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Factors Associated With Negative Conversion Of Rt-Pcr Covid-19

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ABSTRACT

The coronavirus disease 2019 (COVID-19) was declared an International Public Health Emergency (PHEIC) on 30 January 2020 by WHO. Many studies have analysis the clinical, radiological, laboratory profile, and treatment strategies of COVID-19, but there are still no studies on the factors that influence the negative conversion (NCT) of RT-PCR for COVID-19 in Indonesia. This type of study was cross-sectional and included 25 confirmed COVID-19 inpatients at Aminah Hospital Sidoarjo with a median NCT 8 days from initial hospital admission. Men need longer conversion time than women (p-value 0.044). The severity of COVID-19 and patient comorbidities were significantly associated with NCT of COVID-19 RT-PCR (p-value 0.035). Patients with severe severity at baseline had a longer conversion time (9 days) than those with moderate (8 days) and mild (7 days). Patients with comorbidities (diabetes, hypertension, or both) required longer NCT of RT-PCR (9 days) than those without comorbidities (7 days). The results of the log-rank test of age, leukocyte count, platelets, hemoglobin, lymphocyte and granulocyte percentages did not have a significant relationship with NCT of RT-PCR. Gender, case severity, and comorbid history of COVID-19 patients are factors that are associated with COVID-19 RT-PCR conversion time.

Keywords: Factor, Conversion, RT-PCR COVID-19

INTRODUCTION

On December 12, 2019, 27 cases of unknown cause of pneumonia were reported in Wuhan, Hubei Province, China (WMHC, 2020). On February 11, 2020, the World Health Organization (WHO) officially named this disease as corona virus disease 2019 (COVID-19) with an etiology that has been identified and named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2). As the pandemic continues to grow, COVID-19 has spread rapidly to approximately 210 countries and regions around the world and becoming a global threat with the highest risk impact (Our World in Data, 2020).

Many studies have focused on analyzing the epidemiology, clinical profile, laboratory, radiological characteristics, and treatment strategies (Ai et al., 2020; Chen et al., 2020; Cortegiani et

al., 2020; Huang et al., 2020; Xing. et al., 2020; Zhu et al., 2020). However, there is still no research on the factors that affect the negative conversion time of the COVID-19 RT-PCR in Indonesia.

METHODS

This study is a cross-sectional study and data collection retrospectively through the medical records of COVID-19 isolation patients in the period from July to August 2020 at Aminah Hospital Sidoarjo. The variables used were the length of time for conversion of positive to negative RT-PCR, name, age, gender, comorbidities, respiratory rate (RR), oxygen saturation on admission, and laboratory results. The collected data are then organized for analysis. To analyze the time period from the initial positive to negative PCR results by looking at the variables of the influencing factors, the Kaplan Meier Analysis and Survival Curve test was used (Hu et al., 2020).

The inclusion criteria in this study were: (1) all COVID 19 patients with mild to critical symptoms, (2) positive PCR at the time of initial hospital admission, and (3) conversion to negative before discharge from the hospital. Exclusion criteria in this study were (1) patients who were PCR negative at the time of initial hospital admission, (2) patients who were referred to other hospitals before conversion of PCR results occurred, (3) patients who died before conversion of PCR results occurred, and (4) patients who still get positive results before being discharged.

Confirmed case of COVID-19 is based on the gold standard examination, which is real-time reverse transcription-polymerase-chain-reaction (RT-PCR) analysis for SARS CoV-2 on nasopharyngeal swabs. RT-PCR conversion time was defined as the duration between the first day of positive RT-PCR result until the first day of negative RT-PCR result with one test of RT-PCR. In contrast to previous studies which the RT-PCR conversion time was defined as the duration from the first day of positive RT-PCR result till the first day showing negative on nucleic acid testing successively (Hu et al., 2020). According to the latest WHO recommendations, the criteria for completing the isolation of COVID-19 patients do not require repeat PCR. Symptomatic patients are declared cured or have completed isolation provided that 10 days after the onset of complaints plus a minimum of 3 days without complaints and for asymptomatic patients are 10 days after being confirmed positive for SARS-CoV-2 (WHO, 2020). In the assessment of severity on admission, critical cases are patients with Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, severe cases were defined accordingly as patients who met one of the following criteria: respiratory rate 30 breaths/min; oxygen saturation 93% with room air; and severe respiratory distress. Patients with moderate cases are patients with clinical signs of pneumonia (cough; fever; shortness of breath; and rapid breathing) with oxygen saturation 93% with room air. Mild cases were defined as symptomatic patients without evidence of viral pneumonia or without hypoxia, presenting with fever, cough, fatigue, anorexia, shortness of breath, myalgia, nausea, vomiting, and anosmia (PDPI et al., 2020).

