm associated with severe COVID-19 infection. The possibility of serious adverse effects in the utilization of tocilizumab, though rare, cannot be excluded. The presence of a latent or chronic infection that can undergo reactivation should be a consideration for appropriate screening or prophylaxis before administering tocilizumab.

Keywords: *COVID-19, Tocilizumab, Herpes Zoster, adverse drug effects, cytokine storm.*

INTRODUCTION

The COVID-19 pandemic has become a concern for the world community. In handling it, various therapeutic modalities are used both for emergency reasons and research purposes. Tocilizumab, a monoclonal antibody against the IL-6 receptor from the immunoglobulin G1k group, is one of the immunomodulators that has been used for a long time in the therapy of Rheumatoid Arthritis. It is known that IL-6 levels increase sharply in COVID19 patients with critical symptoms, even up to ten times higher, triggering a cytokine storm.[1] Tocilizumab can bind to both membrane-bound IL-6 receptor (mIL-6R) and soluble IL-6 receptor (sIL-6R).[2] The potential benefits of Tocilizumab in suppressing cytokine storms that occur in COVID-19 patients make it an option that is starting to get a lot of attention.[3]

This case report presents a patient with severe pneumonia caused by SARS-CoV-2. During hospitalization, the patients was given Tocilizumab (Actemra) to alleviate symptoms associated with cytokine storm. The patient later

developed a skin rash due to reactivation of the herpes zoster virus which was suspected to be a side effect that occurred after using Tocilizumab.

CASE ILLUSTRATION

A 43-year-old woman presented to the emergency room on July 12, 2020 with a complaint of severe shortness of breath that started three days prior. Approximately seven days before being admitted to the hospital, the patient developed a fever accompanied by dry cough, malaise, and decreased appetite but denied any abdominal pain. There are no complaints of headache, sore throat, diarrhea, or skin rash. Any history of contact with confirmed or suspected cases of COVID-19 was denied. Patient also disproved any travel history prior to symptoms.

On initial examination, rapid shallow breathing was found at a rate of 34 breaths per minute. On auscultation, there were crackles found in both lung fields and the oxygen saturation was 84% on ambient air. Other physical examinations were within normal limits. Blood tests showed lymphopenia (lymphocyte count was 14.4% of total leucocytes), an increase in the value of D-dimer (> 4 µg/mL) and ferritin (1493.55 ng/mL). SARS-COV-2 Antibody Rapid Test was also conducted using immunochromatography methods which showed reactive results to both IgG and IgM. IL-6 examination was not carried out due to limitations in health facilities. The patient had a history of poorly controlled type-2 diabetes mellitus with HbA1c at 10%. The presence of other comorbidities was refuted.

A Thoracic Computed Tomography scan (CT Scan) at admission provided images of multiple consolidation and multifocal ground glass opacities extending to the periphery, which correspond to the features commonly found in COVID-19 infection in severe classification (**Figure 1**). RT-PCR examinations were carried out using the Kit for 2019 Novel Coronavirus RNA with the RT-PCR ABI 7500 method. Samples for the RT-PCR examination were obtained from naso-oropharyngeal swabs and the result came out as positive. The patient was thus a COVID-19-confirmed case and was then being treated in the isolation room.

Based on the clinical presentations, laboratory, and radiological examination results, the patient showed manifestations of severe pneumonia. In accordance with the guidelines for handling COVID-19 that were in effect at the time, at the beginning of treatment, the patient was given a standardized supportive therapy which consisted of empirical antibiotics therapy, high-dose vitamin C, glucocorticoids, anticoagulants, and lopinavir-ritonavir as antiviral. [4-5]

On the eighth and ninth day of treatment, the patient manifested trend towards worsening of the respiratory distress with her oxygen saturation being below 90% despite oxygen supplementation with CPAP (continuous positive airway pressure) device. Settings of the CPAP device were by itself under clinical judgment from our fellow anesthetist. On the tenth day, based on the deteriorating clinical presentations which suggested a cytokine storm might be going on, tocilizumab therapy was initiated. Tocilizumab was given twice with the first dose of 400 mg, followed by the second dose of 200 mg given 12 hours after the first dose. At the time of the administration of the tocilizumab, no significant adverse reaction was observed.

Improvements were apparent during the next few days. Shortness of breath and the oxygen requirement gradually decreased and the patient no longer needed any oxygen supplementation in one week after receiving tocilizumab. The extent of pneumonia also showed coherent reduction on the follow-up chest x-ray performed three days later. The follow-up CT scan conducted one month afterwards also showed satisfactory result (**Figure 2**). Inflammation markers also showed improvement, with the ferritin value of 842.9 ng/mL and the D-dimer value declined to 1.54 µg/mL in ten days after tocilizumab administration. Follow-up SARS-COV-2 RT-PCR with negative results were obtained after 25 days of hospitalization.

However, on the eleventh day after tocilizumab administration, a sensation of prickling pain was felt at the area around the right upper torso. The pain was then followed by the appearance of a reddish rash with multiple clusters of papules and vesicles (**Figure 3**). The erythematous rash began to appear on the

fourth day since the complaints of pain emerged, multiplied and expanded, spreading to the back within ten days. The patient denied having experienced such complaints before.

The clinical appearance clearly indicated a herpes zoster infection. The patient was then treated with acyclovir, gabapentin, gentamicin ointment applied on ruptured vesicles, and salicylic powder for intact vesicles. After the initiation of therapy, no new papules or vesicles emerged, leaving erosions, crusts, and hyperpigmented macules (**Figure 4**). However, the pain persisted even after the patient had been given combination of analgesic therapy (gabapentin and NSAIDs).

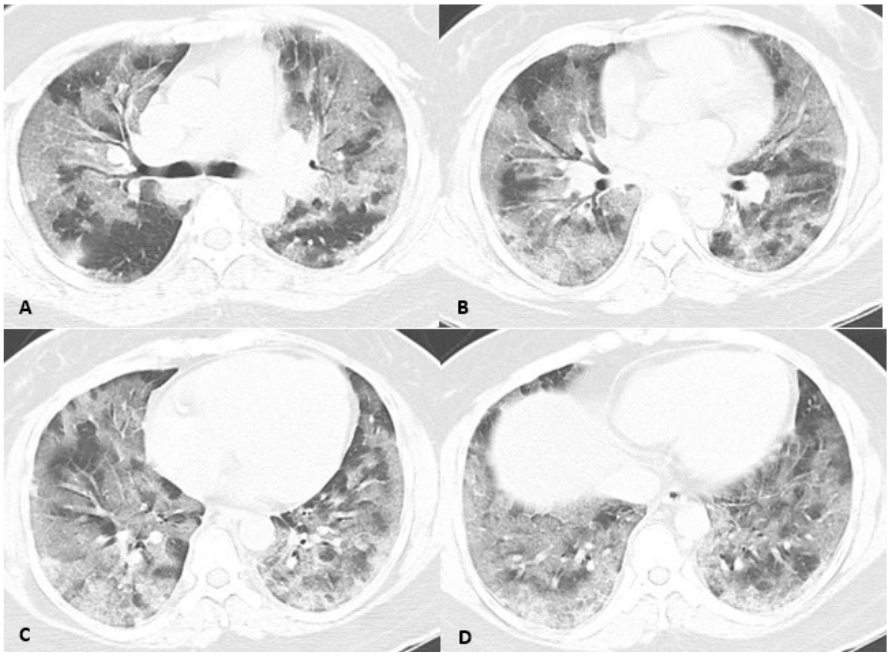

Figure 1. (A-D) Multifocal ground glass opacities and extensive consolidation was found on thoracic computed tomography scan examination on the first day of admission.

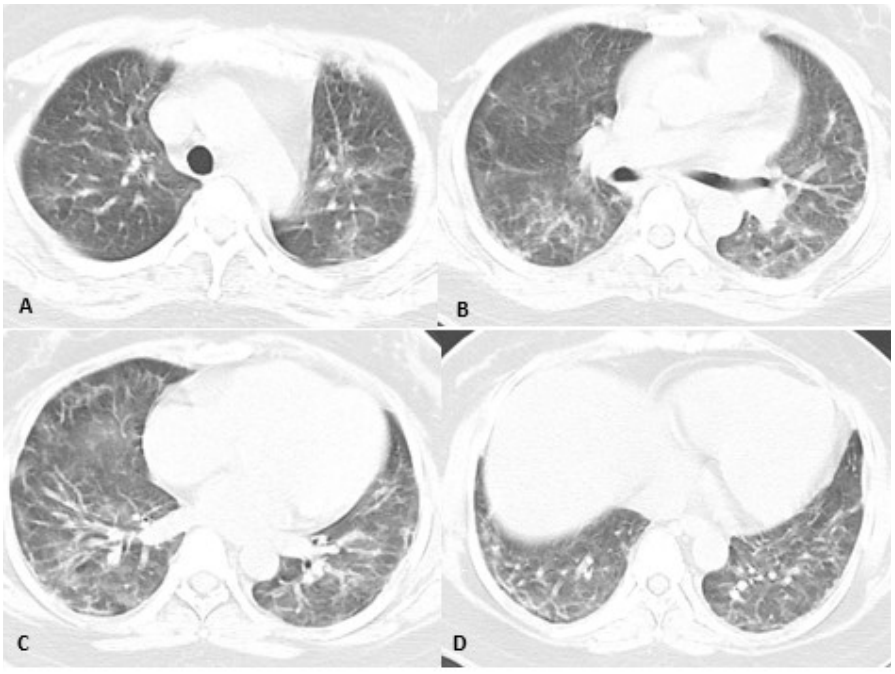

Figure 2. (A-D) A follow-up CT scan conducted one month apart showed a satisfactory reduction of ground glass opacities and consolidation, accompanied by increasing linear opacities indicating the process of lung fibrosis.

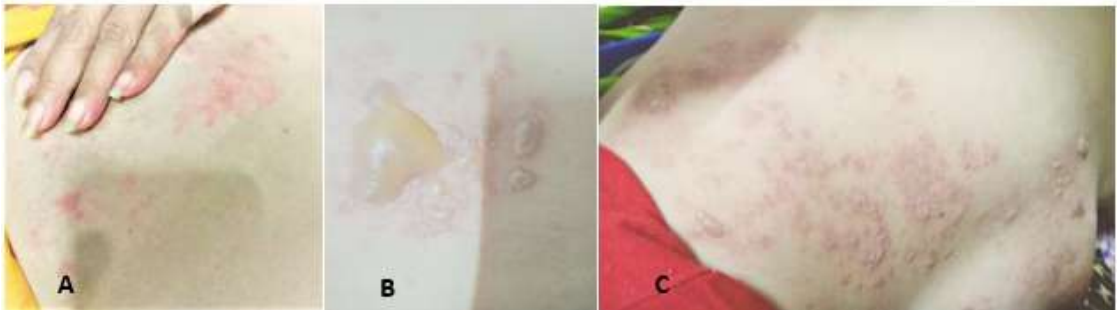

Figure 3. Multiple papules and vesicles initially appeared on the area around the right breast (A) on the fourth day since the first complaint of pain, followed by bullae which appeared (B) on the seventh day, and spread to the back (C) on the tenth day.

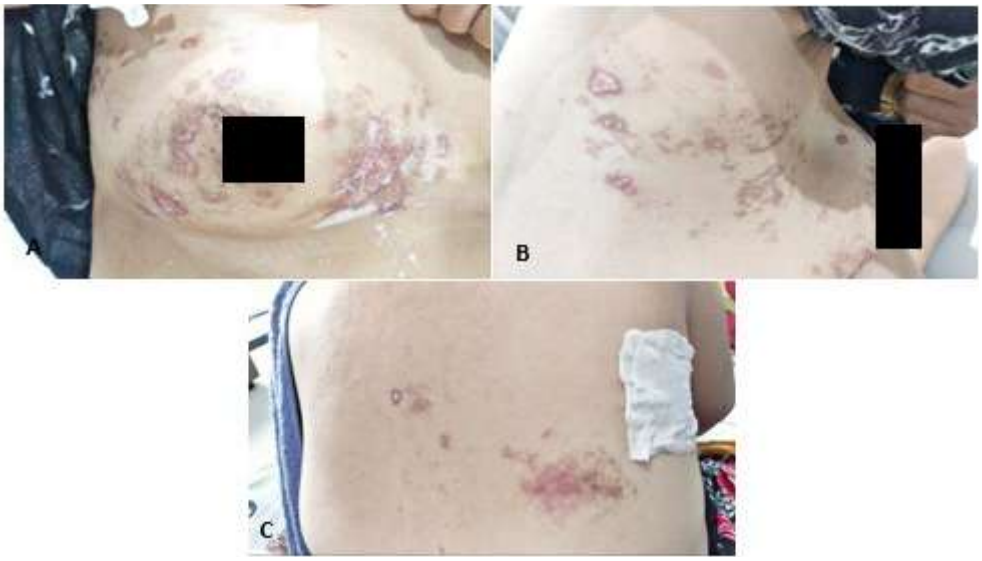

Figure 4. (A-C) The appearance of the lesions on day 20 showed that the rash subsided after therapy

DISCUSSION

As is the case with SARS and MERS-CoV infection, COVID-19 caused by SARS-CoV-2 has also been proven to increase levels of inflammatory cytokines. This cluster of inflammatory messengers which includes IL-6, IL-2, IL-7, IL-10, G-CSF, IFN- γ, macrophage inflammatory protein 1α, and TNF-α could indicate the occurrence of a cytokine storm.[6-7] IL-6 plays a role in T cell activation and proliferation, B cell differentiation, and regulation of acute phase response and hormonal activity.[6] Levels of IL-6 increased sharply in COVID-19 patients with critical symptoms, even up to ten times higher.[1,7-8]

Tocilizumab (TCZ) is an anti-inflammatory therapy that has a potential to suppress cytokine storms triggered by MAS (macrophage-activating syndrome) and has shown benefit in the management of COVID-19 in several case studies[9-12] and recent systematic reviews.[13-14] Majority of clinical experiences support the use of TCZ in COVID-19 because the TCZ group is associated with significant reduction in mortality rate compared to that of standard therapy group, despite some of the studies being retrospective observational studies.

Tocilizumab is a monoclonal recombinant antibody against IL-6 receptors from the immunoglobulin G1k group which binds well to membrane-bound IL-6 receptors (mIL-6R) and soluble IL-6 receptors (sIL-6R). On top of that, TCZ is even said of having the capability to shift IL-6 that has been sitting on its receptor.[2] TCZ effectively ameliorates severe clinical symptoms in critically-ill COVID-19 patients and averts the unstoppable series of vicious cycle in a cytokine storm. Thus, TCZ can be used

as a therapeutic strategy in severe and critical COVID-19 infections, if given immediately at a severe stage.[10,12,15]

Serum IL-6 value was not assessed in the presented case due to laboratory limitations in health care facility. However, judging from the rapid clinical deterioration and respiratory distress, clinicians made the decision to administer TCZ in two doses. TCZ was approved for use in Japan for the first time in 2005 as a therapy for Castleman's disease, a lymphoproliferative disease that involves the expansion of the plasma cell count which is a very rare condition.[16] Other indications include the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, giant-cell arteritis, and cytokines release syndrome induced by chimeric antigen receptor (CAR) T-cell therapy.[17] Its usefulness is also being studied in other conditions such as Crohn's disease, SLE, Takayasu arteritis, polymyalgia rheumatica, and refractory adult-onset Still diasease.[2]

The main consideration for using TCZ is its immunosuppressive effect, which is a gratifying solution when given at the appropriate time to combat an excessive immune response. However, TCZ use is often linked with the risks of infection and its negative effect on lipid profile is well known. The most commonly reported adverse effects of TCZ use are infections, particularly upper respiratory tract infections, nasopharyngitis, cellulitis, herpes, and pneumonia. Gastrointestinal disorders such as nausea, abdominal pain, oral mucosal ulcers, and gastritis are also common adverse effects. Transient neutropenia is also a common adverse effect but it is less common.[16,18-19]

The occurrence of serious adverse effects with TCZ use was 3.8% in a study where TCZ was administered every 4 weeks for 24 weeks as indicated by rheumatoid arthritis. The most common side effect is infection (34.4%), with 4.1% of which is skin infection. The occurrence of herpes infection, although not commonly found, can occur in TCZ users.[17,19-20]

A study in Italy analyzed the use of Tocilizumab in COVID-19 patients with severe symptoms and found that Tocilizumab showed a reduced risk of mechanical ventilation or death. The side effects seen in the study were mostly infection (13%), with one subject (<1%) experiencing severe reactions 12 days after subcutaneous administration of Tocilizumab, with severe liver failure due to reactivation of HSV-1, resulting in death. These patients received high doses of glucocorticoids after administration of TCZ.[21] Because the possibility of severe to life-threatening reactions exist, many clinicians have emphasized the importance of screening for possible reactivation of latent infections especially when glucocorticoids are used.

In other studies, no side effects of moderate or severe magnitude were observed on using Tocilizumab in COVID-19 patients with severe symptoms. However, the patients included in this particular study were only followed-up for 14 days post administration.[11] Many patients who experienced side effects of infection did not show fever, and the increase in CRP was insignificant or even absent due to IL-6 inhibition and suppression of the inflammatory response.[19] Reactivation of tuberculosis is also a challenge, especially in developing countries where the burden of tuberculosis infection is still high.[15] A prophylactic strategy is probably needed to diminish the risk of reactivation of infection in people with tuberculosis or who are Hepatitis B carriers.[23-24]

Although TCZ is only used in the acute phase of COVID-19 cases, unlike the long term of TCZ use for other indications, adverse effects remain a matter of concern. As has been described in this case report, the patient received TCZ at a dose of 8 mg/kg of body weight intravenously in two divided doses and the adverse effect appeared ten days later even though previous history of herpes zoster was denied.

In general, Tocilizumab is well tolerated, and most adverse effects are usually mild or moderate, temporary in nature, and does not lead to discontinuation of therapy. [16,18-19]

CONCLUSION

Tocilizumab use seems to be effective in mitigating cytokine storm associated with severe COVID-19 infection. It can be concluded that the possibility of serious adverse effects, though rare, cannot be excluded. The presence of a latent or chronic infection that can undergo reactivation

should be a consideration for appropriate screening or prophylaxis before administering TCZ. COVID-19 patients with severe and critical symptoms are by themselves more susceptible to infection. The ideal goals of therapy are always to save lives, prevent life-threatening inflammation, and minimize possible adverse effects.

REFERENCES

1. Chen X, Zhao B, Qu Y, et al. Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill patients with COVID-19. Clin Infect Dis [Internet]. 2020;71(8):1937-1942. Available from: https://doi.org/10.1093/cid/ciaa449.
2. Sheppard M, Laskou F, Stapleton PP, Hadavi S, Dasgupta B. Tocilizumab (Actemra). Human Vaccines & Immunotherapeutics [Internet]. 2017, 13(9), 1972-1988. Available from: https://doi.org/10.1080/21645515.2017.1316909.
3. Gatti M, Fusaroli M, Caraceni P, Poluzzi E, Ponti FD, Raschi E. Serious adverse events with tocilizumab: Pharmacovigilance as an aid to prioritize monitoring in COVID-19. Br J Clin Pharmacol [Internet]. 2020;1–8. Available from: https://doi.org/10.1111/bcp.14459.
4. Kementerian Kesehatan RI. Pedoman Pencegahan dan Pengendalian Coronavirus Disease (COVID-19). Juli 2020.
5. Perhimpunan Dokter Paru Indonesia. Panduan Praktik Klinik (PPK) Tatalaksana Pasien COVID-19. April 2020.
6. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents [Internet]. 2020;55(5):105954. Available from: http://www.sciencedirect.com/science/article/pii/S0924857920301047.
7. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet [Internet]. 2020; 395: 497–506. Available from: https://doi.org/10.1016/S0140-6736(20)30183-5.
8. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet [Internet]. 2020;395(10223):507-513. Available from: https://doi.org/10.1016/S0140-6736(20)30211-7.
9. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol [Internet]. 2020;92(7):814–8. Available from: https://doi.org/10.1002/jmv.25801.
10. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A [Internet]. 2020;117(20):10970–5. Available from: https://doi.org/10.1073/pnas.2005615117.
11. Sciascia S, Apra F, Baffa A, et al. Pilot prospective open, single-arm multicentre study on off-label use of tocilizumab in patients with severe COVID-19. Clin Exp Rheumatol [Internet]. 2020;38(3):529–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/32359035.
12. Keske S, Tekin S, Sait B, et al. Appropriate use of tocilizumab in COVID-19 infection. Int J Infect Dis [Internet]. 2020;99:338–43. Available from: http://www.sciencedirect.com/science/article/pii/S1201971220305804.
13. Boregowda U, Perisetti A, Nanjappa A, Gajendran M, Kutti Sridharan G, Goyal H. Addition of Tocilizumab to the standard of care reduces mortality in severe COVID-19: A systematic review and meta-analysis. Front Med [Internet]. 2020;7:586221. Available from: https://pubmed.ncbi.nlm.nih.gov/33123544.
14. Kaye AG, Siegel R. The efficacy of IL-6 inhibitor Tocilizumab in reducing severe COVID-19 mortality: a systematic review. PeerJ [Internet]. 2020 Nov 2;8:e10322–e10322. Available from: https://pubmed.ncbi.nlm.nih.gov/33194450.
15. Dastan F, Saffaei A, Haseli S, et al. Promising effects of tocilizumab in COVID-19: A non-controlled, prospective clinical trial. Int Immunopharmacol [Internet]. 2020 Nov;88:106869. Available from: https://doi.org/10.1016/j.intimp.2020.106869.
16. Higuchi T, Nakanishi T, Takada K, et al. A case of multicentric Castleman's disease having lung lesion successfully treated with humanized anti-interleukin-6 receptor antibody, tocilizumab. J Korean Med Sci [Internet]. 2010;25(9):1364–7. Available from: https://doi.org/10.3346/jkms.2010.25.9.1364.
17. Khiali S, Khani E, Entezari-Maleki T. A Comprehensive Review of Tocilizumab in COVID-19 Acute Respiratory Distress Syndrome. J Clin Pharmacol [Internet]. 2020/07/27. 2020;60(9):1131–46. Available from: https://pubmed.ncbi.nlm.nih.gov/32557541.
18. Jones G, Sebba A, Gu J, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. Ann Rheum Dis [Internet]. 2010;69(1):88–96. Available from: https://doi.org/10.1136/ard.2008.105197.
19. Alexeeva EI, Baranov AA, Denisova RV, et al. Retrospective analysis of efficacy and safety of Tocilizumab treatment in patients with severe systemic-onset juvenile idiopathic arthritis followed for 12 months. Hellstrom I, Boon A, Kapsogeorgou EK, editors. ISRN Immunol [Internet]. 2013;2013:548312. Available from: https://doi.org/10.1155/2013/548312.
20. Alattar R, Ibrahim TBH, Shaar SH, et al. Tocilizumab for the treatment of severe coronavirus disease 2019. J Med Virol [Internet]. 2020;1–8. Available from: https://doi.org/10.1002/jmv.25964.

21. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol [Internet]. 2020;2(8):e474–84. Available from: https://doi.org/10.1016/S2665-9913(20)30173-9.
22. Cantini F, Nannini C, Niccoli L, Petrone L, Ippolito G, Goletti D. Risk of Tuberculosis reactivation in patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis receiving non-anti-TNF-targeted biologics. Mediators Inflamm [Internet]. 2017;2017:8909834. Available from: https://doi.org/10.1155/2017/8909834.
23. Lin CT, Huang WN, Hsieh CW, et al. Safety and effectiveness of Tocilizumab in treating patients with rheumatoid arthritis – A three-year study in Taiwan. J Microbiol Immunol Infect [Internet]. 2019 Feb;52(1):141-150. Available from: https://doi.org/10.1016/j.jmii.2017.04.002.
24. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med [Internet]. 2020;8(5):475–81. Available from: http://www.sciencedirect.com/science/article/pii/S2213260020300795.

Peripancreatic Tuberculosis Lymphadenopathy: The Role of Endoscopic Ultrasound for Diagnosis

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ABSTRACT

Pancreatic and peripancreatic tuberculosis is a rare abdominal tuberculosis. Diagnosis for pancreatic tuberculosis can be challenging. Conventional imaging tools may show mass or malignancy in the pancreas. Endoscopic ultrasound (EUS) is an excellent tools for evaluating pancreas and peri pancreas region. It also allows us to obtain tissue sample for cytology and histopathology. Here we present a case of peripancreatic tuberculosis lymphadenopathy that mimic pancreatic mass. His symptoms were also nonspecific (weight loss, epigastric pain, and irregular fever). From EUS evaluation we found that there was no mass but multiple lymphadenopathy around the pancreas and then performed FNA. The result of the cytology was granuloma inflammation and caseous necrosis which is compatible with tuberculosis infection. From this case illustration we conclude that EUS is an important diagnostic tool for pancreatic lesion to avoid unnecessary surgery.

Keywords**:** *Pancreas; tuberculosis; lymphadenopathy; endoscopic ultrasound*

INTRODUCTION

Tuberculosis is still endemic in Indonesia but abdominal tuberculosis especially pancreatic and peripancreatic tuberculosis is a very rare disease.[1] Pancreatic and peripancreatic tuberculosis is difficult to diagnose. It can mimic mass or other malignancy in the pancreas. Patient usually come to doctor with symptoms of gastrointestinal malignancy (e.g. weight loss, loss of appetite and chronic abdominal pain) and the imaging are often non-conclusive. Patient usually diagnosed with pancreatic tuberculosis after surgical resection. Endoscopic ultrasound (EUS) is non-invasive tool that allow us to evaluate more detail in the pancreas and surrounding tissue. We can also obtain tissue sample for cytology or histopathology diagnosis using EUS.

CASE ILLUSTRATION

Male, 54 years old referred to our hospital with pancreatic head mass. Patient complains persistent epigastric pain for 3 months before admission. Patient also have nausea, sometime vomiting and 10 kg of weight loss in 3 months. Since 1-month patient also have irregular fever. Patient was admitted to other hospital and was performed EGD and MRCP. The result of EGD was gastritis. The MRCP from other hospital result was pancreatic head mass without dilatation of bile duct and pancreatic duct.

On physical examination we found the patient was alert. Patient appearance was cachectic. Hemodynamic was stable. Temperature was 37°C. There were rales at the base of the lungs. Heart sounds were normal. Abdominal

examination we found no abdominal tenderness, no mass, normal liver and spleen.

From laboratory examination we found microcytic hypochromic anemia (Hb: 9.36). Tumor markers were not elevated (CEA:0.72, Ca-19-9:12.9, AFP:0.5).

We perform abdominal CT in our hospital. The result was multiple lesion in the pancreas with multiple lymphadenopathy at the aorta at the level of renal artery, celiac trunk, common hepatic artery and splenic artery suspected malignancy.

Endoscopic ultrasound (EUS) with linear endoscope was performed after we obtain abdominal CT. From EUS we found no mass at the head or the body of pancreas, no dilatation of CBD and PD. There was multiple lymph node enlargement from the hillus of spleen, tail and body of pancreas. We performed fine needle aspiration with 22-gauge FNA needle.

The result of the cytopathology from the lymph nodes was chronic granulomatous inflammation with caseating necrosis suggestive for tuberculosis infection.

We treat the patient with anti-tuberculosis drugs for 9 months. After 1 month of anti-tuberculosis therapy, patient was feeling well. He has weight gain 2 kg after 1 month and increasing appetite.

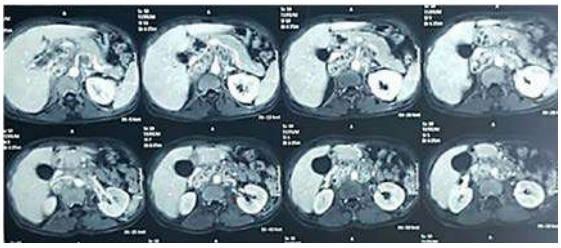

Figure 1. MRCP image showed pancreatic head mass (4x5x6 cm) suspected malignant lesion

Figure 2. Multiple lymph node enlargement peri pancreas was seen and no mass was found from EUS (linear echoendoscope). Fine needle aspiration was performed.

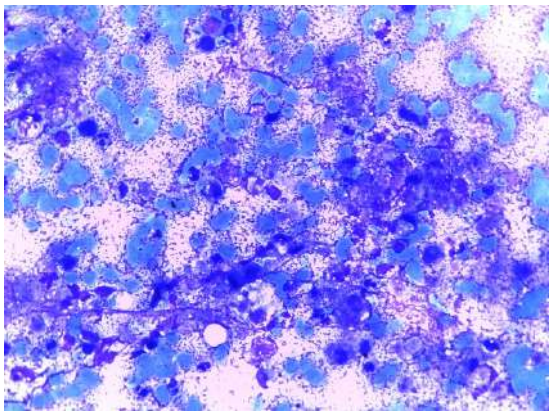

Figure 3. Caseating necrosis was found on cytopathology (Giemsa staining 400x)

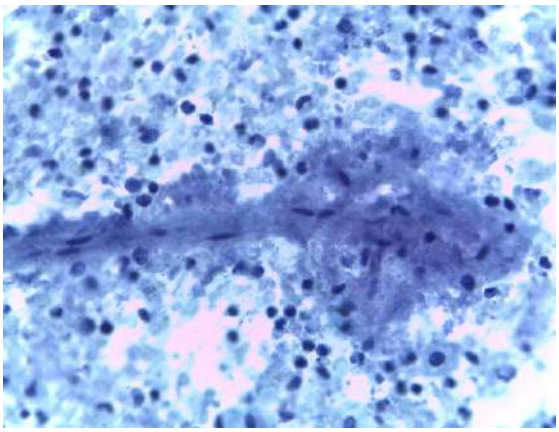

Figure 4. Granuloma inflammation (PAP smear 400x)

DISCUSSION

All abdominal organ can be infected by *M. tuberculosis*. Most gastrointestinal tuberculosis involves the ileocecal and intestinal segment. Pancreas is one of the rare organ that can be infected by tuberculosis. Study by Bhansali from 300 confirmed surgical cases of abdominal tuberculosis found no pancreatic tuberculosis.[1] Most reports for pancreatic and peri-pancreatic tuberculosis are case reports and case series.[2-4] The transmission route of *M.tuberculosis* to pancreas is not fully understood. Hematogenous, lymphatic dissemination or direct spread from another location is the possible mechanism.

There are no specific clinical symptoms for pancreatic or peri pancreatic tuberculosis. Study from Rao et al described that the most common symptom from 14 patients with pancreatic tuberculosis was abdominal pain localized to the epigastrium, fever and weight loss.[3] Data

from Song et al also found abdominal pain/ discomfort and weight loss are the most common symptoms.[5] This patient came to hospital with chronic epigastric pain, weight loss and with sub febrile temperature which also can be found on malignancy.

Imaging evaluation for this patient which is MRCP and abdominal CT showed a possible mass or malignancy in the pancreas. From previous studies showed that 50-75% patient with pancreatic tuberculosis have imaging result of pancreatic mass or malignancy.[2-4] A differential diagnosis off malignant lymphoma lymphadenopathies must also be considered from imaging.

EUS is an excellent tool for evaluating the pancreas and surrounding tissue. EUS is more effective for differentiating malignant or non-malignant lesions in pancreas compared to CT or ultrasound.[6] Recent meta-analysis from Best et al from 54 studies involving 31.196 subjects found EUS-FNA has sensitivity of 0.73(95% CI 0.01 -1.00) and specificity of 0.94(95% CI 0.15-1.00) for differentiating cancerous or precancerous versus benign lesions.[7] Study from Song et al showed that EUS-FNA can accurately diagnose pancreas or peri-pancreatic tuberculosis in 76.2% patient and consequently avoid unnecessary surgery.[5] Using linear echoendoscope with needle also allows us to obtain tissue (fine needle aspiration (FNA) or fine needle biopsy (FNB)). We performed linear EUS for this patient and it became more apparent that what the other imaging see as a mass was actually multiple lymphadenopathy around pancreas that can mimic pancreatic mass.

Diagnosis of pancreatic or peripancreatic tuberculosis can be achieved using FNA or FNB. Tuberculous granuloma in the histology result is the most important finding for diagnosis especially in endemic area. Aljafri et al reported that the specificity of histology diagnosis for tuberculosis in non-endemic area is 88-100%.[8] If the result of cytopathology or histopathology is not specific, *Ziehl-Neelson* staining, culture of *M tuberculosis,* or PCR obtain from FNA can also help for diagnosis. From this case we found granulomatous inflammation with caseating necrosis which highly specific for tuberculosis especially in endemic region.[9]

CONCLUSION

From this case report we conclude that abdominal tuberculosis is one important deferential diagnosis in gastroenterological diseases especially in Indonesia. Combining clinical, laboratory, standard imaging (CT or MRI) and EUS-FNA is essential for diagnosing pancreatic lesions (benign or malignant) to avoid unnecessary surgery.

REFERENCES

1. Bhansali SK. Abdominal tuberculosis. Experiences with 300 cases. Am J Gastroenterol. 1977;67(4):324-37.
2. Franco-Paredes C, Leonard M, Jurado R, Blumberg HM, Smith RM. Tuberculosis of the pancreas: report of two cases and review of the literature. Am J Med Sci. 2002;323(1):54-8.
3. Rao RN, Pandey R, Rana MK, Rai P, Gupta A. Pancreatic and peripancreatic tuberculosis presenting as hypoechoic mass and malignancy diagnosed by ultrasound-guided fine-needle aspiration cytology. Journal of Cytology / Indian Academy of Cytologists. 2013;30(2):130-5.
4. Chen CH, Yang CC, Yeh YH, Yang JC, Chou DA. Pancreatic tuberculosis with obstructive jaundice--a case report. Am J Gastroenterol. 1999;94(9):2534-6.
5. Song TJ, Lee SS, Park DH, et al. Yield of EUS-guided FNA on the diagnosis of pancreatic/peripancreatic tuberculosis. Gastrointestinal Endoscopy. 2009;69(3 Pt 1):484-91.
6. Chang KJ, Nguyen P, Erickson RA, Durbin TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. Gastrointestinal Endoscopy. 1997;45(5):387-93.
7. Best LM, Rawji V, Pereira SP, Davidson BR, Gurusamy KS. Imaging modalities for characterising focal pancreatic lesions. Cochrane Database Syst Rev. 2017;4:Cd010213.
8. Aljafari AS, Khalil EA, Elsiddig KE, et al. Diagnosis of tuberculous lymphadenitis by FNAC, microbiological methods and PCR: a comparative study. Cytopathol. 2004;15(1):44-8.
9. Majeed MM, Bukhari MH. Evaluation for granulomatous inflammation on fine needle aspiration cytology using special stains. Pathol Res Int. 2011;2011.

The role of SpyGlass Direct Visualization System on Patient with Indeterminate biliary strictures: A case report

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ABSTRACT

Biliary strictures diagnosis has become a challenge where benign conditions could mimic a malignant process. Recently, SpyGlass DS overcame the limitations by allowing direct visualization of the biliary tree. A 65 years old Indian patient complaints of jaundice with total and direct bilirubin of 23.3 mg/dL and 16.2 mg/dL, respectively. Liver function test, gamma-glutamyltransferase and CA 19-9 were increased. Transabdominal ultrasound and abdominal CT supported dilatation of common bile duct (CBD) with abrupt narrowing showing periductal enhancement at supra pancreatic level and stricture. Endoscopic ultrasound showed intrahepatic CBD stricture with dilated proximal CBD and sludge ball. Endoscopic retrograde cholangiopancreatography showed mid CBD stricture. Although brush cytology results suggested low grade dysplasia and no definite evidence of malignancy, cholangioscopy using SpyGlass DS found nodularity with abnormal vascularity seen in mid of CBD suggesting malignancy, confirmed with histopathology as cholangiocarcinoma. We reported additional value of SpyGlass DS for detecting cholangiocarcinoma in an indeterminate biliary stricture patient.

Key words**:** *Biliary stricture, cancer, SpyGlass DS, cholangiocarcinoma.*

INTRODUCTION

Diagnosis of biliary strictures became a challenge due to the number of indeterminate biliary strictures after preoperative evaluation being up to 20%, thus definitive diagnosis demands further evaluation through surgical resection and pathologic examination.[1] Moreover, some benign conditions could mimic a malignant process, for example, post-inflammatory strictures and primary sclerosing cholangitis.[2] Iatrogenic injury, and post liver transplantation, post cholecystectomy are the most common cause of benign biliary strictures, whilst pancreatic cancer and cholangiocarcinoma are foremost sources of malignancy.[3] In general, around 13-24% of biliary strictures patients referred for surgery are ultimately found to be benign[4] and surgical resection proved that 20% of the suspected malignant strictures were actually benign conditions.[5] Excessive surgery for benign biliary disease should be avoided but delayed treatment of malignancy was also harmful for the patient. Therefore, preoperative evaluation plays an essential role to exclude malignancy.

Single ideal test regarding sensitivity, specificity and accuracy have not existed, despite

the variability of tests available recently. Several methods have been used as a diagnostic tool in the evaluation of biliary tree strictures, such as percutaneous trans-hepatic cholangiography or bile aspiration during endoscopic retrograde cholangiopancreatography (ERCP). Later, development of biliary tract brush cytology method has been commonly applied and became the method of choice. Brush cytology used air-dried cytologic material for Diff-Quick stain or fixed in ethanol for Papanicolaou staining. Recently, commercial SpyGlass direct visualization system from Boston Scientific Corporation (Marlborough, Massachusetts, United States) was available to overcome the limitations of previous methods. The system directly visualizes the biliary tree for diagnostic and therapeutic purposes by using a disposable digital scope with 120 degrees field of view. A tapered tip, a four-way tip deflection system, and a dedicated channel for water irrigation was featured on the scope, which enable unrestrained observation and biopsy procedures.[6] Direct visualization of the lesion with improved image quality and the ability to take targeted biopsies could be achieved by digital cholangioscopy. In malignancy, the findings obtained dilated of tumor vessels, tortuous vessels, infiltrative stricture, irregular margins with partial occlusion of the lumen, irregular surface, and easy oozing.[7] We reported a case in an Indian patient with indeterminate biliary strictures who underwent ERCP and SpyGlass DS examination.

CASE ILLUSTRATION

A 65 years old male, with Indian ethnicity was admitted to the hospital for jaundice 1 week ago. He also complaints of decreased appetite and generalized weakness. Past illness showed a history of chronic liver disease associated with chronic ethanol intake and cholecystectomy surgery. Physical examinations revealed a yellowish pigmentation of the sclera. The thorax was normal, abdominal was soft, non-tender with no ascites. The skin was turning yellow.

His routine blood investigations showed normal hemogram. Total, direct and indirect bilirubin were 23.3 mg/dL, 16.2 mg/dL and 7.1 mg/dL, respectively. The biochemical analysis revealed SGPT was 120 U/L, SGOT 127 U/L with increased alkaline phosphatase level (270 U/L). Total protein, albumin, globulin and renal function test were at normal limits. Hepatitis and acquired immunodeficiency syndrome (AIDS) viral markers were negative, alpha-fetoprotein of 4.5 ng/ml, gamma-glutamyltransferase of 986 U/L and CA 19-9 of 119.1 U/mL.

Transabdominal ultrasound showed post cholecystectomy state, increased and coarse echotexture of liver with minimal irregular contour suggested early change of hepatic parenchymal disease, common bile duct (CBD) and pancreatic duct were dilated (9.2 mm and 4 mm, respectively) with marginal splenomegaly. Triphasic abdominal CT showed atrophied left lobe with hypertrophy of caudate lobe and prominent fissures of liver with splenomegaly, suggesting chronic liver disease. Dilated CBD (1.54 cm) with abrupt narrowing showing periductal enhancement at supra pancreatic level and stricture.

Upper GI Endoscopy showed antral gastritis with superficial duodenal ulcers. Endoscopic ultrasound showed intrahepatic CBD stricture with dilated proximal CBD and sludge ball. ERCP showed mid CBD stricture, brush cytology was performed distal of CBD stricture with a CBD stent placed. Post procedure, patient was stable without any complications. He was treated with intravenous fluids, proton pump inhibitor and other supportive treatments. He was discharged in stable condition and advised to be in close follow up.

One week later, brush cytology result showed most of the cells are benign with round to oval nucleus and moderate amount of cytoplasm. Results suggest low grade dysplasia and no definite evidence of malignancy, suggesting repeat for definitive evaluation. ERCP was performed followed by cholangioscopy using SpyGlass DS (Figure 1). We found nodularity with abnormal vascularity seen in mid of CBD suggesting malignancy, and endobiliary biopsy was taken. CBD stent was placed.

Histopathology reported dysplastic cells with vesicular to hyperchromatic nucleus with inconspicuous nucleoli and moderate eosinophilic cytoplasm, and moderate nuclear

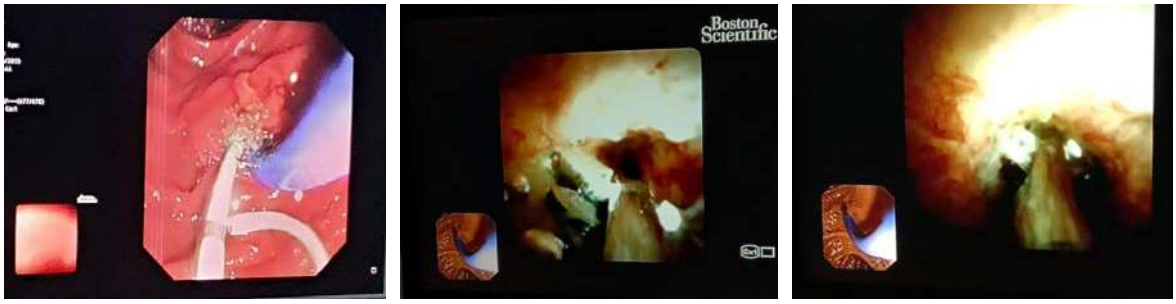

Figure 1. Cholangioscopy using SpyGlass DS showed nodularity with abnormal vascularity suggesting malignancy.

atypia suggesting positive for malignancy-cholangiocarcinoma. We plan further treatment for cholangiocarcinoma. Nature of underlying disease, long term prognosis and its complications has been explained in detail to patient and his attendants.

DISCUSSION

When the basic work-up (transabdominal imaging, endoscopic retrograde cholangiopancreatography and routine cytologic brushing) did not give diagnostic result, it is considered as an indeterminate biliary stricture.[8] We report an indeterminate biliary stricture patient and was diagnosed with cholangiocarcinoma using SpyGlass DS, previously detected as low-grade dysplasia by brush cytology. Although brush cytology of pancreatobiliary strictures has been the most common technique in the carcinoma diagnosis, the sensitivity was low from 48-68%.[9, 10] In contrast, a higher accuracy was observed in several studies used cholangioscopy. Using SpyGlass, a study in the United States reported sensitivity 97%, specificity 96%, positive predictive value 94%, and negative predictive value 98% for patients with indeterminate stricture.[11] The retrospective multicenter study also indicated a high sensitivity of 85% in 44 patients with indeterminate biliary strictures.[12] Asia-Pacific Expert Consensus suggested to use cholangioscopy in patients with indeterminate biliary strictures with Grade A recommendation.[13]

The presence of tumor vessels, indicative of malignancy are highly specific due to the different morphology with the normal vessels of the biliary mucosa that could be detected by experienced cholangioscopists.[14] Based on cholangioscopy findings, bile duct adenocarcinoma can be classified into nodular, papillary and infiltrative. A study reported that in 61% of patients with biliary malignancy, irregularly dilated and tortuous vessels was found but none were detected in benign biliary strictures cases.[15] Furthermore by using biopsy to confirm the malignancy, sensitivity was 80% and specificity was 100%. Thus, cholangioscopy finding is considered to be a general endoscopic marker for biliary malignancy. In contrast, a cholangioscopy biopsy as supplementary of tumor vessel observation might improve the ability to differentiate between benign and malignant biliary stricture. Although study found that tumor vessel ability to confirm malignancy only 61% for sensitivity and 100% for specificity, when tumor vessels and biopsy was combined, the sensitivity and specificity was improved to 96% and 100%, respectively.[15] Further, some studies suggested the on-site pathological evaluation for increasing the diagnostic yield of specimens.[12] Rapid on-site evaluation touch imprint cytology to cholangioscopy improve the sensitivity to 100%, specificity to 88.9%, with 86.7% of positive predictive value, 100% of negative predictive value, and 93.5% for the diagnostic accuracy.[16] Another issue is about biopsy size. The relatively low sensitivity of histology diagnosis was due to several limitations; inadequate number of obtained specimens, small size of the specimens as the result of SpyBite design and the absence of on-site cytology evaluation.[17]

Comprehensive evaluation on the patient's history is helpful to distinguish benign or malignant biliary strictures. A history of liver transplant, AIDS and cholecystectomy are the

frequent causes of benign biliary stricture. Careful observation on the clinical presentation is important because the patients may appear asymptomatic, but may also show abnormal liver test, pruritus, jaundice, right upper quadrant and fever (in several cases).[18] Weight loss and extensive fatigue symptoms require special attention because it may suggest a malignancy.

Transabdominal ultrasound examination provides low reliability of the distal part of the common bile duct due to the bowel gas interference. Meanwhile, abdominal CT may also useful but the sensitivity for early tumor was low. Magnetic resonance cholangiopancreatography ability to differentiate benign or malignant stricture was also problematic, even though it was relatively safe. Cholangiopancreatography procedure by magnetic resonance does not require injection of contrast, thus decreased the risk of cholangitis.[8] EUS-FNA was considered as a reliable option for vessels, perihilar lymph nodes, extrahepatic biliary tree and hilar masses, if the ERCP finding was inconclusive.[5] Serology diagnosis are also still controversial. Only 40% of the patients have elevated gamma glutamyltransferase and serum alkaline phosphatase and 40% of them are non-icteric.[19] As reported in a meta-analysis including 348 patients, another cancer marker CA 19-9 only yields has 69% sensitivity.[20] Importantly, increased serum CA 19-9 levels also occurred in benign cases including cirrhosis, cholestasis and cholangitis. Therefore, for cholangiocarcinoma diagnostic purpose, it is not recommended to use CA 19-9 as a single diagnostic tool but it could be combined with other tools to improve the reliability.[5] In general, ideal clinical, serological marker or radiological features was not available to distinguish benign and malignant biliary strictures accurately.

Several problems associated with cholangioscopy warrant precaution. Although it has detected similar complication rates of pancreatitis and perforation, cholangioscopy showed significantly higher cholangitis rate compared to ERCP (1% vs 0.2%).[21] The additional training required, cost and time consumed, limited area of working channel and inadequate image quality should also be noted.[3] The interobserver agreement was slight to poor,[22] suggesting validation and standardization of criteria for visual interpretation to improve the diagnosis of indeterminate biliary stricture.

CONCLUSION

We observed an additional value of SpyGlass DS for detecting cholangiocarcinoma in an indeterminate biliary stricture patient.

REFERENCES

1. Bowlus CL, Olson KA, Gershwin ME. Evaluation of indeterminate biliary strictures. Nat Rev Gastroenterol Hepatol. 2017;14(12):749.
2. Nguyen Canh H, Harada K. Adult bile duct strictures: differentiating benign biliary stenosis from cholangiocarcinoma. Med Mol Morphol. 2016;49(4):189-202.
3. Singh A, Gelrud A, Agarwal B. Biliary strictures: diagnostic considerations and approach. Gastroenterol Rep (Oxf). 2015;3(1):22-31.
4. Baillie J. Distinguishing malignant from benign biliary strictures: can confocal laser endomicroscopy close the gap? Gastrointest Endosc. 2015;81(2):291-293.
5. Xie C, Aloreidi K, Patel B, et al. Indeterminate biliary strictures: a simplified approach. Expert Rev Gastroenterol Hepatol. 2018;12(2):189-199.
6. Committee AT, Komanduri S, Thosani N, et al. Cholangiopancreatoscopy. Gastrointest Endosc. 2016;84(2):209-221.
7. Monga A, Ramchandani M, Reddy DN. Per-oral cholangioscopy. J Interv Gastroenterol. 2011;1(2):70-77.
8. Victor DW, Sherman S, Karakan T, Khashab MA. Current endoscopic approach to indeterminate biliary strictures. World J Gastroenterol. 2012;18(43):6197-6205.
9. Govil H, Reddy V, Kluskens L, et al. Brush cytology of the biliary tract: retrospective study of 278 cases with histopathologic correlation. Diagn Cytopathol. 2002;26(5):273-277.
10. Navaneethan U, Njei B, Lourdusamy V, Konjeti R, Vargo JJ, Parsi MA. Comparative effectiveness of biliary brush cytology and intraductal biopsy for detection of malignant biliary strictures: a systematic review and meta-analysis. Gastrointest Endosc. 2015;81(1):168-176.
11. Shah RJ, Raijman I, Brauer B, Gumustop B, Pleskow DK. Performance of a fully disposable, digital, single-operator cholangiopancreatoscope. Endoscopy. 2017;49(7):651-658.
12. Navaneethan U, Hasan MK, Kommaraju K, et al. Digital, single-operator cholangiopancreatoscopy in the diagnosis and management of pancreatobiliary disorders: a multicenter clinical experience (with

video). Gastrointest Endosc. 2016;84(4):649-655.
13. Hu B, Sun B, Cai Q, et al. Asia-Pacific consensus guidelines for endoscopic management of benign biliary strictures. Gastrointest Endosc. 2017;86(1):44-58.
14. Karagyozov P, Boeva I, Tishkov I. Role of digital single-operator cholangioscopy in the diagnosis and treatment of biliary disorders. World J Gastrointest Endosc. 2019;11(1):31-40.
15. Kim HJ, Kim MH, Lee SK, Yoo KS, Seo DW, Min YI. Tumor vessel: a valuable cholangioscopic clue of malignant biliary stricture. Gastrointest Endosc. 2000;52(5):635-638.
16. Varadarajulu S, Bang JY, Hasan MK, Navaneethan U, Hawes R, Hebert-Magee S. Improving the diagnostic yield of single-operator cholangioscopy-guided biopsy of indeterminate biliary strictures: ROSE to the rescue? (with video). Gastrointest Endosc. 2016;84(4):681-687.
17. Turowski F, Hugle U, Dormann A, et al. Diagnostic and therapeutic single-operator cholangiopancreatoscopy with SpyGlassDS: results of a multicenter retrospective cohort study. Surg Endosc. Sep 2018;32(9):3981-3988.
18. Altman A, Zangan SM. Benign Biliary Strictures. Semin Intervent Radiol. 2016;33(4):297-306.
19. Mizumoto R, Ogura Y, Kusuda T. Definition and diagnosis of early cancer of the biliary tract. Hepatogastroenterology. 1993;40(1):69-77.
20. Burnett AS, Bailey J, Oliver JB, Ahlawat S, Chokshi RJ. Sensitivity of alternative testing for pancreaticobiliary cancer: a 10-y review of the literature. J Surg Res. 2014;190(2):535-547.
21. Sethi A, Chen YK, Austin GL, et al. ERCP with cholangiopancreatoscopy may be associated with higher rates of complications than ERCP alone: a single-center experience. Gastrointest Endosc. 2011;73(2):251-256.
22. Sethi A, Doukides T, Sejpal DV, et al. Interobserver agreement for single operator choledochoscopy imaging: can we do better? Diagn Ther Endosc. 2014;2014:730731.

Pancreas Divisum as a Rare Etiology of Recurrent Pancreatitis: A Rare Case Ever Documented and Reported In Indonesia

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ABSTRACT

Pancreas divisum is an abnormal condition of pancreas duct that occurs from organogenesis. This abnormal condition defined as a failure in fusion between dorsal and ventral part of the pancreas. The incidence reported 4%-14% in general population. Majority patient with pancreas divisum will not present with any sign or symptom, but in some cases may present with signs of pancreatitis. We illustrate a case of 39 years old male with pancreas divisum presenting as acute recurrent pancreatitis. Diagnosis of pancreas divisum was determined through magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP). Patient treated by sphincterotomy and dilation using Soehendra's dilator catheter. This is the first pancreas divisum case successfully treated and reported in Indonesia.

Keywords: *ERCP, pancreas divisum, pancreatitis, sphincterotomy.*

INTRODUCTION

Pancreas divisum is the most common congenital anomaly of pancreas that can be found in around 4%-14% of the population by cadaveric examination.[1] In Asia, this congenital anomaly was reported in 0.5%-3.7% patients who underwent endoscopy.[2] Pancreas divisum is defined as an abnormal condition caused by failure of fusion between two embryonic parts of the pancreas. In normal development, ventral bud of pancreas will rotate around foregut and fuse with dorsal bud of pancreas during the seventh gestational week. This fusion allows the ventral duct and dorsal duct to form the main pancreatic duct or Wirsung duct and later will join with the common bile duct and drains into the major papilla, which in normal pancreas drains through this duct. The remaining dorsal duct known as Santorini duct, will drain through minor papilla. In pancreas divisum, ventral and dorsal buds fail to fuse and form anomaly that are classified into three major types. Type I (classic pancreatic divisum) is a complete failure of fusion identified by non-union of ventral and dorsal ducts. Type II pancreatic divisum is characterized by the absence of ventral duct with dominant dorsal drainage. Type III or incomplete pancreas divisum described as a presence of rudimentary communication between dorsal duct and ventral ducts.[3] This is a case report of pancreas divisum which presents with recurrent pancreatitis.