SARS-CoV-2 detection in respiratory specimens was carried out by laboratory personnel in biosafety level 2 facilities using a kit that has been approved by the Indonesian Ministry of Health. Nasopharyngeal swab was performed when the patient first came to the hospital and 3 days after the general condition improved before being considered for discharge.

In this study, the negative conversion of viral RNA from the initial positive RT-PCR result to the negative RT-PCR result, as time to event data, was the outcome measure presented by the Kaplan-Meier curve. Then proceed with the log-rank test to test whether or not there are differences in the Kaplan-Meier survival curve on variables that have two or more categories (Kleinbaum and Klein, 2005). There are 5 factors studied, namely gender, age, comorbid variables, type of case severity, and laboratory.

RESULTS AND DISCUSSION

A total of 25 hospitalized patients with COVID-19 met the inclusion criteria in this study. The characteristics of COVID-19 patients being treated can be identified based on gender, age, comorbidities, type of case severity, and laboratory results. There were 36% (9) male patients with confirmed COVID-19 and 64% (16) were female. The majority of patients are between 30 – 39 years old 32% (8). More than half of the patients (14) had no comorbidities, and 44% (11) of the patients had comorbidities, such as type 2 diabetes mellitus 24% (6), hypertension 1 (4%), or both 16% (4). Most of the patients presented on their first admission to the hospital with moderate condition 52% (13) and only 1 patient was in critical condition. More detailed information is listed in table 1.

Tabel 1. Clinical characteristics of 25 patients with confirmed SARS-CoV-2 infection

Variable	Total (%)	Median		*P* Value
Sex		≤8 days (n=15)	>8 days (n=10)	0,044
Male	9 (36%)	3 (20%)	6 (60%)	
Female	16 (64%)	12 (80%)	4 (40%)	
Age (years)				0,254
20 – 29	5 (20%)	2 (13,3%)	3 (30%)	
30 – 39	8 (32%)	6 (40%)	2 (20%)	
40 – 49	4 (16%)	4 (26.7%)	0 (0%)	
50 – 59	4 (16%)	3 (20%)	1 (10%)	
≥60	4 (16%)	0 (0%)	4 (40%)	
Comorbidities				0,035
No	14 (56%)	7 (46.7%)	8 (80%)	
Yes	11 (44%)			

Type 2 Diabetes Mellitus (T2DM)	6(24%)	4 (26.7%)	2 (20%)	
Hypertension	1 (4%)	1 (6.6%)	0 (0%)	
DMT2 and hypertension	4 (16%)	4 (26.7%)	0 (0%)	
Severity				0,027
Moderate	13 (52%)	6 (40%)	7 (70%)	
Severe	11 (44%)	8 (53.3%)	3 (30%)	
Critical	1 (4%)	1 (6.6%)	0 (0%)	
Laboratory				
Leucocyte count				0,700
< 4.000	5 (20%)	4 (26.7%)	1 (10%)	
4.000 - 11.000	19 (76%)	10 (66.7%)	9 (90%)	
>11.000	1 (4%)	1 (6.6%)	0 (0%)	
Lymphocyte percentage				0,188
< 25.0	20 (80%)	13 (86.6%)	7 (70%)	
25.0 - 33.0	3 (12%)	2 (13,3%)	1 (10%)	
>33.0	2 (8%)	0 (0%)	2 (20%)	
Granulocytes percentage				0,667
< 54.0	1 (4%)	0 (0%)	1 (10%)	
54.0 – 62.0	2 (8%)	2 (13,3%)	0 (0%)	
>62.0	22 (88%)	14 (93.3%)	8 (80%)	
Haemoglobin (g/dL)				
<11.5	4 (16%)	2 (13,3%)	2 (20%)	0,851
11.5 – 16.0	21 (84%)	13 (86.6%)	8 (80%)	
>16.0	0 (0%)	0 (0%)	0 (0%)	
Platelets Count				0,719
< 150.000	0 (0%)	0 (0%)	0 (0%)	
150.000 – 450.000	24 (96%)	15 (100%)	9 (90%)	
>450.000	1 (4%)	0 (0%)	1 (10%)	