CASE ILLUSTRATION

A 39 years old male presents with abdominal pain referred by a secondary healthcare facility. He had been diagnosed with pancreatitis twice within the last 1 year prior to his current admission and has undergone magnetic resonance

cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP). His pancreatic enzymes were almost 3000 IU/mL and imaging findings from ERCP and MRCP (**Figure 1**) demonstrated dilatation of dorsal duct that drains to the minor papilla, suggestive of complete pancreas divisum, which unfortunately was not diagnosed by doctors in the secondary healthcare facility. ERCP was done, showing a stenosis in the minor papilla. Treatment is directed towards relieving outflow obstruction at the level of the minor papilla by sphincterotomy and dilation using Soehendra's dilator catheter with successful results. After several days of follow up, his condition improved, the pancreatic enzymes were back to normal levels and the patient was discharged from the hospital without abdominal pain.

DISCUSSION

Pancreatic divisum is a congenital anatomical anomaly of the pancreas that occurs during the seventh-eighth week of fetal development. Normally, the dorsal pancreatic section will join the ventral pancreatic section to form the main pancreatic duct, then merging with the common bile duct at the hepatopancreatic ampulla and drain into the major duodenal papilla. In this case, we found that the dorsal duct mainly drains to the minor papilla and there is no sign of ventral duct or remnant communication. This type of pancreas divisum (Type II) comprise about 34.4%-37% of pancreas divisum cases. The most common type is the classic pancreas divisum (Type I) which identified in 44.4%-47.8% of pancreas divisum cases.[4,5]

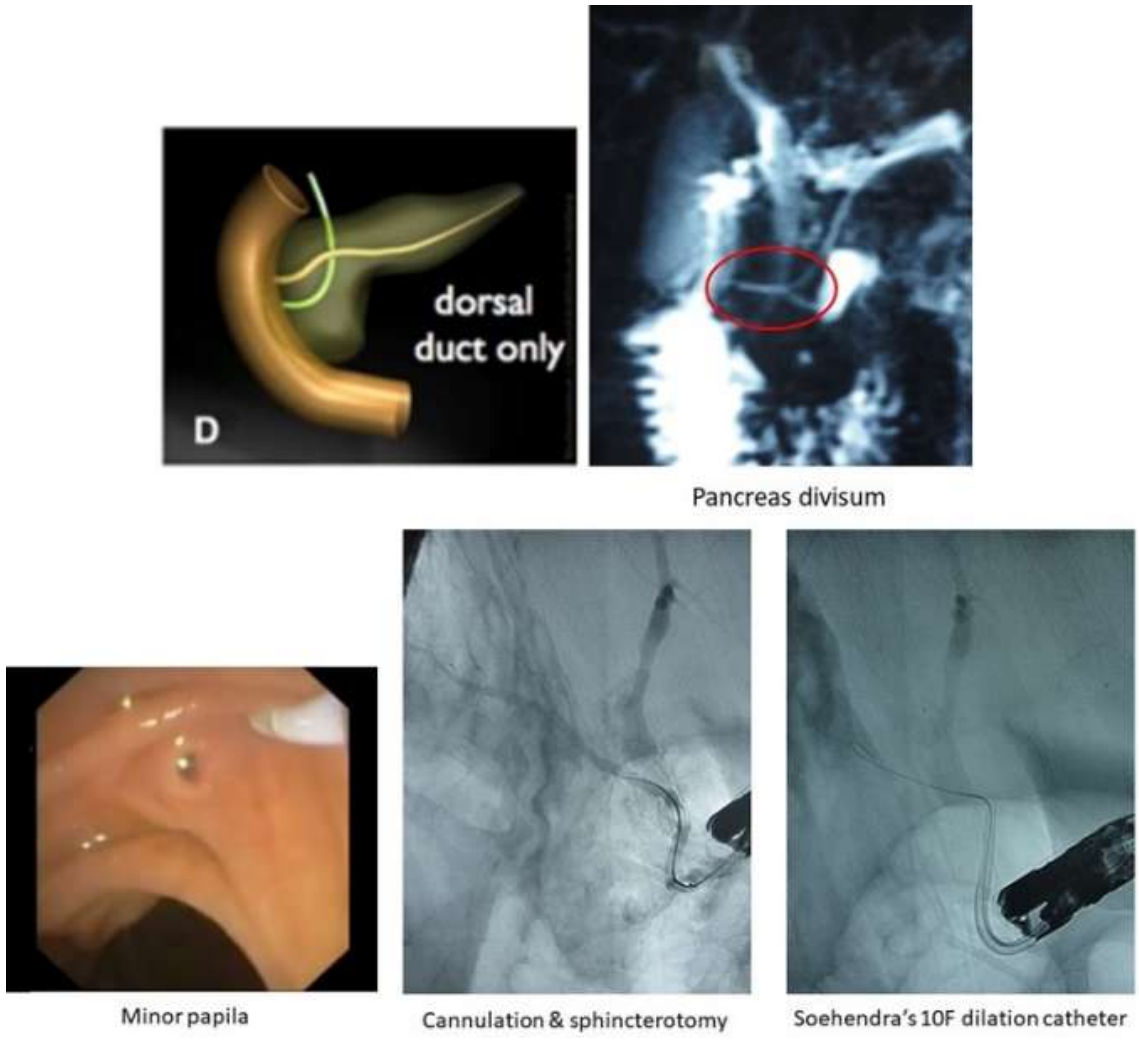

Figure 1. MRCP and ERCP images.

Stenosis at the minor papilla in this patient may cause recurrent pancreatitis. In general case of pancreas divisum, they are commonly asymptomatic. Acute pancreatitis is defined as presence of two out of three criterion: abdominal pain, elevated pancreatic enzyme level, and sign of inflammation from imaging.[6] A study reported cases of pancreas divisum were more associated with acute recurrent pancreatitis, found in 55.26% patients, compared to acute and chronic pancreatitis.[7] Another study also showed evidence that a case of pancreas divisum with recurrent pancreatitis could progress to chronic pancreatitis, which can be identified by calcification findings on imaging.[6] In this case, the patient only presented with sign of recurrent acute pancreatitis with no other significant findings.

Definitive diagnosis is made with either ERCP or MRCP. In routine settings, ERCP is considered to be reliable modality to diagnose pancreas divisum. However, ERCP is an invasive procedure which has higher possibility of complication. MRCP is a less invasive procedure which can evaluate biliary and pancreatic duct by actual visualization. S-MRCP is an advanced method by adding secretin to increasing ductal volume and secretion from exocrine pancreas in addition to improving the visualization. S-MRCP has been reported have better specificity in diagnosing pancreas divisum compares to MRCP. The specificity and sensitivity between ERCP and S-MRCP have been shown to have no significant difference in diagnosing pancreas divisum. Although ERCP still remains as gold standard for diagnostic tools in pancreas divisum, MRCP can be used as an alternative for patient who cannot undergo ERCP.[7,8]

The aim of the treatment in this case is to increase pancreas drainage through minor papilla by removing the obstruction. The option of treatment for symptomatic pancreatic divisum is a sphincterotomy of the minor duodenal papilla with or without dilatation. Studies show the successful rate of endotherapy reported more than 70%.[9–11] A study shows the response rate for all patient who underwent endoscopic intervention with pancreatitis symptom was 75.8%.[11] Specifically, patient with acute recurrent pancreatitis has better response rate compared to patient with chronic pancreatitis.[10,11] Intraprocedural bleeding only occurred in 4.8% patients and none of them required blood transfusion.[11] Successful treatment on this patient was reported with improved clinical condition and no sign of complication.

CONCLUSION

Patient with acute recurrent pancreatitis or chronic pancreatitis should be considered for further examination to eliminate the possibilities of congenital anomaly. MRCP and ERCP have been proven as reliable diagnostic tools to diagnose pancreas divisum. Management of this case can be performed by endoscopic intervention with promising response rate and minimal risk of complication. This is the first pancreas divisum case reported and successfully treated in Indonesia.

REFERENCES

1. Kuzel AR, Lodhi MU, Rahim M. Pancreatic divisum: an unusual cause of chronic pancreatitis in a young patient. Cureus. Cureus Inc. 2017;9(11):e1856.
2. Liao Z, Gao R, Wang W, et al. A systematic review on endoscopic detection rate, endotherapy, and surgery for pancreas divisum. Endoscopy. 2009;41(05):439–44.
3. Türkvatan A, Erden A, Türkoğlu MA, et al. Congenital variants and anomalies of the pancreas and pancreatic duct: imaging by magnetic resonance cholangiopancreaticography and multidetector computed tomography. Korean J Radiol. Korean Soc Radiol. 2013;14(6):905–13.
4. Adibelli ZH, Adatepe M, Imamoglu C, et al. Anatomic variations of the pancreatic duct and their relevance with the Cambridge classification system: MRCP findings of 1158 consecutive patients. Radiol Oncol. De Gruyter Open. 2016;50(4):370–7.
5. Adibelli ZH, Adatepe M, Isayeva L, et al. Pancreas divisum: A risk factor for pancreaticobiliary tumors – an analysis of 1628 MR cholangiography examinations. Diagn Interv Imaging. Elsevier Masson. 2017;98(2):141–7.
6. Kuzel AR, Lodhi MU, Rahim M. Pancreatic Divisum: An Unusual Cause of Chronic Pancreatitis in a Young Patient. Cureus. Cureus Inc. 2017;9(11):e1856.
7. Wang D-B, Yu J, Fulcher AS, et al. Pancreatitis in patients with pancreas divisum: imaging features at MRI and MRCP. World J Gastroenterol. Baishideng Publishing Group Inc. 2013;19(30):4907–16.
8. Mosler P, Akisik F, Sandrasegaran K, et al. Accuracy

of magnetic resonance cholangiopancreatography in the diagnosis of pancreas divisum. Dig Dis Sci. 2012;57(1):170–4.
9. Mariani A, Di Leo M, Petrone MC, et al. Outcome of endotherapy for pancreas divisum in patients with acute recurrent pancreatitis. World J Gastroenterol. 2014;20(46):17468.
10. Kanth R, Samji NS, Inaganti A, et al. Endotherapy in symptomatic pancreas divisum: A systematic review. Pancreatology. Elsevier. 2014;14(4):244–50.
11. Rustagi T, Golioto M. Diagnosis and therapy of pancreas divisum by ERCP: A single center experience. J Dig Dis. John Wiley & Sons, Ltd. 2013;14(2):93–9.

Xanthogranulomatous Pyelonephritis with Pyonephrosis and Renal Abscess in a Young Adult: A Consequence of Neglected Urinary Tract Infection Leading to Nephrectomy

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ABSTRACT

Xanthogranulomatous pyelonephritis (XGP) is a rare form of chronic pyelonephritis, which is challenging to diagnose because its clinical presentation mimics other entities and is commonly associated with a history of urinary tract obstruction. We report a case of XGP in a young adult without nephrolithiasis and urinary tract obstruction. A 23-year-old woman presented with intermittent abdominal pain in the right upper quadrant persisting for the last ten months. The pain was dull, poorly localized, and started spreading to the right back, right shoulder, and right thigh in the last three months. Other complaints included fever, chills, pain during urination, and nausea. The patient had a history of infrequent urination, recurrent urinary tract infections (UTIs), and a low fluid intake. A physical examination revealed that the patient had right upper quadrant abdominal tenderness and right costovertebral angle tenderness. Laboratory findings showed leukocytosis and neutrophilia. The radiological examination revealed a round mass in the superior pole of the right kidney with mixed cystic and solid components, and a well-defined margin. It further enlarged from 4.5 cm to 10.6 cm in diameter in three months. The urologist performed a total right nephrectomy. The histopathological examination showed XGP with renal abscess. Proteus mirabilis was identified from the pus specimen culture. XGP should be considered in the diagnosis of patients having chronic UTI presented with or without the findings of urinary tract obstruction.

Keywords: *Xanthogranulomatous pyelonephritis, pyonephrosis, renal abscess, urinary tract infection, nephrectomy.*

INTRODUCTION

Xanthogranulomatous pyelonephritis (XGP) is a rare form of chronic pyelonephritis, which has a challenging diagnosis because of its resemblance to other renal pathologies. XGP is a rare entity that accounts for only 0.6% of histologically documented cases of chronic pyelonephritis.[1] Women are predominantly affected (the male-to-female ratio ranges between 0.5 and 0.6), usually in mid-life years (40–50 years).[2,3] This chronic destructive granulomatous inflammation of renal parenchyma often results in a complete loss of function in the affected kidney.

The exact etiology of XGP is still yet to be discovered, but it appears to be multifactorial. Two of the most common risk factors are urinary tract obstruction and infection, with obstruction playing a major role in pathogenesis.[4] The most common cause of obstruction is nephrolithiasis, which is present in 90% of XGP cases.[2,3] Other causes include ureterolithiasis, ureteral stenosis, pyelo-ureteral junction abnormalities, and pyelo-ureteral duplicities. XGP is also associated with recurrent urinary infections, with common pathogens such as *Escherichia coli* and *Proteus mirabilis*.

The difficulty of diagnosing XGP arises from the fact that it may closely mimic other diseases of the kidney. This nature has earned XGP the title of "the great imitator".[5] The findings in XGP are often difficult to distinguish from pyonephrosis, renal abscess, renal tuberculosis, and renal cell carcinoma by the clinical symptoms, physical examination, and radiological findings.[6] The diagnosis is only confirmed by pathological examination after nephrectomy.

We present an unusual case of XGP in a young adult without urinary obstruction and nephrolithiasis. The case was complicated by pyonephrosis and renal abscess caused by *Proteus mirabilis*, which further led to nephrectomy. A pathological examination confirmed XGP after total nephrectomy.

CASE ILLUSTRATION

A 23-year-old woman presented with intermittent abdominal pain in the right upper quadrant persisting for the last ten months. The pain was dull and poorly localized, with a score of 8 out of 10 on the numerical rating scale. The abdominal pain started spreading to the right back, right shoulder, and right thigh from the past three months. The patient also complained of fever, chills, pain during urination, and nausea. There were no complaints of darker or turbid urine. The medical history was significant for untreated recurrent urinary tract infections (UTI) for the last three years. She said that she rarely drank water in the last five years, only consuming around 400 mL a day. She denied any history of drug use, sexual activity, and urine catheter usage. She usually changed underwear twice a day and kept the genital area clean. The physical examination revealed that the patient had normal vital signs, right upper abdominal tenderness, and right costovertebral angle tenderness.

Complete blood counts showed leukocytosis ($12.05 \times 10^3/\mu L$) and neutrophilia (76.8% neutrophils). Urea and creatinine blood levels were within normal limit, 18 mg/dL and 0.52 mg/dL respectively. The estimated glomerular filtration rate (eGFR) was 133 ml/min/1.73 m^2. Other laboratory tests were also within the normal range. An ultrasound examination revealed a round mass, 4.5 cm in diameter, in the superior pole of the right kidney with cystic and solid components, and well-defined margins (**Figure 1**). Three months later, a computed tomography scan of the abdomen showed a cystic mass measuring $10.6 \times 8 \times 7.72$ cm in the superior pole of the right kidney suppressing the pelvicocalyceal system, accompanied by perirenal fat obliteration and lymphadenopathy (**Figure 2**).

The patient was diagnosed with a kidney mass. Therefore, a urologist performed a total nephrectomy because of the non-functional kidney. Surprisingly, the kidney was found to be enlarged and contained approximately 200

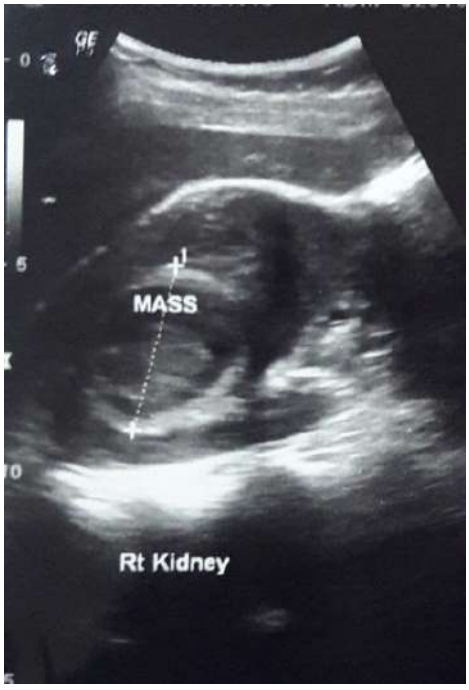

Figure 1. Ultrasonography of the right kidney showed a round, well-defined, mixed cystic and solid mass with a diameter of 4.5 cm in the superior pole of the right kidney

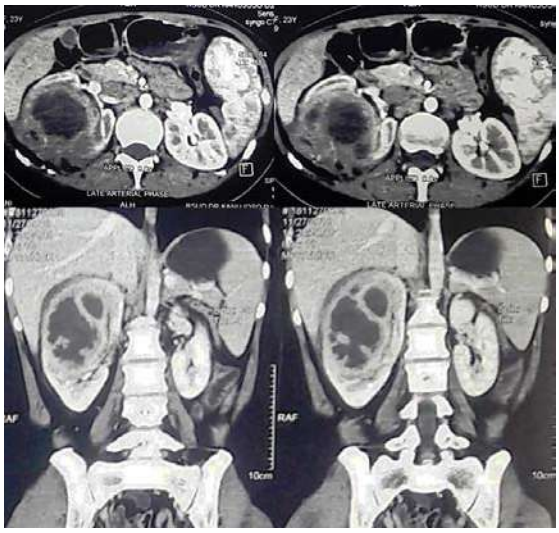

Figure 2. A contrast abdominal CT scan in the transverse plane (upper) and coronal plane (lower) showed a kidney mass measuring 7.72 × 8 × 10.6 cm in the superior pole of the right kidney suppressing the pelvico-calyceal system with perirenal fat obliteration and lymphadenopathy

ml of pus intraoperatively. The histopathological examination revealed the granulomatous inflammation of the renal parenchyma comprising many xanthomatous histiocytes with foamy cytoplasm and multinucleated giant cells surrounded by lymphocytes, areas of hemorrhage, and necrosis with numerous neutrophil infiltrations, thereby indicating XGP with renal abscess (**Figure 3**). Cultures from the

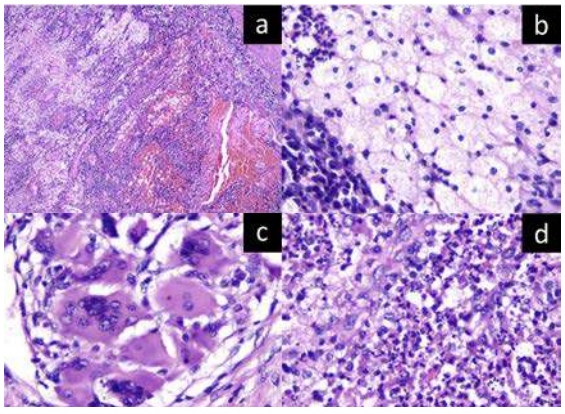

Figure 3. Histopathologic examination showed: (a) granulomatous inflammation of the renal parenchyma, xanthomatous histiocytes with foamy cytoplasm, and areas of hemorrhage and necrosis, indicating xanthogranulomatous pyelonephritis with renal abscess (hematoxylin and eosin (HE) stain, 40x). Higher magnification showed (b) xanthomatous histiocytes (HE, 400x), (c) foreign body-type histiocytic giant cells (HE, 400x), and (d) collection of inflammation cell infiltrate, dominated by neutrophils with few lymphocytes, plasma cells, and histiocytes (HE, 400x).

pus showed *Proteus mirabilis* infection without any antibiotic resistance. The patient was treated with parenteral ceftriaxone and metronidazole postoperatively. The patient was followed up thrice after the nephrectomy. The patient was discharged from the hospital one week after the surgery, when the operation wound was already dry; however, there was still a feeling of pain in the wound area. One week after the first follow-up, the pain had lessened and the wound edges appeared to have fused on inspection. Three months after the surgery, the surgical wound had healed completely and the patient did not have symptoms of any kind. Follow-up of renal function test showed normal results (blood urea 26 mg/dL, blood creatinine 0.8 mg/dL, and eGFR 103 ml/min/1.73 m^2).

DISCUSSION

The diagnosis of XGP is rare and challenging because of its non-specific clinical symptoms, laboratory, and radiologic findings. This condition typically occurs in middle-aged women and is mainly linked to urinary obstruction. However, the patient described in the presented case was a 23-year-old woman without any evidence of underlying urinary obstructions, thus presenting a potential diagnostic pitfall.

Epidemiologically, xanthogranulomatous pyelonephritis is seldom found in a young, otherwise healthy adult. An XGP case in a young adult has been reported previously but it was in the presence of a staghorn calculi.[7] The absence of urinary obstruction further makes the occurrence of XGP more unlikely in the previously described patient. Ultrasonography and CT scans did not find nephrolithiasis or ureterolithiasis. No anatomical abnormalities were observed from radiology and intraoperative assessment. Despite the low possibility, XGP was confirmed in the patient. Similar conditions have also been reported in other case reports where XGP was confirmed by the histopathologic examination in patients who did not have a history of urinary tract obstruction.[8,9]

The only related risk factor found in the aforementioned patient was recurrent and chronic urinary infections. XGP can arise as a complication of chronic urinary infections.[2]

The recurrence and chronicity of the urinary infections could occur from the patient's low fluid intake and infrequent urination. Drinking less than 1.5 L of total fluid daily is associated with more frequent urinary infections in women.[10] Adequate treatment and related lifestyle changes can prevent complications from a urinary infection.

The management of XGP depends on the extent of the disease. The current treatment of choice for diffuse XGP is surgical intervention. If XGP is found in the earlier stages with a smaller lesion size, then antibiotics can be attempted as an initial treatment.[6] The diffuse XGP in the patient had resulted in a non-functioning kidney; hence, the urologist decided treatment with nephrectomy. An early recognition and management of recurrent UTIs and the XGP itself might have prevented the necessity of nephrectomy in the described patient.

CONCLUSION

XGP is a rare type of chronic pyelonephritis, whose diagnosis is challenging preoperatively. The described patient was quite atypical as she was a young adult with no evidence of urinary obstruction. The only risk factor found in the patient was recurrent UTIs. Clinicians should still consider XGP in patients with recurrent UTIs in all ages, even without the presence of risk factors. An early diagnosis and treatment of the UTIs and XGP could prevent unnecessary morbidity caused by nephrectomy.

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REFERENCES

1. Dell'Aprovitola N, Guarino S, Del Vecchio W, et al. Xanthogranulomatous pyelonephritis mimicking a renal cell carcinoma: a unique and challenging case. Acta Radiol Short Reports. 2014;3(1):1-4.
2. Ichaoui H, Saadi A, Chakroun M, et al. Xanthogranulomatous pyelonephritis in adults: clinical, biological, radiological and therapeutic main findings in diffuse and focal forms. About 42 cases. Tunis Med. 2018;96(8):495–500.
3. Kundu R, Baliyan A, Dhingra H, et al. Clinicopathological spectrum of xanthogranulomatous pyelonephritis. Indian J Nephrol. 2019;29(2):111–5.
4. Gupta S, Araya CE, Dharnidharka VR. Xanthogranulomatous pyelonephritis in pediatric patients: case report and review of literature. J Pediatr Urol. 2010;6(4):355–8.
5. Chlif M, Chakroun M, Ben Rhouma S, et al. Xanthogranulomatous pyelonephritis presenting as a pseudotumour. Can Urol Assoc J. 2016;10(1–2):36.
6. Kim SW, Yoon BI, Ha US, et al. Xanthogranulomatous pyelonephritis: clinical experience with 21 cases. J Infect Chemother. 2013;19(6):1221–4.
7. Morales C, Opazo V, Bassa C, et al. Xanthogranulomatous pyelonephritis: A case report. Urol Case Reports. 2018;19(1):65–6.
8. El Abiad Y, Dehayni Y, Qarro A, et al. Xantogranulomatous pyelonephritis: The missed diagnosis. Int J Surg Case Rep. 2016;18:21-3.
9. El-Asmar JM, Ghanem R, Ghandour R, et al. Postpartum xanthogranulomatous pyelonephritis: a case report. Case Reports in Women's Health. 2019;22:e00112.
10. Hooton TM, Vecchio M, Iroz A, et al. Effect of increased daily water intake in premenopausal women with recurrent urinary tract infections: a randomized clinical trial. JAMA Intern Med. 2018;178(11):1509–15.

Clinical Application of Stem Cell Therapy for Liver Cirrhosis: Progress, Pitfalls, and Prospects

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ABSTRACT

Liver cirrhosis is the advanced stage of liver disease accounting for high morbidity and mortality rates worldwide. Liver transplantation for liver cirrhosis has several limitations, rendering stem cell transplantation as a potential therapy. Clinical trials of stem cell applications for liver cirrhosis are being established using various types of stem cells. This review will provide a current report of the achievements, limitations, and future directions of stem cell transplantation. Current progress of clinical trials is valuable in defining the best type of stem cells, mode of delivery, the number and frequency of cells to be injected, and determining potential candidates for cell therapy. Some of the encountered pitfalls are the limited homing and differentiation potential of stem cells, the use of non-xenofree culture system, and the risk for tumorigenesis in certain types of stem cells. The prospective developments of liver stem cell transplantation are the generation of genetically modified stem cells and the formation of liver organoids for treating liver cirrhosis.

Keywords**:** *stem cells, liver cirrhosis, regenerative medicine.*

INTRODUCTION

Distortion of liver architecture due to viruses, toxins, or chemical agents in the case of liver cirrhosis results in declining function of the liver. Deaths due to severe complications of liver cirrhosis are estimated to reach 1.03 million people annually worldwide.[1] While hepatitis B and C infections are the main cause of liver cirrhosis, alcoholic liver disease also contributes to the development of liver cirrhosis.

Anti-fibrotic drugs are being investigated for the management of patients with liver cirrhosis. These drugs may bring clinical improvements for the patients with no overt reversal of the fibrotic state.[2] Orthotopic liver transplantation remains the feasible solution for replacing the cirrhotic liver. However, several limitations are encountered: the shortage of the organ, the high procedural cost, and the risk for organ rejection. The development of cell therapy has emerged as a promising alternative for the management of liver cirrhosis. Hepatocytes have been studied for cell therapy.[3] In spite of that, due to the limited donor organ, inability to be expanded in cell culture, poor engraftment, and proneness for damage under cryopreservation, the finding of another source of cells is of the utmost importance. The proliferation and differentiation ability of stem cells bring benefits for ameliorating liver cirrhosis. Unfortunately, there is still a long way

to go before standard clinical application of stem cell transplantation can be established. Exactly how these cells can restore or replace cirrhotic areas also remains inconclusive. This review will provide a current report of the progress, pitfalls, and prospects of liver stem cell transplantation to give clinical insights and possible future directions in the treatment of liver cirrhosis.

STEM CELLS FOR LIVER CIRRHOSIS

The liver consists of various types of cells including hepatocytes, Kupffer cells, and hepatic stellate cells. Some residing cells in the liver may serve as endogenous stem cell sources that can be activated during injury. Any injury to the liver may cause inflammation and promote the activation of hepatic stellate cells to produce fibrin that accumulates in the space of Disse leading to liver fibrosis.[4]

In the context of exogenous stem cells, there are three large classifications of stem cells that comprise embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). ESCs and iPSCs represent prospective sources of stem cells due to their robust ability in differentiation. Ethical consideration then hampers the use of ESCs that are taken from embryonic tissue. We expect iPSCs to be the ideal source for treating liver cirrhosis, however teratogenicity potential has become the hurdle of developing this cell line, therefore, the study of cell biology in the case of iPSCs are still under investigation. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are ASCs that are commonly used in the clinical trials of treatment for liver cirrhosis.

ASCs can be harvested from healthy donors (allogeneic cells) or from the patients themselves (autologous cells). Allogeneic stem cells may provide good quality of stem cells at the cost of the difficulty to match the human leukocyte antigen (HLA) antigens to prevent any rejection. Autologous stem cells seem to be easier to gain. Stem cells are proposed to have several mechanisms in the treatment of liver fibrosis: mediation of inflammatory microenvironments by paracrine effect, inhibition of hepatic stellate cell activation, apoptosis induction of hepatic stellate cells, the differentiation capacity into hepatocytes, and facilitation in regeneration of residual hepatocytes.[2]

PROGRESS

Clinical studies have emerged in elucidating the potential of stem cells in treating liver cirrhosis.

Type of Stem Cells

ESCs have the ability to differentiate into hepatocyte-like cells and serve as a potential source of cells for treating liver cirrhosis, unfortunately these cells were not further recommended due to ethical consideration. ASCs, comprising of HSCs, MSCs, and endothelial progenitor cells (EPCs), have arisen as potential cells for stem cell therapy. HSCs can be obtained from bone marrow[5,6] and peripheral blood,[7] whereas MSCs can be isolated from adipose tissue, umbilical cord, and bone marrow.[1,8,9]

Bone marrow is the main source of HSCs that can be identified from the expression of cluster of differentiation (CD)34^+ as their surface marker. Peripheral mobilization by granulocyte colony stimulating factor (GCSF) makes it possible to obtain the cells from peripheral apheresis and was shown to be safe in patients with end stage liver disease.[10] Injection of HSCs in patients with liver disease was expected to facilitate liver regeneration by differentiating into functioning hepatocytes and genetic reprogramming of resident hepatocytes by cell fusion. Nevertheless, the advantage of HSCs injection is more likely due to the paracrine effect and the role of macrophages in phagocyting dead cells and producing collagenase.[11]

MSCs serve as the best potential source of stem cells for liver cirrhosis. They can be expanded ex vivo while maintaining their differentiation potential. MSCs have also the ability to migrate into injured areas and the immunomodulatory properties to escape immune recognition. MSCs were shown to differentiate into hepatocytes in animal models of liver cirrhosis, however, the paracrine mechanism through the secretion of cytokines and growth factors seem to dominate their function in liver regeneration.[11]

Administration of bone marrow-derived MSCs in patients with hepatitis B-associated

liver cirrhosis facilitated an increase in regulatory T (Treg) cells and a decrease in helper T (Th)17 cells. This regulation of Treg/Th17 cell balance could mediate improvement of liver function following stem cell transplantation.[12] **Figure 1** depicts possible interaction of hepatic stellate cells and MSCs in term of MSCs transplantation upon liver cirrhosis.[13,14,15]

Liver-derived stem cells consist of oval cells and hepatoblasts that are involved in liver regeneration. Hepatoblasts are bipotent cells that can differentiate into duct cells or hepatocytes. Their small population in liver architecture, with oval cells constituting 0.3-0.7% and hepatoblasts only 0.1% of the liver, makes this source of stem cells not practical.[16]

Mode of Delivery

Stem cells are transplanted in several ways that include peripheral injection,[5] intrahepatic artery injection,[7] intrasplenic vein injection,[8] intraportal vein injection,[7,17] and percutaneous intrahepatic injection.[18] Intrahepatic and intrasplenic injection of bone marrow-derived MSCs have comparable safety and a short-term efficacy profile.[8] A study of MSCs biodistribution following intravenous injection of MSCs was performed with the use of ^{111}In-oxide labelling. Immediately after transfusion, there was accumulation of radioactivity in both lungs of all patients. On the 10th day of observation, radioactivity increased in the liver and spleen along with reduction of radioactivity in the lung. Spleen accumulation of MSCs was found to be higher in quantity compared to the liver in all patients.[19]

Cells Number and Frequency

The number of the cells to be injected and the frequency of injection remains the question to be answered in cell therapy. An average of $2x10^7$ bone marrow-derived MSCs could be injected safely to patients with liver cirrhosis.[8] Autologous bone marrow-derived mononuclear cells of $1.6\text{-}13.1x10^8$ were injected through the intrahepatic artery of patients with advanced liver disease.[6] Another study injected different doses of peripheral blood-derived $CD34^+$ cells with range of $1x10^6$ to $2x10^8$ cells via portal vein and hepatic artery routes.[7] Suk et al. performed transhepatic artery injection of bone marrow-derived MSCs for alcoholic cirrhosis patients revealing that two injections did not improve patients' condition compared to single injection.[20]

Published Clinical Trials

A meta-analysis of stem cells therapy for liver cirrhosis has been published recently.[21] This meta-analysis showed that MSCs therapy could improve laboratory parameters of patients with liver cirrhosis. There were improvements in the level of albumin, alanine aminotransferase, total bilirubin, prothrombin time, and end-stage liver disease score; however, there were no significant changes in terms of cholinesterase level, prothrombin activity, and international normalized ratio. This meta-analysis suggested the safety profile and efficacy of stem cells injection for the patients with liver cirrhosis in the first 6 months. Multiple injections of stem cells did not result in beneficial effects compared to single injection. Intraarterial injection was shown to bring better efficacy in comparison to other modes of delivery. In terms of stem cell source, bone marrow-derived stem cells provided better improvement of liver cirrhosis.

Caution should be taken into consideration when interpreting the results of clinical studies as some studies did not provide a control group of comparable cirrhotic patients[6] with only short-term periods of follow-up. Publications derived from small-scale studies with peripheral injection may result in minimal cell delivery toward the liver. The choice of undifferentiated or differentiated bone marrow-derived MSCs injection showed no significant difference in the efficacy.[9] One clinical study showed safety and efficacy of bone marrow $CD34^+$ cells during 12 months follow-up.[22] Long-term follow-up of cirrhotic patients receiving bone marrow MSCs did not present favorable outcomes.[23]

Although phase I-II clinical trials of stem cell treatments prove the safety profile,[22] stem cells transplantation is not without risk. Treatment related therapy should be also taken into consideration. Intrahepatic injection of stem cells could result in lethal side effects, for instance the development of radiocontrast nephropathy leading to hepatorenal syndrome.[24] A larger scale of randomized controlled trial comparing

the effects of HSCs infusions to GCSF treatment showed no greater beneficial effects received by cirrhotic patients and the patients are even more likely to suffer from treatment-related adverse events compared to standard care.[25]

Defining Potential Candidates for Therapy

Before applying stem cell therapy for patients with liver cirrhosis, it is very crucial to determine the patients that potentially will get the most benefit from the transplantation. Conditioning regimen for preparing stem cell transplantation may include the use of immunosuppressive therapy that could put the patients at risk of infection. Current clinical studies of stem cells show that stem cells are able to improve clinical conditions and laboratory parameters of cirrhotic patients but are yet to reverse the cirrhotic state. Therefore, this method may be beneficial for those waiting for liver transplantation or as an add-on therapy of standard medications. A small-scale study showed that the injection of bone marrow-derived $CD133^+$ may enhance hepatic regeneration before performing hepatectomy in the case of hepatocellular carcinoma or metastatic liver mass.[17] Cirrhotic patients of Child Pugh C aged less than 60 can be considered as the candidates for stem cell therapy. Those patients with advanced complications of cirrhotic liver (moderate to severe hepatic encephalopathy and recurrent variceal bleeding), bleeding tendency, active infection condition, hepatocellular carcinoma, and hepatic/portal/splenic thrombus are considered as non-favorable patients for stem cell transplantation. Strict cut-off point of creatinine level should be decided to prevent patients with underlying kidney problems from receiving contrast for the delivery stem cells that may lead to contrast-induced nephropathy.[24]

PITFALLS

This part covers the limitations of stem cell therapy in alleviating liver cirrhosis.

Homing

Once stem cells are injected into the patients with liver cirrhosis, homing efficacy will be the first aspect to consider. Following cell transplantation, only 1-3% of the liver was repopulated by stem cells.[26] In order to give the best effect, stem cells should remain in the liver. Intravenous injection is one of the most common methods in the administration of stem cells. Unfortunately, stem cells may be phagocyted by reticuloendothelial cells in the circulation. Furthermore, these cells will pass through the capillary bed of the lung before reaching hepatic circulation, putting up an obstacle to achieve effective homing. Peripheral injection of bone marrow MSCs did not give beneficial effect for cirrhotic patients compared to control.[5] Therefore, although technically more difficult, intraarterial injection is preferred to distant intravenous injection.[21]

To promote engraftment, intrahepatic injection of stem cells seems to be the best way to deliver cells since there will be less cell entrapment in the circulation. Cell culture conditioning, for instance hypoxic preconditioning, also contributes in improving the homing ability of stem cells. Preventing high cell confluence during passaging will also maintain homing efficacy as high confluence of culture can increase the production of tissue inhibitor metalloproteinase-3. The use of cells from early passages may also provide better homing efficacy.[1] Homing may also be enhanced by modifying MSCs by liver specific receptors.[11]

Differentiation Potential

Although the injections of stem cells to patients with liver cirrhosis were shown to improve their clinical condition, differentiation potential of the stem cells into hepatocytes still is in question. Autologous bone marrow infusion can stimulate the activation and differentiation of hepatic progenitor cells. However, this effect was not sustained more than 6 months as liver biopsy showed histological pictures returning to the baseline before infusion.[27] Transdifferentiation of stem cells into hepatocytes may be driven by some transcription factors and cellular signalling. Hepatocyte nuclear factor (HNF)3β and HNF4α are among transcription factors that play an important role in hepatic differentiation. HNF3β is a family of forkhead box transcription factors that promote hepatic differentiation of stem cells as evidenced by the production of alpha fetoprotein, albumin, and tyrosine aminotransferase. HNF4α induces the

differentiation into the hepatocyte phenotypes. Wnt signalling inhibition may also drive hepatic differentiation of stem cells. Another concern is that transplanted MSCs may be differentiated into myofibroblast-like cells that may further worsen the liver's fibrotic state.[26]

Non-xeno-free culture system

The preparation of stem cells, from the isolation of cells to the transplantation, needs several reagents processed from animal products. This process brings the potential of infection transmission and increased risk of cells rejection. Techniques for preparing xeno-free culture system have been developed.[28] Unfortunately, the xeno-free system cannot be applied widely because the preparation of the cells in the beginning step requires several growth hormones which are animal-derived products.

Tumorigenesis

The risk for teratoma formation is one of the major hurdles for the application of iPSCs. This tumor may arise from the undifferentiated cells injected along with differentiated ones. Accordingly, there is a method to purge and separate the undifferentiated from differentiated cells. The problem is that even single cells remaining in the injection process may cause the teratoma formation. Furthermore, genome instability of stem cells may cause mutation of cells during culturing, increasing the possibility to become teratogenic cells. One of the solutions for this issue is the method of the viral vector transduction system to insert a suicide gene and oncolytic virus to kill the tumorigenic cells.[29]

The teratogenic potential of MSCs transplantation should also be considered as MSCs may promote tumor growth by transdifferentiating into malignant cells, suppressing the anti-tumor immune system, and secreting growth factors for cancer cells as well as promoting the secretion of angiogenetic factors, for instance fibroblast growth factor, vascular endothelial growth factor, and platelet-derived growth factor.[26]

PROSPECTS

The study of cell biology related to stem cells has been evolving. In the future, there will be new discoveries playing important roles for cell therapy.

Genetically modified stem cells

Stem cells can be modified in such a way that some genes will be transferred and will influence how the cells behave in case of injury. Genetic modification may increase survival and function of stem cells once they are transplanted. Genetically modified-stem cells are expected to be able to protect themselves in term of inflammation following transfusion, immune rejection, hypoxia condition, and apoptosis. Gene modification can be obtained by transgenic methods using specific vectors, Cre/Lox P system to knock out target gene, antisense inhibition, small interfering RNA (siRNA) gene silencing, microRNA technology, or the use of cell specific promoters.[30]

Liver organoids

Liver organoids are expected to possess the ability to replace the diseased tissue. The study of liver organoids is now under the preclinical stage. Hepatocytes are the main cells responsible for liver regeneration following injury of the liver. In addition, there are also ductal populations that may differentiate into bi-potent progenitor. During liver regeneration, several signalling pathways are needed to direct the differentiation that may include Notch (Sattwika et al., unpublished work), Wnt, hepatocyte growth factor, and fibroblast growth factor. In order to create supportive microenvironments to develop liver organoids, an interaction between embryonic endoderm and mesoderm is needed.[31] Therefore, ASCs alone are not enough to produce such complex interaction. Another solution is to prepare co-cultures of iPSCs-derived hepatocytes with MSCs. Takebe et al. has shown outstanding formation of liver organoids from iPSCs.[32] In the future, liver organoids are expected to replace cirrhotic areas in patients with liver cirrhosis.

CONCLUSION

Current basic researches and clinical trials of stem cell therapy for liver cirrhosis show that stem cell transplantation may be a potential alternative to liver transplantation. From the perspective of clinicians, big-scale randomized

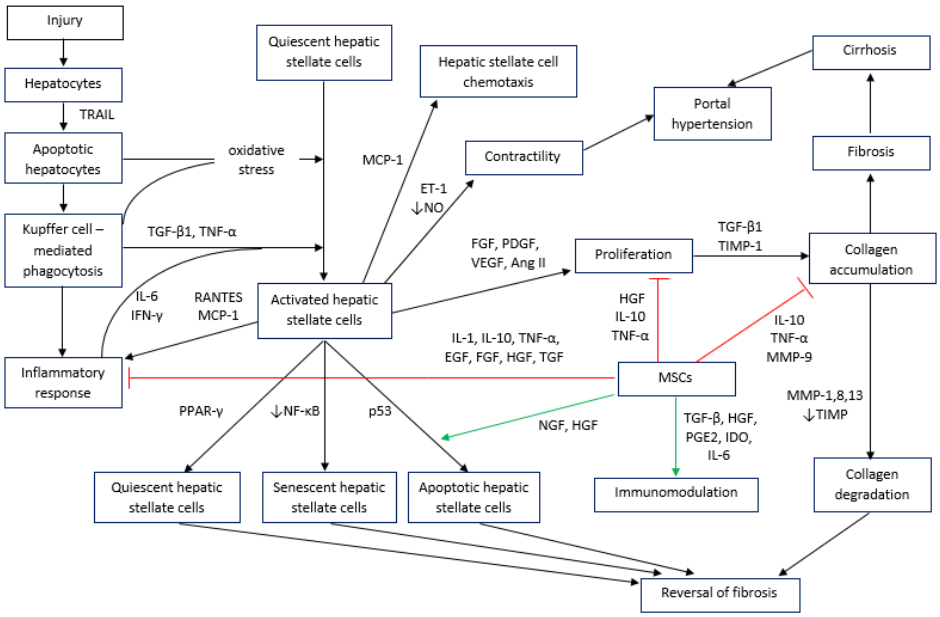

Figure 1. Schematic diagram of the proposed interaction between hepatic stellate cells and mesenchymal stem cells (MSCs) during liver injury. Vulnerable hepatocytes undergo apoptosis when exposed to injury. Apoptotic bodies of hepatocytes are engulfed by resident Kupffer cells. Activated Kupffer cells then promote inflammatory response and activation of hepatic stellate cells. Transformation of quiescent to activated hepatic stellate cells (myofibroblasts) also results from oxidative stress as a consequence of formation of apoptotic bodies. Activated hepatic stellate cells further enhance inflammatory response due to their chemotactic, contractile, and proliferative properties. Contractility of hepatic stellate cells results in an increase in portal pressure, whereas proliferative property is responsible for increased production and accumulation of collagen leading to liver fibrosis. Fibrotic state may eventually result in liver cirrhosis. Cessation of injury, clearance of activated hepatic stellate cells (i.e. by transformation into quiescent, senescent, or apoptotic hepatic stellate cells), and degradation of collagen are all expected to reverse fibrosis. MSCs may contribute to improve the fibrotic state through their paracrine mechanism to suppress inflammatory condition, inhibit the activation of hepatic stellate cells, and induce the apoptosis of hepatic stellate cells.[13,14,15] TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; TNF-α: tumor necrosis factor alpha; TGF-β: transforming growth factor beta; IFN-γ: interferon gamma; IL-6: interleukin-6; RANTES: regulated upon activation, normal T cell expressed and secreted; MCP-1: monocyte chemotaxis protein-1; PDGF: platelet derived growth factor; EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; VEGF: vascular endothelial growth factor; ET-1: endothelin 1; NO: nitric oxide; Ang II: angiotensin II; MMP: matrix metalloproteinase; TIMP: tissue inhibitors of metalloproteinase; HGF: hepatocyte growth factor; NGF: nerve growth factor; IL-1: interleukin-1; IL-10: interleukin-10; PGE2: prostaglandin E2; IDO: indoleamine 2,3-dioxygenase; PPAR-γ: peroxisome proliferator-activated receptor gamma; NF-κB: nuclear factor kappa B; p53: tumor protein 53.

controlled trials will be definitely needed to elucidate the best type of stem cells, the best route, and the best cell number and frequency of injection. From the aspect of scientists, further studies to explore the mechanisms underlying the role of stem cells in the case of liver cirrhosis is of importance, as well as to answer the limitation of stem cell therapy as discussed above. The discovery of genetically modified stem cells and liver organoids may be beneficial for liver cirrhosis patients in the next several decades.

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CONFLICT OF INTERESTS

The author(s) declare that they have no conflict of interests.

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REFERENCES

1. Zhang Y, Li Y, Zhang L, et al. Mesenchymal stem cells: potential application for the treatment of hepatic cirrhosis. Stem Cell Res Ther. 2018;9(1):59.
2. Berardis S, Sattwika PD, Najimi M, Sokal EM. Use of mesenchymal stem cells to treat liver fibrosis: current situation and future prospects. World J Gastroenterol. 2015;21(3):742–758.
3. Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. J Hepatol. 2015;62(S1):S157–S169.

4. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2010;134(6):1655–69.
5. Mohamadnejad M, Alimoghaddam K, Bagheri M, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. Liver Int. 2013;33(10):1490–6.
6. Lyra AC, Soares MB, da Silva LF, et al. Feasibility and safety of autologous bone marrow mononuclear cell transplantation in patients with advanced chronic liver disease. World J Gastroenterol. 2007;13(7):1067–73.
7. Gordon MY, Levičar N, Pai M, et al. Characterization and clinical application of human CD34$^+$ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells. 2006;24(7):1822–30.
8. Amer M-EM, El-Sayed SZ, El-Kheir WA, et al. Clinical and laboratory evaluation of patients with end-stage liver cell failure injected with bone marrow-derived hepatocyte-like cells. Eur J Gastroenterol Hepatol. 2011;23(10):936–41.
9. El-ansary M, Abdel-aziz I. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in egyptian patients with HCV induced liver cirrhosis. Stem Cell Rev Rep. 2012;8:972–81.
10. Gaia S, Smedile A, Omedè P, et al. Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease. J Hepatol. 2006;45(1):13–9.
11. Kwak K-A, Lee S-P, Yang J-Y, Park Y-S. Current perspectives regarding stem cell-based therapy for liver cirrhosis. Can J Gastroenterol Hepatol. 2018;2018:1–14.
12. Xu L, Gong Y, Wang B, et al. Randomized trial of autologous bone marrow mesenchymal stem cells transplantation for hepatitis B virus cirrhosis: regulation of Treg/Th17 cells. J Gastroenterol Hepatol. 2014;29(8):1620–8.
13. Cohen-Naftaly M, Friedman SL. Current status of novel antifibrotic therapies in patients with chronic liver disease. Therap Adv Gastroenterol. 2011;4(6):391–417.
14. Lin H, Xu R, Zhang Z, et al. Implications of the immunoregulatory functions of mesenchymal stem cells in the treatment of human liver diseases. Cell Mol Immunol. 2011;8(1):19–22.
15. Eom YW, Shim KY, Baik SK. Mesenchymal stem cell therapy for liver fibrosis. Korean J Intern Med. 2015;(2):510–6.
16. Nicolas C, Wang Y, Luebke-Wheeler J, Nyberg S. Stem cell therapies for treatment of liver disease. Biomedicines. 2016;4(1):2.
17. am Esch JS, Knoefel WT, Klein M, et al. Portal application of autologous CD133$^+$ bone marrow cells to the liver: a novel concept to support hepatic regeneration. Stem Cells. 2005;23(4):463–70.
18. Bahk JY, Piao Z, Jung JH, Han H. Treatment of the end stage liver cirrhosis by human umbilical cord blood stem cells: preliminary results. In: Stem Cells in Clinic and Research, Dr Ali Gholamrezanezhad (Ed). InTech. 2011. p. 469–500.
19. Gholamrezanezhad A, Mirpour S, Bagheri M, Mohamadnejad M. In vivo tracking of ^{111}In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol. 2011;38(7):961–7.
20. Suk KT, Yoon J-H, Kim MY, et al. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: phase 2 trial. Hepatology. 2016;64(6):2185–97.
21. Zhao L, Chen S, Shi X, Cao H. A pooled analysis of mesenchymal stem cell-based therapy for liver disease. Stem Cell Res Ther. 2018;9:1–13.
22. Levicar N, Pai M, Habib NA, et al. Long-term clinical results of autologous infusion of mobilized adult bone marrow derived CD34$^+$ cells in patients with chronic. Cell Prolif. 2008;41(1):115–25.
23. Peng L, Xie D, Lin B, et al. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. Hepatology. 2011;820–8.
24. Mohamadnejad M, Namiri M, Bagheri M, et al. Phase 1 human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. World J Gastroenterol. 2007;13(24):3359–63.
25. Newsome PN, Fox R, King AL, et al. Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. Lancet Gastroenterol Hepatol. 2017;1253(17):4–6.
26. Liu WH, Song FQ, Ren LN, et al. The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases. J Cell Mol Med. 2015;19(3):511–20.
27. Kim JK, Park YN, Kim JS, et al. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. Cell Transplant. 2010;19(10):1237–46.
28. Shahjalal HM, Shiraki N, Sakano D, et al. Generation of insulin-producing beta-like cells from human iPS cells in a defined and completely xeno-free culture system. J Mol Cell Biol. 2014;6(5):394–408.
29. Mitsui K, Ide K, Takahashi T, Kosai KI. Viral vector-based innovative approaches to directly abolishing tumorigenic pluripotent stem cells for safer regenerative medicine. Mol Ther Methods Clin Dev. 2017;5(June):51–8.
30. Phillips MI, Tang YL. Genetic modification of stem cells for transplantation. Adv Drug Deliv Rev. 2008;60(2):160–72.
31. Huch M, Koo B-K. Modeling mouse and human development using organoid cultures. Development.

2015;142(18):3113–3125.
32. Takebe T, Sekine K, Kimura M, et al. Massive and reproducible production of liver buds entirely from human pluripotent stem cells. Cell Rep. 2017;21(10):2661–70.

Molecular Mechanism of Acute Sarcopenia in Elderly Patient with COVID - 19

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Case fatality rate has been on the rise among older adults. Muscle loss is a consequence of several chronic diseases (chronic sarcopenia) and recent theory also suggested that acute sarcopenia may caused by acute significant stressor such as an acute illness, surgery, infections, trauma or burns including COVID-19 infection leading to further muscle loss in elderly. Cytokine storm, the hallmark of COVID-19 pathogenesis will induce various pro-inflammatory cytokine such as IL-1 and IL-6 causing acute sarcopenia by activating negative regulators like NF-κB, atrogin-1, MURF-1. Long standing chronic inflammation also known as inflammaging along with acute inflammation during COVID-19 in elderly will cause reticulum endoplasmic and mitochondria stress activating caspase and finally increase both cytosolic and nuclear levels of AIF and EndoG to induce acute sarcopenia. Several precipitating factors shared same molecular pathway like physical inactivity and hormonal dysregulation which act through IGF-1-AKT-mTOR pathway. Physical inactivity during COVID-19 infection also induced myostatin and Atrogin-1/ MaFbx/ MuRF pathway. This review provides recent research advances dealing with molecular pathway modulating muscle mass in acute sarcopenia during COVID-19 infection.

Keywords**:** *COVID-19, acute sarcopenia, inflammation, aging.*

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2). It was first reported in December 2019 in Wuhan, China and has spread quickly to all over the world.[1] The number of confirmed COVID-19 cases has reach more than 233 million worldwide and currently has mortality rate 1.71%. [2] Indonesia has 4.2 million confirmed cases up to October 2021 including 142.115 total death.[3] COVID-19 has high mortality and morbidity in elderly patients with 38.6% death case among elderly patients in Indonesia. Men have higher proportion of both confirmed and death cases compared to women in elderly population.[4] Case fatality rate has been on the rise among older adults. According to statistical data, up to 80-

90% of deaths was mostly happened in elderly patients (≥60 years old).[5]

Globally, elderly population is growing faster than the number of people in all younger age groups each year. In 2019, aging population have reached 703 million worldwide.[6] Indonesia is one of the most populated countries and its elderly population is estimated to be 25.64 million in 2019. Elderly people will experience decrease of organ function such as loss of muscle strength. Decrease of muscle mass and muscle function will lead to sarcopenia in elderly. Sarcopenia is a syndrome characterized by loss of skeletal muscle mass and function.[7] Acute sarcopenia is defined as changes in muscle mass and muscle function less than 6 months due to significant stressor such as an acute illness, surgery, infections, trauma or burns.[8] In COVID-19 cases, acute sarcopenia was associated with increased mortality risk, longer ICU admission and risk of higher mechanical ventilator.[9,10,11,12,13] The combination of high inflammation, malnutrition, and immobilization in critically ill COVID-19 will increase the probability of elderly developing acute sarcopenia.[14] The underlying mechanism of acute sarcopenia in elderly with COVID-19 is a complex process but several cellular mechanisms are thought to be involved in the acute sarcopenia pathogenesis. This review aims to understand further about the complexity of acute sarcopenia molecular pathway in elderly with COVID -19, identify the contributing factors and the implications so that clinicians can try the best measures to prevent and treat this acute and long-term, disabling condition.