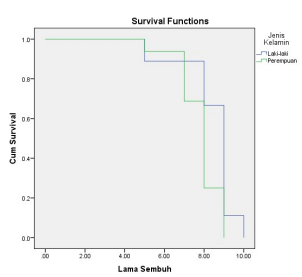
To determine the survival characteristics of COVID-19 patients based on factors that are thought to affect the RT-PCR conversion time, ages, gender, comorbidities, type of case severity, and laboratory results, the Kaplan Meier survival curve was used in accordance with previous studies (Hu et al., 2020). Based on the survival time analysis, overall it can be seen that the median RT-PCR conversion time for COVID-19 patients is 8 days. With a 95% confidence interval, it shows that COVID-19 patients will get negative RT-PCR results within 7 to 9 days. In contrast to the previous study which took 14 days to get the RT-PCR conversion to be negative (Hu et al., 2020), while from

the study of Ling et al., 2020, the average duration of virus disappearance was 9.5 days. This difference may be due to differences in patient characteristics, timing of RT-PCR sampling to symptom onset, and type of case severity.

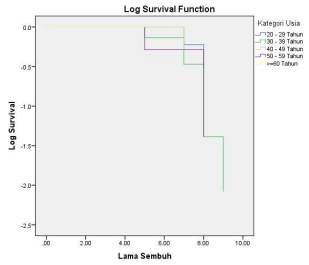
Tabel 2. Means and Medians for Survival Time

Mean[a]				Median			
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
8.040	.241	7.567	8.513	8.000	.272	7.467	8.533

a. Estimation is limited to the largest survival time if it is censored.



(a)



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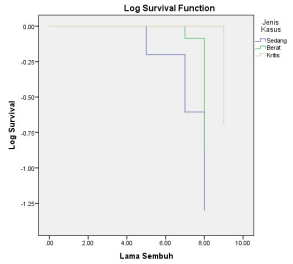

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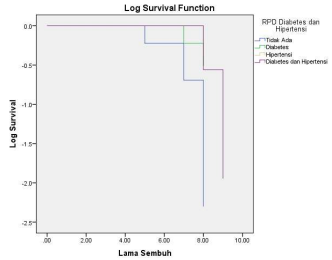

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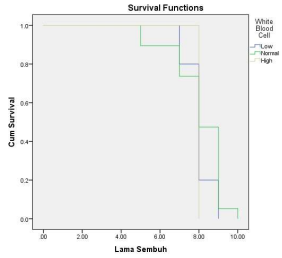
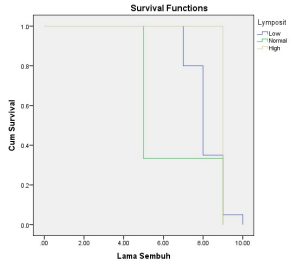


(e)



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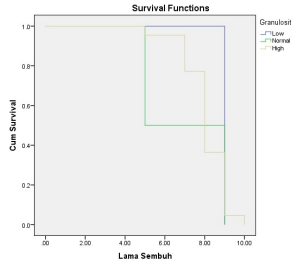
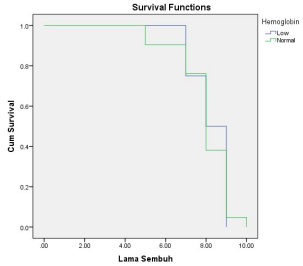


(g)



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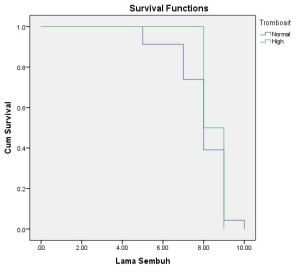

(i)

Figure 1. Length of Hospitalization (LOH) for COVID-19 Patients by (a) Gender, (b) Ages, (c) Case Severity, (d) Comorbidities, (e) Leucocyte Count, (f) Lymphocyte Percentage, (g) Granulocytes Percentage, (h) Haemoglobin Count, (i) Platelet Count

Seen in (Fig. 1a) female COVID-19 patients have faster negative conversion RT-PCR than male patients. This is evidenced in the Kaplan-Meier chart, the female sex curve is below the male curve, meaning that the female patient group has a faster chance of recovering than the male patient, so that if the patient has a faster chance of recovering, the positive cases of COVID-19 will decrease. The median survival time with 95% confidence level showed that male patients would show negative RT-PCR conversion results after 9 days, while female patients would show a negative RT-PCR conversion after 8 days. To prove the hypothesis, then proceed with log rank testing. significant results were obtained, namely with a p-value of 0.044 ($\alpha<0.05$). These results are consistent with previous studies that males have prolong shedding of SARS-CoV-2 RNA (Xu et al., 2020). The relationship between sex and SARS-CoV-2 infection is still unclear, this may be related to hormones that affect the immune system. Women are reported to produce more humoral and cellular immune reactions, so they are more resistant to certain infections (Annechien et al., 2005).