AGING, COVID-19 INFECTION, AND ACUTE SARCOPENIA

Aging is accompanied by remodeling of the immune system including decreasing immune system capability to induce antibody and cellular response to fight against infection. This phenomenon is known as *imunosenescence*, a multifactorial condition which influences innate and adaptive immunity, especially T-Lymphocyte cells. The hallmark of immunosenescence is the reduced ability to respond to new antigens, the accumulation of memory T cells, and long standing low-grade inflammation termed as inflammaging.[15] Aging has been associated with chronic inflammation and increase of inflammation markers such as *C-reactive protein* (CRP) and interleukin-6 (IL-6).[16] Aging is also risk factor for poor outcome in COVID – 19 infection. Aging will cause decrease of inhaled particle clearance in respiratory tract due to diminished ciliary amount in respiratory tract. Moreover aging also affects upper respiratory tract size which reduces its size especially in men and finally increase the risk of upper respiratory tract collapse.[17] SARS-CoV-2 S spike protein plays a crucial role in binding the human cell receptor ACE2 as entry point of the virus. In lung cells, by cleaving residues of AngII, ACE2 produces Ang I-VII which reduces the inflammatory effect of AngII. The spike protein of the SARS-CoV-2 virus causes internalization and degradation of ACE2 which exacerbates lung damage. New study found that younger subjects are prone to have higher probability for COVID-19 infection, whereas in elderly who has lower amount of ACE2 was associated with more severe symptoms in COVID-19 infection. Lower expression of ACE 2 does not protect against viral invasion because SARS-CoV-2 has a high intrinsic affinity for the ACE 2 receptor. ACE 2 deficiency is accompanied by viral downregulation of ACE 2 causing imbalance between ACE/Ang II/AT1R receptors and ACE 2/ Angiotensin 1-7 receptors. At the lung level, such dysregulation will greatly facilitate the development of the inflammatory process and hypercoagulation resulting from the hyperactivity of Ang II.[18] These phenomenon is associated with more severe alveolar damage and mechanical ventilator support.[19]

Age is not the only factor that affects the increase of disease severity. Epidemiological studies also show differences of higher incidence and mortality of COVID-19 infection in men compared to women. The number of T cells and B cells decrease significantly in elderly men and there was an increase in CD8 memory effector T cells compare to women. Elderly men showed an inverse CD4/CD8 ratio compared to women. The proliferative and secretory capacity of T cell cytokines also decreases more rapidly in male.[17] Sex hormones also play a role and explained why

men are more susceptible compared to women. Research on mice have showed differences in ACE2 expression according to sex. One study also reported higher expression of ACE2 in female mice in comparison to male mice. SARS-CoV-2 uses the cell surface enzyme ACE2 and the transmembrane serine protease 2 (TMPRSS2) for providing virus cell entry and priming. Estrogen seems play as protective factor in women. Estrogen receptor alpha (ERα) has an effect on T cells such as Th1, Th2, Th17, and T regulatory cells, as well as follicular helper T (TFH) cells. Therefore, estrogen, stands out as a key biological factor making women's immune system more active against the virus. Estrogen also has anti inflammatory and antioxidative effect on the effectors of the renin-angiotensin system.[20] On the other hand, male sex hormone might contribute to severity of COVID-19 in men compared to women. TMPRSS2 is an androgen-mediated protein that plays a critical role in priming the virus spike proteins for entry into the host cell as one of the first steps involved in infection and its activation is dependent on androgens.[21]

Sarcopenia is a syndrome characterized by progressive loss of muscle mass and muscle strength.[7,22] The definition of sarcopenia based on the European Working Group on Sarcopenia in Older People (EWGSOP) 2 is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with increasing risks such as physical disability, poor quality of life and death. Acute sarcopenia based on EWGSOP2 is defined as incident sarcopenia within six months, normally following a stressor event and if sarcopenia persists for more than six months it will be described as chronic.[22] In order to diagnose acute sarcopenia, we must obtained measurements of muscle mass and muscle function either pre-illness or during early stages of an illness.[8] **Figure 1** shows how acute sarcopenia secondary to hospitalization can lead to worsening chronic sarcopenia and this is often seen in elderly patients infected with COVID-19. Sarcopenia in elderly patients has implications not only when the patient is hospitalized, but also functional and physical decline after COVID-19 recovery.[23]

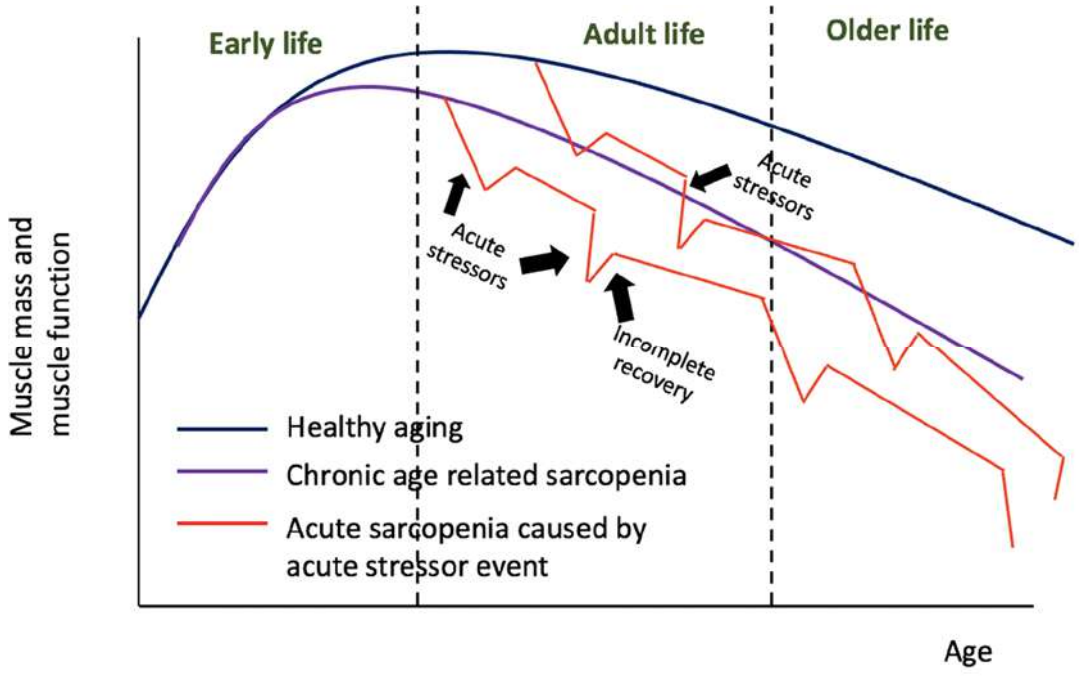

Figure 1. Acute sarcopenia, chronic sarcopenia, healthy aging illustration.[8,22]

ACUTE SARCOPENIA IN ELDERLY WITH COVID-19 INFECTION: PREDISPOSING AND PRECIPITATING FACTORS

In order to effectively prevent the development of acute sarcopenia, it is important to know predisposing and precipitation factors. Some predisposing factors play a major role on determining the outcome of acute sarcopenia in elderly with COVID – 19 infection even after recovering from COVID – 19 infection. Predisposing factors for acute sarcopenia is a complex process in which many factors can contribute. Aging is the main predisposing factor of sarcopenia, but other factors also contribute to the loss of muscle mass such as reactive oxygen species (ROS). In the context of aging, increased activity of ROS has been implicated in the processes underlying aging and, in all species, tissues (including skeletal muscle) of aged organisms contain increased amounts of oxidative damage to lipids, DNA and proteins. Aberrant ROS generation and oxidative damage have been associated with many aspects of mitochondrial dysfunction in skeletal muscle aging.[24] Sarcopenia is an example of impaired physical function that is thought to have complex relationships with individual long-term conditions and multimorbidity, including bidirectional effects. Many studies proved that elderly who developed sarcopenia often has multimorbidity. Study conducted by Richard et al, found that almost half of the sample (44.5%) had multimorbidity (two or more categories of long-term conditions). This was more common in those with sarcopenia (64.8%) than those without (43.4%).[25]

Since elderly are often at risk for both the development of sarcopenia and obesity, a double burden exists. Obesity remains one of predisposing factors that might cause elderly prone to develop sarcopenia and makes it not very surprising that the two conditions often coexist which is known as sarcopenic obesity. An important risk factor for both sarcopenia and obesity is the lower rate of energy expenditure with age, which is a result of lower physical activity, as well as a fat free mass related-lower basal metabolic rate, which is often seen with older age. Obesity may create resistance towards anabolic stimuli, such as, growth factors, hormones, amino acids, and exercise, a phenomenon called anabolic resistance. Finally, obesity is responsible for causing systemic low-grade inflammation, particularly by visceral fat, which excretes several different pro-inflammatory cytokines, such as IL-6)and TNF-α and inflammation is also one of predisposing factor for sarcopenia as well.[26] Besides obesity, malnutrition was one of the remaining problem in elderly and found to be associated with sarcopenia in several populations, including hospitalized patients. The association between malnutrition and severe sarcopenia could be explained by a lower intake of key nutrients such

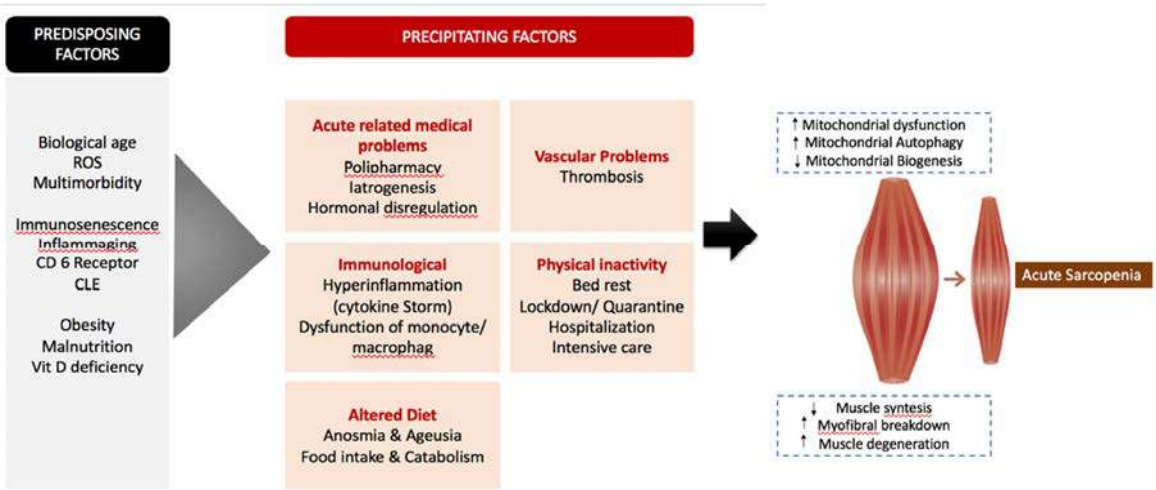

Figure 2. Predisposing and precipitating factors of acute sarcopenia in elderly with COVID-19 infection.[22-27]

as protein, vitamin D and calcium, amongst other factors, which affects preservation of muscle mass and subsequently muscle strength and physical performance. [22,27]

Acute sarcopenia is usually related to an acute illness or injury, while chronic sarcopenia is likely to be associated with chronic and progressive conditions and increases the risk of mortality. Long before elderly experienced critical illness, they often have chronic sarcopenia as consequence of aging. At one point, acute illnesses could worsen the degree of sarcopenia and this condition could be termed as an acute-on-chronic sarcopenia phenotype.[28] Some precipitating factors are playing crucial role in the accelerated development of acute on chronic sarcopenia in elderly infected with COVID-19, such as altered diet during hospitalization including anosmia and ageusia, acute medical problems including COVID -19 itself, immunological events such as cytokine storm which is the hallmark of COVID-19 pathogenesis, vascular problems, and physical inactivity.

Immobilization that occurs due to COVID-19 is very different from immobilization due to other diseases such as fractures, CHF FC IV or severe pneumonia. Elderly with COVID-19 will be more susceptible to suffering from acute sarcopenia. Study conducted by Mayer et al found that prolonged bed rest in the elderly with COVID-19 who used high-flow oxygen therapy or was treated in the ICU for COVID-19 reduced rectus femoris muscle mass by 18.5% on day 7 of treatment compared to the first day of hospital admission. Other effects include post intensive care syndrome (PICS) with symptoms of muscle weakness and some conditions, including fatigue, anxiety, depression, and sleep disturbances.[29] Immobilization during hospitalization and due to government policy to restrict mobilization will increase the probability of elderly getting a thrombotic event and longer bedrest. Loss of muscle mass does not only start as early as the second decade of life, but it is exacerbated by muscle inactivity.

The complex interaction between predisposing and precipitating factors would lead to mitochondrial dysfunction, mitochondrial autophagy and mitochondrial biogenesis and finally causing decrease of muscle syntesis, increase of myofibral breakdown and muscle degeneration. **Figure 2** showed some of predisposing and precipitating factors that might contributed in elderly with acute sarcopenia.

MOLECULAR MECHANISM OF ACUTE SARCOPENIA IN ELDERLY WITH COVID-19 INFECTION

Elderly are known to be particularly vulnerable to the effects of the illness, with age being associated with not only mortality but may cause more severe clinical presentation leading to development of acute sarcopenia. It has now become clear that survivors of COVID-19 are still at increased risk of acute sarcopenia.

Biological Age and ROS

Aging has many effects towards our body, including endoplasmic reticulum (ER) stress caused by ROS and harmful protein accumulation. Mitochondria induces apoptosis via both caspase-mediated or caspase-independent pathways. Initiator caspases such as caspase-8, caspase-9, caspase-12 will be activated if there is stimuli and leading to the activation of effector caspases (caspase-3, caspase-6, caspase-7) which is responsible for cellular degradation and DNA fragmentation via a caspase-activated DNase (CAD). The stimuli to activate pro caspase to become caspase can divided into two pathways, firstly the extrinsic apoptotic signaling that is initiated by death receptors located on the cell surface, such as the tumor necrosis factor receptor (TNF-R) and the Fas receptor and intrinsic pathways of caspase activation include those triggered by the endoplasmic reticulum (ER) and the mitochondrion. Recently, higher Fas expression on CD4 + T and CD8 + Tcells has been reported in COVID-19 patients than in healthy controls but it needs further study. Under ER stress conditions, ER-specific procaspase-12 can be activated by m-calpain, leading to caspase-3 activation. Along with aging, the caspase-independent apoptotic pathways are expressed more compare to younger age leading to sarcopenia.[30]

Immunosenescence

Aging has been associated with chronic inflammation and increase of inflammation markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) which is well known as a phenotype predictive factor for body composition change, homeostasis and immunosenescence.[16] As humans age, the presence of systemic basal inflammatory mediators increases independently of acute immune challenges in a phenomenon known as inflammaging. Long standing chronic inflammation has been speculated to be main contributor to immunosenescence, a term defined as overall changes to the immune system in elderly, including a reduced ability to combat new infections.[31] Primary goals of the innate immune system in response to a SARS-CoV-2 infection are (1) to initiate a local inflammatory response to activate and recruit immune cells, (2) to directly eliminate virally infected cells and (3) to prime the adaptive immune response. As in elderly, those abilities are either diminished or dysregulated.

Mitochondria plays a central role in the induction and regulation of programmed cell death. A study in experimental animal models has showed a proapoptotic shift in the expression pattern of Bcl-2 proteins (increased Bax and decreased Bcl-2 levels) that was observed in muscles of aged rodents. The release of mitochondria-specific apoptotic mediators is the process of mitochondrial outer membrane permeabilization (MOMP). Once MOMP has occurred, the release of apoptogenic factors stored in the mitochondrial intermembrane space ensues, initiating the series of events that result in cell death. Several studies also showed that caspase-independent apoptotic pathways are activated in elderly. Translocation of mitochondrial EndoG to the nucleus was increased in the soleus muscle of old mice. In addition, aging will increase both cytosolic and nuclear levels of AIF and EndoG and this was was observed in the rat gastrocnemius muscle. Moreover, AIF gene expression progressively increased during aging in the rat plantaris muscle, and was correlated with the progression of sarcopenia. The aforementioned findings support the role of mitochondrial caspase-independent pathway of apoptosis in the pathophysiology of sarcopenia.[32]

Hyperinflammation (Cytokine Storm)

COVID-19 is an infectious disease characterized by an increase in inflammatory cytokines known as cytokine storms. Hyperactivation of the nuclear factor kappa-light-chain- enhancer of activated B cells (NF-κB) pathway has been implicated in the pathogenesis of the severe/critical COVID- 19 infection. NF-κB activation plays major role to the acute respiratory RNA virus-induced cytokine storm. In humans, SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor and with the help of the cellular serine protease TMPRSS2 will trigger endocytosis into the host cell. Within the endosomes, RNA from single-stranded RNA virus is known to activate the Toll-like receptors TLR7 and TLR8. However, as a second major effect the activation of the TLRs can trigger—via various intermediates—the activation of IKK (IκB kinases) resulting in phosphorylation of the cytoplasmic inhibitor factor IκBα triggering its ubiquitination followed by degradation by the 26S proteasome, thereby NF-κB (a heterodimer complex consisting of protein subunits p50 and p65) is released from IκBα.NF-κB transcription factors activation promotes the gene expression of wide variety of cytokines such as IL-1, IL-6, IL-12, TNF-α, LT- α, chemokines, adhesion molecules, acute phase proteins, and inducible effector enzymes. Activation of NF-κB will induce the upregulation of Muscle Ring Finger 1 (MuRF1), a mediator of muscle atrophy and finally result in acute sarcopenia condition.[8]

On the other hand, Caspase-8 which was previously viewed exclusively as an apoptotic caspase, has now emerged as a master regulator of the three major cell death pathways, including apoptosis, pyroptosis, and necroptosis. Some studies found that SARS-CoV-2 induces caspase-8 activation to trigger cell apoptosis and directly activates some inflammatory factors such as pro-IL-1β. The IL-1β is then secreted through the SARS-CoV-2-triggered necroptosis pathway. The caspase-8-mediated apoptosis activation and inflammatory responses

in infected lung epithelial cells may induce downstream immune pathogenesis in the lung tissue. In line with the phenomenon, massive infiltration of inflammatory cells, necrotic cell debris, and pulmonary interstitial fibrosis were observed in the postmortem lung sections of fatal COVID-19 patients.[33] The caspase-8 is an initiator caspases that will activate the effector caspase-3 which are responsible for the cellular degradation and DNA fragmentation via a caspase-activated DNase (CAD) and will cause acute sarcopenia.

Physical Inactivity

Regional quarantine policies and activity restrictions for the purpose of reducing infection transmission also affect the mobility of patients, especially the elderly.[34] Bed rest is associated with decreased muscle quantity, strength and endurance. Bed rest reduces muscle protein synthesis by altering expression of ubiquitin ligases (MuRF-1 and MAFbx).[27] Kortebein et al. found that in 10 days of immobilization in elderly aged 67 ± 5 years, lower extremity mass was decreased by 6.3%, isokinetic strength decreased by 15.6%, ability to climb stairs decreased by 14%, and VO2 max decreased by 2%.[35] The molecular mechanisms that have been implicated in the development of disuse muscle atrophy are Atrogin-1/ Muscle atrophy F-Box (MaFbx)/ Muscle ring finger 1 (MuRF1) pathway, the IGF-1-AKT-mTOR pathway and the Myostatin pathway.[8]

MuRF1 is the only family member shown to be associated with muscle atrophy and to result in the attenuation of muscle loss when deleted. Similar to MuRF1, MAFbx expression is selective to striated muscle. Regulation of MuRF1 and MAFbx expression in skeletal muscle. Skeletal muscle atrophy is induced by a number of stressors. These stressors can lead to the increase in the expression of a number of transcription factors, including the forkhead transcription factors (FOXO1 and FOX03a), NF-κB transcription factors (p65, c-Rel, RelB, p52, and p50), CCAAT/enhancer-binding protein-β (C/EBPβ), kruppel-like factor-15 (KLF-15), and/or activation of the glucocorticoid receptor. These transcriptional mediators can bind to the promoter regions of either the MuRF1 or MAFbx genes, leading to an increase in their expression levels within the muscle. MaFbx and MuRF1 mRNA levels rise in rodent models of immobilisation and associated with increases in proteolysis but not inhibition of protein synthesis.[8]

Some studies has proved the importance of IGF-1 expression in the maintenance of muscle mass. When binding to IGF-1, IGF-1 receptor (IGF-1R) phosphorylates an intracellular adaptor protein insulin receptor substrate-1 (IRS-1), which recruits and phosphorylates phosphoinositide 3-kinase (PI3K) followed by Akt phosphorylation. The PI3K/Akt pathway plays a critical role in myotube hypertrophy, and activation of Akt in rat muscle prevents denervation-induced atrophy. Mammalian target of rapamycin (mTOR) is a downstream target of Akt. The IGF-1/Akt/mTOR pathway has been shown to be play major role in promoting muscle hypertrophy.[36] Immobilization is a negative regulator in IGF-1/Akt/mTOR pathway which inhibited during disuse (unloading)-induced atrophy. IGF-1 also affects protein synthesis via myostatin signalling.

Myostatin is a member of TGF-β family, its expression mainly from skeletal muscle, and negatively regulates muscle mass. IGF-1 and myostatin counteract each other. Myostatin signalling is activated by activin type II receptors (ActRIIA and ActRIIB) and activin type I receptors (ALK4 and ALK5), leading to phosphorylation of Smad proteins (Smad2 and -3). Smad2/3 form a complex with Smad4, which is also a co-mediator of the bone morphogenic protein (BMP) signalling pathway. When myostatin expression is downregulated, Smad4 becomes more available to BMP signalling and will cause muscle hypertrophy. Akt activation is downregulated by ActRIIB and balancing the activation of IGF-1, myostatin, and BMP pathways are critical to maintain muscle mass.[36]

Hormonal Dysregulation

After the age of 60 years, a variety of hormones that promote the growth of muscle cells, such as testosterone, growth hormone (GH), and Insulin-like growth factor 1 (IGF-1) are decreasing. Sex steroids such as estrogen and testosterone decline with aging and contribute to

muscle loss. Testosterone blocks the production of myostatin and ROS, inhibit apoptosis, potentiate myosatellite stem cells, accelerating muscle insulin growth factor-1 (IGF-1) expression, regulate skeletal muscle metabolism and increase muscle protein synthesis rate and muscle mass in elderly man. IGF-1 decrease by 50% by the age of 60. Growth hormone (GH) decrease in aging will also lower muscle mass.[37] Low expression of GH/IGF-1 level in elderly will cause a decrease of protein anabolism in skeletal muscle cells, which ultimately leads to changes in the structure and function of skeletal muscle cells. Study conducted by Ioannis Ilias et al (2021) found that IGF-1 was higher in Covid-19 survivors compared to non-survivors (-0.96 ± 1.89 vs -2.05 ± 2.48, respectively, p=0.030) but no significant differences were noted in GH between the groups. These results suggest that there might be an association between low IGF1 (and possibly GH) and poor outcome in patients with COVID-19.[38]

IGF-1 receptor (IGF-1R) binds to IGF-1and phosphorylates an intracellular adaptor protein insulin receptor substrate-1 (IRS-1), which recruits and phosphorylates phosphoinositide 3-kinase (PI3K) followed by Akt phosphorylation. The PI3K/Akt pathway will induce myotube hypertrophy and activation of Akt in rat muscle prevents denervation-induced atrophy. Activation of Akt activates mammalian target of rapamycin (mTOR) and its activity is tightly regulated by amino acid availability to the cells. Amino acids are necessary to build proteins, nucleic acid, glucose, and ATP in the body, mTOR activity is highly correlated with the anabolic or catabolic balance. The effect of Akt on mTOR is indirect, Akt inhibits the tuberous sclerosis complex (TSC) proteins 1 and 2, which act as a GTPase activating protein (GAP) to inhibit the small G protein Ras homolog enriched in brain (Rheb) which activates mTOR signaling. mTOR consist of two different protein complexes, the rapamycin-sensitive mTORC1 and the rapamycin-insensitive mTORC2. TORC2 is necessary for Akt phosphorylation and activation. mTORC1 phosphorylates S6 kinase (S6K), which in turn phosphorylates the ribosomal protein S6 and other factors involved in translation initiation and elongation, thus stimulating protein synthesis. The IGF-1/Akt/mTOR pathway is very important in promoting muscle hypertrophy. Therefore, decreased level of GH/ IGF-1 plays a key role in the loss of skeletal muscle mass.[39]

ROS, reactive oxygen species; CAD, caspase-activated DNase; DNA, deoxyribonucleic acid; Endo G. endonuclease G; AIF, Apoptosis-inducing factor; TNF, Tumor necrosis factor;

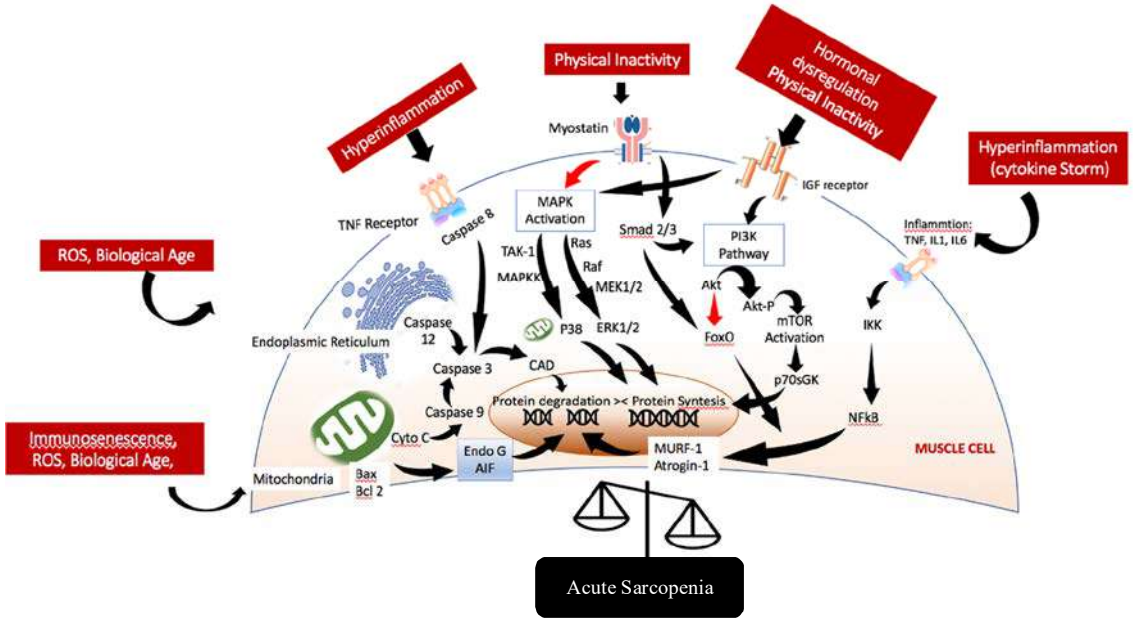

Figure 3. Proposed model of molecular mechanism of acute sarcopenia in elderly with COVID-19 infection.[8,16,30-32,36-39]

TAK-1, transforming growth factor-β-activated kinase 1; MAPKK, Mitogen-activated protein kinase kinase; MEK 1/2, Mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinases; PI3K, Phosphoinositide 3-kinases; FoxO, The forkhead box O; Akt-P, Phosphorylated Akt; mTOR, mammalian target of rapamycin; IL, Interleukine; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; MURF-1, muscle RING-finger protein-1.

IMPLICATION OF ACUTE SARCOPENIA IN ELDERLY WITH COVID – 19

Decreased quality of skeletal muscles is the main cause of poor quality of life, immobilization, disability, falls, fractures, hospitalization, length of stay, hospital readmission, morbidity and mortality in the elderly. Hospitalization, even for a short period of time, is associated with an increased risk of nosocomial infection in sarcopenia patients and a significant decrease in muscle strength and functional capacity.[37]

ICU Admission

Acute sarcopenia is associated with an increased risk of ICU admission and mechanical ventilation. A study conducted by Giraudo et al., (2021) found muscle mass loss was a predictor of patients admitted to the ICU. Patients who lost muscle mass were significantly older (73.4±10 years) and with higher CRP values (71.5±71).[12] Low muscle mass on CT scan results is associated with higher risk of ICU admission and mortality.[40,11] Acute sarcopenia also consider as one of risk factors for difficulty in ventilator weaning.[11] Intensive Care Unit-Acquired Weakness (ICUAW) is a well-known complication following admission to the Intensive Care Unit (ICU). Most relevant risk factors that associated with ICUAW is the severity of underlying critical illness and inflammation. Both of these factor was present in elderly with COVID – 19.[8]

Length of Stay

Research conducted by Sousa et al., (2016) found that acute sarcopenia patients had a longer length of stay. Patients with sarcopenia are less likely to return home and also have longer time in bed.[31] The study by Martone et al., (2017) in hospitalized patients showed that the mean time of acute sarcopenia patients in bed was 5.1 days compared to 3.2 days in non-sarcopenia patients. Muscle protein synthesis is also impaired due to lack of nutrition and physical exercise.[41]

Frailty

Sarcopenia and frailty are two conditions that often coexist. Frailty is a multisystem organ decline characterized by an increase in the patient's susceptibility to stressors.[42] In frailty, there is a decline in the immune, metabolic, and neuromuscular systems. COVID-19 virus binds to ACE2 receptors present in various organs such as the lungs, heart, liver, kidneys, and intestines to enter human cells. COVID-19 patients experienced organ damage where 14% had respiratory failure, 15% had heart damage, 15.7% had liver damage, and 13.7% had kidney problems. Because of this organ damage, COVID-19 patients are prone to becoming frail, especially in elderly patients. Woolford et al., reported that COVID-19 patients had a 1.4 times higher risk in becoming frail.[43] Frailty was significantly associated with an increase in poor outcomes, mortality and morbidity, severity of COVID-19, likelihood of being admitted to the ICU, mechanical ventilation, and length of stay.[44] Research by Zhang et al., (2021) found that frailty was a predictor of mortality in COVID-19 patients. The prevalence of frailty is 51%, with HR 1.99 and OR 2.48.[42] Hewitt et al., (2020) studied frailty in COVID-19 patients and they found that frailty was detected in 49.4% of patients. The 7-day mortality rate increased in frailty patients with an OR of 1.22. Frailty is also associated with longer treatment.[45]

Malnutrition

COVID-19 affects patients of all ages, but has more severe clinical consequences in elderly patients. Some factors might be associated why the elderly with COVID-19 has higher chance to become malnourished. Morphine that is administered to relieve pain associated with breathing and to facilitate respiration will slow gastro-intestinal transit inducing nutritional related complaints like nausea and constipation. Dexamethasone in COVID-patients who require oxygen therapy to improve outcome, often

increase plasma glucose levels necessitating tailored nutrition and insulin therapy which can be a challenge in patients who are already diabetic. COVID-19 has a profound negative impact on nutritional status already before admission. This is proven in several studies showing that in 22% of the patients, mainly ICU patients, lost more than 5 kg extra body weight during hospital stay. It has been demonstrated that in-hospital malnutrition is associated with hospital length of stay (LOS), in-hospital mortality, and re-admission rate.[46] COVID-19 itself results in multiple nutrition-related problems such as changes in appetite, taste and energy expenditure and changes in taste that might further worsen the level of malnutrition.

Malnutrition and sarcopenia often coexist and develop clinically through a combination of decreased nutritional intake, weight loss, then decreased mass, muscle strength, and physical function. This syndrome is called Malnutrition-Sarcopenia Syndrome (MSS). Both conditions are independently associated with morbidity, mortality, decreased quality of life, rehospitalization, length of stay, and costs.[47] A study by Cederholm et al. found a significant difference in mortality after discharge from home between malnourished patients compared to good nutrition at 44% versus 18% respectively.[48] Community studies in patients with unexpected weight loss and low body mass index (BMI) had an increased risk of death at 3 years by 1.67 times.[49] A study by Newman et al. found that even 5% weight loss was a significant predictor of mortality in the elderly in the community.[50] Malnutrition is associated with a decrease in the functional capacity of treated patients based on Mini-Nutritional Assessment (MNA) values <24.[51] Malnourished elderly patients were more likely to continue treatment in nursing homes compared to returning home. This condition is also accompanied by disability, use of assistive devices, loss of muscle mass, risk of metabolic disease, and increased risk of falls and fractures.[47]

CONCLUSION

COVID-19 infection causes acute sarcopenia with various predisposing and precipitating factors such as inflammation, biological age, ROS, altered diet, physical inactivity, hormonal dysregulation and many more. Molecular mechanism of acute sarcopenia in elderly infected with COVID-19 is a complex process involving various molecular pathways. Immunosenescence, ROS, and biological age cause acute sarcopenia through endoplasmic reticulum and mitochondria stress and via caspase pathway. Hyperinflammation and cytokine storm requires IL-6, IL-1, NFkB and MURF-1 to induce acute sarcopenia. IGF-1 is the main pathway involved in acute sarcopenia due to hormonal dysregulation whereas physical inactivity has three different pathways to induce acute sarcopenia, they are Atrogin-1/ MaFbx/ MuRF1 pathway, the IGF-1-AKT-mTOR pathway and the Myostatin pathway. Sarcopenia conditions can also worsening COVID-19 infection and vice versa and leading to longer length of stay including treatment in the ICU, immune dysregulation, frailty conditions, and the occurrence of malnutrition.

REFERENCES

1. Susilo A, Rumende CM, Pitoyo CW, et al. Coronavirus disease 2019: tinjauan literatur terkini Coronavirus disease 2019. 2020;7:45–67.
2. World Health Organizarion. WHO Coronavirus disease (COVID-19). Dashboard. [accessed 2nd October 2021]. URL: https://covid19.who.int/
3. Satgas penanganan Covid-19. Data sebaran. [accessed 2nd October 2021]. URL: https://covid19.go.id/4.
4. Muhammad Khifzhon Azwar, Siti Setiati, Aulia Rizka, Ika Fitriana, Siti Rizny F. Saldi, Eka Dian Safitri. Clinical profile of elderly patients with COVID-19 hospitalised in Indonesia's National General Hospital. Acta medica Indonesiana 52, 199–205.
5. Kang SJ, Jung SI. Age-related morbidity and mortality among patients with COVID-19. Infect Chemother. 2020;52:154.
6. United Nations. World population ageing 2019 highlights. New York: United Nations (2019).
7. Setiati, S., Dwimartutie, N. Buku ajar ilmu penyakit dalam. Jilid III. Edisi VI. Jakarta: Interna Publishing; 2014. p. 3719–27.
8. Welch CK, Hassan-Smith ZA, Greig CM, et al. Acute sarcopenia secondary to hospitalisation - an emerging condition affecting older adults. Aging and disease. 2018;9:151.
9. Zhang XM, et al. Sarcopenia as a predictor of mortality among the critically ill in an intensive care unit: a systematic review and meta-analysis. BMC Geriatr.

2021;21:339.
10. Weijs P.J, et al. Low skeletal muscle area is a risk factor for mortality in mechanically ventilated critically ill patients. Crit Care. 2014;18:R12.
11. Kou HW, et al. Sarcopenia is an effective predictor of difficult-to-wean and mortality among critically ill surgical patients. PLoS ONE. 2019;14:e0220699.
12. Giraudo, C. et al. Reduced muscle mass as predictor of intensive care unit hospitalization in COVID-19 patients. PLoS ONE. 2021;16:e0253433.
13. C. F., Isirdi A, Brusasco C, et al. Low diaphragm muscle mass predicts adverse outcome in patients hospitalized for Covid-19 pneumonia. 2020.
14. Welch C, Greig CA, Masud T, Pinkney T, Jackson TA. Protocol for understanding acute sarcopenia: a cohort study to characterise changes in muscle quantity and physical function in older adults following hospitalisation. BMC Geriatr. 2020;20:239.
15. Aiello A, et al. Immunosenescence and its hallmarks: How to oppose aging strategically? A review of potential options for therapeutic intervention. Front. Immunol. 2019;10:2247.
16. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol Series A: Biol Sci Med Sci. 2014;69:S4–S9.
17. Perrotta F, et al. COVID-19 and the elderly: insights into pathogenesis and clinical decision-making. Aging Clin Exp Res. 2020;32:1599–608.
18. Diaz JH. Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. J Travel Med. 2020;27:taaa041.
19. Baker, SA, Kwok S, Berry GJ, Montine TJ. Angiotensin-converting enzyme 2 (ACE2) expression increases with age in patients requiring mechanical ventilation. PLoS ONE. 2021;16:e0247060.
20. Rehman S, et al. Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol. 2021;12:680845.
21. Ory J, et al. Understanding the complex relationship between androgens and SARS-CoV2. Urology. 2020;144:1–3.
22. Cruz-Jentoft AJ, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age and Ageing. 2019;48:16–31.
23. Domingues R, Lippi A, Setz C, Outeiro TF, Krisko A. SARS-CoV-2, immunosenescence and inflammaging: partners in the COVID-19 crime. Aging. 2020;12:18778–89.
24. Thoma A, Akter-Miah T, Reade RL, Lightfoot AP. Targeting reactive oxygen species (ROS) to combat the age-related loss of muscle mass and function. Biogerontol. 2020;21:475–84.
25. Dodds RM, Granic A, Robinson SM, Sayer AA. Sarcopenia, long-term conditions, and multimorbidity: findings from UK Biobank participants. Journal of Cachexia, Sarcopenia and Muscle. 2020;11:62–8.
26. Cleasby ME, Jamieson PM, Atherton PJ. Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. Journal of Endocrinology. 2016;229:R67–R81.
27. Welch C, Greig C, Masud T, Wilson D, Jackson TA. COVID-19 and acute sarcopenia. Aging and disease. 2020;11:1345.
28. Conti P. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by COVID-19: anti-inflammatory strategies. J Biol Regul Homeost Agents. 2020;34:1.
29. Stam H, Stucki G, Bickenbach J. Covid-19 and post intensive care syndrome: A call for action. J Rehabil Med. 2020;52:jrm00044.
30. Marzetti E, et al. Multiple pathways to the same end: Mechanisms of myonuclear apoptosis in sarcopenia of aging. Sci World J. 2010;10:340–9.
31. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol. 2018;15:505–22.
32. Dupont-Versteegden EE. Apoptosis in muscle atrophy: Relevance to sarcopenia. Experimental Gerontology. 2005;40:473–81.
33. García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. Front Immunol. 2020;11:1441.
34. Piotrowicz K, Gąsowski J, Michel JP, Veronese N. Post-COVID-19 acute sarcopenia: physiopathology and management. Aging Clin Exp Res. 2021;33: 2887–98.
35. Kortebein P, et al. Functional impact of 10 days of bed rest in healthy older adults. J Gerontol Series A: Biol Sci Med Sci. 2008;63:1076–81.
36. Yoshida T, Delafontaine P. Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. Cells. 2020;9:1970.
37. Ali AM, Kunugi H. Physical frailty/sarcopenia as a key predisposing factor to Coronavirus disease 2019 (COVID-19) and its complications in older adults. BioMed. 2021;1:11–40.
38. Ilias I, et al. Covid-19 and growth hormone/insulin-like growth factor 1: Study in critically and non-critically ill patients. Front Endocrinol. 2021;12:644055.
39. Guertin DA, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCα, but Not S6K1. Developmental Cell. 2006;11:859–71.
40. Schiaffino S, et al. CT-derived chest muscle metrics for outcome prediction in patients with COVID-19. Radiology. 2021;300:E328–E336.
41. Martone AM, et al. The incidence of sarcopenia among hospitalized older patients: results from the Glisten study: Hospitalization and risk of incident sarcopenia. Journal of Cachexia, Sarcopenia and Muscle. 2017;8: 907–14.

42. Zhang XM, et al. Frailty as a predictor of mortality among patients with COVID-19: a systematic review and meta-analysis. BMC Geriatr. 2021;21:186.
43. Woolford SJ, et al. COVID-19 and associations with frailty and multimorbidity: a prospective analysis of UK Biobank participants. Aging Clin Exp Res. 2020;32:1897–905.
44. Yang Y, et al. The impact of frailty on COVID-19 outcomes: a systematic review and meta-analysis of 16 cohort studies. J Nutr Health Aging. 2021;25: 702–9.
45. Hewitt J, et al. The effect of frailty on survival in patients with COVID-19 (COPE): a multicentre, European, observational cohort study. The Lancet Public Health. 2020;5:e444–e451.
46. Hudson L, Chittams J, Griffith C, Compher C. Malnutrition identified by academy of nutrition and dietetics/american society for parenteral and enteral nutrition is associated with more 30-day readmissions, greater hospital mortality, and longer hospital stays: a retrospective analysis of nutrition A. J Parent Enter Nutr. 2018;42:892–7.
47. Vandewoude MFJ, Alish CJ, Sauer AC, Hegazi RA. Malnutrition-sarcopenia syndrome: is this the future of nutrition screening and assessment for older adults? J Aging Res. 2012;2012:1–8.
48. Cederholm T, Jägren C, Hellström K. Outcome of protein-energy malnutrition in elderly medical patients. Am J Med. 1995;98:67–74.
49. Locher JL, et al. Body mass index, weight loss, and mortality in community-dwelling older adults. J Gerontol Series A: Biol Sci Med Sci. 2007;62: 1389–92.
50. Newman AB, et al. Weight change and the conservation of lean mass in old age: the health, aging and body composition study. Am J Clin Nutr. 2005;82:872–8.
51. Neumann SA, Miller MD, Daniels L, Crotty M. Nutritional status and clinical outcomes of older patients in rehabilitation. J Hum Nutr Diet. 2005;18: 129–36.

Pulmonary Embolism in Hospitalized Patient with Coronavirus Disease 2019 (COVID-19)

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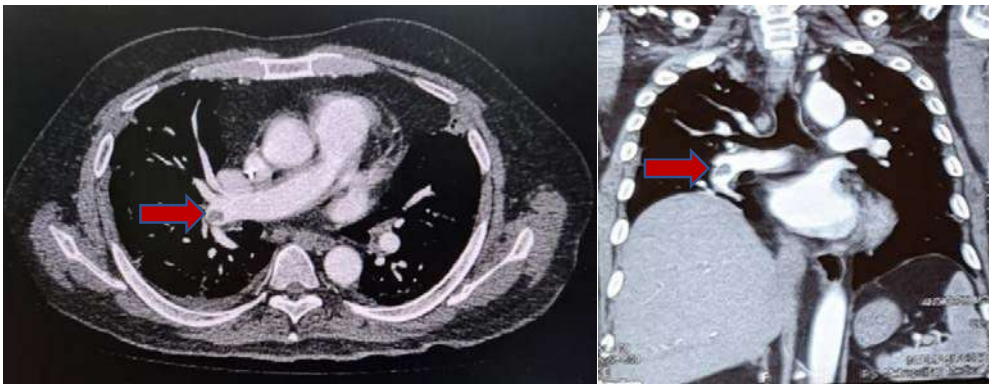

Figure 1. (A) and (B) CT-pulmonary angiography showed intra-luminary hypodense lesion in posterior and basal segment of right pulmonary artery suggesting pulmonary embolism.

Coronavirus disease 2019 (COVID-19) pandemic has affected healthcare policy globally more than ever. For the first time, a global pandemic was studied meticulously by scientists and clinicians worldwide. The virus was initially considered to be a respiratory disease. Meanwhile, several discoveries have been made, including the impact of this infectious disease on coagulation. Early studies showed that patients with COVID-19 had elevated D-dimer levels.[1] These findings were confirmed by autopsies which showed thrombosis in various sites in patients that died from COVID-19.[1]

Thrombosis occurs in up to one-third of hospitalized patients with COVID-19,[2] it happens at many sites by the diffuse activation of coagulation cascade. Furthermore, pulmonary embolism have been reported to be the most dangerous thrombotic event which greatly increases mortality in COVID-19.[2] However, the diagnosis of this condition is frequently overlooked. Pulmonary angiogram or Computed Tomography (CT) Pulmonary Angiogram is often difficult to obtain, meanwhile, echocardiogram and electrocardiogram might show pathognomonic abnormalities suggesting pulmonary embolism but lack sensitivity and specificity. Therefore, the demonstration of intraluminal filling defect by CT pulmonary angiogram or fluoroscopic pulmonary angiogram is considered the gold standard of diagnosis.

A 54-year-old male was presented to the emergency department and complained of worsening dyspnea associated with fever and cough 9 days before admission. The nasopharyngeal and oropharyngeal SARS-CoV-2 polymerase chain reaction (PCR) swab test was performed and the result was positive. The patient was hospitalized at COVID-19 isolation intensive care unit (ICU) with ventilatory support due to acute respiratory distress syndrome (ARDS) for 9 days. Fortunately, a good clinical response was achieved without endotracheal intubation and mechanical ventilation, i.e., good chest X-ray response without duplex pneumonia, and tested negative to the nasopharyngeal and oropharyngeal PCR swabs. Afterwards, the patient was transferred to non-COVID-19 ICU and later to the general ward after 3 days. However, in the inpatient ward, a sudden incidence of dyspnea, cough, and nervousness were observed. On physical examination, the patient was alert but moderately ill, blood pressure was 140/90 mmHg, heart rate 110x/ minute, respiratory rate 20x/ minute, and afebrile. Furthermore, peripheral O_2 saturation was 90% and improved to 98% with 15 L/ minute oxygen supplementation via non-rebreathing mask, while the laboratory values include Hb 14.5 g/dL, white blood cells (WBC) 9,810 cells/ μL, and platelets 276,000 cells/μL. Liver and renal function tests were within normal limits as well as random blood glucose and electrolytes. D-dimer was extremely elevated to 14,150 ng/ mL (reference value: <500 ng/mL), while chest X-ray imaging showed no infiltrates in both lungs. A doppler ultrasound was performed on both extremities and the result indicated chronic venous insufficiency, while CT-pulmonary angiography showed intra-luminary hypodense lesion in the posterior and basal segment of right pulmonary artery. The diagnosis of post-severe COVID-19 pneumonia-ARDS pulmonary embolism was established.

Low molecular weight heparin (LMWH): Enoxaparin 6,000 IU (0.6 mL) bid was administered subcutaneously to the patient. After 10 days, the clinical condition improved, the patient was discharged with direct oral anticoagulant Rivaroxaban 15 mg bid for 21 days and 20 mg qd for 3-6 months.

Hospitalized patients with COVID-19 are at high risk of thromboembolic complications such as deep vein thrombosis (DVT) and pulmonary embolism (PE).[3] A systematic review and meta-analysis by Suh, et al[4] reported that pooled incidence rates of PE and DVT in patients with COVID-19 were 16.5% (95%CI: 11.6-22.9) and 14.8% (95%CI: 8.5-24.5) respectively. Incidence of PE was higher in patients admitted to the ICU i.e. 24.7% (95% CI: 18.6-32.1) compared to 10.5% (95% CI: 5.1-20.2) in patients admitted to the general ward.[4]

About two centuries ago, German physician Rudolph Ludwig Karl Virchow described 3 factors that contribute to thrombosis namely endothelial vessel wall injury, venous stasis, and hypercoagulability widely known as the "Virchow's triad".[5] The primary function of the endothelium is maintenance of nonturbulent blood flow with homeostatic mechanisms to prevent thrombosis.[6] Meanwhile, viral particles of the SARS-CoV-2 virus penetrate the cells through the binding of spike-like protein (S-protein) with the angiotensin-converting enzyme 2 (ACE2) receptor. The ACE2 receptor for SARS-CoV-2 is also present in endothelial cells. This endothelial invasion by SARS-CoV-2 leads to endothelial dysfunction and activates coagulation cascade, thereby contributing to thrombosis.[7] Furthermore, immobilization due to dyspnea and hypoxia induces thrombosis due to venous stasis and increasing blood viscosity.[8] Meanwhile, COVID-19-related hypercoagulable state and thrombotic complications are usually due to the increased thrombo-inflammation secondary to infection. This leads to severe hemostatic instability as typically seen in septic patients which promotes coagulation. Other complications include weakening of anticoagulation and fibrinolysis.[9]

Pulmonary embolism is a part of venous thromboembolism together with deep vein thrombosis (DVT).[10] As described above, COVID-19 hypercoagulable state leads to activation of the coagulation cascade. This process occurs throughout systemic circulation. In a classic case of pulmonary embolism, about 70% of patients have lower extremity

DVT.[10] However, in this study, lower extremity ultrasound did not find any evidence of DVT. The pathophysiology of hypercoagulation in COVID-19 differs from classic cascade activation caused by other infections. Given that pulmonary involvement is common in COVID-19, researchers have postulated a hypothesis on the pathogenesis of this coagulation process, which is termed pulmonary intravascular coagulation (PIC).[11] In the PIC process, the binding of SARS-CoV-2 to ACE 2 receptor on type II pneumocytes and endothelium increases procoagulant activity and activates the coagulation cascade.[11] This mechanism is assumed to be responsible for the formation of microthrombi in the lung capillaries. This process continues and promotes systemic coagulation. The thrombus produced travel through the systemic circulation and clog the pulmonary artery.

Several available modalities are used to diagnose pulmonary embolism. Point of care testing such as electrocardiography and bedside echocardiography aid pulmonary embolism diagnosis. However, these techniques lack sensitivity and specificity.[10] The classic McGinn White ($S_1Q_3T_3$) pattern occurs rarely. The electrocardiogram is useful in exploring the possibility of other differential diagnoses such as acute myocardial infarction and pericarditis.[10] Similarly, echocardiography is used to evaluate indirect signs of pulmonary embolism. The thrombus is rarely demonstrated in the echocardiogram.

Lung scintigraphy or ventilation-perfusion scan was used extensively in patients suspected of pulmonary embolism. However, perfusion defects might also be caused by other pathologies such as lung consolidation, fibrosis, and malignancy. In this COVID-19 pandemic era, CT pulmonary angiography is a versatile modality. Aside from being used to diagnose or exclude pulmonary angiography, the lung window also provides information on the extent of damage caused by COVID-19 to the lungs i.e., the amount of ground glass opacities. This modality also provides information on consolidation, tumors, and pleural diseases.

In acutely ill patients at general wards, initial treatment with LMWH have advantages in terms of minimal drug–drug interactions and risk of rapid clinical deterioration compared to oral treatment. Current guidelines by the International Society of Thrombosis and Hemostasis (ISTH) suggest LMWH for thromboembolism prophylaxis in hospitalized COVID-19 patients.[12] This is due to the simplicity in administration and low risk of drug-drug interaction. In cases of acute VTE, anticoagulants are given in therapeutic doses. In this study, the patient was treated with enoxaparin 6.000 IU (0.6 mL) bid subcutaneously.

Direct Oral Anti Coagulants (DOACs) provide advantages over vitamin K antagonists (VKAs), especially in the post-hospital setting, because it is safer, and does not require routine monitoring. Nevertheless, in hospitalized COVID-19 patients, DOAC is not recommended due to potential drug-drug interaction with antivirals.[13] Furthermore, VTE in COVID-19 patients is induced by a reversible risk factor, therefore, a treatment duration of 3 months is advised. An initial LMWH. Enoxaparin 0.6 mL was administered twice daily to the patient and then Rivaroxaban after discharge for 3 months.[14] Rivaroxaban and other DOACs are considered safe after discharge given that the patient had already finished the prescribed antibiotics and antivirals.

COVID-19 is often accompanied by thromboembolic complications. Meanwhile, pulmonary embolism occurs in COVID-19 patients without DVT. Based on the results, parenteral anticoagulant followed by DOAC is the mainstay of treatment in COVID-19 coagulopathy.

REFERENCES

1. Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood. 2020;136(4):489-500.
2. Loo J, Spittle DA, Newnham M. COVID-19, immunothrombosis and venous thromboembolism: biological mechanisms. Thorax. 2021;76(4):412-20.
3. Cuker A, Tseng EK, Nieuwlaat R, et al. American Society of Hematology 2021 guidelines on the use of anticoagulation for thromboprophylaxis in patients with COVID-19. Blood Adv. 2021;5(3):872-88.
4. Suh YJ, Hong H, Ohana M, et al. Pulmonary embolism and deep vein thrombosis in COVID-19: A systematic review and meta-analysis. Radiology

2021;298(2):E70-e80.
5. Mehta JL, Calcaterra G, Bassareo PP. COVID-19, thromboembolic risk, and Virchow's triad: Lesson from the past. Clin Cardiol. 2020;43(12):1362-7.
6. Gąsecka A, Borovac JA, Guerreiro RA, et al. Thrombotic complications in patients with COVID-19: pathophysiological mechanisms, diagnosis, and treatment. Cardiovasc Drugs Ther. 2021;35(2):215-29.
7. Ahmed S, Zimba O, Gasparyan AY. Thrombosis in Coronavirus disease 2019 (COVID-19) through the prism of Virchow's triad. Clinical Rheumatology. 2020;39(9):2529-43.
8. Sakr Y, Giovini M, Leone M, et al. Pulmonary embolism in patients with coronavirus disease-2019 (COVID-19) pneumonia: a narrative review. Annals of Intensive Care. 2020;10(1):124.
9. Ozsu S, Gunay E, Konstantinides SV. A review of venous thromboembolism in COVID-19: A clinical perspective. Clin Respir J. 2021;15(5):506-12.
10. Riedel M. Acute pulmonary embolism 1: pathophysiology, clinical presentation, and diagnosis. Heart. 2001;85(2):229-40.
11. Belen-Apak FB, Sarıalioğlu F. Pulmonary intravascular coagulation in COVID-19: possible pathogenesis and recommendations on anticoagulant/thrombolytic therapy. J Thromb Thrombol. 2020;50(2):278-80.
12. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020.
13. Hashemi A, Madhavan MV, Bikdeli B. Pharmacotherapy for prevention and management of thrombosis in COVID-19. Seminars in Thrombosis and Hemostasis. 2020;46(7):789-95.
14. Flaczyk A, Rosovsky RP, Reed CT, Bankhead-Kendall BK, Bittner EA, Chang MG. Comparison of published guidelines for management of coagulopathy and thrombosis in critically ill patients with COVID 19: implications for clinical practice and future investigations. Crit Care. 2020;24(1):559.