Ages 50 to 59 years had a COVID-19 RT-PCR conversion time of 7.5 days, faster than others (Fig. 1b). It is shown that the purple line representing patients aged 50-59 years is the lowest compared to the others. Age 60 years had the longest time for negative RT-PCR conversion, which was 9 days. In the log rank test, the age variable showed insignificant results with respect to the RT-PCR conversion time, p value 0.254 ($\alpha<0.05$). According to previous research, this is because there is no increase in virus clearance or shedding time with age. Older age was not an independent predictive factor of viral RNA shedding (Bennasrallah et al., 2020). However, according to another study, older people have a longer conversion time than young people, this is due to the high incidence of pre-existing comorbidities and a decrease in the function and number of T cells which results in the inability to control viral replication (Hu et al., 2020; Mo et al., 2020, Liu, K. et al., 2020).

In this study, moderate severity cases had a faster negative RT-PCR conversion time than patients with severe and critical cases. This is indicated by a blue line which represents patients with moderate case severity at the bottom compared to others. Similar to previous studies, patients with severe and critical cases had a longer conversion time compared to mild and moderate cases (Hu et al., 2020). A study of the viral dynamics showed that patients with severe COVID-19 tended to have a high viral load and a long virus-shedding period (Liu Y et al. 2020).

The patients were divided into 4 groups according to the comorbidities. Compared with patients with comorbidities, patients without comorbidities had a negative conversion time of 7 days, faster than those with comorbidities, either type 2 diabetes mellitus (T2DM) (9 days), hypertension (9 days), or both (9 days). This is evidenced by the blue line which represents patients without comorbidities at the bottom compared to others. The log-rank test showed that comorbid

variables had a significant relationship with the negative conversion time of RT-PCR. Older COVID-19 patients are indirectly associated with increased comorbidities, and tend to have decreased T cell function and number leading to a lack of ability to control viral replication (Hu et al., 2020). Moreover, age-related comorbidities may result in prolonged viral shedding among the elderly (Liu et al., 2020a). Patients with comorbidities, especially cardiovascular/cerebrovascular, respiratory system, endocrine and other more severe disease at initial hospital admission had a longer negative RT-PCR COVID-19 conversion time compared to the short negative conversion time group (<18 days). Patients with long conversion times had higher leukocyte, neutrophil, aspartate amino transferase (AST), creatinine kinase and erythrocyte sedimentation rate (ESR) counts, and had lower CD3+ CD4+ lymphocytes and albumin (Mo et al., 2020).

The laboratory inspection section showed that 76% (19) of the patients had normal leukocyte count, and 80% (20) of patients had lymphocytopenia. These results are similar to previous studies, that most COVID-19 patients have low lymphocyte counts (Mo P et al., 2020). More than half patients have high granulocytes count 88% (22). Low lymphocyte count due to SARS-CoV infection may be related to the following mechanisms. 1) SARS-CoV-2 can directly invade immune organs, continuously proliferate, and infect more lymphocytes. 2) NK cells may be consumed early than T lymphocytes as the virus attacks the immune system, leading to the failure of the replenishment of the cells, resulting in lower counts. 3) Increased expression of the inhibitory cytokine, interleukin 10 (IL-10), and the inhibitory molecules, programmed cell death protein 1 (PD-1) and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), on the cell surface and their subsequent inhibitory effects 4) Studies have also suggested that activation of the p53 signaling pathway may cause lymphocyte depletion in patients 5) SARS-CoV-2 replicate rapidly after infection and it makes mass death of epithelial and endothelial cells, vascular leakage, activates a large number of pro-inflammatory cytokines and chemokines. In other words, it leads to the so-called "cytokine storm." Overexpression of TNFα can induce T cell apoptosis by binding to tumor necrosis factor receptor 1 (TNFR1). The normal hemoglobin and platelets counts were in 84% (21) and 96% (24) of patients, respectively. All laboratory data were analyzed using the log-rank test and the results were not significant with the negative RT-PCR conversion time (Table 1). These results are supported by previous studies by Yang et al., 2021 and Mo et al., 2020 that there is no significant relationship between laboratory results of COVID-19 patients and Negative Conversion Time (NCT).

CONCLUSION

This study was able to identify that gender, case severity, and comorbid history of COVID-19 patients were factors related to the time of negative conversion RT-PCR of COVID-19. We hope that these factors can provide early clues to identify patients with longer negative RT-PCR COVID-19 conversion times and can provide more optimal treatment strategies and isolation protocols.\

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