Effectiveness of Convalescent Plasma Therapy in Treating COVID-19: an Evidence-based Case Report

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ABSTRACT

Background: *Convalescent plasma is a potentially beneficial, tolerable, and available additional treatment option for COVID-19. This study aims to evaluate whether the administration of convalescent plasma therapy leads to improved clinical outcomes in COVID-19 patients compared to standard medical therapy.* ***Methods:*** *We conducted a search of Pubmed, Cochrane, and EBSCO for studies assessing the clinical question using inclusion and exclusion criteria. Selected studies were critically appraised, and the results were summarized.* ***Results:*** *A meta-analysis of 10 randomized clinical trials (RCTs), an RCT, a case-control clinical study were selected and assessed. Only the case-control clinical study showed that convalescent plasma administration improved the clinical outcomes of patients with COVID-19, including all-cause mortality, hospital length of stay, and the need for mechanical ventilation. On the contrary, the other two studies of a higher level of evidence showed no significant clinical outcome improvement with convalescent plasma therapy.* ***Conclusion:*** *The effectiveness of convalescent plasma therapy in improving clinical outcomes of patients with COVID-19 was still inconclusive due to several study limitations and other possible causes.*

Keywords: *convalescent plasma, coronavirus, COVID-19, SARS-CoV-2*

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been an ongoing pandemic for more than a year. According to current guidelines, the treatment of COVID-19 is still mainly supportive with additional drugs such as antibiotics or antiviral agents with limited evidence.[1,2] SARS-CoV-2 entry into host cells is facilitated by the spike (S) protein, which has two functional subunits (S1 and S2). The receptor-binding domain (RBD) within S1 binds the angiotensin-converting enzyme 2 (ACE2) receptor, enabling viral attachment to the target cell's surface.[3–5]

Convalescent Plasma (CP) is a type of passive immunization obtained through apheresis from survivors of prior infections by pathogens of interest. A key component of CP is the neutralizing antibodies (NAbs). NAbs in CP from COVID-19 survivors can bind S1-RBD, inhibiting viral attachment and entry to host cells.[3,6] In addition, complement activation, antibody-dependent cellular cytotoxicity, and phagocytosis are other antibody-mediated pathways thought to contribute to viral clearance

by CP.[6] CP also contains non-neutralizing antibodies (Non-NAbs) that may contribute to prophylaxis or even enhance recovery through mechanisms still unknown.[7] Apheresis may also yield other proteins including anti-inflammatory cytokines, coagulation factors, natural antibodies, and defensins, that might provide additional benefits such as immunomodulatory effects by relieving cytokine storm in severe COVID-19.[6]

CP has been considered an emergency intervention in pandemics in the past, such as the Spanish flu and SARS-CoV, where early administration of CP resulted in reduced mortality in severe acute respiratory infections with no adverse events.[6] CP is rapidly available, requires relatively low technology, is easily scalable as long as donors are available, and requires low cost compared to other options such as the interleukin-6 inhibitors.[8,9] Considering its potential benefit, tolerability, and availability, CP is a good candidate for COVID-19 therapy, especially in low-to-middle income countries.[3] This report aims to assess the effectiveness of CP therapy in treating COVID-19.

CASE ILLUSTRATION

Case 1

A 57-year-old female patient was admitted with a fever since four days before admission. She had shortness of breath, dry cough, throat discomfort, nausea, and a bitter taste in her mouth, leading to decreased appetite. The patient lived in an area with local transmission of COVID-19. The patient had type 2 diabetes mellitus and hypertension with routine oral medication of glibenclamide, acarbose, metformin, amlodipine. On initial examination, the patient was alert but had tachypnea, rales on bilateral lungs, and oxygen desaturation; then, she was confirmed to have COVID-19 by RT-PCR. On her third day of admission (day 7 of symptom onset), the patient had just been transferred to the high-care unit (HCU) due to oxygen desaturation and diabetic ketoacidosis (DKA). The DKA had resolved, but the difficulty breathing and a dry cough remained. The patient was alert with a blood pressure of 133/69 mmHg, pulse 91 bpm, respiratory rate 18 rpm, temperature 36°C, SpO_2 85% on 15 LPM oxygen via non-rebreathing mask (NRM).

Lab results showed leukocytosis (10580 / mcL) with high neutrophil (85.6%) and low lymphocyte (10.5%) counts (NLR 8.16), a respiratory alkalosis and oxygen desaturation (pH 7.508, pCO_2 23.4 mmHg, pO_2 43.7 mmHg, HCO_3 18.7 mmHg, SaO_2 85.1%). Prothrombin time (PT) was 10.6 s (control 11.4 s), aPTT was 34.4 s (control 31.1 s), and D-Dimer level was 420 ng/mL, IL-6 level was 207 pg/mL. Chest X-ray showed inhomogenous opacities, some nodular, at upper-middle-lower segments of both lungs suggestive of pneumonia.

The patient was assessed with the diagnoses below:

1. Confirmed severe COVID-19 (day 7 of symptom onset)
2. Moderate-severe Acute Respiratory Distress Syndrome (ARDS)
3. Type 2 Diabetes Mellitus on Insulin regulation with history of DKA
4. Controlled Hypertension
5. Hypercoagulable state

Therapies administered to the patient included 15 LPM oxygen via NRM, ceftriaxone 2 g i.v. q.d., levofloxacin 750 mg i.v. q.24h, remdesivir 200 mg i.v. (D1), then 100 m.g. i.v. q.d., Other therapies include IV insulin, a prophylactic dose of heparin, candesartan, vitamin D3, and acetylcysteine.

Case 2

A 49-year-old female patient was admitted with shortness of breath since three days before admission, accompanied by dry cough, fatigue, and nausea. She had no past medical history of other diseases. Physical examination and blood tests were done, and the patient was assessed with acute respiratory distress syndrome (ARDS), severe confirmed COVID-19, and had elevated liver transaminase levels. At the time of this report, the patient was in her third day of hospital stay in the standard COVID-19 ward and considered to be transferred to the HCU due to unresolved shortness of breath and desaturation after two days of standard therapy, including an antiviral agent, an antibiotic, and an intravenous corticosteroid (dexamethasone 5 mg

i.v. b.i.d.). The patient was alert; blood pressure was 140/100 mmHg, pulse 90 bpm, respiratory rate 22 rpm, temperature 36.5 °C. The patient was already on a high-flow nasal cannula (HFNC) with FiO2 90% 60 LPM and a SpO2 of 93-94%.

CLINICAL QUESTION

Does the administration of Convalescent Plasma (CP) Therapy lead to improved clinical outcomes (such as reduced mortality, length of hospital stay, and need for mechanical ventilation) in COVID-19 patients compared to standard medical therapy alone?

METHODS

A search of Pubmed, Cochrane, and EBSCO was performed on March 29, 2021, using the search terms "COVID-19" and "convalescent plasma" along with their synonymous and related terms. The search strategy used, results, inclusion criteria, and exclusion criteria can be seen in **Figure 1**. Selected articles include completed clinical trials performed on human subjects, systematic reviews or meta-analyses of these trials, published within two years. These articles were then critically appraised based on the appraisal tools from the Centre for Evidence-Based Medicine, University of Oxford.

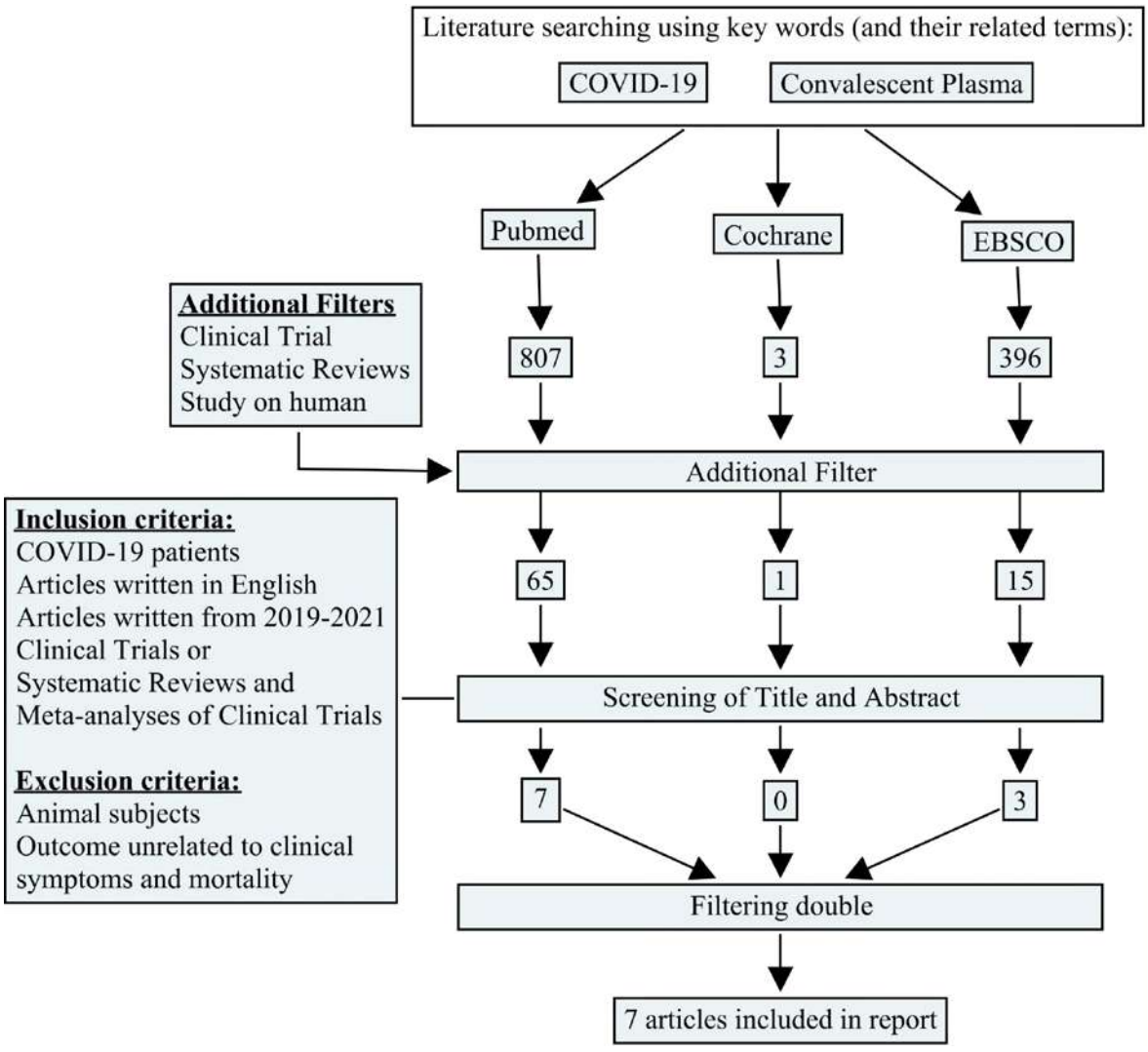

Figure 1. Flow chart of the search strategy.

RESULTS

The search criteria, additional filters, and screening of title and abstract described in **Figure 1**, 10 articles fulfilled the inclusion and did not meet the exclusion criteria. Three of the studies were duplicates; hence we only included seven articles in the report. The articles include a meta-analysis by Janiaud et al.[10], five randomized controlled trials (RCTs), and a non-randomized multicenter clinical trial. Four of the RCTs, written by Simonovich et al.[11], Libster et al.[12], Li et al.[13], and the PLACID trial by Agarwal et al.[14] were already reviewed and analyzed in the meta-analysis by Janiaud et al.[10], hence will not be discussed separately in this report as the two remaining studies by Balcells et al.[15] and Abolghasemi et al.[16] Critical Appraisal of the meta-analysis and clinical trials are described in **Table 1** and **Table 2**. The studies were considered applicable to the patient in this report with the same diagnosis of COVID-19, and the patient had agreed to the use of convalescent plasma with no contraindication of the therapy.

The meta-analysis by Janiaud et al.[10] consisted of primary analysis of 4 peer-reviewed RCTs and secondary analysis including additional 6 RCTs not published in peer-reviewed journals. The analyses were conducted for clinical outcomes of all-cause mortality, length of hospital stay, and mechanical ventilation use (**Figure 2**). Certainty of evidence in all-cause mortality was low in the primary analysis but moderate in the secondary analysis. The RECOVERY trial dominated the evidence since it accounted for most of the weight (90.2%) in the meta-analysis and the number of patients.[10] Results of the three studies are summarized in **Table 3**.

DISCUSSION

The treatment of COVID-19 has continued to be extensively studied since the start of its pandemic, but there is still limited evidence in therapies currently used in guidelines and clinical practice. Convalescent plasma (CP) therapy has proved to be a rapidly obtainable and useful emergency treatment option in previous

Table 1. Critical appraisal of the study by Janiaud et al.[10]

Articles	Validity					Level of Evidence
	PICO	Relevant studies included	Appropriate search criteria	Quality assessment of trials	Heterogeneity	
Jainaud et al.	+	+	+	+	+	1

Table 2. Critical appraisal of articles by Abolghasemi et al.[16] and Balcells et al.[15]

Articles	Time Published	Study Design	No. of Patients	Validity					Importance			Level of Evidence
				Randomization	Similarity at Start of Trial	Equal Treatment	Intention-to-treat	Blinding	Measurement of Outcome	Treatment Effect Result	Point Estimate (CI)	
Abolghasemi et al.14	Jul-20	Case control	189	-	+	+	+	-	Comparative study	+	-	4
Balcells et al.13	Mar-21	RCT	58	+	+	+	+	-	OR	+	+	2

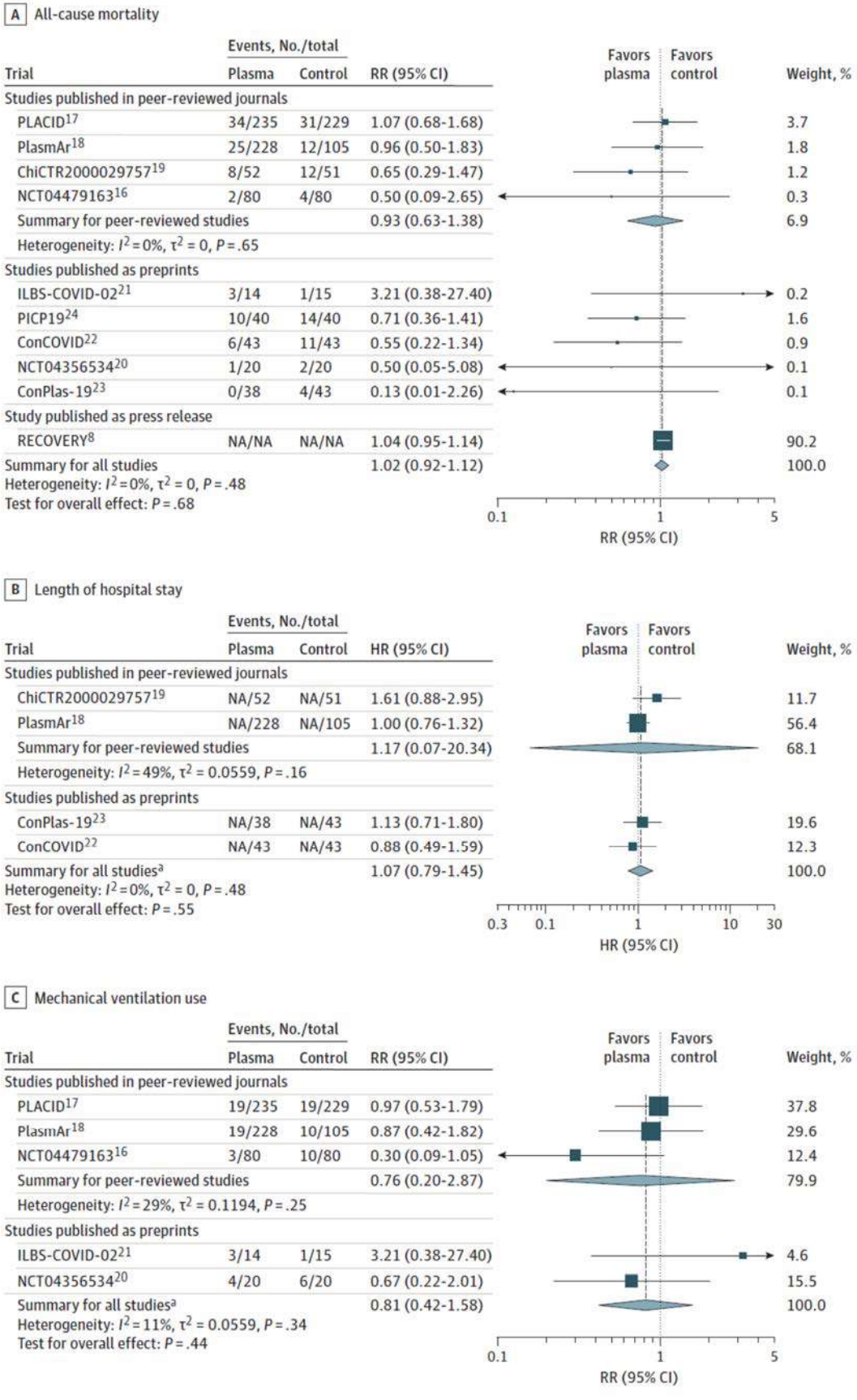

Figure 2. Forest plot of the association between convalescent plasma therapy and clinical outcomes in the meta-analysis by Janiaud et al.[10]

Table 3. Results of the three studies.

Authors	Endpoints	Result	Summary
Janiaud et al. [10]	All-cause mortality, length of hospital stay, mechanical ventilation use	Primary analysis of 4 peer-reviewed RCTs: - Relative risk (RR) for all-cause mortality with convalescent plasma: 0.93 (95% CI 0.63 to 1.38; P = 0.60), absolute risk difference: -1.21% (95% CI, -5.29% to 2.88%) - Hazard Ratio (HR) for length of hospital stay with convalescent plasma: 1.17 (95% CI 0.07 to 20.34; P = 0.61) - Relative risk (RR) for mechanical ventilation with convalescent plasma: 0.76 (95% CI 0.20 to 2.87; P = 0.35), absolute risk difference: -2.56% (95% CI, -13.16% to 8.05%) Secondary analysis including additional 6 pre-published RCTs (a total of 10 RCTs): - Relative risk (RR) for all-cause mortality with convalescent plasma: 1.02 (95% CI 0.92 to 1.12; P = 0.68) - Hazard Ratio (HR) for length of hospital stay with convalescent plasma: 1.07 (95% CI 0.79 to 1.45; P = 0.87) - Relative risk (RR) for mechanical ventilation with convalescent plasma: 0.81 (95% CI 0.42 to 1.58; P = 0.88), absolute risk difference: -2.21% (95% CI, -8.94% to 4.51%)	Treatment with convalescent plasma in addition to standard of care compared to standard of care only or standard placebo in addition to standard of care was not significantly associated with any of the clinical outcome benefits (all-cause mortality, length of hospital stay, mechanical ventilation use) among COVID-19 patients
Abolghasemi et al. [16]	All-cause mortality, length of hospital stay, need for mechanical ventilation	- All-cause mortality: plasma 14.8% vs control 24.3% (p = 0.09), absolute reduction of 9.5% - Length of hospital stay: plasma 9.54 days vs control 12.88 days (p = 0.002) - Need for mechanical ventilation: plasma 7% vs control 20.3% (p = 0.006)	Statistically significant reduction in length of hospital stay and need for mechanical ventilation in plasma group compared to control, but not in all-cause mortality
Balcells et al. [15]	Composite and individual outcomes of in-hospital death, use of mechanical ventilation, and length of hospitalization >14 days (prolonged hospital stay)	- Composite of the three outcomes of early vs deferred plasma group: 32.1% vs 33.3%, OR 0.95, 95% CI 0.32-2.84, p > 0.999 - In-hospital death of early vs deferred plasma group: 17.9% vs 6.7%, OR 3.04, 95% CI 0.54-17.17, p = 0.246 - Use of mechanical ventilation in early vs deferred plasma group: 17.9% vs 6.7%, OR 3.04, 95% CI 0.54-17.17, p = 0.246 - Length of hospital stay >14 days (prolonged hospital stay) in early vs deferred plasma group: 21.4% vs 30.0%, OR 0.64, 95% CI, 0.19-2.10, p = 0.554	No significant difference in either the composite or individual outcomes (in-hospital death, use of mechanical ventilation, and prolonged hospital stay) between the early and deferred plasma group

pandemics.[6] Studies have also shown that CP therapy has a good safety profile in patients with COVID-19.[17,18] More than a hundred ongoing clinical trials were still studying CP in COVID-19 patients.[19]

Based on the three studies we analyzed, only one study (a case-control clinical study) showed that CP therapy improved the clinical outcomes of patients with COVID-19, including all-cause mortality, hospital length of stay, and the need for mechanical ventilation.[16] On the other hand, a systematic review and meta-analysis of 10 RCTs and another open-label RCT not included in the prior study found no significant association between CP therapy and those clinical outcomes. The reasons for these results have been elaborated in the studies themselves.[10,15]

The meta-analysis by Janiaud et al. mentioned five of its limitations. First, three out of ten RCTs analyzed had some concerns or a high risk

of bias. Still, they only contributed to 10.8% of weight in all-cause mortality metanalysis (majority dominated by RECOVERY trial). Second, the RECOVERY trial was insufficient and inconsistent in using definitions and relevant details in reporting clinical outcomes besides all-cause-mortality. Third, the data were too limited for meaningful subgroup analyses. Fourth, in all but 1 RCT with outpatients, all patients were hospitalized, indicating moderate to critically ill COVID-19 patients. Therefore, results in patients with milder COVID-19 remained unclear. Lastly, the study also mentioned the many ongoing trials studying CP therapy in COVID-19 patients.[10]

The open-labeled RCT by Balcells et al. mentioned limitations that might also apply in other studies. The NAbs were not determined in donor plasma before transfusion. Evidence regarding the dose of CP needed to sufficiently increase antibodies to neutralize the virus was limited. The study found no significant difference in seropositivity conversion rates (measured by IgG titer at baseline, day 3, and day 7) in the early plasma and the deferred plasma group, suggesting insufficient dose. The study was also open-labeled; therefore, cointerventions such as steroids may unintendedly have influenced outcomes. They found it challenging to find patients admitted to the hospital in the early stages of the disease and stated that CP dose (volume and antibody titer levels) must be optimized.[15]

The two reported cases showed patients with severe COVID-19 with clinical conditions not improved after days of standard medical therapy alone, including an intravenous corticosteroid. In such patients, the use of additional therapy such as convalescent plasma (CP) therapy, IL6-inhibitors, and intravenous immunoglobulin therapy (IVIG) should be considered. However, IL-6 inhibitors and IVIG are not readily available and costly. Additionally, both patients were still within ten days of symptom onset. Considering these factors, we decided to administer convalescent plasma, with a dose of 400 mL divided into two administrations of 200 mL each on the same day, to both patients. The CP's antibody titer level was not known since the donor criteria did not include its measurement. Not more than one day after receiving CP, both patients showed clinical improvements, including decreased shortness of breath and a lowered supplemental oxygen needed to maintain SpO_2. The first patient, who had a high circulating IL-6 level (207 pg/mL) and initial SpO_2 85% with 15 LPM oxygen with standard therapy, could maintain SpO_2 of 96% with 15 LPM oxygen via NRM after CP therapy. The second patient who needed HFNC to reach SpO_2 of 93-94%, after CP therapy, only needed supplemental oxygen of 15 LPM via NRM to maintain SpO_2 of 98%. Both patients' oxygen supplementation could be titrated down to nasal cannula 3 to 4 days after CP therapy.

CONCLUSION

In conclusion, the results of studies assessing the effectiveness of convalescent plasma (CP) therapy in improving the clinical outcomes of patients with COVID-19 were still inconclusive. Differences in dose, interval of administration, time of administration since symptom onset, plasma titer level of convalescent plasma therapy may have caused the difference in outcomes of the patients. Limited data, inconsistency in details, and varying cointerventions may also have affected the studies' results. More than a hundred ongoing clinical trials studying CP therapy in COVID-19 patients may yield different and more conclusive results. Nevertheless, convalescent plasma therapy is still a widely and rapidly available, low-cost, and safe option of additional therapy in COVID-19 patients whose clinical conditions have not yet improved with standard medical therapy alone. CP therapy will continue to be used and studied in the COVID-19 pandemic, especially in countries where other high-cost therapies such as IL-6 inhibitors and IVIG might not be available.

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REFERENCES

1. Rajendran K, Krishnasamy N, Rangarajan J, Rathinam J, Natarajan M, Ramachandran A. Convalescent plasma transfusion for the treatment of COVID-19: Systematic review. J Med Virol. 2020;92:1475–83. doi: 10.1002/jmv.25961
2. Burhan E, Susanto AD, Nasution SA, et al. Pedoman tatalaksana COVID-19. 3rd ed. In: Burhan E, Susanto AD, Isbaniah F, et al., editor. Jakarta: PDPI, PERKI, PAPDI, PERDATIN, IDAI, IDAI; 2020.
3. Zuo Z, Wu T, Pan L, et al. Modalities and mechanisms of treatment for Coronavirus disease 2019. Front Pharmacol. 2021;11:1–19. doi: 10.3389/fphar.2020.583914
4. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271-280.e8. doi: 10.1016/j.cell.2020.02.052
5. Franchini M, Liumbruno GM. Convalescent plasma for the treatment of severe COVID-19. Biol Targets Ther. 2021;15:31–8. doi: 10.2147/btt.s272063
6. Rojas M, Rodríguez Y, Monsalve DM, et al. Convalescent plasma in Covid-19: Possible mechanisms of action. Autoimmun Rev [Internet]. 2020;19:102554. Available from: https://doi.org/10.1016/j.autrev.2020.102554 doi: 10.1016/j.autrev.2020.102554
7. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. J Clin Invest. 2020;130:2757–65. doi: 10.1172/JCI138745
8. Pérez-Cameo C, Marín-Lahoz J. Serosurveys and convalescent plasma in COVID-19. E Clinical Medicine. 2020;23:10–1. doi: 10.1016/j.eclinm.2020.100370
9. Atal S, Fatima Z. IL-6 Inhibitors in the treatment of serious COVID-19: A promising therapy? Pharmaceut Med [Internet]. 2020;34:223–31. Available from: https://doi.org/10.1007/s40290-020-00342-z doi: 10.1007/s40290-020-00342-z
10. Janiaud P, Axfors C, Schmitt AM, et al. Association of convalescent plasma treatment with clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. JAMA - J Am Med Assoc. 2021;325:1185–95. doi: 10.1001/jama.2021.2747
11. Simonovich VA, Burgos Pratx LD, Scibona P, et al. A randomized trial of vonvalescent plasma in Covid-19 severe pneumonia. N Engl J Med. 2020;619–29. doi: 10.1056/nejmoa2031304
12. Libster R, Pérez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe Covid-19 in older adults. N Engl J Med. 2021;384:610–8. doi: 10.1056/nejmoa2033700
13. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening covid-19: a randomized clinical trial. JAMA - J Am Med Assoc. 2020;324:460–70. doi: 10.1001/jama.2020.10044
14. Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P. Convalescent plasma in the management of moderate covid-19 in adults in India: Open label phase II multicentre randomised controlled trial (PLACID Trial). BMJ. 2020;371:1–10. doi: 10.1136/bmj.m3939
15. Balcells ME, Rojas L, Le Corre N, et al. Early versus deferred anti-SARS-CoV-2 convalescent plasma in patients admitted for COVID-19: A randomized phase II clinical trial. PLoS Med. 2021;18:e1003415. doi: 10.1371/journal.pmed.1003415
16. Abolghasemi H, Eshghi P, Majid A, et al. Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: Results of a multicenter clinical study. Transfus Apher Sci. 2020;59(5): 102.
17. Joyner MJ, Wright RS, Fairweather DL, et al. Early safety indicators of COVID-19 convalescent plasma in 5,000 patients. medRxiv. 2020;1–26. doi: 10.1101/2020.05.12.20099879
18. Joyner MJ, Bruno KA, Klassen SA, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. Mayo Clin Proc. 2020;95:1888–97. doi: 10.1016/j.mayocp.2020.06.028
19. U.S. National Library of Medicine. Clinicaltrials.gov [Internet]. 2021 [cited 2021 Mar 29]. Available from: https://www.clinicaltrials.gov/ct2/results?recrs=&cond=Covid19&term=convalescent+plasma&cntry=&state=&city=&dist=

The Comparison of Point Prevalence Survey (PPS) and Gyssens Flowchart Approach on Antimicrobial Use Surveillance in Indonesian National Referral Hospital

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ABSTRACT

The antimicrobial resistance (AMR) rate in Indonesia is steadily rising, despite the existing national action plan in 2014. In line with the Global Action Plan on AMR, proper surveillance on antimicrobial usage and resistance are needed. At present, antimicrobial surveillance (AMS) data in Indonesia is heterogeneous, fragmented, and localized. The common method of antimicrobial surveillance (AMS) in referral hospitals is by implementing Gyssens flowchart during Antimicrobial Resistance Control Program Committee clinical rounds. However, the recent method of AMS with Point Prevalence Survey (PPS) offers many advantages include its concise and simple protocol, large data collection, shorter required time, comprehensive data outcomes, real-time data, and standardized parameters. In low-middle income countries such as Indonesia with its restricted resources in AMS, PPS is superior compared to the 'traditional' hospital clinical round in generating representative and homogenous outcomes that can be compared to data from other centers worldwide.

Keywords**:** *Antimicrobial, point prevalence survey, Gyssens flowchart, Indonesia.*

INTRODUCTION

Indonesia, the fourth most populous country in the world, underwent a rapid increase in infectious diseases and antimicrobial usage (AMU) up to 54-84%, therefore potentiating rise in antimicrobial resistance (AMR).[1–6] Despite the existing national action plan toward AMR in 2014, the AMR rate in Indonesia remained high[4,5] and caused an increase in mortality, length of hospital stays, and hence costs of hospitalization. Furthermore, the imbalance of newer antimicrobial invention was lagging

behind intense microbial mutation.[7,8]

Consistent with the 2011 Jaipur Declaration[9] which aimed to tackle AMR, in 2015, World Health Assembly adopted the Global Action Plan on Antimicrobial Resistance, which concentrated on global surveillance and research.[10] Numbers of regional surveillance programs had been undergone mostly in high-income countries (HICs), such as the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR)[11], European Point Prevalence Survey by European Centre for Disease Prevention and Control (ECDC)[12], European Antimicrobial Resistance Surveillance Network (EARS-Net)[13], and Latin American Antimicrobial Resistance Surveillance Network (ReLAVRA)[14]. Regardless of the success of data collection over years, these networks had a variety of standards for methods, data-sharing, and coordination at local and global levels. Therefore in 2015, World Health Organization (WHO) established Global Antimicrobial Resistance Surveillance System (GLASS)[15] and consequently Global Point Prevalence Survey (Global-PPS) which encompassed over 80 participating countries and more than 800 participating hospitals.[16]

Unfortunately, AMU surveillance data in Indonesia are heterogeneous, fragmented, sporadic, with most only performed by referral hospitals and did not connect to the national network.[2] This data was commonly obtained by implementing Gyssens flowchart[17], either through Antimicrobial Resistance Control Program (Program Pengendalian Resitensi Antimikroba/ PPRA) Committee clinical rounds or incidental antimicrobial audit researches.[4,18–22] The recently popular surveillance method by PPS offers a simpler method and a more thorough data collection on AMU and AMR, thus guided local and national ASP.[23,24] Overseas studies were familiar with PPS[3,12,24–26] however Indonesia had only carried out one antimicrobial surveillance research up to now.[27]

This review aims to observe the comparison of Gyssens flowchart application to PPS for AMU surveillance method in Indonesian National Referral Hospital.

METHOD OF ANTIMICROBIAL SURVEILLANCE IN INDONESIA

Regulation on Antimicrobial Resistance Control Program in Indonesia was authorized in 2015, which mainly focused on microbial resistance and antimicrobial surveillance.[28] It recommended the extraction of antimicrobial quantity data from medical or pharmacy records and quality data from antimicrobial usage form. Data was analyzed afterward using Gyssens flowchart by Antimicrobial Resistance Control Program panelists during the clinical round. Any disagreement on antimicrobial assessment will be discussed among panelists, consisted of infectious disease specialists, pharmacologists, clinical microbiologists, clinical pathologists, therapy-pharmacists, clinical pharmacists, nurses, attending physician, and Infection Prevention Control (IPC) members.[28–30]

This method of surveillance was widely implemented in referral hospitals, one of which was Cipto Mangunkusumo National Referral Hospital in Jakarta. The clinical round was usually performed weekly among all clinical departments proposing one or two complicated clinical cases. These cases were discussed for 2-3 hours by panelists who examined the quality of antimicrobial prescribing with Gyssens flowchart. The outcomes of the analysis were commonly formed as an assessment of antimicrobial conformity with the clinical case and also further recommendations toward the patient.

Recently in 2020, PPS on antimicrobial prescribing was also performed in Cipto Mangunkusumo Hospital.[31] Data collection from patients on antimicrobial consumption was completed in 12 days by five field enumerators. Enumerators were medical doctors or hospital staff who received one-day training in data collection guidelines.[5] As different from the previous method, PPS succeeded in gathering a larger amount of data from 244 patients who were on antimicrobial consumption. The outcomes of this PPS were characteristics of adult inpatients, antibiotic usage profile, and microbial resistance profile.

GYSSENS FLOWCHART

Criterion on the antimicrobial prescribing quality audit was developed by Kunin, et al. in 1973. This criterion was applied and performed by infectious disease specialists, and then further evolved and modified by other authors throughout time. In 1992, Gyssens flowchart was developed to assess the quality of individual antimicrobial prescriptions. The flowchart is read from top to bottom to evaluate the process outcome (**Figure 1**).[32]

Gyssens flowchart was ideally performed by experts handling authoritative criteria or comparison of agreement with local, national, or international guidelines or standards. The outcomes measurements were explained in terms: data not sufficient, not indicated, not appropriate (efficacy, toxicity, cost, broadness of spectrum), not appropriate in the duration of treatment, not appropriate in dosage (dose, dose interval, administration, and not appropriate in timing (too late/ early). To conclude this, experts evaluation was needed.[32] Moreover, Gyssens flowchart was commonly assessed retrospectively, hence missing medical record data was common.[33]

POINT PREVALENCE SURVEY (PPS)

Point prevalence survey is a cross-sectional study that identifies a number of people with

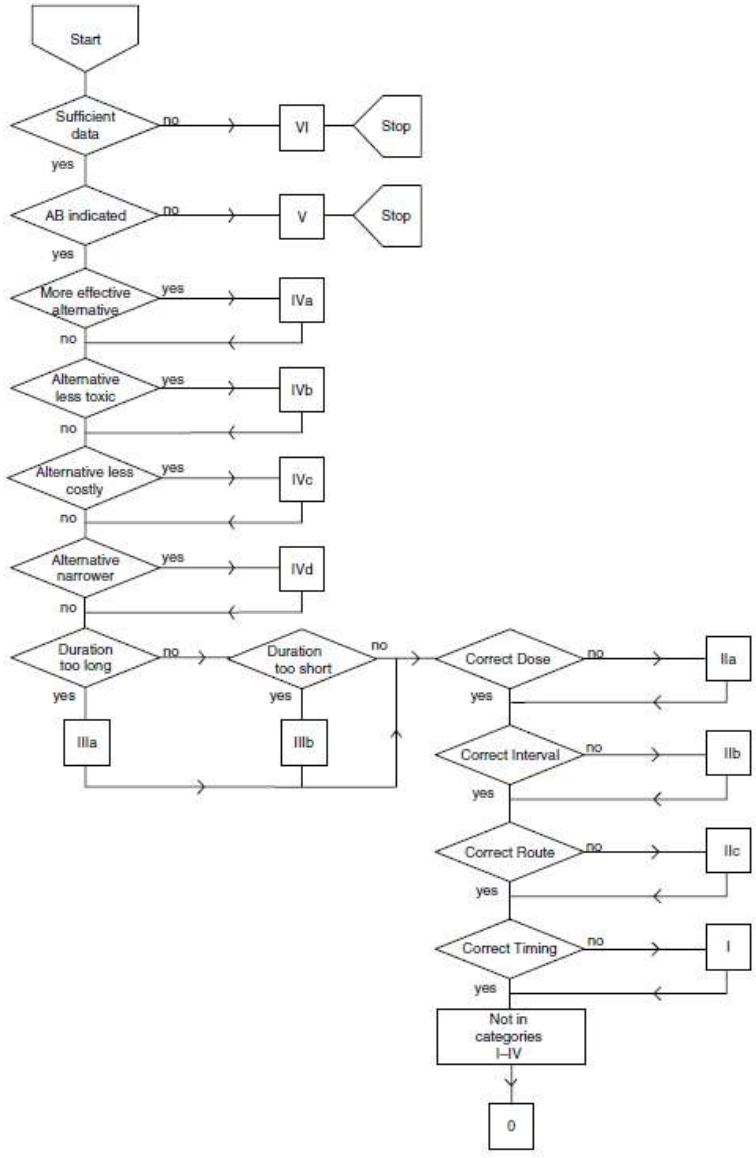

Figure 1. Gyssens flowchart.

disease or condition at one point in time.[34] One widely known protocol by Global PPS WHO performed data collection by retrieving information at ward level (as the denominator) and patient level (as the numerator) within 4 weeks. The departments involved in the survey were grouped into the medical and surgical adult department, adult intensive care units (ICUs), pediatric and neonatal department. Each ward will be alternately assessed in only one day. A multidisciplinary team will collect the data at 8 a.m. from all inpatients admitted on the ward and on the consumption of antimicrobial agents.[35]

Point prevalence survey gave snapshot real-time data on basic information from medical records and associated patient documentations. The included data were the type of ward and available beds, the number of admissions and antimicrobial consumption, patient's characteristics (age, body mass index, gender), biomarkers, culture (blood, urine, wound, sputum), antimicrobial data (include duration, start and stop date, indication, route, diagnosis, frequency, guideline compliance, review date, type of treatment), and any additional variables due to research preference. Accordingly, PPS was able to summarize quantitative and qualitative data on the prevalence of AMU, types of infection by sites and by location (community, hospital), and also quality indicators of antimicrobial, within a short duration of the study.[35,36] Figure 2 shows the concise flowchart of PPS.

Prevalence surveys on infections had been published since the 1970s in Italy and Sweden.[37,38] Hereinafter, the surveillance methods evolved into PPS on infection and related antimicrobial consumption.[39] Within a decade, national PPS had been vastly implemented in HICs.[26,40–43] Recently, PPS has been the latest trend in antimicrobial surveillance, not only because it allowed a thorough extraction of data, but also was able to generate uniform and comparable outcomes among one study to another, especially in the availability of global PPS protocol by WHO.[24,26,44]

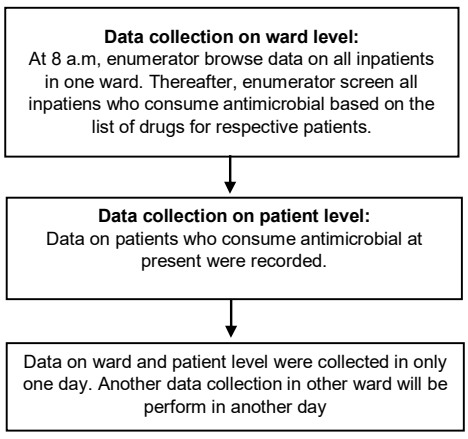

Figure 2. Flowchart of point prevalence survey (PPS) method

In years, LMICs such as Indonesia struggled with data collection and analysis on antimicrobial consumption, due to the high workload and level of resources needed for regular monitoring. PPS proposed a simpler method, therefore it could be repeatedly performed to maintain sustainability in surveillance.[26] The first PPS in Indonesia was studied by Limato, et al.[27] and was published in 2021.

POINT PREVALENCE SURVEY (PPS) VERSUS GYSSENS FLOWCHART

Based on multicenter surveys in six referral hospitals in Jakarta[27], we observed that PPS was a concise yet comprehensive method for antimicrobial surveillance in referral hospitals in Indonesia. The key of PPS method was in its study protocol which was easy and simple to be performed, even by general practitioners.[26,35] In comparison, hospital clinical rounds used Gyssens flowchart that had to be discussed among a group of multi-department experts, consequently demand bigger effort and resources.[32]

Point prevalence survey method was also capable of gathering a large database within a brief duration of the study. Surveys in two large teaching and referral hospitals in Jakarta were completed within only 12 days, respectively. In total, the duration of surveys in six referral hospitals was 40 days, conducted by 3 – 5 field enumerators, and comprised of 993 patients on antibiotics. In general, every enumerator took approximately 20-25 minutes for respective patients and was responsible for 6 – 8 patient's data every day.[27] This method of antibiotic audit resulted in faster and larger data collection compared to Gyssens flowchart implementation during clinical rounds, which was only able

Table 1. Comparison of point prevalence survey and Gyssens flowchart.

Point Prevalence Survey	Gyssens flowchart
- Concise and simple protocol study can be applied by trained general practitioners - Large data collection within a brief duration - Required time for data collection of one case/ patient was approximately 15-20 minutes - Outcomes of quantitative and qualitative data were available (included antimicrobial quality indicators) - Standardized protocols were available, therefore data on outcomes were mostly homogenous - Analysis of real-time data	- Require discussion from multi-department experts: infectious disease specialists, pharmacologists, clinical microbiologists, clinical pathologists, therapy-pharmacists, clinical pharmacists, nurses, Infection Prevention Control (IPC) members - Required time for case evaluation of one case/patient was approximately 60-120 minutes - Outcome focused on quality of antimicrobial prescribing, without data on other quality indicators - Analysis of retrospective data

to evaluate approximately 11-12 cases in 90 days. The outcome data in PPS was also comprehensive, in which it included patients' baseline characteristics, the profile of antibiotic use (prevalence, type, purpose, indication), the profile of culture and resistance, and also the presence and compliance to clinical pathway among a group of patients.[35,36] An alike data was not available from clinical rounds with Gyssens flowchart practice, albeit clinical rounds were able to analyze most complex cases compared to PPS. The outcomes of clinical rounds were also usually limited to the quality analysis of antibiotic prescribing which was specific for certain cases.[32]

Another superiority of PPS over Gyssens flowchart was its homogeneity in study outcomes. In the presence of standardized protocol by WHO[35] and Global-PPS[36], many countries all over the world performed PPS by referring to these protocols, hence the outcomes of PPS were able to be accumulated and compared from one another centers.[26] In contrast, antibiotic audit data from clinical rounds were usually fewer and heterogenous among centers, therefore outcomes collection and comparison were difficult.

In addition to that, unlike PPS which collected real-time data, an antibiotic audit by clinical round evaluated retrospective data, therefore increased concern on missing outcomes.[18,19,33,45,46] On top of that, PPS was appropriate for continuous surveillance in LMICs, including Indonesia, in consideration of its simple, repeatable, relatively low-cost practice, yet resulted in comprehensive data.[47] One study in Makassar stated that lack of manpower specialized in antimicrobial surveillance was the principal obstacle in ASP, therefore PPS supposedly ideal to overcome it.[30] **Table 1** shows differences in antimicrobial audit between PPS dan Gyssens flowchart.

CONCLUSION

Point prevalence survey was an appropriate method for antimicrobial prescribing audit and surveillance in LMIC such as Indonesia. Audit with PPS offered a concise and simple method, yet resulted in comprehensive data on quantity and quality of antimicrobial use. This method was also superior compared to the 'traditional' hospital clinical round in generating representative and homogenous outcomes that can be compared to data from other centers worldwide. Based on our analysis, we emphasize the importance of routine antimicrobial surveillance with PPS method at referral hospitals in Indonesia. The data from PPS had been proven useful for many institutions and countries, therefore it is time for Indonesia to perform adequate antimicrobial surveillance.

REFERENCES

1. Klein EY, Van Boeckel TP, Martinez EM, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci USA. 2018;115(15):E3463–70.
2. Parathon H, Kuntaman K, Widiastoety TH, et al. Progress towards antimicrobial resistance containment and control in Indonesia. BMJ. 2017;358:31–5.
3. Ansari F, Erntell M, Goossens H, Davey P. The european surveillance of antimicrobial consumption (ESAC) point-prevalence survey of antibacterial use in 20 European hospitals in 2006. Clin Infect Dis. 2009;49(10):1496–504.
4. Hadi U, Duerink DO, Lestari ES, Nagelkerke NJ, Keuter M, Huis D. Audit of antibiotic prescribing in two governmental teaching hospitals in Indonesia. Clin

Microbiol Infect. 2008;14(7):698–707. Available from: http://dx.doi.org/10.1111/j.1469-0691.2008.02014.x
5. Guterres H, Nelwan EJ, Chen LK, Nugroho P. Point prevalence survey of antibiotics use among inpatient in national referral hospital in Indonesia. Int J Infect Dis. 2021;101:2021.
6. Lestari E, Severin J, Verbrugh H. Antimicrobial resistance among pathogenic bacteria in Southeas Asia. Southeast Asian J Trop Med Public Heal. 43(2):385–422.
7. World Health Organization. Antimicrobial resistance: global report on surveillance. 2014;35–41.
8. The Review on Antimicrobial Resistance (Chaired by Jim O'Neill). Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. 2016;(May):1–80.
9. World Health Organization. JAIPUR: Jaipur declaration on antimicrobial resistance. 2011; Available from: http://www.searo.who.int/%0Aentity/world_health_day/media/2011/whd-11_amr_jaipur_declaration_.pdf
10. World Health Organization. Global Action Plan on Antimicrobial Resistance. 2015;
11. World Health Organization (WHO). Central Asian and Eastern European Surveillance of Antimicrobial Resistance. 2018;39. Available from: http://www.euro.who.int/pubrequest%0Ahttp://fossportal/departments/Management/Annual Report/Annual Report English/FOSS AS Annual Report 2017.pdf
12. European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011–2012. 2019;3.1.2.
13. European Centre for Disease Prevention and Control. European Antimicrobial Resistance Surveillance Network (EARS-Net) [Internet]. [cited 2021 Mar 7]. Available from: https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net
14. Pan American Health Organization. Latin American Network for Antimicrobial Resistance Surveillance - ReLAVRA [Internet]. [cited 2021 Mar 7]. Available from: https://www.paho.org/en/topics/antimicrobial-resistance/latin-american-network-antimicrobial-resistance-surveillance
15. World Health Organization (WHO). Global Antimicrobial Resistance Surveillance System. WHO. 2015;36.
16. Global PPS. Global point prevalence survey: scientific publications. 2020; Available from: %0Awww.global-pps.com
17. Gyssens I, van den Broek P, Kullberg B, van der Meer J. Optimizing antimicrobial therapy: a method for antimicrobial drug use evaluation. J Antimicrob Chemother [Internet]. 1992;30:724–7. Available from: https://academic.oup.com/jac/article-abstract/30/5/724/849889?redirectedFrom=fulltext
18. Magdalena R, Bachtiar A. Antimicrobial Resistance Control Program on the Rational Use of Antibiotics in Eka Hospital Pekanbaru, Indonesia. Proc Int Conf Appl Sci Heal. 2018;(3):374–80.
19. Karuniawati H, Yulianti T, Aini DK, Nurwienda FI. Impact of antimicrobial stewardship program on the use of antibiotics in pneumonia patients at teaching hospital in surakarta Indonesia. Int J Appl Pharm. 2021;13(special issue 1):20–3.
20. Yoanitha N, F. Wirakusumah F, Arev Sukarsa MR. Gambaran Rasionalitas Penggunaan Antibiotik berdasarkan Kriteria Gyssens di Bangsal Obstetri dan Ginekologi RSUP Dr. Hasan Sadikin Bandung. Indones J Obstet Gynecol Sci. 2018;1(2):111–6.
21. Setiawan S, Widyati W, Harijono P. Antibiotic Usage Profile after Antibiotic Stewardship Program Implementation in Intensive Care Unit of dr. Ramelan Naval Hospital Surabaya. Indones J Clin Pharm. 2018;7(1):30–7.
22. Komite Pengendalian Resistensi Antimikroba RSCM. Kajian resistensi antimikroba di rumah sakit. Jakarta; 2017.
23. Aldeyab MA, Kearney MP, McElnay JC, Magee FA, Conlon G, Gill D, et al. A point prevalence survey of antibiotic prescriptions: Benchmarking and patterns of use. Br J Clin Pharmacol. 2011;71(2):293–6.
24. Vandael E, Latour K, Goossens H, Magerman K, Drapier N, Catry B, et al. Point prevalence survey of antimicrobial use and healthcare-associated infections in Belgian acute care hospitals: Results of the Global-PPS and ECDC-PPS 2017. Antimicrob Resist Infect Control. 2020;9(1):1–13.
25. Porto APM, Goossens H, Versporten A, Costa SF. Global point prevalence survey of antimicrobial consumption in Brazilian hospitals. J Hosp Infect. 2020;104(2):165–71.
26. Frenette C, Sperlea D, German GJ, Afra K, Boswell J, Chang S, et al. The 2017 global point prevalence survey of antimicrobial consumption and resistance in Canadian hospitals. Antimicrob Resist Infect Control. 2020;9(1):1–9.
27. Limato R, Nelwan EJ, Mudia M, Brabander J de, Guterres H, Enty E, et al. A multicentre point prevalence survey of patterns and quality of antibiotic prescribing in Indonesian hospitals. 2021;
28. Kementerian Kesehatan Indonesia. Peraturan Menteri Kesehatan Republik Indonesia nomor 8 tahun 2015 tentang program pengendalian resistensi antimikroba di rumah sakit. 2015; Available from: www.bphn.go.id
29. Tambunan T. Kebijakan pengendalian resistensi antimikroba. 2019;4–7. Available from: http://parceiro.vitalatman.com.br/cgi-bin/content/view.php?data=kebijakan_pengendalian_resistensi_antimikroba&filetype=pdf&id=3e98db39a5ab9e39f94acd5e9f47c5c2
30. Rukmini, Siahaan S, Sari ID. Analisis implementasi kebijakan program pengendalian (studi kasus di RSUP Dr . Wahidin Sudirohisudo, Makassar). Bul Penelit Sist

Kesehat. 2019;22(2 April 2019):106–16.
31. Guterres H. Point prevalence survey penggunaan antibiotik pada pasien dewasa rawat inap di RSUPN Cipto Mangunkusumo. Thesis. Universitas Indonesia; 2020.
32. Gyssens IC. Audits for monitoring the quality of antimicrobial prescriptions. Antibiot Policies Theory Pract. 2005;197–226.
33. Katarnida SS, Murniati D, Katar Y. Evaluasi penggunaan antibiotik secara kualitatif di RS penyakit infeksi Sulianti Saroso, Jakarta. Sari Pediatr. 2016;15(6):369.
34. Centers for Disease Control and Prevention. Introduction to public health surveillance [Internet]. 2021 [cited 2021 Mar 15]. Available from: https://www.cdc.gov/training/publichealth101/surveillance.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fpublichealth101%2Fsurveillance.html#:~:text=Public health surveillance is "the,health surveillance systems and methods.
35. World Health Organization (WHO). WHO methodology for point prevalence survey on antibiotic use in hospitals. 2018; Available from: http://apps.who.int/iris
36. Goossens H. Protocol: Global Point Prevalence Survey of antimicrobial consumption and resistance. 2020;(May):2–10.
37. Moro M, Stazi M, Marasca G, Greco D, Zampieri A. National prevalence survey of hospital-acquired infections in Italy, 1983. J Hosp Infect. 1988;8:72–85.
38. Bernander S, Hambraeus A, Myrback K, Nystrom B, Sundelof BO. Prevalence of hospital-associated infections in five Swedish hospitals in November 1975. Scand J Infect Dis. 1978;10(November 1975):66–70.
39. Vincent JL, Bihari DJ, Suter PM, et al. The prevalence of nosocomial infection in intensive care units in Europe: results of the European prevalence of infection in intensive care (EPIC) study. JAMA J Am Med Assoc. 1995;274(8):639–44.
40. Gharbi M, Doerholt K, Vergnano S, et al. Using a simple point-prevalence survey to define appropriate antibiotic prescribing in hospitalised children across the UK. BMJ Open. 2016;6(11):1–8.
41. Xie D shuang, Xiang L li, Li R, Hu Q, Luo Q qin, Xiong W. A multicenter point-prevalence survey of antibiotic use in 13 Chinese hospitals. J Infect Public Health [Internet]. 2015;8(1):55–61. Available from: http://dx.doi.org/10.1016/j.jiph.2014.07.001
42. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. Lancet. 2005;365(9459):579–87.
43. Lee C, Walker SAN, Daneman N, et al. Point prevalence survey of antimicrobial utilization in a Canadian tertiary-care teaching hospital. J Epidemiol Glob Health [Internet]. 2015;5(2):143–50. Available from: http://dx.doi.org/10.1016/j.jegh.2014.06.003
44. Willemsen I, Groenhuijzen A, Bogaers D, Stuurman A, Van Keulen P, Kluytmans J. Appropriateness of antimicrobial therapy measured by repeated prevalence surveys. Antimicrob Agents Chemother. 2007;51(3):864–7.
45. Yuniar I, Karyanti MR, Tambunan T, Rizkyani NA. Evaluasi penggunaan antibiotik dengan kartu monitoring antibiotik Gyssens. Sari Pediatr. 2016;14(6):384.
46. Sitompul F, Radji M, Bahtiar A. Evaluasi penggunaan antibiotik dengan metode Gyssens pada pasien stroke rawat inap di RSUD Koja secara retrospektif (Periode KJS dan BPJS). J Kefarmasian Indones. 2016;6(1):30–8.
47. World Health Organization. Antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries: a WHO practical toolkit [Internet]. Vol. 1, JAC-Antimicrobial Resistance. 2019. Available from: https://academic.oup.com/jac/article-abstract/30/5/724/849889?redirectedFrom=fulltext